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Aims and scope

The Journal of Associated Medical Sciences belongs to Faculty of Associated Medical Sciences (AMS), Chiang Mai University, Thailand. The journal specifically aims to provide the platform for medical technologists, radiologic technologists, occupational therapists, physical therapists, speech-language pathologists and other related professionals to distribute, share, discuss their research findings, inventions, and innovations in the areas of:

1. Medical Technology
2. Radiologic Technology
3. Occupational Therapy
4. Physical Therapy
5. Communication Disorders
6. Other related fields

Submitted manuscripts within the scope of the journal will be processed strictly following the double-blinded peer review process of the journal. Therefore, the final decision can be completed in 1-3 months average, depending on the number of rounds of revision.

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The Journal of Associated Medical Sciences aims to publish integrating research papers in areas of Medical Technology, Physical Therapy, Occupational Therapy, Radiologic Technology, and related under peer-reviewed via double-blinded process by at least two internal and external reviewers.

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Manuscripts may be submitted in the form of review articles, original articles, short communications, as an approximate guide to length:

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- **Original articles** must not exceed 15 journal pages (not more than 3,500 words), including 6 tables/figures, and 40 reference (maximum 40, recent and relevant).
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Correlation between high-sensitivity cardiac troponin I, lactate levels, and clinical outcomes in on-pump coronary artery bypass grafting

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ABSTRACT

Background: On-pump coronary artery bypass graft (CABG) causes myocardial damage and hypoperfusion. However, it is unknown how varied timings of combining serum high-sensitivity cardiac troponin I (hs-cTnI) and lactate levels in on-pump CABG surgery would affect clinical outcomes.

Objectives: This study aims to evaluate serum hs-cTnI and lactate levels and their influence on postoperative clinical outcomes in patients with on-pump CABG.

Materials and methods: Eleven coronary artery disease (CAD) patients were included for on-pump CABG surgery. The biomarkers were collected at four stages of on-pump CABG: before sternotomy (T0, pre-cardiopulmonary bypass (pre-CPB)), 5 minutes before aortic cross-clamp of CPB (T1, preaortic cross-clamp), after aortic cross-clamp (T2, postaortic cross-clamp), and 24 hrs post-surgery in an intensive care unit (T3, ICU at 24 hrs).

Results: Correlation analysis revealed that during the study period, average hs-cTnI is positively associated with lactate levels (r=0.775, *p*=0.005). However, 24 hours after surgery, lactate levels return more quickly than hs-cTnI levels. The average hs-cTnI and lactate levels were positively correlated with CPB time and aortic clamp time. Regarding clinical outcomes, average hs-cTnI, and lactate levels were positively associated with a length of ICU stay (r=0.717 and 0.612, *p*=0.013 and 0.045, respectively). However, only the lactate levels were associated with ventilator support time (r=0.674, *p*=0.023).

Conclusion: We demonstrated that hs-cTnI and lactate levels are important markers of myocardial injury in association with hypoperfusion during on-pump CABG, and it could be used to monitor the postoperative outcome.

Introduction

On-pump coronary artery bypass graft (CABG) with a heart-lung machine remains the most common heart surgery procedure and an effective revascularisation strategy for coronary artery disease (CAD).[1] CAD is caused by the buildup of atherosclerotic plaque in the coronary arteries, reducing or occluding the myocardium's blood flow.[2] Myocardial revascularization with CABG has been advocated to improve cardiac function. During the bypass, blood is redirected around a blocked or narrowed lesion in the coronary artery. This restores blood flow and oxygen supply to the ischemic myocardium and relieves anginal symptoms.[3] Severe perioperative myocardial injury or

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cardiac dysfunction, which may be caused by ischemia-reperfusion injury, surgical injury, and inadequate myocardial protection during cardiopulmonary bypass (CPB), is related to an increased incidence of adverse events.[4] Furthermore, myocardial ischemic reperfusion injury in CABG is both a sequence and a cause of cardiac dysfunction.[5] Consequently, it is important to think about biomarkers to identify myocardial injury and hypoperfusion and lower the risk of morbidity and mortality. If any predicted indicators are identified, the negative impacts of clinical outcomes may also be avoided and managed.

CPB has adverse effects associated with postoperative morbidity and mortality caused by using a surgical technique with a heart-lung machine that leads to hyperlactatemia.[6] The tissues' usage of anaerobic metabolism results in hyperlactatemia when there is a lack of oxygen. The consequence leads to a conversion of pyruvate to lactate, which is used as a biomarker for hypoperfusion. This phenomenon leads to tissue hypoxia caused by an imbalance between the organ's oxygen supply and demand.[7]

Elevated blood lactate levels are widely used in clinical settings as indicators of tissue hypoxia and postoperative pediatric cardiac surgery.[8] Previous study has shown that serum lactate is a significant predictive indicator in cardiogenic shock treated with temporary mechanical circulatory support.[9] Moreover, blood lactate is a recognized prognostic indicator in patients in intensive care units and in septic shock.[10]

High-sensitivity cardiac troponin I and T (hs-cTn I and T) are highly sensitive and specific to myocardial ischemia and damage.[11, 12] However, cardiac troponin T is also expressed in skeletal muscle.[13] Cardiac troponin regulates the calcium-mediated interaction between actin and myosin.[14] The low sensitivity of conventional cTn assays during patient presentation is a major disadvantage. Furthermore, hs-cTn I assays can detect troponin at 10- to 100-fold lower concentrations than conventional assays.[15] A clinical study found that troponin I levels increased in CAD. Moreover, hs-cTnI is a valuable biomarker for predicting short- and long-term prognoses and the probability of death in patients with acute coronary syndromes.[16]

The association between serum hs-cTnI and lactate levels and clinical outcomes, has not been well established. Therefore, we aimed to investigate serum hs-cTnI and lactate levels and their association with postoperative clinical outcomes in patients with on-pump CABG.

Materials and methods

Study design and protocol

Eleven patients with CAD were included for on-pump CABG surgery at the Cardiac Center, Naresuan University Hospital, Faculty of Medicine, Naresuan University, between June 2020 and May 2021. This study was approved by the Naresuan University Institutional Review Board (COA No.160/2020). The study design was prospective and random. The exclusion criteria were emergency operation, a permanent pacemaker, and off-pump CABG.

Surgery protocol and cardiopulmonary bypass

All patients received anesthesia using a standard protocol, including morphine 0.1 mg/kg and induction with fentanyl, etomidate, and cisatracurium. Anesthesia was maintained with sufentanil, propofol during CPB, and sevoflurane. All patients received standard intraoperative monitoring. Heparin was administered at 300 U/kg for a target-activated coagulation time of 480 second.[17] The bypass circuit was primed with 1,300 mL of total priming solution, including 500 mL of crystalloid Ringer acetate solution (Acetar), 500 mL of colloid hydroxyethyl starch (Voluven), 250 mL of mannitol, and 50 mL of sodium bicarbonate. After heparinisation, a heart machine (Stöckert, Munich, Germany) and oxygenator (Dideco; Mirandola, Italy) were set up with a heater-cooler device (Stöckert, Munich, Germany). During CPB, all patients were treated with mild hypothermic CPB (32-34 °C), non-pulsatile flow at 2.2-2.4 L/min/m^2, and hematocrit (Hct) >20%. Mean arterial pressure during CPB was monitored and maintained at 50-70 mmHg, and the average urine output flow rate was >0.5 mL/kg/hr.[18] For conventional ultrafiltration (CUF), a hemoconcentration device (BRIZIO BHC-110G, Nipro Medical, Sorocaba, Brazil) was coupled to the recirculation line connecting the oxygenator to the venous reservoir as AV-CUF. The blood flow rate across the hemoconcentration was 100-200 mL/min. Myocardial protection was achieved using blood cardioplegia solution, 4:1 (St. Thomas), with the antegrade and retrograde cannula. Patients were weaned from CPB with standard criteria. After CPB was terminated, protamine was administrated to neutralize heparin to return the activated coagulation time (ACT) to baseline values.

Measurement of hs-cTnI and lactate levels

Blood samples were collected at four stages of on-pump CABG: before sternotomy (T0, pre-CPB), 5 minutes before aortic cross-clamp of CPB (T1, preaortic cross-clamp), after aortic cross-clamp (T2, postaortic cross-clamp), and 24 hours post-surgery in an intensive care unit (T3, ICU at 24 hrs). Levels of hs-cTnI were measured using the VIDAS high-sensitive troponin I assay (bioMérieux, Marcy L'Etoile, France). Two hundred microliter of heparinized plasma was obtained and analyzed using an immune analyzer. Lactate levels were measured by the i-STAT CG4+ cartridge on the Abbott i-STAT (Abbott Point of Care, Princeton, NJ, USA) analyzer.

Clinical outcomes

The clinical outcomes were recorded, including the amount of postoperative packed red cell (PRC) volume, total thoracic drainage fluid 24 hrs after surgery, postoperative mechanical ventilation time, length of intensive care unit (ICU) stay, and postoperative stay in the hospital.

Statistical Analysis

The Shapiro–Wilk test was used to evaluate the normal distribution. Continuous variables were presented

as mean±SD for normally distributed variables or median±interquartile range (IQR) for skewed variables. Categorical variables were presented as numbers (n) and percentages (%). An ANOVA with repeated measures or Friedman's test was used to compare continuous data in each stage of CPB. The association between quantitative variables was analyzed using Pearson's or Spearman's correlation. A *p*-value of less than 0.05 was considered statistically significant.

Results

A total of 11 patients, of which 63.64% were males, with a median age of 65.00±3.50 years, were included in the study. The mean preoperative left ventricular ejection fraction was 48.44±15.13%. Each patient with on-pump CABG received three grafts. The patients had an average surgical duration of 306.27±82.83 minutes, an average CPB time of 156.27±37.12 minutes, and an average aortic clamp time of 108.73±30.06 minutes. The median and IQR of conventional ultrafiltrate volume is 1,200.00±1,400 mL. The patients required an average postoperative packed red cell volume of 525.09±187.95 mL and a median total thoracic drainage fluid of 800.00±100.00 mL. The median mechanical ventilator support time was 24.00±49.00 hrs, the median length of ICU stay was 6±11 days, and the median postoperative stay in the hospital was 10±15 days. Patient demography, surgery variables, and clinical outcomes are listed in Table 1.

Monitoring data analysis revealed that hs-cTnI concentration significantly increased in postaortic cross-clamp (T2) when compared with pre-CPB (T0), (p=0.004). In addition, hs-cTnI levels were significantly higher in ICU at 24 hrs (T3) than pre-CPB (T0) (p=0.0001) and preaortic cross-clamp (T1) (p=0.001) (Figure 1). Lactate levels were significantly higher in postaortic cross-clamp (T2) (p=0.030) than pre-CPB (T0) (Figure 2). Both hemoglobin and hematocrit were lower in preaortic cross-clamp (T1) and postaortic cross-clamp (T2) than pre-CPB (T0) and significantly higher in postaortic cross-clamp (T2) than preaortic cross-clamp (T1). Hemoglobin and hematocrit

Table 1 Characteristics of patients, surgery parameters, and clinical outcomes.

Variables	N=11
Characteristic of patients	
Age (years)	*65.00±3.50*
Male: Female (n [%])	7:4 (63.64:36.36)
Weight (kg)	59.55±13.42
Height (cm)	159.00±8.75
BSA (m^2)	1.60±0.21
Hypertension (n [%])	6 (55.54%)
Diabetes (n [%])	4 (36.36%)
Chronic kidney disease (n [%])	2 (18.18%)
Dyslipidemia (n [%])	4 (36.36%)
Smoking (n [%])	5 (45.45%)
Alcohol (n [%])	3 (27.27%)
Left ventricular ejection fraction (%)	48.44±15.13
Characteristics of surgeries	
Number of patients using 3 grafts (n [%])	11 (100%)
Surgical time (min)	306.27±82.83
CPB time (min)	156.27±37.12
Aortic clamp time (min)	108.73±30.06
Conventional ultrafiltrate volume (mL)	*1,200.00±1,400*
Clinical outcomes	
Postoperative packed red cell volume (mL)	525.09±187.95
Total thoracic drainage fluid (mL)	*800.00±100.00*
Mechanical ventilator support time (hr)	*24.00±49.00*
Length of ICU stay (day)	*6.00±11.00*
Postoperative stay in the hospital (day)	*10.00±15.00*

BSA: body surface area, CPB: cardiopulmonary bypass (CPB), ICU: intensive care unit; Italic indicated median ± interquartile range, where other normal text indicated mean±SD

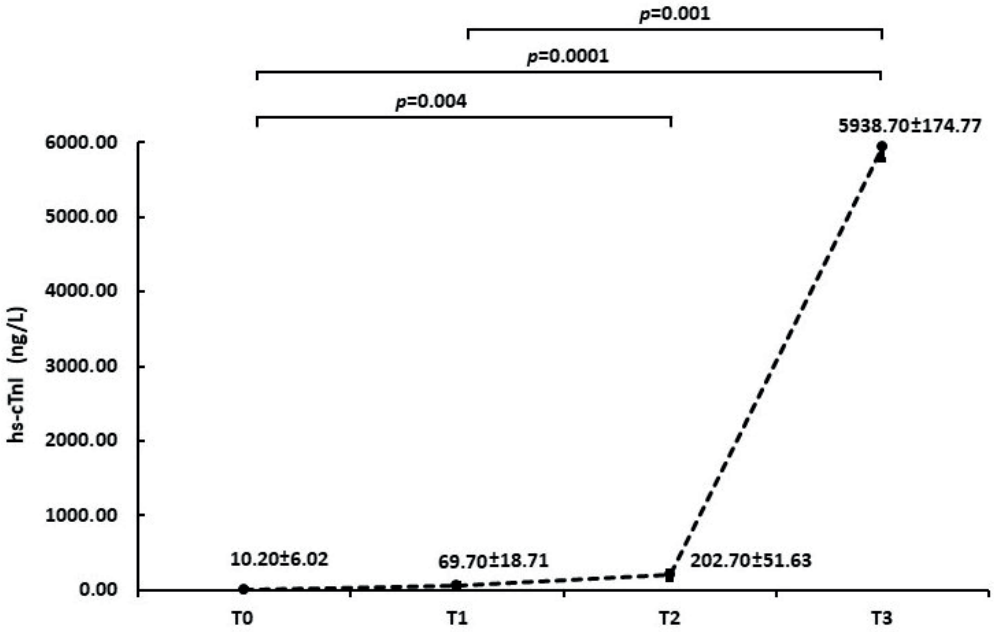

Figure 1. *Dynamic change of hs-cTnI, Pre-CPB (T0), preaortic cross-clamp (T1), postaortic cross-clamp (T2), and ICU at 24 hrs (T3).*

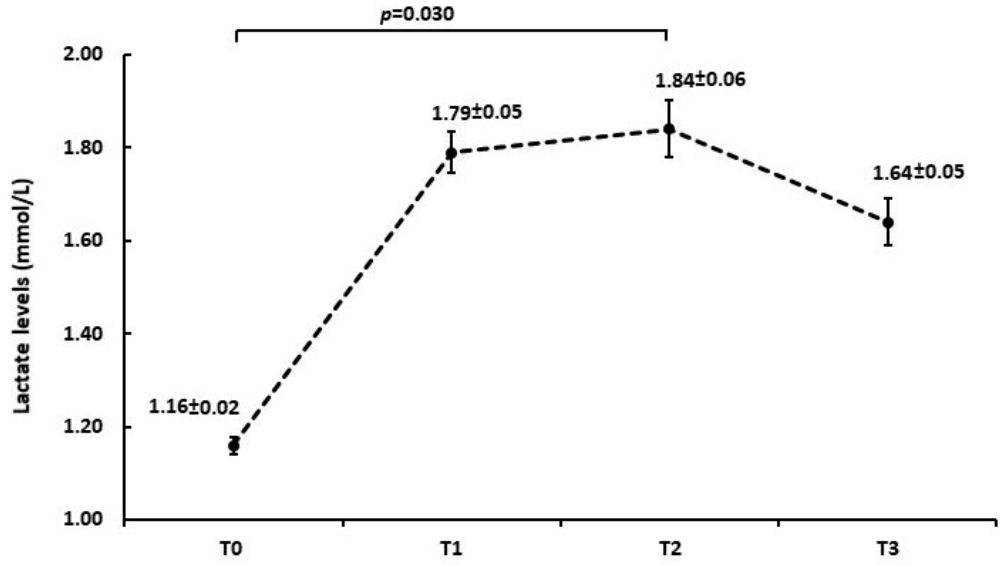

Figure 2. *Dynamic change of lactate levels, Pre-CPB (T0), preaortic cross-clamp (T1), postaortic cross-clamp (T2), and ICU at 24 hrs (T3).*

were significantly higher in ICU at 24 hrs (T3) than preaortic cross-clamp (T1) and postaortic cross-clamp (T2) (Figure 3, 4). The temperature in postaortic cross-clamp (T2) was significantly lower than that in pre-CPB (T0) (p=0.01) and significantly higher in ICU at 24 hrs (T3) than preaortic cross-clamp (T1) (p=0.030) and postaortic cross-clamp (T2) (p=0.001) (Figure 5). The results of correlation analysis showed that average hs-cTnI is positively associated with lactate levels (r=0.775, p=0.005) (Figure 6).

Univariate analysis showed that the average hs-cTnI was positively correlated with CPB time (r=0.811, p=0.002), aortic clamp time (r=0.673, p=0.023), and the length of ICU stay (r=0.717, p=0.013). The average lactate was also positively correlated with CPB time (r=0.835, p=0.001), aortic clamp time (r=0.706, p=0.015), and the length of ICU stay (r=0.612 p=0.045). Only the average lactate levels were significantly associated with mechanical ventilator support time (r=0.674, p=0.023). Other analyses showed that average hs-cTnI and lactate levels did not correlate with postoperative packed red cell volume, total thoracic drainage fluid, or postoperative hospital stay (Table 2).

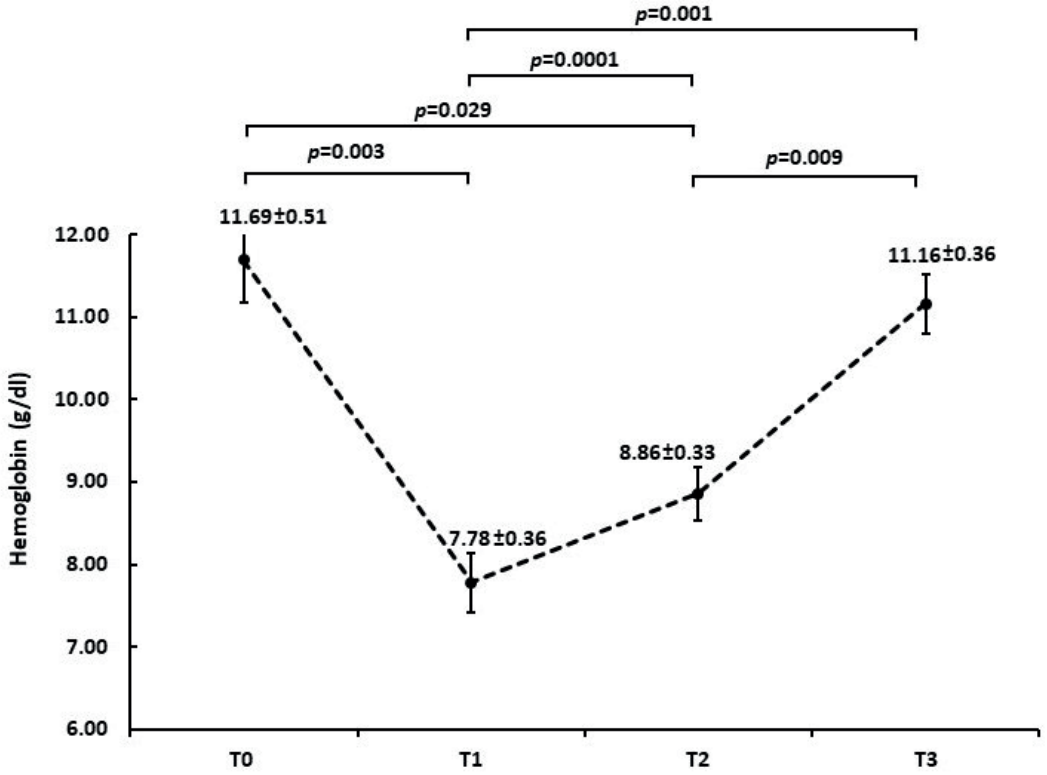

Figure 3. *Dynamic change of hemoglobin, Pre-CPB (T0), preaortic cross-clamp (T1), postaortic cross-clamp (T2), and ICU at 24 hrs (T3).*

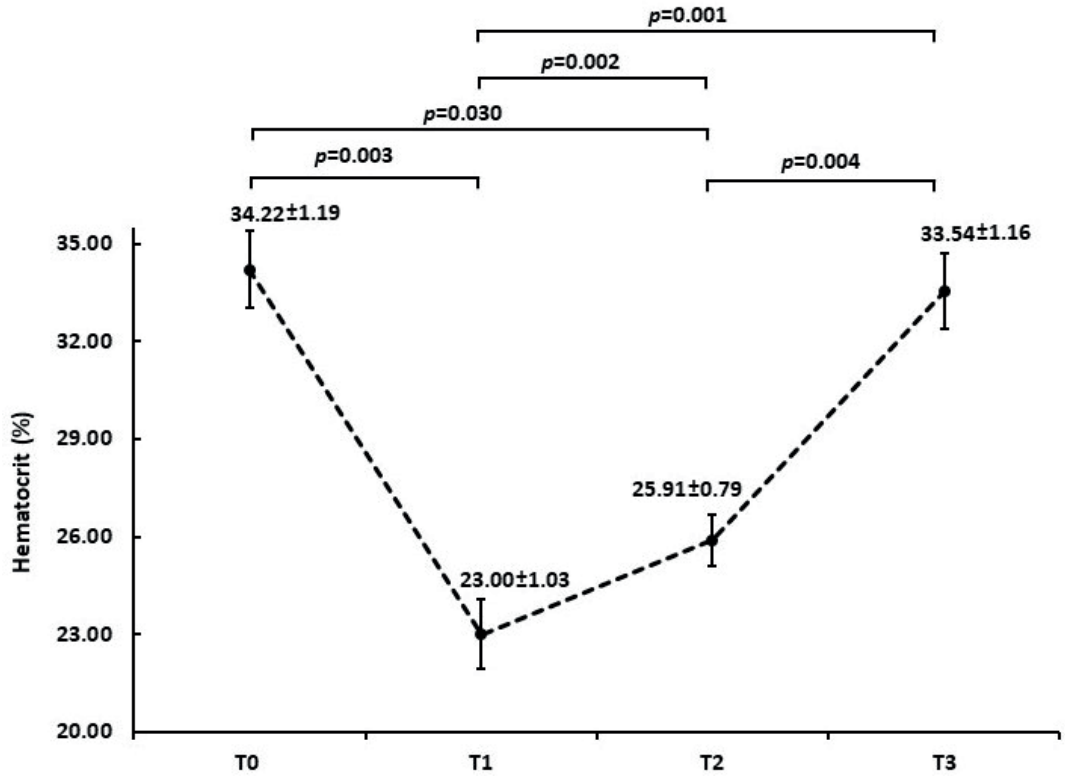

Figure 4. *Dynamic change of hematocrit, Pre-CPB (T0), preaortic cross-clamp (T1), postaortic cross-clamp (T2), and ICU at 24 hrs (T3).*

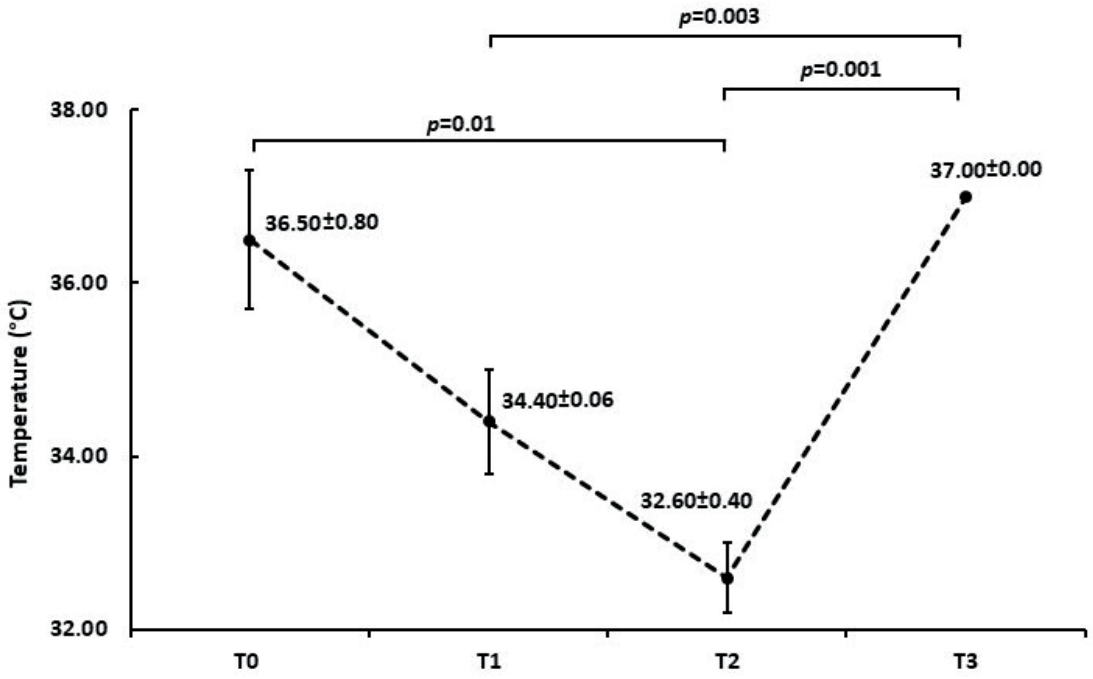

Figure 5. *Dynamic change temperature, Pre-CPB (T0), preaortic cross-clamp (T1), postaortic cross-clamp (T2), and ICU at 24 hrs (T3).*

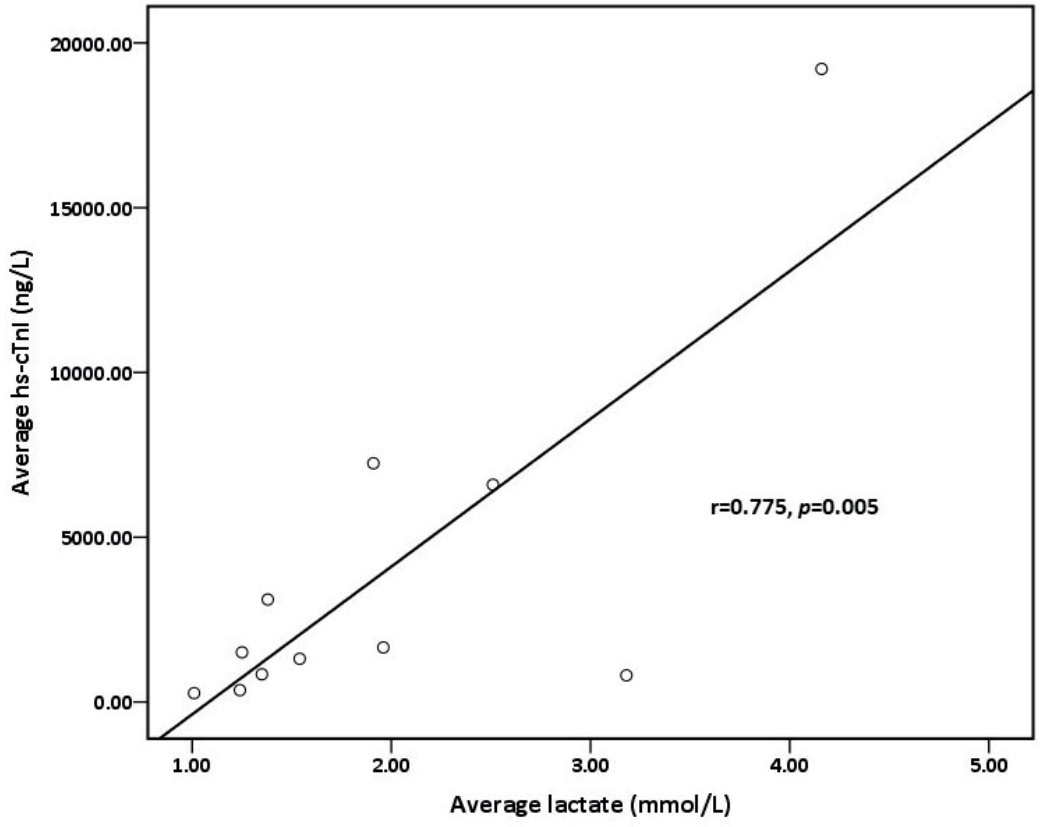

Figure 6. *A graph showing the correlation between the average all-time point of high-sensitivity cardiac troponin I (hs-cTnI) and lactate level.*

Table 2. Correlation between average hs-cTnI, average lactate levels, CPB time, aortic clamp time, and postoperative clinical outcomes.

Variables	Average hs-cTnI		Average lactate levels	
	r	*p value*	r	*p value*
CPB time	0.818	0.002*	0.835	0.001*
Aortic clamp time	0.673	0.023*	0.706	0.015*
Postoperative packed red cell volume	-0.198	0.559	-0.229	0.498
Total thoracic drainage fluid	-0.272	0.418	-0.319	0.339
Mechanical ventilator support time	0.384	0.244	0.674	0.023*
Length of ICU stay	0.717	0.013*	0.612	0.045*
Postoperative stay in the hospital	0.165	0.628	0.236	0.485

CPB: cardiopulmonary bypass (CPB), hs-cTnI: high-sensitivity cardiac troponin I, ICU: intensive care unit

Discussion

The findings of this study revealed that hs-cTnI concentration was significantly higher at postaortic cross-clamp (T2) and 24 hrs post-surgery in the ICU (T3) than pre-CPB (T0). A positive correlation was found between CPB time, aortic cross-clamp time, and hs-cTnI concentration. Serum hs-cTnI level is well known as one of the most sensitive myocardial biomarkers for detecting myocardial injury and myocardial infarction in heart surgery.[19,20] Serum hs-cTnI concentration often increases after CABG, possibly resulting from myocardial damage during surgery and ischemic time.[21,22] Ischemic reperfusion injury occurs after aortic cross-clamp, and this maneuver is the main cause of the increase in hs-cTnI concentration in on-pump CABG. Ischemic reperfusion injury induces calcium overload and the synthesis and release of oxidative stressors, which induce endothelial dysfunction, inflammatory response, and mitochondrial dysfunction, leading to myocardial apoptosis. [23] This mechanism stimulates hs-cTnI release into the blood. Januzzi *et al.* reported increased cTnI levels within 4 hours of myocardial ischemia, with the peak reaching 24-48 hrs.[24] Furthermore, preoperative myocardial infarction with poor left ventricular ejection fraction also affected hs-cTnI levels.[25]

Serum lactate is a biomarker of poor tissue perfusion monitoring during CPB. A study reported that serum lactate is superior to oxygen delivery and oxygen consumption to predict clinical outcomes in septic shock.[26] Lactate level indicates inadequate perfusion for tissue ischemia and is monitored during CPB.[27] Our study found that lactate levels increased significantly in postaortic cross-clamp. Our results also showed that hemoglobin and hematocrit decreased in preaortic cross-clamp (T1) and postaortic cross-clamp (T2) when compared with pre-CPB (T0). When using the heart-lung machine with the hemodilution technique, low hemoglobin and hematocrit levels resulted from the CPB circuit's priming solution. This phenomenon demonstrates that hemodilution with poor perfusion induces an increased lactate level in the blood.[28] Furthermore, it may be impaired during CPB, where non-pulsatile flow and pump flow are generally adjusted based on the body surface area of the patient and the cardiac index range of 2.0-2.4 L/min/m^2.[29] This is another reason that may induce inadequate blood flow. Because during CPB, perfusionists usually maintain more than 7 g/dL of hemoglobin and 22% of hematocrit.[30] The hemoglobin and hematocrit levels were significantly higher in postaortic cross-clamp (T2) than in preaortic cross-clamp (T1) because, during the operation, the CUF was routinely used to reduce hemodilution after aortic cross-clamp during CPB. CUF is used to remove extra fluid from plasma and induce an increase in hemoglobin and hematocrit concentration.[31] Aortic cross-clamp and CPB times are correlated with serum hs-cTnI and lactate levels. Previous research also demonstrated this parameter's significant positive association. Long ischemia times and inadequate perfusion cause ischemic myocardial damage and an increase in the anaerobic pathway, which increases the synthesis of hs-cTnI and lactate.[32-34] Notably, hematocrit and hemoglobin levels in the ICU at 24 hours (T3) significantly returned to normal levels due to post-CUF and blood transfusion in the ICU. According to Levraut *et al.*, continuous CUF could remove only 3% of the lactate produced.[35] Hypothermia during open-heart surgery is another possible cause of increased serum lactate levels. Hypothermia during CPB (T2, T3) induces vasoconstriction, decreasing the microcirculation of tissues and thereby leading to lactate production.[36]

Regarding the clinical outcomes, we discovered a good correlation between serum lactate and the duration of mechanical ventilator support and ICU admission, which might be a sign of postoperative care. A more extended stay in the intensive care unit is required for patients with higher average lactate levels. We also showed that higher average hs-cTnI patients also need more time to stay in the ICU. This could indicate that the patients require time to recover from myocardial injury and infarction during surgery.[12, 37, 38] Interestingly, the levels of hs-cTnI were still high after 24 hours of surgery, while the lactate levels started to decrease. These findings may imply that hypoperfusion, which impacted systemic health, was recovering. In contrast, specific organ damage to the heart required more time to recover because it was directly impacted during surgery.

Study limitations

Even though we established a time series that could reveal the association between the levels of myocardial damage and hypoperfusion biomarkers and the clinical outcomes, the study was performed in a single center,

and the sample size was small. Therefore, confirming the association between hs-cTnI, lactate levels, and clinical outcomes in large sample sizes and multicenter studies will be valuable. In long-term follow-up, further studies are also needed to evaluate other tissue hypoxia and myocardial infarction biomarkers.

Conclusion

The average hs-cTnI levels positively correlated with lactate levels in patients with on-pump CABG and correlated with the length of ICU stay and ventilator support time. Therefore, we suggest that hs-cTnI and lactate levels are valuable parameters to indicate myocardial damage and hypoperfusion during on-pump CABG. It would also probably benefit as a clinical outcome management strategy.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical approval

The study was conducted following the Declaration of Helsinki and approved by the Naresuan University Institutional Review Board (Protocol code 160/2020).

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References

[1] Diodato M, Chedrawy EG. Coronary artery bypass graft surgery: the past, present, and future of myocardial revascularisation. Surg Res Pract. 2014; 1: 1-6. doi: 10.1155/2014/726158

[2] Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005; 111(25): 3481-8. doi.org/10.1161/CIRCULATIONAHA.105.537878

[3] Hawkes AL, Nowak M, Bidstrup B, Speare R. Outcomes of coronary artery bypass graft surgery. Vasc Health Risk Manag. 2006; 2(4): 477-84. doi: 10.2147/vhrm.2006.2.4.477

[4] De Hert S, Moerman A. Myocardial injury and protection related to cardiopulmonary bypass. Best Pract Res Clin Anaesthesiol. 2015; 29(2): 137-49. doi: 10.1016/j.bpa.2015.03.002.

[5] Ferguson TB. Ischemia/reperfusion injury in coronary artery bypass grafting: Time to revisit? J Thorac Cardiovasc Surg. 2011; 141(1): 1-2. doi: 10.1016/j.jtcvs.2010.04.008

[6] Naik R, George G, Sathappan K. Hyperlactatemia in patients undergoing adult cardiac surgery under cardiopulmonary bypass: Causative factors and its effect on surgical outcome. Ann Card Anaesth. 2016; 19(4): 668-75. doi: 10.4103/0971-9784.191579.

[7] Kushimoto S, Akaishi S, Sato T, *et al*. Lactate, a useful marker for disease mortality and severity but an unreliable marker of tissue hypoxia/hypoperfusion in critically ill patients. Acute Med Surg. 2016; 3(4): 293-7. doi: 10.1002/ams2.207.

[8] Stephens EH, Epting CL, Backer CL, Wald EL. Hyperlactatemia: an update on postoperative lactate. World J Pediatr Congenit Heart Surg. 2020; 11(3): 316-24. doi: 10.1177/215013512090397

[9] Scolari FL, Schneider D, Fogazzi DV, Gus M, Rover MM, Bonatto MG, *et al*. Association between serum lactate levels and mortality in patients with cardiogenic shock receiving mechanical circulatory support: a multicenter retrospective cohort study. BMC Cardiovasc Disord. 2020; 20(1): 1-10. doi: 10.1186/s12872-020-01785-7

[10] Ryoo SM, Kim WY. Clinical applications of lactate testing in patients with sepsis and septic shock. J Emerg Crit Care Med. 2018; 2(2): 14. doi: 10.21037/jeccm.2018.01.13

[11] Ooi DS, Isotalo PA, Veinot JP. Correlation of antemortem serum creatine kinase, creatine kinase-MB, troponin I, and troponin T with cardiac pathology. Clin Chem. 2000; 46(3): 338-44. doi: 10.1093/clinchem/46.3.338

[12] Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ. 2005; 173(10): 1191-202. doi: 10.1503/cmaj/051291.

[13] Schmid J, Liesinger L, Birner-Gruenberger R, Stojakovic T, Scharnagl H, Dieplinger B, *et al*. Elevated cardiac troponin T in patients with skeletal myopathies. J Am Coll Cardiol. 2018; 71(14): 1540-9. doi: 10.1016/j.jacc.2018.01.070.

[14] Sharma S, Jackson P, Makan J. Cardiac troponins. BMJ Publishing Group; 2004. p. 1025-6. doi: 10.1136/jcp.2003.015420

[15] Garg P, Morris P, Fazlanie AL, Vijayan S, Dancso B, Dastidar AG, *et al*. Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. Intern Emerg Med. 2017; 12: 147-55. doi: 10.1007/s11739-017-1612-1.

[16] Shah AS, Anand A, Strachan FE, *et al*. High-sensitivity troponin in the evaluation of patients with suspected acute coronary syndrome: a stepped-wedge, cluster-randomised controlled trial. Lancet. 2018; 392: 919-28. doi: 10.1016/S0140-6736(18)31923-8.

[17] O'Carroll-Kuehn BU, Meeran H. Management of coagulation during cardiopulmonary bypass. Cont Educ Anaesth Crit Care Pain. 2007(6); 7: 195-8. doi.org/10.1093/bjaceaccp/mkm03

[18] Wahba A, Milojevic M, Boer C, *et al*. 2019 EACTS/EACTA/EBCP guidelines on cardiopulmonary bypass in adult cardiac surgery. Eur J Cardiothorac Surg. 2020; 57(2): 210-51. doi: 10.1093/ejcts/ezz267.

[19] Neumann FJ, Sousa-Uva M, Ahlsson A, *et al*. 2018 ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J. 2019; 40: 87-165. doi.org/10.1093/eurheartj/ehy394.

[20] Greenson N, Macoviak J, Krishnaswamy P, *et al*. Usefulness of cardiac troponin I in patients undergoing open heart surgery. Am Heart J. 2001; 141(3): 447-55. doi: 10.1067/mhj.2001.113071

[21] Domanski MJ, Mahaffey K, Hasselblad V, *et al*. Association of myocardial enzyme elevation and

survival following coronary artery bypass graft surgery. Jama. 2011; 305(6): 585-91. doi: 10.1001/jama.2011.99

[22] Thygesen K, Alper JS, Jaffe AS, *et al*. Fourth universal definition of myocardial infarction (2018). J Am Coll Cardiol. 2018; 72: 2231-64. doi: 10.1016/j.jacc.2018.08.1038.

[23] Piper HM, Meuter K, Schäfer C. Cellular mechanisms of ischemia-reperfusion injury. Ann Thorac Surg. 2013; 75(2): S644-8. doi: 10.1016/s0003-4975(02)04686-6.

[24] Januzzi Jr JL. Troponin testing after cardiac surgery. 2009; 1(3): 22-32.

[25] Chauin A. The Main Causes and Mechanisms of Increase in Cardiac Troponin Concentrations Other Than Acute Myocardial Infarction (Part 1): Physical Exertion, Inflammatory Heart Disease, Pulmonary Embolism, Renal Failure, Sepsis. Vasc Health Risk Manag. 2021; 17: 601-17. doi: 10.2147/VHRM.S327661.

[26] Bakker J, Coffernils M, Leon M, *et al*. Blood lactate levels are superior to oxygen-derived variables in predicting outcome in human septic shock. Chest. 1991; 99(4): 956-62. doi: 10.1378/chest.99.4.956.

[27] Lee YS, Kim WY, Yoo JW, *et al*. Correlation between regional tissue perfusion saturation and lactate level during cardiopulmonary bypass. Korean J Anesthesiol. 2018; 71(5): 361-7. doi: 10.4097/kja.d.17.00002.

[28] Mitchell SC, Vinnakota A, Deo SV, *et al*. Relationship between intraoperative serum lactate and hemoglobin levels on postoperative renal function in patients undergoing elective cardiac surgery. J Card Surg. 2018; 33(6): 316-21. doi: 10.1111/jocs.13713.

[29] Wahba A, Milojevic M, Boer C, *et al*. 2019 EACTS/EACTA/EBCP guidelines on cardiopulmonary bypass in adult cardiac surgery. Eur J Cardiothorac Surg. 2020; 57(2): 210-51. doi: 10.1093/ejcts/ezz267.

[30] Ferraris VA, Ferraris SP, Saha SP, *et al*. Perioperative blood transfusion and blood conservation in cardiac surgery: the Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists clinical practice guideline. Ann Thorac Surg. 2007; 83: S27-S86. doi: 10.1016/j.athoracsur.2007.02.099.

[31] Gaynor JW. Use of ultrafiltration during and after cardiopulmonary bypass in children. J Thorac Cardiovasc Surg. 2001; 122(2): 209-11. doi: 10.1067/mtc.2001.115925.

[32] Minton J, Sidebotham DA, Hyperlactatemia and cardiac surgery. J Extra Corpor Technol. 2017; 49(1): 7-15.

[33] Ranucci M, De Toffol B, Isgrò G, *et al*. Hyperlactatemia during cardiopulmonary bypass: determinants and impact on postoperative outcome. Crit Care. 2006; 10(6): 1-9. doi: 10.1186/cc5113.

[34] Alam SR, Stirrat C, Spath N, *et al*. Myocardial inflammation, injury and infarction during on-pump coronary artery bypass graft surgery. J Cardiothorac Surg. 2017; 12: 1-10. doi.org/10.1186/s13019-017-0681-6.

[35] Levraut J, Ciebiera JP, Jambou P, *et al*. Effect of continuous venovenous hemofiltration with dialysis on lactate clearance in critically ill patients. Crit Care Med. 1997; 25(1): 58-62. doi: 10.1097/00003246-199701000-00013.

[36] Rabie Soliman EF, Belghith M, Abdelmageed T. Conventional hemofiltration during cardiopulmonary bypass increases the serum lactate level in adult cardiac surgery. Ann Card Anaesth. 2016; 19(1): 45-51. doi: 10.4103/0971-9784.173019.

[37] Li Y, Li Y, Hu Q, *et al*. Association of early elevated cardiac troponin I concentration and longitudinal change after off-pump coronary artery bypass grafting and adverse events: a prospective cohort study. J Thorac Dis. 2020; 12(11): 6542-51. doi: 10.21037/jtd-20-1691.

[38] Tevaearai Stahel HT, Do PD, Klaus JB, *et al*. Clinical relevance of troponin T profile following cardiac surgery. Front Cardiovasc Med. 2018; 5: 182. doi.org/10.3389/fcvm.2018.00182.

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The effects of workplace intervention programs to manage work-related musculoskeletal pain among poultry slaughterhouse workers: A randomized controlled trial

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ABSTRACT

Background: The prevalence rate of work-related musculoskeletal pain (WMSP) among poultry slaughterhouse workers (PSW) has been reported in the shoulder, arm, and hand due to the involvement of repetitive and forceful upper limb movements. An intervention program is needed to reduce upper limb pain and improve upper limb functions among PSW.

Objectives: This study aimed to investigate the effectiveness of workplace intervention programs on musculoskeletal pain, upper limb functions, and work ability among PSW.

Materials and methods: A total of 48 participants (21 males and 27 females) who met the inclusion criteria were recruited into the study. The participants were allocated into two groups: The workplace intervention group (WIG, N=24; 10 males; 14 females) and the control group (CG, N=24; 11 males; 13 females). The WIG performed the exercise training with elastic resistance bands (ERB) and stretching exercises for eight weeks, and the CG did not perform any activity. The Visual Analogue Scale (VAS), shoulder reach flexibility test, active range of motion (AROM), the disability of the Arm, Shoulder, and Hand (KKU-DASH), and Thai Work Ability Index (Thai WAI) were used as outcome evaluations. The two-way analyses of variance (ANOVAs) with repeated measures (group x time) were used for statistical analyses, and the statistical significance was set at $p<0.05$.

Results: Approximately 79.1% (N=19) of the participants in WIG reported significantly decreased pain intensity of upper limb 3.06 (0.83 to -5.4) in the right side ($p<0.01$) and 1.96 (1.6 to -5.6) in the left side ($p<0.001$) after the 8-week intervention. Meanwhile, shoulder flexibility in WIG significantly increased ($p<0.001$) to around 3.55 cm. (0.67 to 8.20) on the right side and 5.61 cm. (2.0 to 10.67) on the left side. For AROM in WIG, neck flexion, lateral neck flexion, neck rotation, and left shoulder flexion significantly increased ($p<0.05$), as well as KKU-DASH and Thai WAI scores ($p<0.001$). There was no significant difference within-group in all variables in CG.

Conclusion: The workplace intervention program significantly reduced upper limb pain and increased neck movement and shoulder flexion among PSW. The training programs also improved work ability and reduced disability with upper extremity musculoskeletal conditions among PSW.

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Introduction

Work-related musculoskeletal pain (WMSP) represents the most common occupational diseases that have a direct effect on employee health, work disability, and sickness absence.[1] The poultry industry in Thailand is rapidly

expanding to the global demand for chicken products.[2] Conversely, the constant drive for higher profit and production creates adverse factors (i.e., increased working hours, increased production line speeds, or decreased break time), which contribute to WMSP at the workplace and affect the health and well-being of the PSW.[3] The prevalence of WMSP, especially in slaughtering and meat processing operations, was found in the shoulder, neck, and upper extremities due to a high degree of repetitive and forceful upper limb movements, lack of sufficient recovery, and awkward postures.[3] In a recent cross-sectional study among Thai PSW, approximately 97% of PSW had WMSP in at least one body region.[4] The highest prevalence rate was found at the shoulders and wrists/hands regions around 61.5% and 60.3%, respectively. In comparison, 83% of PSW reported work disability due to upper limb pain, emphasizing the functional consequences of arm, shoulder, and hand on daily work.[4] Some previous studies also reported that PSW had pain and discomfort in at least one of the body regions, which includes the shoulder, neck, upper or lower back, forearm, arm, wrist, and hand.[3, 5-7]

Current evidence to reduce or prevent WMSP at the workplace includes physical exercise, ergonomics, participatory ergonomics, and multifaceted interventions.[8] Previous systematic reviews on WMSP were reported among the general working population, such as sedentary employment, office workers, health care workers, or labor.[8] However, the general working population has vastly different working conditions than slaughterhouse workers, and previous findings may, therefore, not be directly transferable to slaughterhouse workers. Unfortunately, only a few previous studies have investigated the effect of workplace interventions among PSW.[9-11] The studies of Sundstrup *et al* found that strength training exercises in high intensity can improve pain, work disability, and strength of the shoulder and wrist muscles among PSW with upper limb chronic pain.[9-11] However, there are many factors, such as the nature of the work environment, physical demands at work, work organization, or the support of the workplaces that vary among PSW from industry to industry, that affect transferring the findings across studies.[8] On the other hand, industry-specific interventions are suggested to have the better potential to reduce WMSP.[8] Workers, workplaces, and other relevant stakeholders such as employers, health care practitioners, managers, and social care workers have to get knowledge on effective industry-specific interventions to protect and promote the health, safety, and well-being of their workers and the sustainability of their workplace.[12]

Theoretically, increasing physical capacity by resistance training (RT) of the shoulder, arm, and hand muscles may provide an alternative way of reducing chronic pain and work disability in PSW.[9-11] Recently, elastic resistance bands (ERB) have been used for resistance training to improve physical capacity in many populations, not only in athletes but also in injured patients and sedentary people.[13,14] ERB is relatively inexpensive, safe, easy to use, portable, and requires little space, and could represent an attractive and feasible alternative to free weights and training machines for RT, especially at the workplace. Several studies have shown the effects of training with ERB to improve muscle strength and functional ability.[15-17] ERB can provide similar therapeutic outcomes to exercises performed with training equipment, such as resistance training machines or free weights.[17,18]

Further, upper limb pain in PSW has commonly tended to shorten the muscle-tendon unit such as the upper trapezius and levator scapulae which are the most commonly resulting in limited neck and shoulder mobility.[19] Also, there are many studies that supported stretching exercises as a beneficial intervention to manage various musculoskeletal conditions.[19,20] While stretching exercises can improve joint range of motion and flexibility,[19,20] its benefits in managing WMSP among PSW have not been investigated to date. To our knowledge, only a few studies have evaluated the effects of exercise interventions among PSW with WMSP.[9-11] There is a need for using a specific package of exercise programs in the workplace setting, which can be effectively used for relief of neck and shoulder pain, under feasible for implementation. An exercise program that combines resistance training with ERB and stretching exercises would benefit the PSW with WMSP. Therefore, the aim of this study was to investigate the effectiveness of workplace intervention programs on musculoskeletal pain, upper limb functions, and work ability among PSW.

Materials and methods

Study design and setting

The current study was an assessor-blinded, repeated measure, randomized controlled trial (RCT) with two parallel groups. The study protocol was registered in the RCT registry (TCTR20230123001) and approved by the institutional ethical committee according to the standards of the Declaration of Helsinki (Ethical approval number AMSE64EX-112). All participants were informed about the study and signed a written informed consent prior to their participation in the study. Participants were randomly allocated to one of two groups (1:1 ratio): 1) workplace intervention group (WIG) and 2) control group (CG). The randomization was stratified by the severity of upper limb pain (i.e., pain intensity). An internet randomized scheme generator, which generated block randomization with a random sequence of permuted blocks of 4, was used for allocating the participants for the intervention groups. An independent research staff undertook randomization and allocation of the participants in the trial. The group assignment number was written on paper, placed in an opaque envelope, and sealed. The staff members who conducted these processes did not have any roles in data collection or intervention assignments. The opaque sealed envelopes were opened after the completion of the baseline assessment. The outcome assessor was blinded. This study was a continuation of the initiatives from the Sustainable Measures for Assessment and Rehabilitation Drive (SMART Drive) research networking group which had been working to improve musculoskeletal health and

well-being for people at the workplace.

Participant characteristics

The study was conducted in the poultry slaughterhouse factory located in the northern parts of Thailand during the period of November 2022 - March 2023. The factory had 120 workers, including 24 office staff, six storage workers, and 90 PSW. The inclusion criteria of the study were as follows: 1) males and females aged between 18 and 59 years, 2) working at a poultry slaughterhouse factory for at least one year, 3) working in a standard full-time job for at least 7 hours per working day, 4) presence of moderate pain intensity (VAS = 3/10 to 7/10) at least one body region including; shoulder, elbow/forearm, or hand/wrist during the last three months and 5) communicating in Thai fluently. The exclusion criteria include participants who had 1) radicular pain or numbness at upper and lower extremities, 2) impaired sensation at the body and lower extremities, 3) specific spinal disorders or nerve root compression, 4) previous spine surgery or upper and lower extremities surgery, 5) history of injury from an accident in the previous three months, 6) pregnancy, 7) severe medical conditions such as cardiovascular disease, or hypertension, and 8) those who received any pain-relieving medication in the previous three months.

Figure 1 shows the CONSORT flow diagram of PSW recruitment. There were 60 PSW reported WMSP at the upper limb region. Twelve PSW were excluded because 5 PSW have been working at this factory for less than one year, 2 PSW had hypertension condition, 1 PSW had a history of back surgery, 1 PSW had a recent motorcycle accident, and 3 PSW was not willing to participate in the study. The personal characteristics (e.g., age, sex, weight, and height) of the participants were collected using a study form. Furthermore, the study form included additional factors such as work experience, working hours per day, type of task, and tools (i.e., knives and scissors) used. The weight was measured by a mechanical weighing scale (Camry (DT-613), China), and the height was measured by a portable stadiometer (Health-O-Meter Mechanical Beam Scale, United States of America).

Interventions

Two qualified physiotherapists experienced in exercise training provided the interventions. Blinding of participants was not possible due to the nature of the interventions. Participants were advised to inform if they would experience any discomfort during the interventions

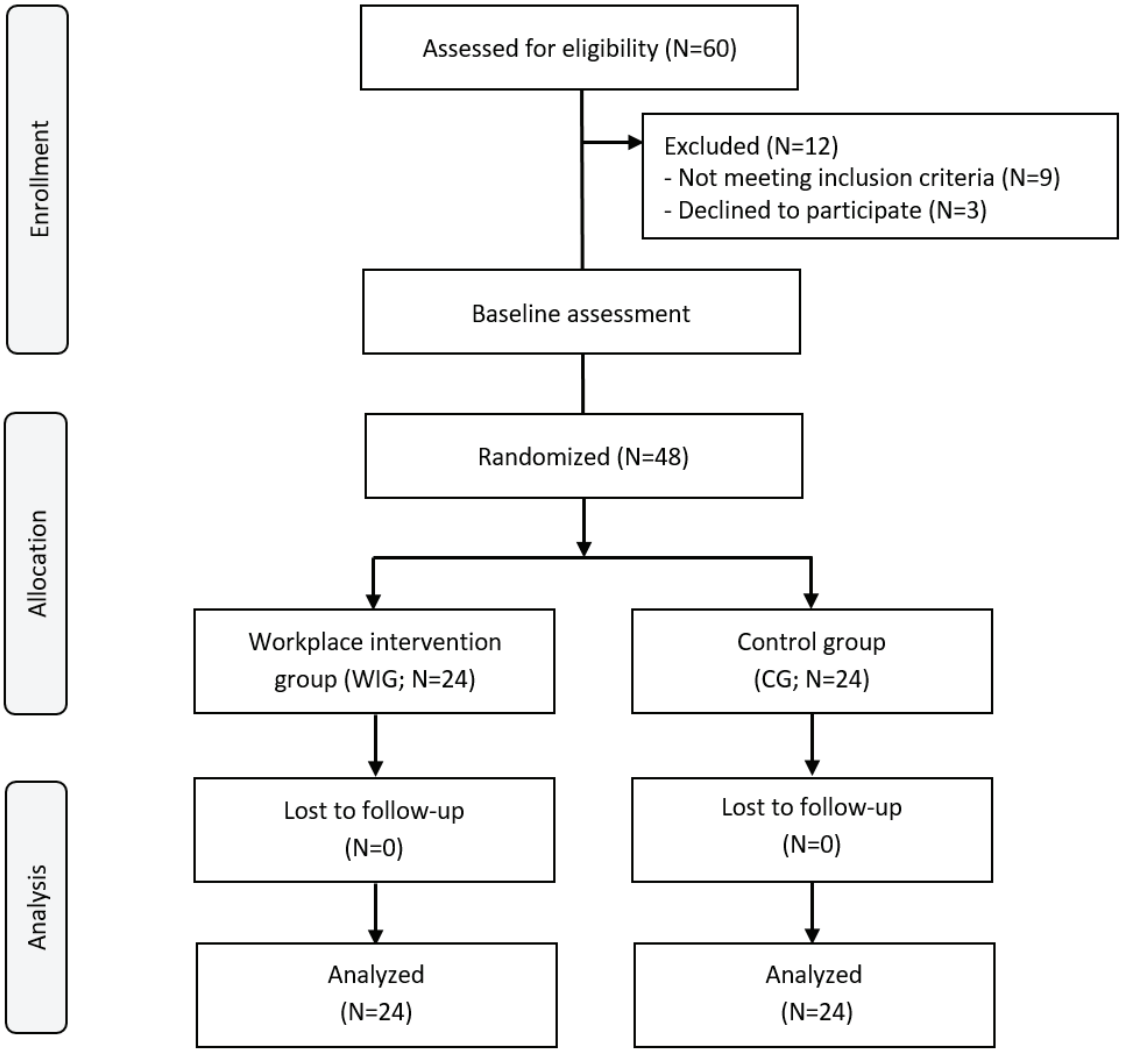

Figure 1. *The Consolidated Standards of Reporting Trials (CONSORT) flow diagram of poultry slaughterhouse workers recruitment.*

and were allowed to leave the experiment if the participants requested to leave the study due to any personal reasons or any reported worsening of symptoms. The participants were allocated to the workplace intervention group (WIG) and control group (CG).

Group 1: Workplace intervention group (WIG)

Participants in this group (N=24; 10 males; 14 females) performed resistance exercise training with ERB and stretching exercises for the upper body part. Table 1 shows a detailed description of the exercise program. The training protocol was accepted in content validity, the Index of Item Objective Congruence (IOC) was 0.94. The elastic bands (Decathlon elastic bands, Decathlon Sports, Thailand) used in this study were three colors for three levels of resistance: green, red, and blue. The elastic band is 1.90 meters long with a difference in resistance of 15 to 25% among the bands.[21] Each individual began with the lowest elastic load (green color) and increased to the medium (red color) and hard resistance (blue color). The participants were advised to choose and progress the resistance of the bands based on the self-perception of effort.[22,23] All participants were instructed to exercise at a moderate intensity level and still maintain the repetition of exercise according to the program.

Table 1 Workplace intervention programs: the combination of exercise training with elastic resistance bands (ERB) and stretching exercises.

Workplace intervention programs*		
Warm-up exercises	1) Arm circles 2) Side bends 3) Shoulder rolls	5 minutes
Exercise training with elastic resistance bands (ERB)	1) Arms stretch (10 repetitions x 2 set) 2) Shoulder diagonals pull (10 x 2) 3) Arms pull-down (10 x 2) 4) Shoulder horizontal abduction-adduction (10 x 2) 5) Elbow extension (10 x 2) 6) Elbow flexion (10 x 2) A 60-second rest interval between sets	15 minutes
Stretching exercises	1) Neck stretching (10 repetitions x 1 set) 2) Shoulder stretching (10 x 1) 3) Triceps stretching (10 x 1) 4) Biceps stretching (10 x 1) 5) Wrists and hands stretching (10 x 1) 6) Chest stretch (10 x 1) Hold each stretch for 20 seconds	15 minutes
Cool-down exercises	1) Deep breath 2) Self-stretching (e.g., back and leg stretch)	5 minutes

**participants performed three sessions (before - during break - after work time) per day, three days per week, for eight weeks.*

All training sessions took place in designated private training rooms at the worksites. Before the training started, all participants were instructed not to take any pain-relieving medication during the interventions. All sessions were supervised by qualified physiotherapists, who instructed the participants to correctly perform the exercises and helped with exercise adjustment when needed. Participants were asked to record the exercise frequency in a logbook and bring them to a researcher at the end of the study to monitor exercise compliance.

Group 2: Control group (CG)

Participants in this group (N=24; 11 males; 13 females) received the usual care provided by the occupational health services at their workplace. The usual care involved healthcare advice and pain medication, if necessary. The participants were re-evaluated after eight weeks. However, all participants received the combination of exercise training with ERB and stretching exercises after completing the study.

Outcome measurements

Primary outcome measures

The intensity of pain in this study was measured at the upper limb using a VAS. The measurement consists of a 0-10 VAS scale, which is a horizontal line anchored with two opposite labels; 'no pain' (0) and 'worst pain imaginable' (10).[24,25] Participants were asked to draw a vertical mark on the horizontal line. The VAS was reported to have excellent reliability (ICC=0.995).[25] The flexibility of the shoulders was measured using the shoulder reach flexibility test. The participant placed one hand behind the back and the other over the shoulder, behind the head, and participants were asked to reach as far as possible.[26,27] The test administrator measured the distance between the tips of two middle fingers using a tape measure. The score was positive when the hands overlapped each other and negative when the hands stayed apart from each other.[27] The test was performed in three times, and the best trial was noted. The interpretation of shoulder reach flexibility test values are reported as good level when

fingertips are touching, fair level when fingertips were not touching but were less than 5 cm. apart, and poor level when fingertips are more significant than 5 cm. apart.[27] A universal goniometer (NIIGATA SEIKI (SK), Japan) was used to measure AROM in the neck region and shoulder, elbow, and wrist joints.[26,28] The test was performed three times, and the best trial was noted in degrees.[28] The reliability of assessors was assessed using the Intraclass correlation coefficients (ICC). The inter-tester reliability of the shoulder reach flexibility test and AROM measurement were 0.96 (range 0.95-0.98), and 0.92 (range 0.91-0.94), respectively. The intra-tester reliability of the shoulder reach flexibility test and AROM measurement were 0.97 (range 0.96-0.98) and 0.95 (range 0.93-0.97), respectively. The level of reliability of assessors can be regarded as excellent.

Secondary outcome measures

The Thai Version of Disability of the Arm, Shoulder and Hand Questionnaire (KKU-DASH) was used to measure disability related to WMSP in the upper extremity region.[29] The total scores of KKU–DASH were summarized in ranging from 0-100 scores. A score of zero '0' represents no disability, and '100' scores represents the most severe disability.[29] The KKU–DASH had been shown to have moderate reliability (ICC= 0.52).[29] The Thai version of Work Ability Index (Thai WAI) was used to measure self-reported ability to work.[30] Possible scores range from 7-49 and are classified as follows: 7-27 (poor), 28-36 (moderate), 37-43 (good), and 44-49 (excellent).[30] The WAI was determined based on the interaction between the health and capacities of the individual, their competence or skill level, their values and attitudes, and the demands of the work they are required to do.[30] The level of reliability of WAI can be regarded as good reliability (ICC=0.89).[30]

Statistical analysis

The sample size was calculated on pain intensity (between-group differences) based on Sundstrup *et al* by G * Power 3.1.9.4. with an effect size of 0.49, 80 % power, and alpha error of 0.05.[11] A total sample size was 48 individuals (24 per group) which included a 10% drop out was determined to be appropriate for the study. The statistical analyses were performed using the SPSS version 24.0 for Windows (SPSS, Inc., Chicago, IL, USA). The normality and homogeneity of variances within the data were confirmed with the Shapiro–Wilk and Levene tests, respectively. The demographic data (e.g., age, sex, weight, height, BMI) were summarized in frequency, mean (M), standard deviation (SD), and percentages. All dependent variables (pain intensity, shoulder flexibility, AROM, KKU-DASH scores, and Thai WAI scores) were analyzed by two-way analyses of variance (ANOVAs) with repeated measures (group x time). Bonferroni correction was used for post hoc pairwise comparisons. Between-group effect sizes (ES) were also calculated with partial eta squared (η_p^2), where $0.01<\eta_p^2<0.06$ constitutes a small effect, $0.06\leq\eta_p^2\leq0.14$ constitutes a medium effect, and $\eta_p^2>0.14$ constitutes a large effect.[31] Statistical significance was set at $p<0.05$.

Results

A total of 48 PSW participated in this study; 43.7% (N=21) were men, and 56.3% (N=27) were women. There was no adverse event reported during the intervention, and all participants completed the exercise compliance. The baseline characteristics of PSW are shown in Table 2. There were no significant differences between groups for all variables ($p>0.05$). The complete results of the study are shown in Table 3. The results showed significant group x time interaction for pain intensity ($p\leq0.05$). After eight

Table 2 Baseline characteristics of poultry slaughterhouse workers (PSW) in workplace intervention group (WIG) and control group (CG).

Characteristics	WIG (N=24) (Mean±SD)	CG (N=24) (Mean±SD)	*p* value
Sex			
Males - N (%)	10 (41.7%)	11 (45.8%)	
Females - N (%)	14 (58.3%)	13 (54.2%)	
Age (years)	39.96±10.42	40.29±7.38	0.118
Weight (kg)	61.42±13.78	57.50±9.65	0.200
Height (centimeters)	161.54±10.68	161.16±8.39	0.451
Body mass index (kg/m²)	23.68±5.56	22.77±3.46	0.089
Work experience (years)	4.40±3.75	4.54±3.70	0.853
Work hours (hours)	10.13±1.62	10.50±1.69	0.604
Job section			
Slaughtering - N (%)	3 (12.5%)	3 (12.5%)	
Evisceration - N (%)	9 (37.5%)	10 (41.7%)	
Cut-up - N (%)	12 (29.5%)	11 (45.8%)	
Tool use (i.e., knives and scissors)			
No - N (%)	11 (45.8%)	12 (50.0%)	
Yes - N (%)	13 (54.2%)	12 (50.0%)	

There were no significant differences between groups for all variables (p>0.05).

Table 3 Between- and within-group differences in pain intensity of upper extremities (UE) (Visual analog scale; VAS 0-10 scales), shoulder flexibility, and active range of motion (AROM) of neck, shoulders, elbows, and wrist regions in the workplace intervention group (WIG) and control group (CG) before and after the 8-week intervention. Data was presented as the mean±SD.

	WIG (N=24)				CG (N=24)				*p* value		Effect size (η_p^2)	
	Baseline		Post-intervention		Baseline		Post-intervention					
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
VAS of UE (cm.)	5.42±1.14	3.38±2.88	2.36±0.84	1.42±1.23	5.27±1.26	3.92±2.75	5.02±1.44	3.95±2.65	0.003* <0.001#	<0.001* <0.001#	0.196	0.154
Shoulder flexibility (cm.)	0.90±2.33	-3.17±3.63	4.45±1.27	2.53±2.11	1.08±2.74	-2.92±2.87	0.95±2.82	-2.71±2.97	<0.001* <0.001#	<0.001* <0.001#	0.126	0.151
AROM (degree)												
Neck flexion	44.33±4.74		48.79±4.91		45.88±3.25		46.11±3.32		<0.001*		0.007	
Neck extension	33.52±2.30		34.09±2.43		33.20±4.32		33.81±4.58		NS		0.002	
Lateral neck flexion	43.80±2.60	39.66±3.67	45.70±1.09	43.49±2.68	42.52±1.75	41.66±2.07	42.77±1.72	41.75±1.63	NS <0.001#	<0.001* 0.009#	0.075	0.138
Neck rotation	54.12±5.40	52.36±4.61	58.51±5.30	56.95±5.00	54.12±5.40	50.27±3.32	53.12±3.28	50.91±3.12	<0.001* 0.024#	0.002* 0.018#	0.055	0.097
Shoulder flexion	173.49±3.30	171.73±1.24	175.70±1.18	175.18±1.31	175.90±1.16	172.80±2.25	175.42±0.91	172.94±2.14	NS	0.014#	0.001	0.125
Shoulder extension	34.41±4.17	32.94±3.59	34.27±3.34	33.73±3.11	34.76±3.83	33.62±3.45	35.19±3.78	33.65±3.42	NS	NS	0.008	0.001
Elbows flexion	155.06±2.77	155.36±2.25	156.02±2.14	155.06±1.87	155.84±1.30	155.52±1.98	156.04±2.81	155.54±1.60	NS	NS	0.009	0.002
Wrist flexion	55.81±1.92	55.01±2.11	55.50±2.19	55.61±2.47	55.38±1.51	54.04±2.12	54.97±1.46	54.33±2.10	NS	NS	0.005	0.003
Wrist extension	41.49±3.12	39.54±4.19	42.23±3.48	40.98±3.47	42.02±4.40	39.40±3.55	42.16±4.47	39.27±3.71	NS	NS	0.001	0.002

*Note: *significantly different within-group (WIG), p<0.05. #significantly different between-group at the post-intervention, p<0.05, There was no significant difference within-group in all variables in CG (p>0.05), and no significant difference between-group at the pre-intervention (p >0.05), NS: no significant difference within-group (WIG) (p>0.05), and no significant difference between-group at the post-intervention (p>0.05),cm: centimeters, η_p^2 : partialmeta squared.*

weeks of intervention, 79.1% (N=19) of the participants in WIG demonstrated a significant reduction in pain scores in both arms compared to CG (p<0.001). The shoulder flexibility also showed a significant group x time interaction (p<0.001). Post-hoc analysis showed that the WIG had significantly increased shoulder flexibility than the CG (p<0.001). Also, there were significant group x time interactions for the active range of motion as follows: neck flexion (p<0.001), lateral neck flexion (left side (p<0.001)), and neck rotation in both sides (right side - (p<0.001) and left side - (p=0.002)). Post-hoc analyses showed that at the end of 8 weeks, the WIG had significantly increased range of motion of lateral neck flexion on both sides (right side - (p<0.001) and left side - (p=0.009)), neck rotation in both sides (right side - (p=0.024) and left side - (p=0.018)) when compared to the CG. Active range of motion between groups of left shoulder flexion in WIG had significantly increased than the CG (p<0.001).

The findings of the secondary outcomes are shown in Table 4. There were significant group x time interactions for upper disability scores (p<0.001). After eight weeks of intervention, the WIG demonstrated a significant reduction in KKU-DASH scores compared to CG (p<0.001). KKU-DASH scores in the WIG were decreased from baseline (p<0.001), while it did not change for CG. Finally, the results showed a significant group x time interaction for the Thai work ability index scores (p<0.001). At the post-intervention, the WIG had significantly increased WAI scores compared to the CG (p<0.001). However, the WAI scores in the CG did not change at the post-intervention.

Table 4 Between- and within-group differences in disability of the Arm, Shoulder and Hand (KKU-DASH scores) and Thai Work Ability Index (Thai WAI scores) in workplace intervention group (WIG) and control group (CG) before and after the 8-week intervention. Data was presented as the mean±SD.

	WIG (N=24)		CG (N=24)		*p* value	Effect size (η_p^2)
	Baseline	Post-intervention	Baseline	Post-intervention		
KKU-DASH Scores	26.71±2.19	15.46±1.57	25.71±2.19	25.93±1.57	<0.001* <0.001#	0.083
Thai WAI Scores	37.62±5.78	43.08±4.92	37.66±3.72	38.12±3.27	<0.001* <0.001#	0.065

**Significantly different within-group (WIG), p<0.05, # Significantly different between-group at the post-intervention, p<0.05*
There was no significant difference within-group in both variables in CG (p>0.05), and no significant difference between-group at the pre-intervention (p>0.05), η_p^2: partialmeta squared

Discussion

Results of the present study demonstrated that the combination of exercise training with ERB and stretching exercises significantly reduced upper limb pain and increased neck, shoulders, and upper limbs functions, which were evaluated using shoulder reach flexibility and AROM test among PSW. Our results showed that 79.1% (N=19) of the participants in WIG demonstrated improvement in pain symptoms; meanwhile, the CG did not. A previous study reported that change in pain intensity is a primary efficacy of interventions for chronic pain.[32] In patients with chronic pain, a change in pain intensity of 2 scales on a 0-10 scale is considered moderately clinically meaningful. In contrast, a change of one is considered a minimal change.[32] The study of Sundstrup *et al* showed that average pain intensity in the resistance training group decreased by 1.8, with 73% of the PSW in the resistance training group reporting some or much improvement in pain.[11] Another study showed that the pain intensity of the hand/wrist decreased by 1.6 in the strength training group, with 41% of PSW reporting a reduction of pain. In the present study, the absolute change in average VAS scores was 3.06 and 1.96 for the right and left upper limb, respectively, with almost 79% improvement in pain with a large effect size (η_p^2>0.14), which showed that effects of the intervention were higher than previous studies.[11] The possible mechanisms of exercise programs to reduce the severity of pain may be that exercise enhances the release of endogenous opioids, such as endorphins hormone, which is the pain-killer substance.[33] Consequently, the endogenous opioids would inhibit the chemical substances (i.e., bradykinin, prostaglandin, histamine) that activate the pain receptor.[33] However, 21% (N=5) of the participants in WIG did not find any changes in pain intensity that were likely due to the effects of other factors, such as psychosocial elements.

The study findings of the intervention program agree with previous research.[9-11] The results of exercise training with ERB in this study have shown an effective reduction in upper limb pain and improving physical capacity among PSW. Evidence supports that exercise training with ERB device utilized in training can produce muscle activation and strength gains that are equivalent to those observed with free weights and conventional-device training.[13,14] ERB is a viable option to conventional resistance training equipment for the exercise's lateral pull-downs and unilateral rows, as it induces similar muscular activations for these exercises.[17] In other studies, ERB also provides additional unique training features including ability to perform exercises similar to a sport-specific pattern, providing eccentric and concentric resistance regardless of gravity,[14] and ability to perform quick motion and change direction.[15] Moreover, previous studies have concluded that exercise training with ERB has also previously been proven to be effective in improving physical capacity, whole-body strength and functional activities.[15-17] Therefore, ERB was used as an easy and feasible choice for the workers to perform their exercise at their workplace.

Moreover, there were significant improvements in workability and disability with upper extremity musculoskeletal conditions among PSW. The results of the current study are consistent with previous findings, which reported that the resistance training intervention decreased work disability as assessed by the DASH questionnaire in PSW.[11] The average change of DASH score improved by a score of 6.5 (13.2 to 0.1). However, the improvement of DASH score in the current study was 11.26 (35.83 to 3.14), with a medium effect size (η_p^2=0.083). Similarly, the WAI score (43.08) reported by the participants was also higher than a WAI score (39.7) reported by a past study that investigated the effects of strength training or usual-care ergonomics among slaughterhouse workers.[9] With workability related to musculoskeletal pain, chronic disease, productivity, sickness absence, early retirement, and all causes of mortality, the improvement in the WAI reported among PSW may be considered as a positive outcome of the intervention.[34]

Additionally, when muscles are repeatedly used, such as while performing repetitive tasks, the muscles contract according to the sliding filament theory. Eventually, the muscle would be shortened and tight[35,] which can lead to increased muscle tension and limited neck and shoulder ROM.[19,20] Thus, stretching exercises would benefit people to maintain the viscoelastic properties of the muscles and to maintain joint flexibility.[36,37] Also, past studies showed that stretching exercises not only gain range of motion but also reduce shoulder tightness pain and dysfunction in workers with chronic neck and shoulder pain.[36,37] Therefore, stretching exercises were designed and included as part of the exercise regime for the intervention in the current study. The results from the intervention program demonstrated that holding each stretch for 20 seconds showed statistically significant improvements in shoulder flexibility neck and shoulder ROM, especially in neck flexion, lateral neck flexion, neck rotation, and shoulder flexion. The acute effect of stretching on flexibility is apparent. Stretching creates an acute increase in joint range of motion that persists for 60 to 90 minutes.[38] Holding stretches for 20 to 30 seconds is a good standard because most stress relaxation in passive stretches occurs in the first 20 seconds.[19] Subjects can feel this decrease in muscle tension when they hold a static stretch.[19,20] Stress relaxation following stretching provides an acute 10-30% decrease in passive tension.[38] All these stretching effects might explain the improved range of motion obtained by the study participants.

The study findings have implications for clinical practice and industry. In this study, we prioritized a training program design based on the concept of industry-specific intervention.[8] The current study was a continuation of the previous part of the project, which investigated the WMSP among PSW.[4] Our training programs were created under the feasibility of the workplace environment, organizations, and support from the workplaces. Moreover, the need of workers were also included. Therefore, our programs were cost-efficient and utilized easy-to-use exercises and training equipment to be implemented in any environment. The worksites can implement our programs based on the easily adopted and inexpensive.

Limitations

The study has some limitations. Participants in this study were recruited from one slaughterhouse industry in northern Thailand. However, the results were generalizable to a broader group of PSW because similar work environments and working conditions were found. The current study did not consider the medium- and long-term effects of the intervention. Hence, the transferability of findings for the long-term benefits is not known. Also, the study did not investigate the lived experience of workers, such as psychosocial or occupational elements, which may contribute to the pain problem. The intervention has room for improvement, especially regarding the biopsychosocial aspects of intervention. Therefore, further studies are needed to evaluate the psychosocial elements (i.e., job satisfaction or job stress) or occupational elements (i.e., working hours, intensity of work, or years of experience). In addition, the modulation of our exercise intensity was not based on a percentage of one-repetition maximum (1-RM) evaluation.[39] However, there was the concern that the 1-RM test could cause a physical overload and trigger a flare-up of symptoms.[22,23] Hence, our modulation of exercise intensity followed the guidelines recommended, based on the self-perception of effort, progressively from low-load with low intensity.[19] The guidelines of the self-perception of effort also described the validity of this scale for the prescription of exercises and suggested its use instead of 1-RM.[22,23,40]

Conclusion

The workplace intervention programs which include resistance exercise training with ERB and stretching exercises, induced clinically relevant improvements in pain symptoms and increased neck movement, shoulders flexion, and upper limb functions among PSW. The training programs also improved work ability and reduced disability related to upper extremities among PSW with WMSP.

Conflict of interest

No conflict of interest to declare.

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References

[1] Tingulstad A, Meneses-Echavez J, Evensen LH, Bjerk M, Berg RC. Effectiveness of work-related interventions for return to work in people on sick leave: A systematic review and meta-analysis of randomized controlled trials. Syst Rev. 2022; 11(1): 192. doi:10.1186/s13643-022-02055-7.

[2] Office of the National Economic and Social Development Council (NESDC). National income of Thailand 2020, chain volume measures [Internet]. 2021 [cited 2022 Nov 16]. Available from: https://www.nesdc.go.th/ewt_dl_link.php?nid=12298&filename=ni_page

[3] Tirloni AS, Reis DC, Borgatto AF, Moro ARP. Association between perception of bodily discomfort and individual and work organisational factors in Brazilian slaughterhouse workers: A cross-sectional study. BMJ Open. 2019; 9(2): e022824. doi:10.1136/bmjopen-2018-022824.

[4] Hancharoenkul B, Joseph L, Khamwong P, Pirunsan U. An investigation of the prevalence of work-related musculoskeletal pain and related disability among poultry slaughterhouse workers: A cross-sectional study. Int Arch Occup Environ Health. 2022; 96(3): 463-72. doi: 10.1007/s00420-022-01940-x.

[5] Reis DCD, Tirloni AS, Ramos E, Moro ARP. Assessment of risk factors of upper-limb musculoskeletal disorders in a chicken slaughterhouse. Jpn J Ergon. 2017; 53 (Supplement2): S458-S61. doi:10.5100/jje.53.S458.

[6] Schulz MR, Grzywacz JG, Chen H, Mora DC, Arcury TA, Marín AJ, *et al*. Upper body musculoskeletal symptoms of Latino poultry processing workers and a comparison group of Latino manual workers. Am J Ind Med. 2013; 56(2): 197-205. doi:10.1002/ajim.22100.

[7] Tirloni AS, Reis DC, Santos JB, Reis PF, Barbosa A, Moro AR. Body discomfort in poultry slaughterhouse workers. Work. 2012; 41 Suppl 1: 2420-5. doi:10.3233/ WOR-2012-0651-2420.

[8] Sundstrup E, Seeberg KGV, Bengtsen E, Andersen LL. A systematic review of workplace interventions to rehabilitate musculoskeletal disorders among employees with physical demanding work. J Occup Rehabil. 2020; 30(4): 588-612. doi: 10.1007/s10926-020-09879-x.

[9] Sundstrup E, Jakobsen MD, Jay K, Brandt M, Andersen LL. High intensity physical exercise and pain in the neck and upper limb among slaughterhouse workers: Cross-sectional study. Biomed Res Int. 2014; 218546. doi:10.1155/ 2014/218546.

[10] Sundstrup E, Jakobsen MD, Brandt M, Jay K, Aagaard P, Andersen LL. Strength training improves fatigue resistance and self-rated health in workers with chronic pain: A randomized controlled trial. Biomed Res Int. 2016; 2016: 4137918. doi: 10.1155/2016/4137918.

[11] Sundstrup E, Jakobsen MD, Andersen CH, Jay K, Persson R, Aagaard P, *et al*. Effect of two contrasting interventions on upper limb chronic pain and disability: A randomized controlled trial. Pain Physician. 2014; 17(2): 145-54.

[12] World Health Organization. Preventing disease through a healthier and safer workspace. 2018 [cited 2020 Sep 15]. Available from: https://www.who.int/publications/i/item/preventing-disease-through-a-healthier-and-safer-workspace.

[13] Aboodarda SJ, Page PA, Behm DG. Muscle activation comparisons between elastic and isoinertial resistance: A meta-analysis. Clin Biomech (Bristol, Avon). 2016; 39: 52-61. doi:10.1016/j.clinbiomech.2016.09.008.

[14] Seguin RC, Cudlip AC, Holmes MWR. The efficacy of upper-extremity elastic resistance training on shoulder strength and performance: A systematic review. Sports (Basel). 2022; 10(2): 24. doi:10.3390/sports10020024.

[15] Hammami M, Gaamouri N, Wagner H, Pagaduan JC, Hill L, Nikolaidis PT, *et al*. Effects of strength training with elastic band programme on fitness components in young female handball players: A randomized controlled trial. Biol Sport. 2022; 39(3): 537-45. doi:10.5114/biolsport.2022.106390.

[16] Iversen V, Vasseljen O, Mork P, Berthelsen I, Børke J-B, Bertheussen G, *et al*. Resistance training in addition to multidisciplinary rehabilitation for patients with chronic pain in the low back: Study protocol. Contemp Clin Trials Commun. 2017; 6: 115-21. doi:10.1016/j.conctc.2017.04.001.

[17] Iversen V, Vasseljen O, Mork P, Gismervik S, Bertheussen G, Salvesen O, *et al*. Resistance band training or general exercise in multidisciplinary rehabilitation of low back pain? A randomized trial. Scand J Med Sci Sports. 2018; 28(9): 2074-83. doi:10.1111/sms.13091.

[18] Sundstrup E, Jakobsen MD, Andersen CH, Jay K, Persson R, Aagaard P, *et al*. Participatory ergonomic intervention versus strength training on chronic pain and work disability in slaughterhouse workers: Study protocol for a single-blind, randomized controlled trial. BMC Musculoskelet Disord. 2013; 14: 67. doi:10.1186/1471-2474-14-67.

[19] Mansoori SS, Moustafa IM, Ahbouch A, Harrison DE. Optimal duration of stretching exercise in patients with chronic myofascial pain syndrome: A randomized controlled trial. J Rehabil Med. 2021; 53(1): jrm00142. doi:10.2340/ 16501977-2781.

[20] Assumpção A, Matsutani LA, Yuan SL, Santo AS, Sauer J, Mango P, *et al*. Muscle stretching exercises and resistance training in fibromyalgia: Which is better? A three-arm randomized controlled trial. Eur J Phys Rehabil Med. 2018; 54(5): 663-70. doi:10.23736/S1973-9087.17.04876-6.

[21] Decathlon Sports Thailand. Pilates equipment. 2022 [cited 2023 Jan 25]. Available from: https://www.decathlon.co.th/en/pilates-equipment/pilates-equipment-15778.

[22] Morishita S, Tsubaki A, Takabayashi T, Fu J. Relationship between the rating of perceived exertion scale and the load intensity of resistance training. Strength Cond J. 2018; 40(2): 94-109. doi:10.1519/SSC.0000000000000373.

[23] Bove A, Lynch A, DePaul S, Terhorst L, Irrgang J, Fitzgerald G. Test-retest reliability of rating of perceived exertion and agreement with 1-repetition maximum in adults. J Orthop Sports Phys Ther. 2016; 46(9): 768-74. doi:10.2519/ jospt.2016.6498.

[24] Boonstra AM, Schiphorst Preuper HR, Reneman MF, Posthumus JB, Stewart RE. Reliability and validity of the visual analogue scale for disability in patients with chronic musculoskeletal pain. Int J Rehabil Res. 2008; 31(2): 165-9. doi:10.1097/ MRR.0b013e3282f-c0f93.

[25] Angthong C, Chernchujit B, Suntharapa T, Harnroongroj T. Visual analogue scale foot and ankle: validity and reliability of Thai version of the new outcome score in subjective. J Med Assoc Thai. 2011; 94(8): 952-7.

[26] Hueter-Becker A, Doelken M, Fründ A. Physical therapy examination and assessment. New York : Thieme; 2015.

[27] Bucht H, Donath L. Sauna yoga superiorly improves flexibility, strength, and balance: A two-armed randomized controlled trial in healthy older adults. Int J Environ Res Public Health. 2019; 16(19): 3721. doi:10.3390/ijerph16193721.

[28] Kim SG, Kim EK. Test-retest reliability of an active range of motion test for the shoulder and hip joints by unskilled examiners using a manual goniometer. J Phys Ther Sci. 2016; 28(3): 722-4. doi:10.1589/ jpts.28.722.

[29] Jianmongkol S, Kosuwon W, Thammaroj T, Boonard M. Validity of the thai version of Disability of the Arm, Shoulder and Hand Questionnaire (KKU-DASH) in patients with brachial plexus injury. J Med Assoc Thai. 2011; 94(1): 71-7.

[30] Kaewboonchoo O, Ratanasiripong P. Psychometric properties of the Thai version of the work ability index (Thai WAI). J Occup Health. 2015;57(4):371-7. doi:10.1539/joh.14-0173-OA.

[31] Mordkoff JT. A Simple Method for Removing Bias From a Popular Measure of Standardized Effect Size: Adjusted Partial Eta Squared. Adv Methods Pract Psychol Sci. 2019; 2(3):228-32. doi:10.1177/ 2515245919855053

[32] Dworkin RH, Turk DC, McDermott MP, Peirce-Sandner S, Burke LB, Cowan P, *et al*. Interpreting the clinical importance of group differences in chronic pain clinical trials: Impact recommendations. Pain. 2009; 146(3): 238-44. doi:10.1016/j. pain.2009.08.019.

[33] Paungmali A, O'Leary S, Souvlis T, Vicenzino B. Naloxone fails to antagonize initial hypoalgesic effect of a manual therapy treatment for lateral epicondylalgia. J Manipulative Physiol Ther. 2004; 27(3):180-5. doi:10.1016/j.jmpt.2003.12.022.

[34] van den Berg T, Elders L, de Zwart B, Burdorf A. The effects of work-related and individual factors on the work ability index: A systematic review. Occup Environ Med. 2009; 66(4): 211-20. doi:10.1136/ oem.2008.039883.

[35] Bhimani R, Hang JY. Prevalence and characteristics of muscle tightness in young adults. Orthop Nurs. 2022; 41(3): 221-6. doi:10.1097/NOR.0000000000000817.

[36] Shariat A, Cleland JA, Danaee M, Kargarfard M, Sangelaji B, Tamrin SBM. Effects of stretching exercise training and ergonomic modifications on musculoskeletal discomforts of office workers: A randomized controlled trial. Braz J Phys Ther. 2018; 22(2): 144-53. doi:10.1016/j.bjpt.2017.09.003.

[37] Ylinen J, Kautiainen H, Wirén K, Häkkinen A. Stretching exercises vs manual therapy in treatment of chronic neck pain: A randomized, controlled cross-over trial. J Rehabil Med. 2007; 39(2): 126-32. doi:10.2340/ 16501977-0015.

[38] Opplert J, Babault N. Acute effects of dynamic stretching on muscle flexibility and performance: An analysis of the current literature. Sports Med. 2018; 48(2): 299-325. doi:10.1007/s40279-017-0797-9.

[39] Westcott W. ACSM strength training guidelines: Role in body composition and health enhancement. ACSMs Health Fit J. 2009; 13(4): 14-22. doi:10.1249/ FIT. 0b013e3181aaf460.

[40] Lagally K, Amorose A. The validity of using prior ratings of perceived exertion to regulate resistance exercise intensity. Percept Mot Skills. 2007; 104(2): 534-42. doi:10.2466/pms.104.2.534-542.

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Development of a communication book for children with cerebral palsy and communication disorders using the partner assisted scanning method: A pilot study

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ABSTRACT

Background: Cerebral Palsy (CP) is a physical disability impacting movement, posture, and communication. For children with CP and severe communication challenges, methods like Partner Assisted Scanning (PAS) are invaluable. Despite its effectiveness, research on PAS in the context of Thailand is scarce.

Objectives: This study aimed to develop and validate a communication book and related materials for children with CP, utilizing the PAS method. Additionally, the study aimed to analyze communication partners' satisfaction levels with the communication book.

Materials and methods: The study involved two phases. First, four instruments were developed and assessed for content validity: The communication Abilities Assessment, the Communication Partners' Satisfaction Survey, the Communication Book Manual, and the Communication Book. In the second phase, these instruments were tested with three families, evaluating partner satisfaction and addressing encountered issues.

Results: The study demonstrated high content validity of the communication book (overall validity index=0.97) as assessed by specialists. Feedback highlighted concerns about the book's format and the time needed for children to grasp its usage. However, communication partners expressed overall high satisfaction (mean=4.67, SD=0.49).

Conclusion: This pilot study establishes the robust content validity of the communication book developed using the PAS method. Moreover, it underscores high satisfaction levels among communication partners, affirming the potential of the communication book to enhance communication skills in children with CP.

Introduction

Cerebral palsy (CP) is a physical or developmental disorder of movement and balance caused by brain pathologies that appear during the development of the brain either before birth, during birth, or after birth, with many factors occurring together.[1] The incidence of children with CP is generally 1.5-3 children per 1,000 live births.[2] The Department of Empowerment of Persons with Disabilities, The Ministry of Social Development and Human Security, has reported situational data as of March 31, 2021, analyzed by type of disability. Movement or physical disability was the first for 1,043,192 people, accounting for 49.85% of all disabilities. Of this number, there were 16,128 people from early childhood (0-5 years) to school age (5-14 years), accounting for 1.54 percent of

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the number of people with mobility or physical disabilities.[3]

Children with CP are at risk for complications and comorbidities such as seizures, speech and language impairment, hearing loss, eating disorders, swallowing problems, visual impairment, psychiatric problems, and malnutrition.[1, 4-6] Studies of children with CP in Europe have shown that 60% of children with CP have communication difficulties, which can range from mild to severe, affecting their language comprehension and expression.[7,8] Therefore, they must receive therapy to improve their communication skills.

Communication is the exchange of information and ideas. It is divided into two types: verbal (written or spoken language) and nonverbal (facial expressions, body movement, and the use of pictures or symbols).[9] Children with CP with movement and communication impairments may not be able to communicate verbally but may be able to communicate non-verbally. As a result, a communication strategy or method with assistive devices may be required.[10]

The American Speech-Language-Hearing Association (ASHA) describes augmentative and alternative communication (AAC) as a method of communication used by children and adults who have temporary or permanent communication challenges, limitations on activities and communication engagement, or significant speech and language problems, including speaking and writing.[11] These are frequently found among children with physical and motor difficulties, autism, and severe intellectual disabilities.[12] The main goal of AAC is to help people with communication disabilities engage independently in their social environment, as well as to improve their ability to communicate their needs and transfer or share information with others for effective interaction and social etiquette.[13]

In general, AAC can be conveyed using simple gestures or body movements (unaided communication), which includes sign language and body language. The use of aided communication is divided into high-tech devices, which include electrical circuits, computers, and sound generators, and low-tech devices that do not include electrical circuits or computers but are typically made from paper or readily available materials such as communication boards, communication books, pictures, real objects, etc.[9] According to Kathryn, Light, and McNaughton, the use of AAC can begin in early childhood with parent involvement to improve communication skills in both language comprehension and language expression, as well as access to appropriate education planning for the future.[14]

The communication book is a tool of AAC, which is low-tech and used for people who cannot speak or have limited speech capability. These people need to point or look at the book to select the items, images, or symbols that they want to communicate. Communication books are suitable for accessing a large vocabulary and can employ various communication access techniques.[15] They also help to expand the vocabulary and knowledge of sentence structure, reduce irritating behavior, and increase role-switching with the interlocutor.[16] Direct and indirect selection and Partner Assisted Scanning (PAS) are methods for obtaining communications through communication books. A systematic search may be performed by utilizing their eyes, buttons, or switches for children with CP who cannot choose, point, press, or type independently due to restrictions in their motor movements and the use of their fingers.[9] PAS is an effective communication approach for those with limitations since it involves communication partners such as parents, caregivers, teachers, or therapists to assist in communication.[17] A systematic search is conducted by the communication partner by pointing to and reading aloud from an ordered list. Moreover, the communication partner does a methodical search by pointing to and reading aloud a list of sequences while simultaneously employing visual and auditory scanning.[18] These systematic sequencing methods can be applied to searches using group-item scanning, which is the most efficient way to search for ordered items in column-row scanning.[9]

For these reasons, researchers working as speech and language pathologists regularly observe that children with CP who have physical and motor deficits in addition to significant communication problems have communication learning limits. They are unable to speak or express their needs. Subsequently, children's communication can be misunderstood by parents, and children can become easily frustrated as a result. Parents frequently use short words or sentences while speaking to their children, and they often rely on a limited range of communication functions or provide choices for the children. Due to their limitations, parents lack the necessary knowledge to help their children communicate efficiently, and children must be rehabilitated by enrolling in a speech training program to correct their speech and boost language and communication development. Therefore, using AAC as part of the treatment is essential. Currently, in Thailand, AAC systems are not widely known or used because SLPs may have prejudices or a lack of understanding due to a lack of training and expertise with AAC systems. Seventy-eight percent of Thai SLPs taught children with nonverbal communication to use gesture communication.[19] There are few studies involving communication books and children with CP who can access tools or devices by pointing or selecting pictures directly. There has also been no study of communication books among children with CP who cannot point or select pictures independently but have sufficient vocabulary for basic communication. As a result, the researcher is interested in establishing and developing a communication book for children with CP who have communication disorders by using the PAS. By establishing and developing an easy-to-use and low-cost communication book, as well as an instruction manual on how to utilize the communication book with PAS, it can be used by parents, caregivers, and multidisciplinary teams to treat communication problems in children with CP.

Materials and methods

This research is an experimental study with a one-group pre-and post-test design. The participants in this

study were three children with CP aged 1-15 years with communication difficulties and three communication partners (parents or caregivers) who were receiving rehabilitation services in augmentative and alternative communication clinics at Rajanagarindra Institute of Child Development, Mae Rim District, Chiang Mai Province.

Inclusion criteria for children with CP

1. Children with CP aged 1-15 years with communication disabilities were diagnosed by a doctor, and the doctor ordered the use of augmentative and alternative communication methods.
2. Having developmental assessment results from the Thai Developmental Skills Inventory for Children from Birth to Five Years (TDSI),[20] which was assessed by nurses in terms of gross motor and fine motor skills, which found that the child has a delay in development or has physical limitations regarding movements, an inability to walk, limitations in the use of fingers to pick up or point objects, or an inability to help themselves in daily activities, and thereby requires assistance from parents or caregivers.
3. Having developmental assessment results from the Thai Developmental Skills Inventory for Children from Birth to Five Years (TDSI),[20] which was assessed by nurses in terms of expressive language and found to be late in childhood, not speaking meaningful words or speaking meaningful words with great difficulty, and in the receptive aspect, the child can understand simple commands and questions and can answer yes or no questions by making a blinking signal or making a refusal or acceptance movement.
4. Having clear vision or wearing visual aids to see clearly, such as glasses in the case of short or long-sightedness, according to a review of their history file.
5. Having hearing within the normal range of less than or equal to 25 decibels or wearing hearing aids that allow for clear hearing, such as wearing hearing aids during testing listed in their profile, and currently responding to commands at a conversation-level volume.
6. Able to understand and use Thai as their primary language and respond by nodding, blinking, smiling, or moving any part of their body.

Inclusion criteria for communication partners

1. Use of Thai as the primary language of communication.
2. Be able to read and write Thai well.
3. Being a parent or caregiver caring for children with CP for at least one year and mainly living with the child.
4. Be a parent or caregiver who can understand and interpret the communication of children in the initial stages, such as if the child accepts or rejects an object by making throat noises or moving any part of his or her body when it is handed to them.
5. Be a parent or caregiver who can communicate, interact, or switch roles with the child.

Exclusion Criteria

Children with CP with diseases that affect movement for communication, such as children with comorbidities e.g., severe intellectual disabilities, seizures that cannot be controlled with medication, etc.

Research instruments

1. An assessment of the communication abilities of children with CP using the PAS method.

There are 10 items in the assessment form that utilize a 3-point Likert rating scale, which includes options for high, moderate, and low ratings.[21] This scale is used to assess the difficulties experienced by the communication partners as follows:

- A score of 1 means that the child cannot communicate with a communication book using the PAS method.
- A score of 2 means that the child can communicate with a communication book using the PAS method to help with some needs or that takes effort to understand, such as guessing from previous experiences.
- A score of 3 means that the child can fully communicate with a communication book using the PAS method to identify needs immediately.

2. An evaluation form for the communication partner's satisfaction in using the communication book with the PAS method.

There are 15 items in this assessment form, which measures satisfaction in five aspects: format, usability, content, reducing frustration or negative behavior, and the communication partner's understanding of children's needs. This assessment employs a 5-point Likert rating scale, i.e., very dissatisfied, somewhat dissatisfied, neither dissatisfied nor satisfied, somewhat satisfied, and very satisfied.[21]

3. Manual for using the communication book for children with CP and communication disorders by applying the PAS method (Appendix 1).

The researcher studied the theory, documentation, and research related to the creation and design of a communication book manual for children with CP such as Children's Aided Language Tools (CHAT-Now)[21] and Pragmatic Organization Dynamic Display (PODD).[22] Therefore, Defining the contents of the manual, providing detailed information on the primary information of the communication book, its components, the details in the communication books, and how to use the communications book in line with the terms, images, patterns, navigation, and command bars, as the researcher designed and reviewed the literature.

4. Communication book (Appendix 2).

A hardcover folder with two holes inserted and an 80-micron hard laminate display page, A4 size, contains pictures of the PCS system symbols, including appropriate and necessary vocabulary for children with CP and their parents to use for communication in daily life, both the core vocabulary and fringe vocabulary.[9,22-24] The researcher

divided the words into 20 main and sub-categories: 1) Main page, 2) Categories lists, 3) Help, 4) Feelings, 5) Likes, 6) Dislikes, 7) the Body, 8) Questions, 9) People, 10) Verbs, 11) Activities, 12) Places, 13) Clothing, 14) Things, 15) Eating, 16) Transportation, 17) Animals, 18) Days and time, 19) Descriptions, and 20) Toilets, which can be searched using a vertical search pattern and navigation links to various categories with a number system in conjunction with the Thai alphabet.

Research methods

The research methods can be separated into two phases:

Phase 1. Development of the tools, including four instruments.

First, the researcher developed four instruments: 1) an assessment of the communication abilities of children with CP using the PAS method, 2) an evaluation form for the satisfaction of the communication partners regarding the use of the communication book with the PAS method, 3) a manual for using the communication book for children with CP and communication disorders by applying the PAS method, and 4) a communication book for children with CP and communication disorders that uses the PAS method. These instruments were designed based on a thorough literature review utilizing the theoretical principles of augmentative and alternative communication, with a particular focus on the PAS method theory. After the development of the four instruments, the researcher sent them to five experts specializing in the field of children with CP and AAC to check the content validity by calculating the Index of Item-Objective Congruence (IOC). After receiving suggestions from experts, the researcher used them to improve the content of the instruments. It was considered that an IOC>0.5 would pass the criteria.[21]

Phase 2. Trial of the tools with three families and examination of the satisfaction level of the communication partners using the communication book.

After the expert evaluation and the revision determined that the content validity test was completed, the researcher selected three families who passed the inclusion criteria for the research from the AAC clinic, Rajanagarindra Institute of Child Development, Department of Mental Health, Ministry of Public Health. They were selected from the order in which they volunteered for research projects. The researcher made an appointment to clarify the details of the research project, including using all four research tools with the communication partners of the children with CP individually for 3 hours per family, one family per day, totaling three days. A lecture and demonstration were used in this clarification by the researcher, who demonstrated to the communication partners how to point and read the symbols for the children to see and hear. When searching for the symbol that the child wants to communicate with, the communication partner will wait for the child to show a response with a movement within limits, such as smiling, blinking, or partial body movements.[17,18] After finishing the lecture session, the researcher asked the communication partners to check the suitability of using a communication book and supplemental materials by asking about the topic from the communication partners' satisfaction assessment form and to provide suggestions for improvement. After providing suggestions and improvements, the researcher distributed the communication partners' satisfaction assessment form for the use of the communication book using the PAS method to the communication partners of the experimental group to assess their satisfaction with the use of the experimental communication book and conduct an analysis of the data.

Results

Phase 1: Content validity

According to the examined IOC, each item of the content validity test passed the criteria, with the results shown in Table 1.

Phase 2. Trial of the tools with three families and examination of the satisfaction level of the communication partners using the communication book.

After the try-out for 3 hours per family, the suggestions were received from all three families, as displayed in Table 2, and the satisfaction levels of the three communication partners in using the communication book for children with CP and communication disorders by applying the PAS method are displayed in Table 3.

Table 1 Content validity of the instruments.

Content Validity			
No.	**Tools**	**IOC**	**Result**
1	Assessment of the communication abilities of children with CP by using the PAS method	0.99	Passed
2	Evaluation form for the satisfaction of the communication partner in the use of the communication book employing the PAS method	0.95	Passed
3	Manual for using the communication book for children with CP and communication disorders by applying the PAS method	0.97	Passed
4	Communication book for children with CP and communication disorders using the PAS method	0.98	Passed
Overall score		**0.97**	Passed

Table 2 Suggestions from the three users in the trial phase.

Consideration list	Suggestion	Improvement
1. Format of the communication book	▪ Change the format.	▪ The format was changed from a desk-calendar communication book to a folder communication book for easy portability and repair if damaged.
2. Content	▪ Some categories may not be used because they have not been taught to children.	▪ The content was kept the same because it is the vocabulary that is necessary for their daily life in the future.
3. Size	▪ Use A4-sized communication books so that the children and communication partners can see clearly.	▪ The size was adjusted according to the suggestion.
4. Symbols	▪ There may be some symbols that children need time to learn.	▪ The symbols were kept the same so that children can learn these symbols in the future.
5. Usability	▪ Some of the samples may not be understood with regard to their usage.	▪ The researcher emphasized that parents should use the communication book in every situation that occurs in daily life and that the children learn all of the content.
6. The manual for using the communication book for children with CP and communication disorders by applying the PAS method.	▪ No problems were found in its usage.	n/a
7. The assessment of the communication abilities of children with CP by applying the PAS method.	▪ No problems were found in its usage.	n/a
8. The evaluation form for the satisfaction of the communication partners in the use of the communication book with the PAS method.	▪ No problems were found in its usage.	n/a

Table 3 Satisfaction of the three communication partners in using the communication book for children with CP and communication disorders by applying the PAS method.

Aspect	List	Mean	SD	Level
1	Format	4.67	0.19	Highest
2	Usability	4.56	0.13	Highest
3	Content	4.80	0.28	Highest
4	Reduction of frustration or negative behavior	4.33	0.47	High
5	Communication partner's understanding of children's needs	4.67	0.28	Highest
Overall		**4.67**	**0.28**	**Highest**

Discussion

Phase 1. Development of tools, including four instruments.

In the content validity test by five experts, passing the criteria required a score of 0.5,[25] as shown in Table 1. Thus, when considering the content validity of all the instruments overall, it was found that all of them passed the criteria in terms of their content validity due to the researcher's review of the related literature and theories. Furthermore, the researcher interviewed communication partners about the words that they used with their children with CP in daily life before setting the contents, as the researcher reviewed the literature such as principles of communication book design,[15,16,26] word selection,[9,26] the PAS method,[17] the Communication Matrix,[27] the Pragmatic Profile for People who use AAC[28], communication books as Children's Aided Language Tools (CHAT-Now),[22] Pragmatic

Organization Dynamic Display (PODD),[23] and other relevant research studies. The researcher also asked for opinions about the vocabulary used in daily life from the parents of children with CP before determining the content of the research tools.

For the information that the experts recommended regarding corrections, in instrument 1, the experts recommended adding the disability characteristics of children with CP and adjusting the method of answering the question "What methods do children use to communicate?" to be optional, as well as adding a description of how to communicate with them. In instrument 2, the experts recommended changing the word "beautiful" to the phrase "clarity and beauty" and adjusting the category page or what to study first to make it easier to navigate links to other pages and to be consistent throughout the book. In instrument 3, the experts suggested adjusting words such as "sub-item" page to "sub-category-item" page, adjusting the details of various components in the manual for improved clarity, and adding illustrations to explain the additional information that will help guide users who are parents or caregivers to act as search assistants more clearly and allow them to understand how to use the communication book more easily. In instrument 4, the experts recommended adapting the symbols to be more contextual and culturally relevant to make them easier to use, such as changing the sign for the word "no" from the circle a with diagonal slash symbol to a cross symbol because it may be misunderstood that it is forbidden. After the researcher corrected all of the data according to the experts' recommendations, the first set of the four tools was obtained, consistent with the content.

Phase 2. Trial of the tools with three families and examination of the satisfaction level of the communication partners when using the communication book.

After the content validity review by experts, the researcher conducted trials involving three children with CP and their communication partners (Table 2). As a result of this phase, the sample groups proposed several improvements. They recommended changing the communication book format from a desk calendar style to a more portable and repairable folder format. Additionally, they suggested using a larger A4-sized communication notebook to ensure clear visibility of words and symbols for both children and communication partners. At first, some of the samples' usage may not be easily understood. For instance, communication partners may initially struggle to understand how to use the communication book, the PAS method, and the suitable duration and timing for their communication book. Therefore, the researcher emphasized that parents use the communication book in every situation in daily life along with reading the manual for the children to learn it well. Moreover, allow parents to repeat the PAS process to see if they can do it correctly until they can do it themselves through explanations and demonstrations.[29,30] Due to the diverse backgrounds of the families participating in this research, it is crucial that apart from a more profound comprehension of cultures and their connections to AAC, the researcher must acquire a heightened sensitivity to the cultural factors that may impact their approaches and suggestions. As a result, families were encouraged to participate in lectures and practice sessions, fostering a shared understanding between researchers and communication partners.[31] The researcher also asked the communication partners to complete an evaluation form for their satisfaction levels with using the communication book with the PAS method. The satisfaction of the three communication partners in using the communication book for children with CP and communication disorders by applying the PAS method (Table 3) was found to have the highest overall satisfaction level (mean=4.67, SD=0.49).

The researcher developed a tool for communication books in the form of an A4 hardcover folder with display pages consisting of 20 categories of symbolic images. There are links from the main page to different categories to support talking about the daily needs of children with CP. Scope stated that a communication book is suitable for people who cannot speak or have limited communication abilities. The communication book can provide a large vocabulary, and thus, it is suitable for discussing the needs of children with CP in daily life.[15] This corresponds to the principles of communication book development that the researcher has reviewed in the literature. Inside the communication book, there are clearly colored symbols contrasting with the white background, and the large and clear symbols also have words above them to stimulate communication through visual means and listening. Beukelman and Mirenda reported that the Picture Communication Symbols (PCS) system is easy to understand and can be used with various people.[9] Fanourgiakis also reported that the development of communication books should take into account the number and size of the symbols so that they are suitable for use, the selection of words in the essential vocabulary category, and the needs of that group of people, which is consistent with the researcher having parents help determine the vocabulary that is used frequently with children to include the vocabulary that meets the users' needs and is suitable for continued use.[26] In addition, both the vocabulary and symbols are appropriate for the Thai cultural context,[24] making it easy for children and communication partners to apply them.

Accessing communication books by children with CP through indirect methods or the PAS method is consistent with Beukelman and Mirenda, who stated that this method is suitable for children with CP or children who cannot select communication lists by themselves.[9] Therefore, an assistant to search for symbols and pictures used in communication is required. Also, Bayldon and Clendon17 and Burkhart and Porter18 describe that the PAS method can be used for people with communication and movement disorders. The communication partner will help to point and read the symbols for the children to see and hear. When searching for the symbol that the children want to communicate, they will show a response with movements within their limits, such as smiling, blinking,

or partial body movements. The PAS method, using visual and auditory content, will help children learn to recognize the symbols and understand more linguistic concepts.

Limitations

Only the results of the tool development process and the communication book trial were included in this research. The trial period in the second phase was relatively short. Therefore, future research should consider extending the trial period to assess the tool's effectiveness more comprehensively.

Conclusion

In the pilot study, it can be inferred that the development of the communication book using the PAS method demonstrated a strong level of content validity, and the communication partners were satisfied with the communication book using the PAS method. However, they suggested changing the communication book's format and size to make it easier to use. Thus, the modified communication book can potentially be used to enhance the communication skills of children with CP in the next phase of the research concerning the efficacy of this communication book using the PAS method.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

Ethics approval

This research gained ethical approval from the Suanprung Psychiatric Hospital, Chiang Mai (SPH.IRB 004/2564 SCs_ful) for research involving human participants. All samples and participants received all of the necessary information related to the research and informed written consent was obtained before enrolling them in the study**.**

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References

[1] Patel DR, Neelakantan M, Pandher K, Merrick J. Cerebral palsy in children: a clinical overview. Transl Pediatr. 2020; 9(Suppl 1): S125-S135. doi. org/10.21037/tp.2020.01.01

[2] Himmelmann K, McIntyre S, Goldsmith S, Smithers-Sheedy H, Watson L. Epidemiology of Cerebral Palsy. In: Miller F, Bachrach S, Lennon N, O'Neil M, Eds. Cerebral Palsy. Cham: Springer International Publishing; 2018. p. 1-16.

[3] The Department of Empowerment of Person with Disabilities, The Ministry of Social Development and Human Security. Report on the disability situation in Thailand; March 31, 2021. [cited 2021 May 25]. Available from: https://dep.go.th/images/uploads/files/situation31mar64.pdf

[4] Hansakunachai T, *et al*. Guideline to the rehabilitation of children with cerebral palsy (Family version). Pathum Thani: Thammasat University Hospital; 2008. (in Thai).

[5] Hansakunachai T. Child Development and Behavior [Bangkok]: Development Behavioral Pediatrics; 2011. (in Thai).

[6] Buntragulpoontawee M. Rehabilitation of pediatric patients with cerebral palsy. Rehabilitation medicine in general practice. Chiangmai, Viangbua Printing; 2018. (in Thai).

[7] Bax M, Tydeman C, Flodmark O. Clinical and MRI Correlates of Cerebral Palsy. JAMA. 2006; 296(13): 1602. doi.org/10.1001/jama.296.13.1602.

[8] Pennington L, Roelant E, Thompson V, Robson S, Steen N, Miller N. Intensive dysarthria therapy for younger children with cerebral palsy. Dev Med Child Neurol. 2013; 55(5): 464-71. doi.org/10.1111/dmcn.12098.

[9] Beukelman DR, Mirenda P. Augmentative and Alternative Communication: Supporting Children and Adults with Complex Communication Needs: Paul H. Brookes Pub.; 2013.

[10] Burkhart L. Multi-modal communication and learning strategies for children who face significant challenges [Internet]. 2016. [cited 2021 Jan 14]. Available from: https://lindaburkhart.com/wp-content/uploads/2016/11/multi-modal-2-day-8-16-AGOSCI-and-ATANZ.pdf.

[11] American Speech-Language-Hearing Association. Augmentative and alternative communication. [Internet]. 2020. [cited 2021 Jan 15]. Available from: https://www.asha.org/public/speech/disorders/aac/.

[12] Balandin S, Sweep A. Functional communication in the classroom [Internet]. Deakin University; 2005 [cited 2021 Jul 15]. Available from: https://hdl.handle.net/10536/DRO/DU:30067011.

[13] Light J. Interaction involving individuals using augmentative and alternative communication systems: State of the art and future directions. Augmentative and Alternative Communication. 1988; 4(2): 66-82. doi.org/10.1080/07434618812331274657.

[14] Kathryn D, Light J, McNaughton D. Effects of AAC interventions on communication and language for young children with complex communication needs. J Pediatr Rehabil Med. 2010; 3: 303-10. doi.org/10.3233/PRM-2010-0141.

[15] Scope. How to design a communication aid. [Internet]. Australia, VIC: Communication & Inclusion Resource Centre; 2015. [cited 2021 Jan 4]. Available from: https://www.scopeaust.org.au/wp-content/uploads/2017/03/Necas-aid-design-handout.pdf.

[16] CATS - Northcott Early Individualhood Assistive

Technology Project. Communication Books. [Internet]. 2008. [cited 2021 Jan 4]. Available from: http://www.complexneeds.org.uk/modules/Module-3.1-Communication-augmentative-and-assistive-strategies/All/downloads/m09p110bcd/using_communication_books.pdf.

[17] Bayldon H, Clendon S. Partner assisted scanning: Enable the unexpected. J Clin Pract Speech Lang Pathol. 2017; 19: 142-150.

[18] Burkhart L, Porter G. Partner-assisted communication strategies for children who face multiple challenges. Proceeding of 12th Biennial Conference of ISAAC; 2006 Jul 29-Aug 4; Düsseldorf, Germany, 2006. Available from: https://lindaburkhart.com/wp-content/uploads/2016/07/Isaac_instructional_06.pdf.

[19] Kamonsitichai W, Goldstein H. Speech-language pathologists' perceptions of augmentative and alternative communication in Thailand. Augment Altern Commun. 2023: 1-11. doi.org/10.1080/07434618.2023.2208222

[20] Samai Sirithongthaworn *et al*. Development of Development Skills Inventory for Children at Birth to 5 years of age, the Ministry of Public Health, Mental Health Department Version. Rajanagarindra Institute of Child Development.; 2009.

[21] Likert R. New patterns of management. New York, NY, US: McGraw-Hill; 1961. ix, 279. (New patterns of management).

[22] Communication Resource Centre: SCOPE. CHAT- Now Manual. Children's Aided language Tools. [Internet]. Box Hill, Australia: Communication Aids and Resource Materials; 2007 [cited 2021 Jan 6]. Available from: http://www.angelman.ie/wp-content/uploads/CHAT-Now-Manual-FINAL.pdf.

[23] Porter, G. Pragmatic organisation dynamic display communication books: Alternative Access Templates. Glen Waverley, VIC: Cerebral Palsy Education Centre.; 2017.

[24] Katkasam, Montarop. Basic vocabulary list pratomsuksa 1-6. Bureau of Academic Affair's and Educational Standards. [Internet]. 2019. Available from: https://sites.google.com/a/phrae2.go.th/nitedph2/thai/baychikhaphunthanp1-6(in Thai).

[25] Ongiem A, Vichitvejpaisal P. Validation of the tests. Thai J Anesthesiol. [Internet]. 2018; 44(1): 36-42. Available from: https://he02.tcithaijo.org/index.php/anesthai/article/view/116944. (in Thai).

[26] Fanourgiakis V. What to Consider When Building a Communication Book. [Internet]. 2016. [cited 2020 Jun 16]. Available from: https://leader.pubs.asha.org/do/10.1044/considerations-for-building-a-communication-book/full/.

[27] Rowland C, Fried-Oken M. Communication Matrix: A clinical and research assessment tool targeting children with severe communication disorders. J Pediatr Rehabil Med. 2010; 3: 319-329. doi.org/10.3233/PRM-2010-0144.

[28] Martin, S., Small, K., and Stevens, R. The Pragmatics Profile for People who use AAC. [Internet]. 2017 [updated 2017 Sep 26; cited 20 Oct 2020]. Available from: http://ace enter.org.uk/resources/pragmatics-profile-people-use-aac/.

[29] Nunes D, Hanline MF. Enhancing the Alternative and Augmentative Communication Use of a Child with Autism through a Parent-implemented Naturalistic Intervention. International Journal of Disability, Development and Education. 2007; 54(2): 177-97. doi.org/10.1080/10349120701330495

[30] Kent-Walsh J, Murza KA, Malani MD, Binger C. Effects of Communication Partner Instruction on the Communication of Individuals using AAC: A Meta-Analysis. Augmentative and Alternative Communication. 2015; 31(4): 271-84. doi.org/10.3109/07434618.2015.1052153

[31] Soto G. Training Partners in AAC in Culturally Diverse Families. Perspectives on Augmentative and Alternative Communication. 2012; 21: 144. doi.org/10.1044/aac21.4.144

Appendix

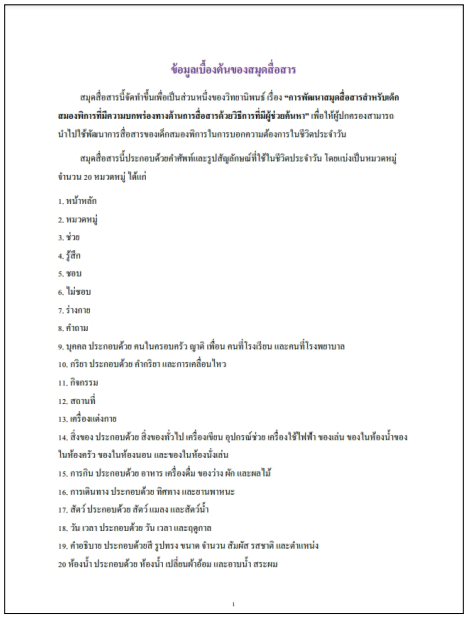
Appendix 1 Example of a manual for using the communication book for children with CP and communication disorders by employing the PAS method.

คู่มือการใช้สมุดสื่อสารสำหรับเด็กสมองพิการ
ด้วยวิธีการที่มีผู้ช่วยค้นหา

วิทยานิพนธ์
การพัฒนาสมุดสื่อสารสำหรับเด็กสมองพิการ
ที่มีความบกพร่องทางด้านการสื่อสารด้วยวิธีการที่มีผู้ช่วยค้นหา

Development of Communication Book for Children with Cerebral Palsy
and Communication Disorders by Using Partner Assisted Scanning Method

คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่

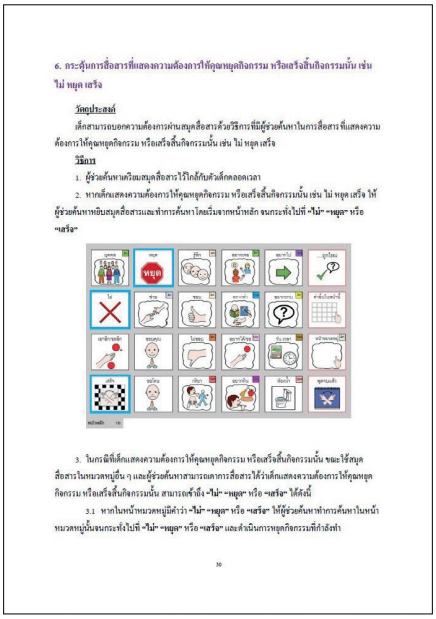


ข้อมูลเบื้องต้นของสมุดสื่อสาร

สมุดสื่อสารนี้จัดทำขึ้นเพื่อเป็นส่วนหนึ่งของวิทยานิพนธ์ เรื่อง **"การพัฒนาสมุดสื่อสารสำหรับเด็กสมองพิการที่มีความบกพร่องทางด้านการสื่อสารด้วยวิธีการที่มีผู้ช่วยค้นหา"** เพื่อให้ผู้ปกครองสามารถนำไปใช้พัฒนาการสื่อสารของเด็กสมองพิการในการบอกความต้องการในชีวิตประจำวัน

สมุดสื่อสารนี้ประกอบด้วยคำศัพท์และรูปสัญลักษณ์ที่ใช้ในชีวิตประจำวัน โดยแบ่งเป็นหมวดหมู่ จำนวน 20 หมวดหมู่ ได้แก่

1. หน้าหลัก
2. หมวดหมู่
3. ช่วย
4. รู้สึก
5. ชอบ
6. ไม่ชอบ
7. ร่างกาย
8. คำถาม
9. บุคคล ประกอบด้วย คนในครอบครัว ญาติ เพื่อน คนที่โรงเรียน และคนที่โรงพยาบาล
10. กริยา ประกอบด้วย คำกริยา และการเคลื่อนไหว
11. กิจกรรม
12. สถานที่
13. เครื่องแต่งกาย
14. สิ่งของ ประกอบด้วย สิ่งของทั่วไป เครื่องเขียน อุปกรณ์ช่วย เครื่องใช้ไฟฟ้า ของเล่น ของในห้องน้ำของในห้องครัว ของในห้องนอน และของในห้องนั่งเล่น
15. การกิน ประกอบด้วย อาหาร เครื่องดื่ม ของว่าง ผัก และผลไม้
16. การเดินทาง ประกอบด้วย พาหนะ และสถานพาหนะ
17. สัตว์ ประกอบด้วย สัตว์เมือง และสัตว์น้ำ
18. วัน เวลา ประกอบด้วย วัน เวลา และอุตุภาล
19. คำอธิบาย ประกอบด้วยสี รูปทรง ขนาด จำนวน สัมผัส รสชาติ และตำแหน่ง
20 ห้องน้ำ ประกอบด้วย ห้องน้ำ เปลี่ยนคำย้อม และอาบน้ำ สระผม



6. กระตุ้นการสื่อสารที่แสดงความต้องการให้หยุดพฤติกรรม หรือเสร็จสิ้นกิจกรรมนั้น เช่น ไม่ หยุด เสร็จ

วัตถุประสงค์

เด็กสามารถบอกความต้องการผ่านสมุดสื่อสารด้วยวิธีการที่มีผู้ช่วยค้นหาในการสื่อสารที่แสดงความต้องการให้หยุดพฤติกรรม หรือเสร็จสิ้นกิจกรรมนั้น เช่น ไม่ หยุด เสร็จ

วิธีการ

1. ผู้ช่วยค้นหาเหรียมสมุดสื่อสารไว้ใกล้กับตัวเด็กตลอดเวลา
2. หากเด็กแสดงความต้องการให้หยุดพฤติกรรม หรือเสร็จสิ้นกิจกรรมนั้น เช่น ไม่ หยุด เสร็จ ให้ผู้ช่วยค้นหาหยิบสมุดสื่อสารและทำการค้นหาโดยเริ่มจากหน้าหลัก จนกระทั่งไปที่ "ไม่" "หยุด" หรือ "เสร็จ"

3. ในกรณีที่เด็กแสดงความต้องการให้หยุดพฤติกรรม หรือเสร็จสิ้นกิจกรรมนั้น ขณะใช้สมุดสื่อสารในหมวดหมู่อื่น ๆ และผู้ช่วยค้นหาสามารถค้นหาการสื่อสารได้ว่าเด็กแสดงความต้องการให้หยุดพฤติกรรม หรือเสร็จสิ้นกิจกรรมนั้น สามารถเข้าถึง "ไม่" "หยุด" หรือ "เสร็จ" ได้ทันที

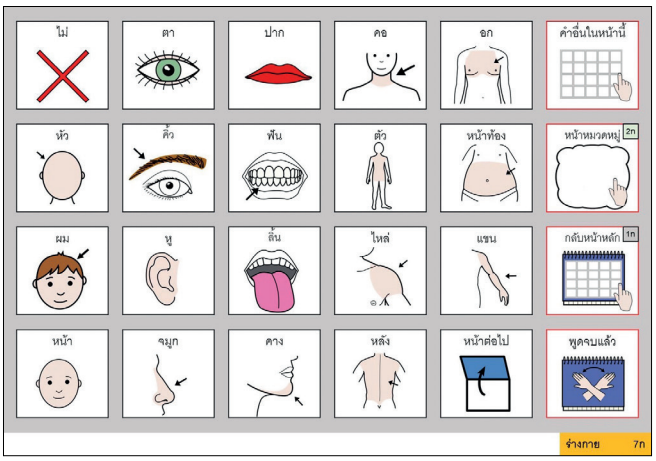
3.1 หากในหน้าหมวดหมู่มีคำว่า "ไม่" "หยุด" หรือ "เสร็จ" ให้ผู้ช่วยค้นหาทำการค้นหาในหน้าหมวดหมู่นั้นจนกระทั่งไปที่ "ไม่" "หยุด" หรือ "เสร็จ" และดำเนินการหยุดกิจกรรมที่กำลังทำ

Appendix 2 Example of the communication book.

สมุดสื่อสารสำหรับเด็กสมองพิการด้วยวิธีการที่มีผู้ช่วยค้นหา

วิทยานิพนธ์
การพัฒนาสมุดสื่อสารสำหรับเด็กสมองพิการที่มีความบกพร่องทางด้านการสื่อสารด้วยวิธีการที่มีผู้ช่วยค้นหา
Development of Communication Book for Children with Cerebral Palsy and Communication Disorders
by using Partner Assisted Scanning Method

คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่



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Radiation dose in radiologist from cerebral angiography using optically stimulated luminescence dosimeter

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ABSTRACT

Background: The number of cerebral angiography procedures is increasing, resulting in higher X-ray radiation doses received by radiologists. Consequently, understanding the radiation doses received by radiologists and the accumulation of radiation in control rooms is crucial for guiding prevention strategies against radiation hazards.

Objectives: This study aimed to measure and evaluate radiation doses to the hands, lenses of the eyes, and thyroids of radiologists performing cerebral angiography procedures, as well as to measure the accumulated radiation dose in the control room.

Materials and methods: OSL dosimeters were placed on the eyeglass frames, thyroids, hands, and legs of radiologists performing 20 cerebral angiography procedures, as well as on the wall and window of the control room.

Results: Radiologists' average radiation doses were measured at specific body parts as follows: left eye (49 µSv), right eye (15 µSv), left hands (34 µSv), right hands (16 µSv), left legs (27 µSv), right legs (7 µSv), and thyroid glands (14 µSv). Notably, the received doses remained well within the maximum radiation dose limit established by the International Commission on Radiological Protection (ICRP). When calculating the maximum number of procedures that can be performed annually, we based it on the limit of the radiation dose that the eyes' lenses should not exceed. Our findings revealed that the permissible number of procedures determined by the lens radiation dose limit, should not surpass 405 cases annually (equivalent to 34 cases per month). The radiation dose from therapeutic angiography procedures was discovered to be up to 5 times higher than that from diagnostic angiography procedures. The maximum accumulated radiation dose in the control room was 1.18 µSv/hr, which remained below the limit of the Department of Medical Sciences (< 3 µSv/hr).

Conclusion: Radiologists receive less radiation from cerebral diagnostic angiography than therapeutic angiography. Organs on the left side were exposed to greater radiation levels than those on the right side. Wearing radiation protection devices during each procedure can reduce radiation exposure and mitigate long-term effects on radiologists. It is recommended to monitor and calculate the accumulated radiation dose of workers to ensure their exposure remains within safety limits.

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Introduction

Currently, a significant number of patients are facing issues related to cerebral vascular diseases. According to the World Stroke Organization (WSO), the global incidence of cerebral vascular diseases is approximately 15 million annually, with at least one death occurring every 6 seconds.[1] Cerebral vascular disease refers to a condition where the brain lacks blood supply due to cerebral artery stenosis, cerebral artery occlusion, or cerebral artery rupture, obstructing blood flow to the brain cells and leading to functional impairment or even death. Cerebral vascular disease stands among the noncommunicable diseases (NCDs), presenting a significant health concern in the Thai population. In 2018, it exhibited the highest mortality rate before the expected age, ranking second to cancer.[2] Therefore, diagnosing and treating cerebral vascular disease is crucial in reducing mortality rates. The treatment options for cerebral vascular disease vary depending on the severity of the disease, including medication, surgery, and endovascular interventions.

Cerebral angiography is a diagnostic and therapeutic procedure used to visualize blood vessels and identify the location of lesions during medical interventions. It involves using a Biplane Digital Subtraction Angiography (DSA) machine, which utilizes X-ray technology. During the procedure, a small catheter or tube is inserted into the artery to access the blood vessels supplying the brain and neck. Then, a contrast agent is injected to enhance visibility in radiographic images. These images help visualize the blood vessels and the lesion area with greater clarity. The procedure of cerebral angiography requires extensive radiographic imaging during the procedure. This includes real-time fluoroscopy, which provides live images of the moving blood vessels, a series of static images, mask images obtained before contrast injection, and image subtraction techniques applied to optimize the visualization of blood vessels. Subsequently, the physician plans the treatment of the affected cerebral blood vessels, which may involve various therapeutic techniques. These may include the application of balloon angioplasty to treat ischemic stroke, stent placement, or coil embolization to treat aneurysms. In cases of vascular blockages, an embolization technique using substances like glue may be employed to occlude the affected blood vessels. Conversely, for cases of arterial stenosis, treatments such as mechanical thrombectomy may be employed to address the condition and restore proper blood flow.[3]

The use of X-ray, known as ionizing radiation, leads to the ionization of atoms within the body, thereby exposing both patients and medical professionals to direct radiation as well as scattered radiation from various angles during diagnostic and therapeutic procedures.[4,5] The amount of radiation received by patients and medical professionals during cerebral angiography procedures depends on several factors. These factors include the distance from the X-ray tube, the duration of exposure during fluoroscopy, the number of radiographic images taken, and the radiation exposure technique used (kV, mAs). Additionally, the complexity of the underlying medical condition and the level of expertise demonstrated by the physician performing the procedure also contribute to the overall radiation dose received.[6] These combined factors may result in radiation doses that fall within permissible ranges[6] or potentially exceed the established safety thresholds for physicians and patients.[7] The effects of radiation include both stochastic and deterministic effects. Stochastic effects occur to a varying degree depending on the accumulated radiation dose received. Prolonged accumulation of radiation in the body over time may have long-term effects, such as the development of leukemia, genetic changes, and cataract formation.[8] Deterministic effects, on the other hand, depend on the amount of radiation received and may result in symptoms such as nausea and erythema. The International Commission on Radiological Protection (ICRP) has set a threshold dose for cataract formation at 0.5 Gy and established annual equivalent radiation dose limits for the lens of the eye for radiation workers not exceeding 20 millisieverts (mSv/y), and for the skin, hands, and feet not exceeding 500 mSv/y.[9] Monitoring and measuring the radiation doses physicians receive can help analyze and assess their radiation exposure for future risk prevention. Radiation measuring devices such as thermoluminescence dosimeters (TLD),[6,7] optically stimulated luminescence dosimeters (OSL)[10], photoluminescence glass dosimeters (PGD)[11] are positioned on the bodies of physicians, medical professionals, patients, or simulated phantoms. These dosimeters, along with personal radiation monitoring devices capable of offering real-time data,[12] serve to record and evaluate the levels of radiation exposure experienced by individuals involved in the procedure. By employing these measurement tools, healthcare providers can effectively monitor and assess radiation doses, thus promoting a safer environment for medical personnel and patients. Moreover, the control room of the cerebral angiography X-ray machine, regularly occupied by radiologic technologists, nurses, and physicians, is categorized as a controlled area with an elevated likelihood of radiation exposure compared to uncontrolled areas. Therefore, it is necessary to assess the effectiveness of the walls and doors in preventing radiation leakage. The Department of Medical Sciences specifies that the radiation dose in controlled areas should not exceed 5 microsieverts per hour (μSv/h), and regular measurements should be conducted every two years.[13] For instance, radiation survey meters or optically stimulated luminescence dosimeters are used to measure radiation levels in radiology work areas[14] or patient accommodation areas where iodine-131 therapy is administered.[15]

This research aims to measure radiation doses from radiologists' hands, feet, eye lenses, and thyroid during cerebral angiography procedures. Additionally, it aims to assess the accumulation of radiation doses in the control room of the X-ray machine used for cerebral angiography.

Materials and methods

Instruments and equipment

The C-Arm fluoroscopy machine used in this study was the Allura Xper FD20/20 Biplane model, manufactured by

Philips in the Netherlands. Radiation measurement devices included the nanoDot™ dosimeter (from Landauer® in the United States) for measuring accumulated radiation doses in various organs of the physicians. The InLight® dosimeter (also from Landauer® in the United States) was used to measure accumulated radiation doses in the environment. The nanoDot™ dosimeter readings were obtained using the microStar® Dosimetry Reader (manufactured by Landauer® in the United States). A radiation dose eraser (provided by the Office of Atoms for Peace, Thailand) removed the signal from OSL nanoDot™ dosimeter. An InLight® reader and annealer device (InLight Auto 200 Dosimetry Reader, manufactured by Landauer® in the United States) were used.

Methods

This research study was approved by the Research Ethics Committee of the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, with the reference number COA 087/2565. The aim was to measure radiation levels and assess the radiation doses received by radiologists during the cerebral angiography procedures.

Measurement of radiation doses received by radiologists.

Before attaching the nanoDot™ radiation dosimeter to the radiologist, baseline values of all nanoDot™ dosimeter badges were read using the microStar® Dosimetry Reader to establish background readings. The nanoDot™ dosimeter badges were individually wrapped in thin plastic sheets to prevent contamination and divided into two sets. One set was kept in a radiation-free area for the radiation background measurement. The other set, consisting of eight nanoDot™ dosimeter badges, was attached at various positions on the radiologist, including both sides of the eyeglass frames to represent the radiation doses at the lenses, the center of the outer and inner sides of the thyroid shield to measure the thyroid dose, both wrists, and both legs. After the completion of the procedure, the eight nanoDot™ dosimeter badges were placed in a light-tight box to prepare them for reading using the dosimetry reader. Once the readings were acquired, the badges underwent processing in the dosimeter reader to erase the residual signal from the nanoDot™ dosimeters. For subsequent procedures, a new set of nanoDot™ dosimeter badges was employed to measure the radiation doses during each procedure (Figure 1).

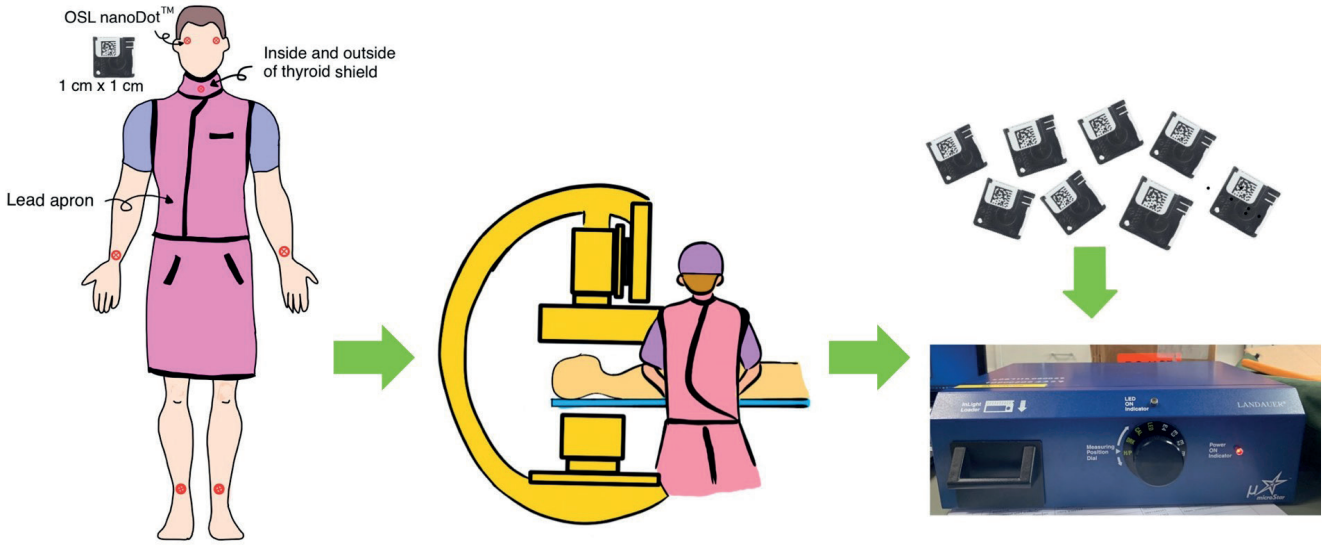


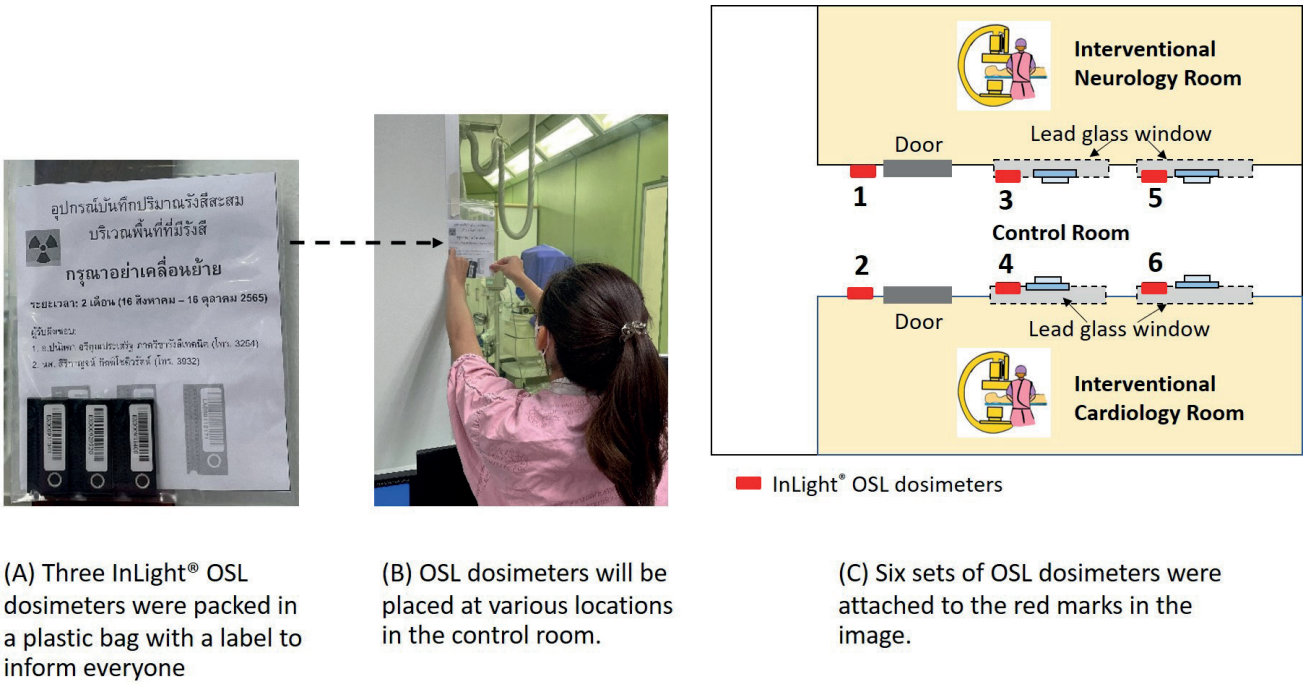
Figure 1. *Illustrates (A) The attachment of OSL nanoDot™ dosimeters to the physician's body. (B) The physician is performing cerebral angiography procedures. (C) The reading of OSL nanoDot™ dosimeters using a Landauer microStar dosimetry reader.*

In addition, this study collected both qualitative and quantitative data. Qualitative data included the type of procedure, pathological conditions, and vascular positions. Quantitative data consisted of fluoroscopic time, patient radiation doses, dose area product (DAP), and relevant parameters for each cerebral angiography procedure. The purpose of collecting this data was to analyze the relationships between radiation doses received and to calculate the maximum number of procedures that could be performed per month or per year. This analysis involved comparing the average radiation doses received by various organs with the established dose limits.

The Measurement of cumulative radiation in the controlled area

To assess the cumulative radiation dose in the controlled area, InLight® radiation dosimeters were placed in designated locations. Before placing the InLight®

dosimeters, their baseline values were obtained by reading them with the InLight® Dosimetry Reader. A set of three InLight® dosimeters was packed in individual plastic bags. One set was stored in an area without radiation to serve as the background radiation value. The remaining sets of InLight® dosimeters were placed in the control room of the angiography room, where the staff regularly performs their duties. These dosimeters were left in place for two months to measure the cumulative radiation dose. After the designated period, the radiation dose accumulated in the InLight® dosimeters was read using the InLight® Dosimetry Reader (Figure 2). At the end of the two months, all the InLight® dosimeters were sealed in light-protected boxes and sent to read using the InLight® Dosimetry Reader. Three readings were conducted to determine the average radiation dose per hour at various positions within the control room. These calculated values were then compared against the established radiation dose limits for the controlled area.



(A) Three InLight® OSL dosimeters were packed in a plastic bag with a label to inform everyone

(B) OSL dosimeters will be placed at various locations in the control room.

(C) Six sets of OSL dosimeters were attached to the red marks in the image.

Figure 2. *Illustrates (A) The preparation of InLight® OSL dosimeters. (B) The attachment of OSL dosimeters in the control room. (C) OSL dosimeters were attached at six positions in the control room.*

Results

The data obtained from 20 patients who underwent cerebral angiography revealed the following average values: the patients' average body mass index (BMI) was 26.11±3.96, the average fluoroscopy time was 19.91±20.81 minutes, and the average dose area product (DAP) was 119,751±99,175 mGy/cm^2. Additionally, the average number of acquired runs was 14±7, and the average number of acquired images was 762±438 (Table 1).

Table 1. Data on cerebral angiography procedures (N=20).

	Max	Min	Average	SD
Patient BMI	36.21	20	26.11	3.96
Fluoroscopy time (minutes)	90.12	3.36	19.91	20.81
DAP (mGy/cm^2)	452,435	33,684	119,751	99,175
Number of acquired runs	42	6	14	7
Number of acquired image	1690	294	762	438

Interventional radiologists' radiation dose during cerebral angiography was measured using nanoDot™ dosimeters at various organs. The dosimeters were attached to the outside and inside of the thyroid shield, both sides of the eyes, both hands, and both legs. Organs on the left side received higher radiation doses than the right side. The left eye received the highest dose at 49±65 µSv (max 289 µSv), followed by the left hand at 34±37 µSv (max 46 µSv). The maximum and minimum values of the measured radiation dose are very different. This may be due to staff height and experience, radiation shield used, OSL efficiency, and angular dependence. Using a thyroid shield reduced radiation dose by approximately three times (Table 2).

Table 2. Radiation dose received by organs during cerebral angiography procedures (N=20).

Organ	Max	Min	Average	SD
Left Eye (µSv)	289	2	49	65
Right Eye (µSv)	54	2	15	14
Inside Thyroid Shield (µSv)	64	0*	5	14
Outside Thyroid Shield (µSv)	73	2	14	16
Left Hand (µSv)	174	2	34	38
Right Hand (µSv)	47	0*	16	15
Left Leg (µSv)	236	0*	27	52
Right Leg (µSv)	21	0*	7	5

*Note: *organs with readings below the background during cerebral angiography are reported as receiving 0 Gy of radiation dose.*

Comparing radiation exposure in various organs during diagnostic and therapeutic cerebral angiography, it was found that therapeutic procedures resulted in higher doses than diagnostic ones (Figure 3). The radiologist's left eye received up to 5 times more radiation during therapeutic procedures, and the left hand received up to 2 times more (Table 3). Moreover, therapeutic procedures require longer fluoroscopy time, leading to higher radiation doses for patients than diagnostic procedures.

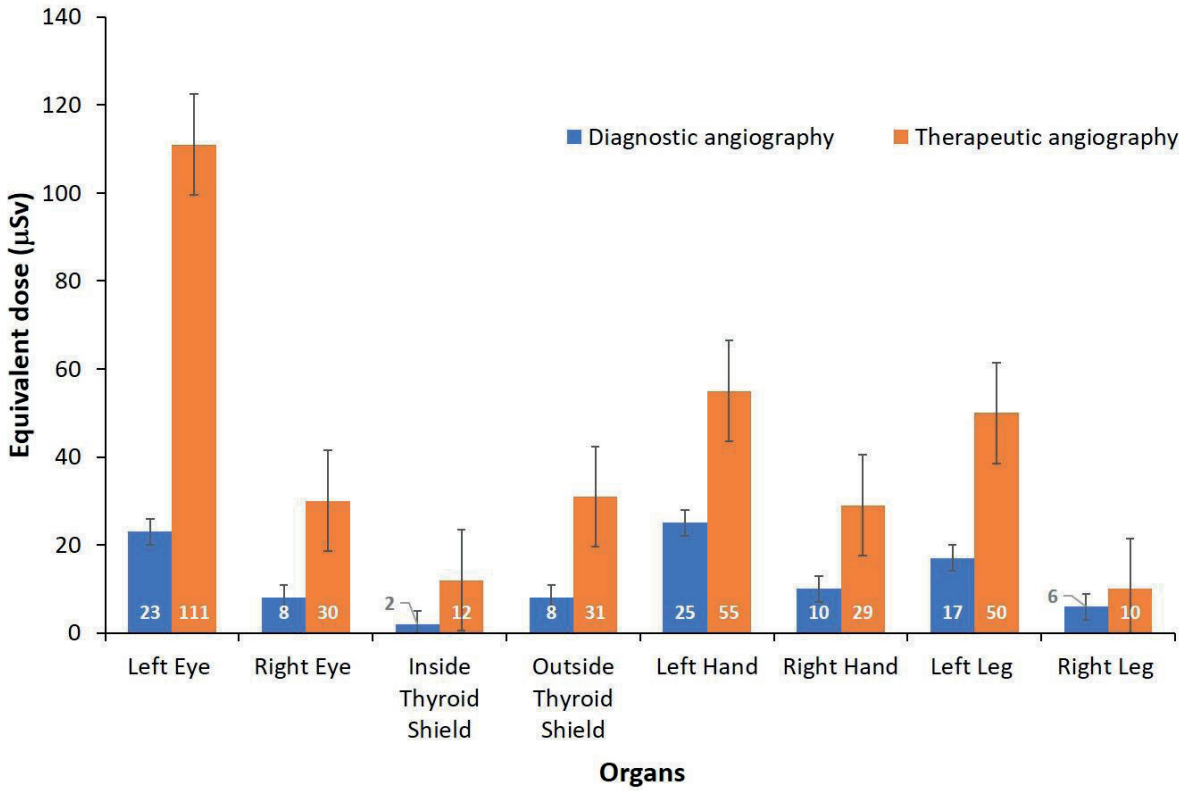


Figure 3. *Compares the radiation equivalent doses between the two procedure types for each organ.*

Table 3. Radiation equivalent doses for radiologists in different organs during diagnostic (N=14) and therapeutic (N=6) cerebral angiography procedures.

Organ	Diagnostic Angiography (N=14)				Therapeutic Angiography (N=6)			
	Max	Min	Average	SD	Max	Min	Average	SD
Left Eye (µSv)	47	2	23	13	289	35	111	96
Right Eye (µSv)	17	2	8	5	54	13	30	18
Inside Thyroid Shield (µSv)	4	0*	2	1	64	0	12	25
Outside Thyroid Shield (µSv)	16	2	8	4	73	9	31	23
Left Hand (µSv)	74	2	25	20	174	12	55	60
Right Hand (µSv)	34	0	10	8	47	9	29	19
Left Leg (µSv)	60	0	17	19	236	4	50	91
Right Leg (µSv)	18	0	6	5	21	2	10	6

*Note: *organs with readings below the background during cerebral angiography are reported as receiving 0 Gy of radiation dose.*

The evaluation of the maximum number of cerebral angiography procedures that radiologists can perform is based on the average radiation quantities received by various organs, measured in millisieverts (mSv). By dividing this average by the organ-specific radiation dose limit per year, we can determine the maximum number of procedures performed annually. Similarly, dividing it by 12 months gives the maximum number of procedures that can be performed per month. Using the radiation quantity received by the left eye, radiologists can perform a maximum of 405 procedures per year or 34 per month during cerebral angiography (Table 4).

Table 4. Displays the maximum number of cerebral angiography procedures that radiologists can perform per year and per month.

Organ	Cerebral Angiography Procedures (N=20)	
	Maximum number of procedures per year	Maximum number of procedures per month
Left Eye	405	34
Right Eye	1,370	114
Inside Thyroid Shield	63,291	5,274
Outside Thyroid Shield	20,711	1,726
Left Hand	14,594	1,216
Right Hand	31,143	2,595
Left Leg	18,625	1,552
Right Leg	67,159	5,597

Comparing diagnostic and therapeutic cerebral angiography procedures, it was found that if physicians performed only diagnostic procedures, the maximum number of procedures per year would be 865 (72 procedures per month). However, if physicians performed only therapeutic procedures, the maximum number of procedures per year would be 181 (15 procedures per month). The radiation doses received by the thyroid, eyes, hands, and legs did not exceed the prescribed limits (Table 5).

Radiation levels in controlled areas

We measured the accumulated radiation levels at various positions in the controlled area. Notably, the position in front of the interventional neurological angiography room door exhibited the highest radiation level, measuring 1.18 µSv/hr. Comparatively, the position in front of the cardiac angiography room door had a radiation level of 0.93 µSv/hr. However, the position between the two angiography rooms and the control room, near the leaded glass window, had a relatively lower radiation level, measuring below 0.68 µSv/hr. Importantly, this value remains below the radiation dose limit specified by the Department of Medical Sciences, Ministry of Public Health (Figure 4).

Table 5. The maximum number of procedures that radiologists can perform per year and month for diagnostic cerebral angiography procedures (N=14) and therapeutic cerebral angiography procedures (N=6).

Organ	Diagnostic procedures (N=14)		Therapeutic procedures (N=6)	
	maximum number of procedures per year	maximum number of procedures per month	maximum number of procedures per year	maximum number of procedures per month
Left Eye	865	72	181	15
Right Eye	2,458	205	674	56
Inside Thyroid Shield	198,113	16,509	24,457	2,038
Outside Thyroid Shield	39,810	3,318	9,772	814
Left Hand	19,836	1,653	9,028	752
Right Hand	47,814	3,985	17,172	1,431
Left Leg	29,313	2,443	10,064	839
Right Leg	79,909	6,659	48,940	4,078

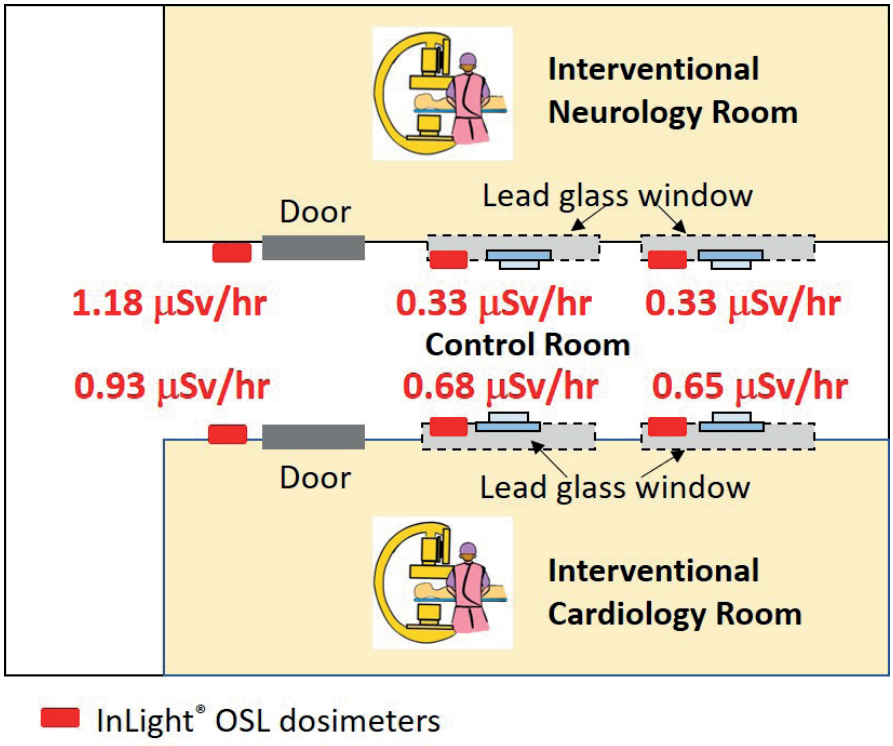


Figure 4. *Illustrates the accumulated background radiation levels at various points in the controlled area.*

Discussion

This study aimed to assess the radiation exposure from interventional radiologists during cerebral angiography procedures using the optical stimulated luminescence (OSL) nanoDot™ dosimeter. The radiation dosimeters were attached to the arms of the glasses to represent the radiation dose to the eye's lens and to the inside and outside of the thyroid shield to represent the radiation dose to the thyroid gland. The dosimeters were also placed on the hands and legs of the physicians performing the procedures. The study included 20 cases, revealing that the left-sided organs, including the left eye, left hand, and left leg, received a notably higher average radiation dose in comparison to their corresponding right-sided counterparts. This observation can be attributed to the fact that during cerebral angiography procedures, interventional radiologists consistently stand on the right side of the patient, bringing their left side closer to the X-ray tube. The radiologist's left eye received a higher radiation dose than other organs, potentially posing a risk of radiation to the eye lens and cataract formation.[16, 17] To prevent exceeding the maximum permissible dose of radiation defined by the International Commission on Radiological Protection (ICRP), it is crucial to utilize lead glasses for lens protection and wear radiation shielding devices during therapeutic cerebral angiography procedures. These measures can reduce the radiation dose the interventional radiologist receives up to three times, consistent with studies in orthopedic surgical procedures[18] and transcatheter arterial chemoembolization for hepatocellular carcinoma treatment.[19] Furthermore, organizations working with radiation should establish monitoring programs to evaluate

personnel's annual cumulative radiation exposure and calculate the cumulative dose over five years.

The main limitation of this study was the inability to attach the nanoDotTM dosimeter to the lens position directly. Instead, it was fixed to the arms of the interventional radiologist's eyeglasses during cerebral angiography. Although this approach may not precisely represent the scattered radiation dose at the lens position, it is a reference point used in other radiation dose studies involving radiologists' lenses,[7,20] where measurement devices were attached near the left and right eye lenses.

Although the radiation dose may not exceed the level that causes deterministic radiation effects, it may pose a risk of stochastic effects, which include a small probability or risk of developing fatal cancer and genetic defects in the future. However, this is a very low-risk probability, ranging from one in a hundred million to one in ten million patients. Radiation oncologists and staff performing interventional radiology procedures must take necessary precautions.[21]

Radiation dose measurement devices such as the nanoDotTM enable the assessment of cumulative radiation dose in controlled areas by calculating dose values per hour. The calculated cumulative radiation dose values represent the actual radiation dose values experienced in real-world applications. Moreover, these devices are valuable for measuring cumulative radiation doses in patient ward areas undergoing treatment with iodine-131 radiation therapy and for measuring radiation in drainage pipes.[15] The Department of Medical Sciences, Ministry of Public Health, recommends conducting regular inspections of controlled areas and verifying the proper functioning of radiation protection equipment at least once a year to ensure adequate radiation protection in accordance with established standards.[13]

Conclusion

During cerebral angiography, the left eye of the radiologist receives the highest radiation doses. Radiation protection devices such as thyroid shields and lead aprons can effectively reduce radiation exposure, and it is advisable to wear them during every cerebral angiography procedure. Organizations working with radiation should establish a monitoring system to track personnel's annual cumulative and cumulative doses over five years, ensuring compliance with prescribed limits for occupational exposure. Controlled areas' cumulative radiation dose should not exceed the limits set by the Department of Medical Science

References

[1] Saposnik G, Galanos LC, Guerrero R, Casagrande F, Adhamidhis E, Gao MMY, *et al*. The World Stroke Academy: A World Stroke Organization global pathway to improve knowledge in stroke care. Int J Stroke. 2022; 17(8): 829-34. doi: 10.1177/17474930221085895.

[2] Department of Medical Sciences, Ministry of Public Health. Report on the Situation of NCDs: Diabetes, hypertension and related risk factors in 2019. 1st Ed. Bangkok: Aksorn Graphic and Design Publishing; 2020. (in Thai)

[3] Haussen DC, Nogueira RG. Primer on cerebrovascular diseases: conventional cerebral arteriograph. 2nd Ed. San Diego: Academic Press; 2017: 707-12.

[4] Chida K, Kato M, Kagaya Y, Zuguchi M, Saito H, Ishibashi T, *et al*. Radiation dose and radiation protection for patients and physicians during interventional procedure. J Radiat Res. 2010; 51(2): 97-105. doi: 10.1269/jrr.09112.

[5] Feygelman VM, Huda W, Peters KR. Effective dose equivalents to patients undergoing cerebral angiography. Am J Neuroradiol. 1992; 13(3): 845-9.

[6] Berthelsen B, Cederblad A. Radiation doses to patients and personnel involved in embolization of intracerebral arteriovenous malformations. Acta Radiol. 1991; 32(6): 492-7. doi: 10.1177/0284185 1910320061.

[7] Lunelli NA, Khoury HJ, Andrade GHV de, Borrás C. Evaluation of occupational and patient dose in cerebral angiography procedures. Radiologia Brasileira. 2013; 46(6): 351–7. doi: 10.1590/S0100-39842013000600007.

[8] Seals KF, Lee EW, Cagnon CH, Al-Hakim RA, Kee ST. Radiation-induced cataractogenesis: A critical literature review for the interventional radiologist. Cardiovasc Intervent Radiol. 2016; 39(2): 151-60. doi: 10.1007/s00270-015-1207-z.

[9] Authors on behalf of ICRP, Stewart FA, Akleyev AV, Hauer-Jensen M, Hendry JH, Kleiman NJ, *et al*. ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs--threshold doses for tissue reactions in a radiation protection context. Ann ICRP. 2012; 41(1-2): 1-322. doi:10.1016/j.icrp.2012.02.001.

[10] Gonzales CAB, Morales AA. Assessment of patient and staff doses in interventional cerebral angiography using OSL. Proceedings of World Congress on Medical Physics and Biomedical Engineering; 2015 Jun 7-12; Toronto, Canada, 2015. doi: 10.1007/978-3-319-19387-8_192.

[11] Moritake T, Matsumaru Y, Takigawa T, Nishizawa K, Matsumura A, Tsuboi K. Dose measurement on both patients and operators during neurointerventional procedures using photoluminescence glass dosimeters. Am J Neuroradiol. 2008; 29(10): 1910-7. doi: 10.3174 /ajnr.A1235.

[12] Castilho AVB, Szjenfeld D, Nalli D, Fornazari V, Moreira AC, Medeiros RB. A study of radiation doses to the patient and medical team at embolization procedures. J Radiat Prot Res. 2019; 44(3): 110-7. doi: 10.14407/jrpr.2019.44.3.110.

[13] Department of Medical Sciences, Ministry of Public Health. Laboratory Radiation Safety Handbook. Bangkok: Beyond Publishing Company Limited; 2020. (in Thai)

[14] Phaorod J, Wongsanon W, Hanpanich P, Dornsrichan P, Awikunprasert P, Sriwicha J, *et al*. The measurement radiation doses to the lens of eye and thyroid gland

from computed tomography brain scans and radiation dose around in CT scan room: phantom study. Srinagarind Med J. 2020; 35(2): 153-60. (in Thai)

[15] Wisetnan C, Molee P, Awikunprasert P, Srichachet K, Pungkun V. A survey of radiation released from patients treated with radioiodine-131 therapy. J Assoc Med Sci. 2021; 54(2): 17-21.doi: 10.14456/jams.2021.12.

[16] Krisanachinda A, Srimahachota S, Matsubara K. The current status of eye lens dose measurement in interventional cardiology personnel in Thailand. Radiol Phys Technol. 2017; 10(2): 142-7. doi: 10.1007/s12194-017-0403-8.

[17] Principi S, Delgado Soler C, Ginjaume M, Beltran Vilagrasa M, Rovira Escutia JJ, Duch MA. Eye lens dose in interventional cardiology. Radiat Prot Dosimetry. 2015; 165(1-4): 289-93. doi: 10.1093/rpd/ncv051.

[18] Palácio EP, Ribeiro AA, Gavassi BM, Di Stasi GG, Galbiatti JA, Junior AD, et al. Exposure of the surgical team to ionizing radiation during orthopedic surgical procedures. Rev Bras Ortop. 2014; 49(3): 227-32. doi: 10.1016/j.rboe.2014.04.008.

[19] Kaewmarong K, Teebumrung N, Awikunprasert P, Kittichotwarat S, Claimon T, Dachviriyakij T. Measurement of radiation dose by a radiologist from Transarterial Chemoembolization (TACE) intervention with optically stimulated luminescence. J Med Health Sci. 2023; 30(1): 44-52. (in Thai)

[20] Kato M, Chida K, Ishida T, Toyoshima H, Yoshida Y, Yoshioka S, et al. Occupational radiation exposure of the eye in neurovascular interventional physician. Radia Prot Dosim. 2019; 185(2): 151-6. doi: 10.1093/rpd/ncy285.

[21] Hidajat N, Wust P, Felix R, Schröder RJ. Radiation exposure to patient and staff in hepatic chemoembolization: risk estimation of cancer and deterministic effects. Cardiovasc Intervent Radiol. 2006; 29(5): 791-6. doi: 10.1007/s00270-005-0247-1.

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Comparison of aberrant behavior profiles across different severity levels of autism symptoms among Thai children aged 2-9 years with autistic spectrum disorder

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ABSTRACT

Background: The incidence of autism spectrum disorder (ASD) has been increasing steadily, thus posing a substantial public health concern in Thailand and globally. Individuals with ASD, who frequently experience challenges related to their behavior and emotions, have neurodevelopmental disorders. The Aberrant Behavior Checklist (ABC) represents one available tool for addressing these issues.

Objectives: This study aimed to explore and compare aberrant behavior profiles across different severity levels of autism among Thai children aged 2-9 years with ASD.

Materials and methods: The participants comprised 71 parents and young children with ASD, recruited online, particularly from an online Facebook community of parents from diverse regions of Thailand. A convenience sampling method was employed for selecting the participants. The research tools consisted of 3 parts for collecting data. Demographic data were used for collecting such information about the participants. A Thai version of the Autism Treatment Evaluation Checklist (Thai-ATEC) was utilized to measure autism symptom severity, and the Aberrant Behavior Checklist-Community (ABC-C) Thai Version was used for evaluating behavioral problems from caregiver administration.

Results: A total of 71 ASD participants were identified, of which 5, 15, and 51 displayed mild, moderate, and severe symptoms of ASD, respectively. The mean scores of aberrant behaviors, as assessed by the ABC-C Thai version, showed an increase across all four subscales and the total score in response to the severity levels of ASD symptoms. The study findings, including reported correlations between aberrant behaviors and severity levels of autism symptoms, revealed predominantly high correlation coefficients (rs ranging from 0.27 to 0.93). Furthermore, a positive correlation was identified between the four subscales of the Thai-ATEC assessment and the five subscales of the ABC-C Thai version, highlighting their interrelated nature. For more detailed analysis, the ASD participants were categorized into two groups: a “mild-moderate” group consisting of 20 participants and a “severe” group comprising 51 participants. Subsequently, a comparison was made between the mean scores of the ABC-C subscales and total scores. The results of this comparison demonstrated significant distinction in all five subscales and the total score of the ABC-C Thai version between the “mild-moderate” and “severe” groups of ASD participants. These findings shed light on the notable differences in aberrant behavior profiles between individuals with varying levels of autism symptom severity.

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Conclusion: The aberrant behaviors increased in response to severe symptoms of ASD. This might be useful for clinical purposes, and assessing aberrant behaviors may help understand behavioral problems and severity levels of ASD and tailoring occupational therapy interventions.

Introduction

Autism spectrum disorder (ASD) is currently recognized as a diverse set of neurodevelopmental disorders, exhibiting a range of characteristics, including challenges in social interaction, communication, and the presence of repetitive behaviors.[1] A retrospective cohort study conducted in 2023 used data from a large pediatric primary care network in the United States, and the prevalence of ASD within the cohort was found to be 3.2%. The frequency of ASD was greater in Asian and non-Hispanic black children and children living in neighborhoods with higher socioeconomic risk.[2] Over the past years, the prevalence of ASD has been increasing consistently and emerging as a significant public health concern. Previous research reported that the incidence rate of ASD among Thai children was 6.94 per 10,000 in 2002, and the number of new cases is expected to continue to rise.[3,4]

ASD is a multifaceted condition that manifests with diverse symptoms and varying levels of severity, which varies from child to child. Additionally, there are numerous associated atypical behaviors, including eating patterns, gastrointestinal symptoms, food intolerance, sleep disturbances, epilepsy, and attention deficit hyperactivity disorder.[5] These additional symptoms make clinical diagnosis of ASD more difficult and add to the difficulty of treating them. Clinical traits outlined by the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 clinical criteria are used to identify ASD symptoms. According to the severity of the impairment, the DSM-5 further categorizes ASD into three levels: levels 1, 2, and 3 require support, significant support, and very substantial support, respectively.[6] Furthermore, challenging, or aberrant behaviors, commonly observed in individuals with ASD, include repetitive actions, self-injurious behaviors, aggression, and hyperactivity. A physician performs comprehensive behavioral examinations to determine the clinical traits associated with ASD.[7] To evaluate the behavioral problems or challenging behaviors displayed by children with ASD, medical professionals use the Aberrant Behavior Checklist (ABC), an evidence-based scale. Agitation/Crying, Irritability, Noncompliance/Hyperactivity, Social Withdrawal/Lethargy, and Stereotypic Behavior are the five dimensions covered by the ABC scale.[8] The prescription of drugs and dosage levels for children with ASD depends on these clinical and behavioral traits.

Early detection of ASD is essential because it enables prompt intervention, which lowers lifelong healthcare expenses. Families, educators, and direct service providers play a crucial role in influencing the lives of individuals with ASD, demonstrating the significant impact of ASD intervention. Occupational therapists are health professionals who contribute by providing insights into present functioning and behavioral issues and subsequently guiding intervention in ASD. However, while the core symptoms of ASD are recognized, a knowledge gap concerning accompanying aberrant symptoms remains, especially within the context of varying levels of symptom severity in the Thai ASD population. This study sought to address this gap through the exploration and comparison of aberrant behavior profiles across different severity levels of autism among Thai children aged 2-9 years. The chosen age range is particularly significant due to its critical developmental window, offering substantial potential for effective early interventions. By bridging the knowledge gap, this study aims to contribute to a more refined understanding of the behavioral challenges associated with different levels of ASD severity, thus enabling more effective and tailored interventions that address specific challenges, facilitate skill development, and enhance these children's overall quality of life.

Materials and methods

Participants and procedures

The research protocol for this study received approval from the Institutional Review Board (IRB) at Mahidol University (MUSSIRB COA No. 2022/011.0202). In this cross-sectional study, anonymous online questionnaires were employed to investigate parental perceptions concerning aberrant behaviors and the severity of autism symptoms in children with ASD. A convenient sampling method was employed for participant selection. Participants initiated the process by expressing their agreement with three screening statements: 1) I am a parent of a child aged between 2 and 9 years; 2) My child has received a diagnosis of autism, ASD, or pervasive developmental disorder not otherwise specified (PDD-NOS); and 3) I affirm that I am at least 18 years old and proficient in reading Thai. Subsequently, only individuals who confirmed agreement with all three screening statements and provided online informed consent were eligible to proceed by responding to the remaining survey sections. A total of 71 parent-child pairs were recruited (Figure 1). The recruitment occurred specifically within an online community on Facebook, which included parents from various regions across Thailand. The demographic characteristics of the children with ASD are presented in Table 1. Their average age was 4.51±1.9 years, comprising 61 boys and ten girls. Most of the children were firstborn. Of the participants, 28 children (39.4%) were not attending school, while 43 (60.6%) were enrolled as students. ASD symptom severity in the participants was classified as mild in 7% of cases (N=5), moderate in 21.1% (N=15), and severe in 71.8% (N=51). Table 2 provides the demographic characteristics of the parents of children with ASD (N=71). The average age of the parents was 35.1±6.5 years, consisting of 20 males and 51 females. Most parents were married, had completed a bachelor's degree, and were self-employed.

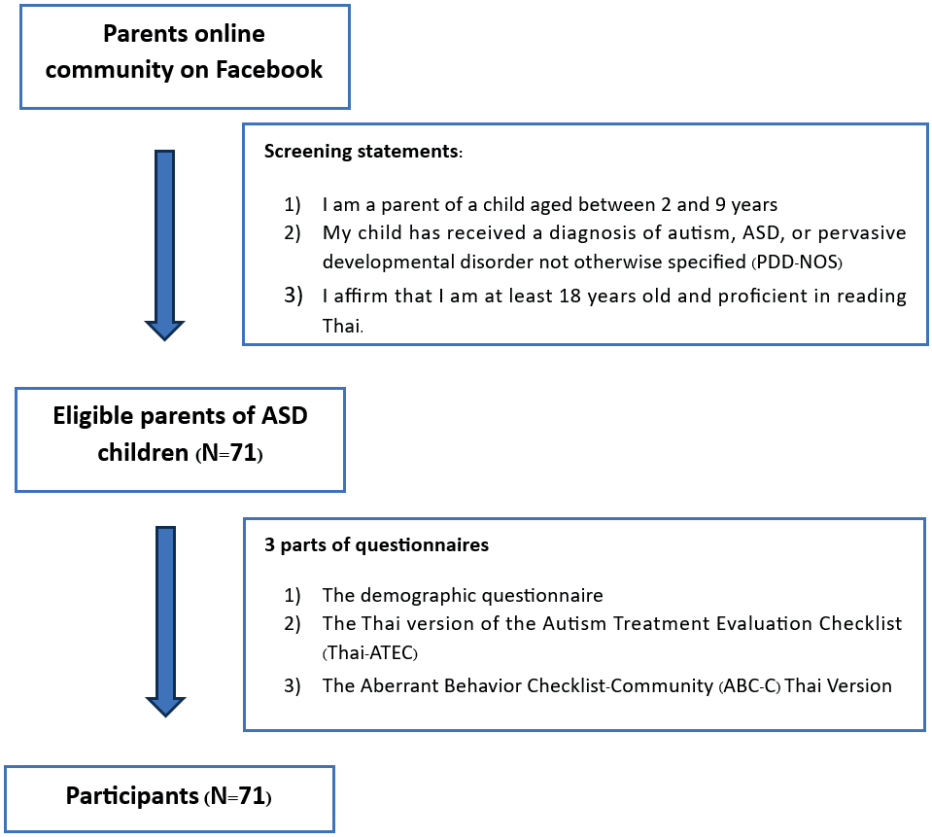

Figure 1. *Flowchart of study protocol.*

Table 1 Demographic characteristics of children with ASD (N=71).

Demographic Variable		N	%
Age, Mean (SD)		4.51 (1.9)	
2.0-2.11		3	4.2
3.0-3.11		25	35.2
4.0-4.11		18	25.4
5.0-5.11		7	9.9
6.0-6.11		6	8.5
7.0-7.11		4	5.6
8.0-8.11		4	5.6
9.0-9.11		4	5.6
Sex			
Male		61	85.9
Female		10	14.1
Birth order			
1st born		53	74.6
2nd born		17	23.9
3rd born		1	1.4
Education			
Not enrolled		28	39.4
Nursery		15	21.1
Preschool		17	23.9
Primary school		11	15.5
Autistic severity level			
ATEC	Mild	5	7.0
	Moderate	15	21.1
	Severe	51	71.8

Table 2 Demographic characteristics of parents of children with ASD (N=71).

Demographic Variable	N	%
Age, Mean (SD)	35.1 (6.5)	
Sex		
Male	20	28.2
Female	51	71.8
Marital status		
Single	2	2.8
Married	63	88.7
Separated	6	8.5
Highest Educational Level		
Less than bachelor	17	23.9
Bachelor	41	57.7
Higher than bachelor	13	18.3
Occupation		
Public	12	16.9
Private	16	22.5
Self-employed	43	60.6
Monthly Income (Baht/month)		
<15,000	8	11.2
15,000-30,000	12	16.9
30,001-45,000	16	22.5
45,000-60,000	16	22.5
>60,0001	19	26.8

Measures

The research tools consisted of 3 parts for collecting data. The demographic questionnaire was used for collecting demographic information on the children and parents. To assess the severity levels of autism symptoms, the Thai version of the Autism Treatment Evaluation Checklist (Thai-ATEC) was utilized and administered by caregivers. The Thai-ATEC provides a total score and four subscale scores. The first three subscales involve questions scored on a scale of 0-2, while the fourth subscale, Health/Physical/Behavior, is scored on a 0-3 point scale. The ATEC can be accessed online or obtained in a printed format. The first subscale, Speech/Language/Communication, comprises 14 items with a score range of 0-28 points. The Sociability subscale consists of 20 items, allowing participants to score 0-40. The third subscale, Sensory/Cognitive awareness, contains 18 items, with a score range of 0-36. Lastly, the Health/Physical/Behavior subscale includes 25 items. The scores from each subscale are combined to calculate a Total Score, which can range from 0 to 179 points. A lower score on the Thai-ATEC indicates lower severity levels of ASD symptoms. In this study, a cut-off point of scores ≤38 was used to differentiate between children with mild ASD symptoms and those with more pronounced ones. The sensitivity of this cut-off point was 94%, indicating its ability to identify children with mild ASD symptoms accurately. In comparison, specificity was 61.9%, suggesting a moderate level of correctly classified children without mild symptoms. The area under the receiver operating characteristic (ROC) curve, which assesses the overall performance of the cut-off point, was found to be 90%. Similarly, another cut-off point of scores ≥68 was employed to distinguish between children with severe ASD symptoms and milder ones. This cut-off point demonstrated a sensitivity of 94% and specificity of 62.8%, indicating its ability to identify children with severe ASD symptoms effectively while moderately classifying those without them. The area under the ROC curve for this cut-off point was 85%. Furthermore, the inter-rater reliability of the Thai-ATEC was found to be very strong, with an intra-class correlation coefficient (ICC) of 0.97. This indicates a high level of agreement between different raters when using the Thai-ATEC to evaluate ASD symptoms.[9]

The Aberrant Behavior Checklist-Community (ABC-C) Thai Version was utilized as the final assessment tool. This instrument primarily focuses on observing and evaluating behavioral problems or challenging behaviors. It consists of 58 items, categorized into five behavior domains:

Irritability, agitation, and crying (15 items), Lethargy and social withdrawal (16 items), Stereotypic behavior (7 items), Hyperactivity and non-compliance (16 items), and Inappropriate speech (4 items). Parents completed the 58-item ABC-C questionnaire, which provided their perception of their child's behaviors over the previous four weeks. Each item was scored on a 4-point scale, ranging from 0 (not a problem) to 3 (severe problem), to calculate raw subscale scores. The ABC-C Thai Version demonstrates high internal consistency, with a Cronbach's alpha coefficient of 0.922. It also exhibits strong inter-rater reliability and test-retest reliability, with intra-class correlation coefficients (ICC) of 0.90 (95% CI: 0.81-0.95) and 0.92 (95% CI: 0.86-0.96), respectively. Furthermore, the ABC-C Thai Version shows a strong positive correlation with the Clinical Global Impression-Severity Scale (CGI-S), indicating good concurrent validity with a correlation coefficient of 0.87 (p<0.01).[10]

Procedures

Varied demographic information was gathered, such as gender, age, education, occupation, and more, from both parents and children with ASD. The ASD children were evaluated using the ABC-C Thai version and Thai-ATEC assessments. This study followed a cross-sectional design and utilized anonymous online questionnaires (Figure 1). Before the survey, researchers conducted demonstrations and made necessary adjustments to ensure the scientific validity, rationality, and relevance of the survey plan. The quality of the questionnaire was maintained by making the core question require an answer. The questionnaire was distributed to participants through an online survey platform (Google Form) and shared via the ASD Thai parent community on social media platforms like Line and Facebook. The survey was conducted between February 2 and September 30, 2022. All participants provided electronic informed consent, which included information about the study's purpose, procedures, potential benefits, voluntary participation, and contact details of the researchers.

Data analysis

The statistical analysis was conducted using SPSS 23.0 software. Descriptive statistics, such as percentages, means, and standard deviations, were calculated for socio-demographic variables and participant characteristics. Mann-Whitney U Test was employed to compare the groups. In this exploratory report, significance was considered at p<0.05.

Results

The autism severity level and aberrant behaviors among ASD participants

Tables 3 and 4 provide a detailed overview of autism severity levels and aberrant behaviors observed in 71 children diagnosed with ASD. Among these children, 5, 15, and 51 exhibited mild, moderate, and severe symptoms, respectively, across all areas assessed by the Thai-ATEC evaluation.

Table 3. Distribution of autism severity level among ASD participants (N=71).

Thai-ATEC Subscale	Autism Severity Level, N (%)			Total (N=71) Range (Mean±SD)
	Mild (N=5) (Mean±SD)	Moderate (N=15) (Mean±SD)	Severe (N=51) (Mean±SD)	
Speech/Language/Communication	3.4±3.4	18.4±8.7	22.6±5.6	0-28 (18.4±8.7)
Sociability	7.4±6.5	25.0±11.2	30.5±7.6	0-40 (25.0±11.2)
Sensory/Cognitive Awareness	6.2±3.8	22.1±10.0	27.1±6.4	1-35 (22.1±10.0)
Health/Physical/Behavior	8.0±2.9	27.7±12.9	32.9±10.6	3-52 (27.7±12.9)
ATEC Total Score	25.0±11.2	93.2±37.9	113.0±22.6	12-147 (93.2±37.9)

Table 4 Distribution of aberrant behaviors among ASD participants (N=71).

ABC-C Thai version Subscale	Autism Severity Level			Total (N=71) Range (Mean±SD)
	Mild (N=5) Mean±SD	Moderate (N=15) Mean±SD	Severe (N=51) Mean±SD	
Irritability, agitation, and crying	2.8±1.8	12.6±10.1	29.7±8.9	0-43 (24.2±12.7)
Lethargy and social withdrawal	4.8±2.9	9.5±7.6	28.0±9.7	0-44 (22.5±12.6)
Stereotypic behavior	1.6±1.5	4.3±4.5	12.4±5.8	0-21 (9.9±6.6)
Hyperactivity and non-compliance	10.4±5.6	13.7±8.2	33.0±8.2	1-46 (27.3±12.2)
Inappropriate speech	2.6±2.6	3.1±2.7	5.7±3.7	0-12 (4.9±3.7)
ABC-C Total Score	21.8±5.3	42.1±28.7	106.9±28.7	7-143 (87.2±42.2)

The scores obtained from the ABC-C Thai version, which measures aberrant behaviors, showed a consistently upward trend in response to the severity levels of ASD symptoms. This pattern was evident across all four subscales and the overall total score. In breaking down the results by specific subscales, it is notable that mean scores for irritability, agitation, and crying were 2.8, 12.6, and 29.7 for mild, moderate, and severe symptoms, respectively. For lethargy and social withdrawal, the respective mean scores were 4.8, 9.5, and 28.0 for mild, moderate, and severe symptoms. Similar trends were observed for stereotypic behavior, with mean scores of 1.6, 4.3, and 12.4 for mild, moderate, and severe symptoms, respectively.

Regarding hyperactivity and non-compliance, mean scores were 10.4, 13.5, and 33.0 for mild, moderate, and severe symptoms, respectively. At the same time, inappropriate speech had respective mean scores of 2.6, 3.1, and 5.7 for mild, moderate, and severe symptoms. Furthermore, the mean total score obtained from the ABC-C Thai version exhibited an incremental rise corresponding to the severity of ASD symptoms. Specifically, the mean total score was 21.8, 42.1, and 106.9 for mild, moderate and notably elevated severe symptoms, respectively.

Comparison of aberrant behavior profiles across different severity levels of autism symptoms

The classification of severity levels in this study involved grouping participants into "mild-moderate" and "severe" categories, a decision shaped by the distribution of individuals within these groups. While the conventional categorization of severity levels in ASD often encompasses three levels, it is important to note that the specific sample in this study had a relatively smaller number of participants exhibiting "mild" and "moderately severe" symptoms. In this study, the 71 ASD participants were divided into two distinct groups: a "mild-moderate" group consisting of 20 individuals and a "severe" group comprising 51. Then. the mean difference in the ABC-C Thai version subscale and total scores was compared. Results of the analysis comparing ABC-C Thai version subscale scores with autism severity levels are presented in Table 5 and Figure 2. When compared to the mild-moderate autism severity group, the mean scores for all ABC-C Thai version subscales were considerably higher in the severe autism severity group (p<0.001). In particular, the severe group outperformed the mild-moderate one considerably in terms of mean scores for Irritability, Agitation, Crying, Lethargy/ Social Withdrawal, Stereotypic Behavior, Hyperactivity/ Noncompliance, and Total Score (p<0.001). In addition, the mean scores for inappropriate speech varied significantly between the two severity groups (p=0.005). These results suggest a linear relationship between the scores of aberrant behaviors assessed by the ABC-C Thai version subscales and the severity levels of autism symptoms.

Discussion

This study aimed to explore and compare aberrant behavior profiles among Thai children with ASD across different severity levels of autism symptoms. The sample size for this study comprised 71 pairs of parents and children with ASD. Among ASD children, 5, 15, and 51 exhibited mild, moderate, and severe symptoms, respectively. For analysis, this study divided participants into two distinct severity groups: mild-moderate and severe. The comparison of mean scores across ABC-C Thai version subscales between these two groups showcased substantial differences. Specifically, the severe group demonstrated markedly higher scores across multiple ABC-C Thai version subscales, including Irritability, Agitation, Crying, Lethargy/Social Withdrawal, Stereotypic Behavior, Hyperactivity/Noncompliance, and Total Score. This statistically significant difference indicates that individuals with more severe autism symptoms exhibit more pronounced aberrant behaviors across various domains. These findings are consistent with previous research that has shown a positive correlation between the severity of autism symptoms and the presence of aberrant behaviors. Individuals with more severe autism symptoms tend to exhibit higher levels of Irritability, Agitation, Social Withdrawal, Stereotypic Behavior, and Hyperactivity/Noncompliance.[11-14]

Significant differences were evident across all five subscales and the overall ABC-C Thai version score when comparing mean scores between the mild-moderate and severe ASD groups. Interestingly, this study demonstrates a smaller disparity in mean scores for Inappropriate Speech between these two severity groups, underscoring how autism severity influences specific aberrant behaviors.

Table 5 Mean difference in ABC-C Thai version subscale and total scores by severity level.

ABC-C Thai version subscale	Autism Severity Level		*p* value
	Mild-Moderate (N=20) Range (Mean±SD)	**Severe (N=51)** Range (Mean±SD)	
Irritability, Agitation, and Crying	10.2±9.7	29.7±8.9	0.000
Lethargy/Social Withdrawal	8.3±6.9	28.0±9.7	0.000
Stereotypic Behavior	3.7±4.1	12.4±5.8	0.000
Hyperactivity/Noncompliance	12.7±7.7	33.0±8.2	0.000
Inappropriate Speech	2.9±2.6	5.7±3.7	0.005
ABC-C Total Score	37.1±26.4	106.9±28.7	0.000

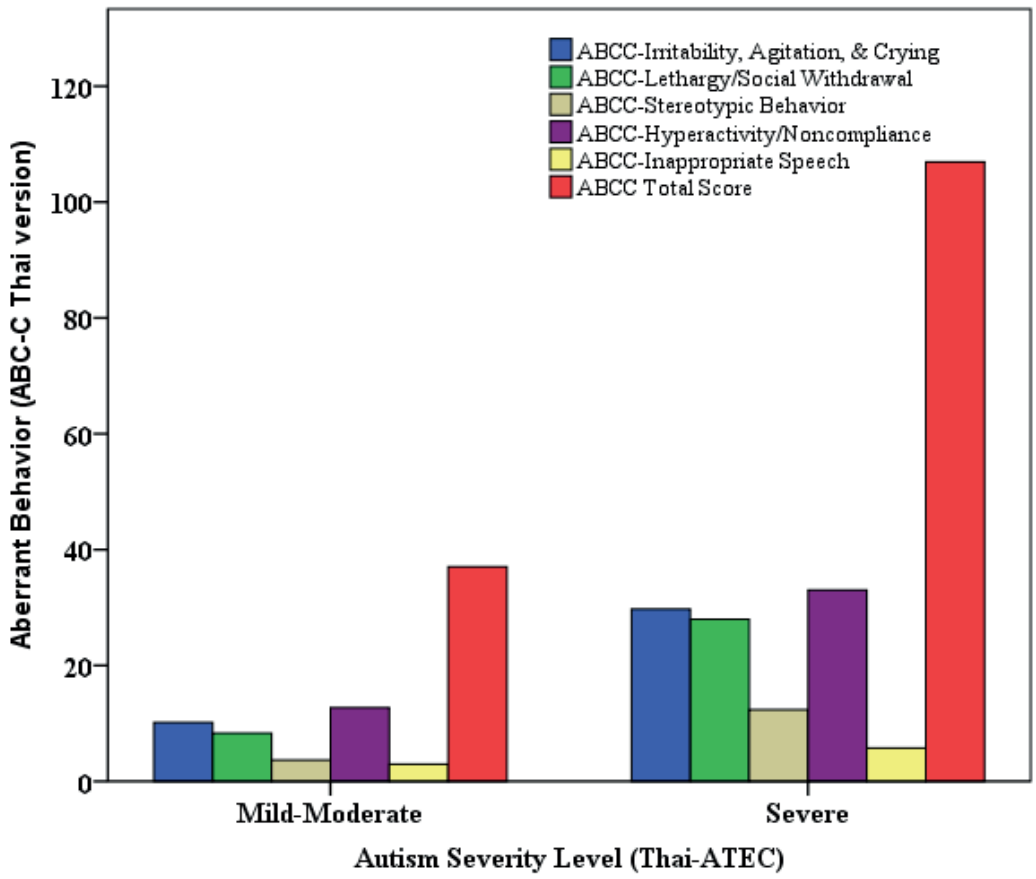

Figure 2. *Comparison of aberrant behavior profiles across different severity levels of autism symptoms.*

Remarkably, despite Inappropriate Speech being comparatively less frequent or intense than other behaviors, it still shows a notable distinction between the two severity groups. Individuals with ASD more frequently exhibit challenging behaviors, such as stereotypies, aggression, property destruction, and self-injury, which persons with ASD often exhibit.[13-15] These actions frequently include extreme severity, endangering the child's physical safety and the safety of people around him or her, and possibly limiting access to community and/or educational opportunities.[16] Additionally, if the behaviors go untreated, they will likely persist into adulthood and increase in severity as the child matures physically.[16]

While this study's findings regarding differences in aberrant behavioral profiles across severity groups in children with ASD were in line with expectations, delving deeper into several dimensions can enrich our comprehension. Notably, this study marks the first exploration of these patterns within the Thai context and carries critical clinical implications. Identifying unique behavioral profiles across severity groups underscores the urgency of tailored interventions that address the specific challenges encountered by individuals with varying autism severity levels. Utilizing the ABC-C Thai version subscales to assess aberrant behaviors offers valuable insights into the severity of autism symptoms. These findings can guide the design of interventions that align closely with the individual needs of those with different severity levels, thereby optimizing their therapeutic progress and overall well-being. This entails adopting a holistic approach encompassing diverse strategies, including sensory modulation techniques, social skills training, adaptive strategies, and cognitive-behavioral interventions.

As occupational therapists, recognizing distinct behavioral patterns across severity groups highlights the imperative of finely tuned interventions that cater to the unique needs of individuals with varying severity levels. By crafting interventions tailored to their specific challenges, occupational therapists play a pivotal role in enhancing functional abilities, independence, and overall quality of life. Moreover, acknowledging the ever-evolving nature of the individuals' needs, interventions must be assessed continually and adapted as individuals traverse the spectrum of autism severity. Ultimately, by aligning therapeutic strategies with the diverse behavioral profiles observed across severity groups, occupational therapists contribute significantly to advancing well-rounded support for individuals with ASD. This holistic and person-centered approach not only benefits the individual but also enriches our collective understanding of the complexities of autism and the effectiveness of tailored interventions.

Limitation

It is important to note that this study has some limitations. First, the sample size was rather small, limiting how broadly the results could be applied. These findings

need to be confirmed in larger, more diverse investigations in the future. Due to the distribution of the questionnaire being through an online survey platform and social media platforms such as Line and Facebook, control over the number of participants in each ASD severity subgroup was limited. Future studies should attempt to recruit a greater and more evenly distributed number of individuals in each severity subgroup to create a more representative sample.

Additionally, it is advised to use various questionnaire dissemination techniques to improve data collecting and guarantee a broader pool of participants. To reach a wider audience and include participants without access to or preference for Internet surveys could entail sending questionnaires via traditional mail in addition to online platforms. Using multiple data collection methods can help capture a broader range of perspectives and improve the reliability and generalizability of the findings. Second, the study relied on caregiver-reported data, which may be subject to bias. Incorporating objective measures and gathering input from multiple sources, including parents, could provide a more comprehensive understanding of aberrant behaviors in ASD.

Conclusion

All individuals with ASD in this study exhibited behavioral problems, and the severity of these problems increased with the severity of ASD symptoms. Strong positive correlations were found between aberrant behaviors and the severity of autism symptoms, which, as they became more severe, aberrant behaviors became more pronounced. Additionally, positive correlations were observed between the subscales of the Thai-ATEC and ABC-C Thai Version, suggesting that these measures capture similar constructs. Furthermore, the results of this study revealed significant differences in all five subscales and the total score of the ABC-C Thai version between the mild-moderate and severe ASD groups. This suggests that the presence of aberrant behaviors is more prominent in individuals with severe ASD symptoms when compared to those with mild-moderate ones. These findings have important implications for occupational therapy interventions, highlighting the need for screening and addressing aberrant behaviors in clinical settings. By assessing aberrant behaviors and tailoring interventions accordingly, occupational therapy services for children with ASD can better address the behavioral problems and severity of ASD symptoms.

The association between autism severity and aberrant behaviors, as measured by the ABC-C Thai version subscales, provides valuable evidence for understanding the relationship between these variables. It underscores the importance of considering and addressing aberrant behaviors as an integral part of comprehensive treatment approaches for individuals with ASD. By targeting and managing these behaviors, clinicians and therapists can improve the overall outcomes and well-being of individuals with ASD.

Conflicts of Interest

The authors declare no conflict of interest.

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References

[1] Association AP. Diagnostic and statistical manual of mental disorders: DSM-5: American psychiatric association Washington, DC; 2013.

[2] Wallis KE, Adebajo T, Bennett AE, Drye M, Gerdes M, Miller JS, *et al*. Prevalence of autism spectrum disorder in a large pediatric primary care network. Autism. 2023:13623613221147396. doi: 10.1177/13623613221147396.

[3] Plubrukarn R, Piyasilp V, Moungnoi P, Tanprasert S, Chutchawalitsakul V. Trend study of autistic spectrum disorders at Queen Sirikit National Institute of Child Health. J Med Assoc Thail. 2005; 88: 891-7.

[4] Hansakunachai T, Roongpraiwan R, Sombuntham T, Limprasert P, Ruangdaraganon N. A new structured interview for children with autism spectrum disorder based on the DSM-IV. J Med Assoc Thail. 2014; 97: S7-S14.

[5] Li C, Liu Y, Fang H, Chen Y, Weng J, Zhai M, *et al*. Study on aberrant eating behaviors, food intolerance, and stereotyped behaviors in autism spectrum disorder. Front Psychiatry. 2020; 11: 493695. doi: 10.3389/fpsyt.2020.493695.

[6] American Psychiatric Association, D. and A. P. Association (2013). Diagnostic and statistical manual of mental disorders: DSM-5, American psychiatric association Washington, DC.

[7] Megerian JT, Dey S, Melmed RD, Coury DL, Lerner M, Nicholls CJ, *et al*. Evaluation of an artificial intelligence-based medical device for diagnosis of autism spectrum disorder. NPJ Digit Med. 2022; 5(1): 57. doi: 10.1038/s41746-022-00598-6.

[8] Aman MG, Burrow WH, Wolford PL. The Aberrant Behavior Checklist-Community: factor validity and effect of subject variables for adults in group homes. Am j ment retard. 1995.

[9] Sunakarach K, Kessomboon, Pattapong. Validity and reliability of the Thai version of the Autism Treatment Evaluation Checklist: A two-phase diagnostic. F1000research. 2018. doi: 10.12688/f1000research.14537.1.

[10] Narkpongphun A, Charnsil C. Reliability and Validity of Aberrant Behavior Checklist-Community, Thai Version. J Child Adolesc Behav. 2018; 6: 373. doi: 10.4172/2375-4494.1000373.

[11] Matson JL, Nebel-Schwalm M. Assessing challenging behaviors in children with autism spectrum disorders: A review. Res Dev Disabil. 2007; 28(6): 567-79. doi: 10.1016/j.ridd.2006.08.001.

[12] Mudford OC, Arnold-Saritepe AM, Phillips KJ, Locke JM, Ho I-CS, Taylor SA. Challenging behaviors. Clinical assessment and intervention for autism spectrum disorders: Elsevier; 2008: 267-97.

[13] Horner RH, Carr EG, Strain PS, Todd AW, Reed HK, disorders d. Problem behavior interventions for young children with autism: A research synthesis. J Autism Dev Disord. 2002; 32: 423-46. doi: 10.1023/A:1020593922901.

[14] Militerni R, Bravaccio C, Falco C, Fico C, Palermo MT. Repetitive behaviors in autistic disorder. Eur Child Adolesc Psychiatry. 2002; 11: 210-8. doi: 10.1007/s00787-002-0279-x.

[15] Symons FJ, Sperry L, Dropik P, Bodfish J. The early development of stereotypy and self-injury: a review of research methods. J Intellect Disabil Res. 2005; 49(2): 144-58. doi: 10.1111/j.1365-2788.2004.00632.x.

[16] Sigafoos J, Arthur M, O'Reilly M. Challenging behaviour and developmental disability. Baltimore: Paul H Brookes Publishing Co; 2003.

[17] Murphy GH, Beadle-Brown J, Wing L, Gould J, Shah A, Holmes N. Chronicity of challenging behaviours in people with severe intellectual disabilities and/or autism: A total population sample. J Autism Dev Disord. 2005; 35(4): 405-18. doi: 10.1007/s10803-005-5030-2.

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Non-transfusion dependent HbE/β^{O}-thalassemia as the results of co-existent SEA-α^{O} thalassemia, Hb Constant Spring, and XmnI-$^{G}\gamma$ site: Thai family studies

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ABSTRACT

Background: Four university students of northern Thai descent were found to be HbE/β^{O}-thalassemia. However, they all had a mild form of this disease, categorized as Non-Transfusion Dependent Thalassemia.

Objectives: To analyze involvement of types of β-globin mutations, α-thalassemia, and *Xmn*I-$^{G}\gamma$ site in mild clinical symptoms observed in four Thai non-transfusion dependent HbE/β^{O}-thalassemia cases.

Materials and methods: EDTA blood samples were collected from the patients and their family members after signing the informed consent. Automated complete blood count with blood smear examination, hemoglobin typing, molecular analysis for α and β-globin mutations, β-globin gene haplotypes, and *Xmn*I-$^{G}\gamma$ site were performed on all blood samples. In addition, nucleotide sequencing of β-globin gene and globin chain separation were performed for patient#3 and their parents.

Results: The first three patients had hemoglobin levels ranging 8.5-11.2 g/dL, while the fourth patient had hemoglobin level of 6.7 g/dL. The first and fourth patients were compound heterozygote for β^{E} (HBB:c.79G>A) and β^{17} (HBB:c.52A>T) alleles with typical hemoglobin pattern of EF. The second patient was compound heterozygote for β^{E} and $\beta^{41/42}$ (HBB:c.126_129delCTTT) alleles also with typical hemoglobin pattern of EF. The third patient was compound heterozygote of β^{E} and $\beta^{IVS1\text{-}1}$(HBB:c.92+1G>T), however, with atypical hemoglobin pattern of EE. Family analysis found co-inheritance of Hb Constant Spring (HBA2:c.427T>C) and the *Xmn*I-$^{G}\gamma$ site (T at rs7482144) in the first two patients, of SEA-α^{O} thalassemia (NG_000006.1:g.26264_45564del19301) and *Xmn*I-$^{G}\gamma$ site in the third patient, and of only *Xmn*I-$^{G}\gamma$ site in the fourth patient.

Conclusion: These family studies proved the fact that co-existence of SEA-α^{O} thalassemia and Hb Constant Spring in HbE/β^{O}-thalassemia could lead to mild clinical severity. Minimal effect of *Xmn*I-$^{G}\gamma$ site on clinical symptoms of this disease was emphasized. This information should be useful in prenatal diagnosis of HbE/β-thalassemia.

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Introduction

HbE/β-thalassemia is the syndrome resulting from co-segregation of β^{E}-gene (HBB:c.79G>A) and β-thalassmia gene.[1] Surveys in Thailand have shown that the affected patients had different clinical symptoms. Some patients had anemia, requiring regular blood transfusions while others in contrast are not anemic and do not need any blood

transfusion throughout their life.[2] At least 3 determinants have been shown to be linked to mild clinical symptoms of the HbE/β-thalassemia. These include 1). types of β-globin gene mutations, 2). co-existence of α-thalassemia, and 3). co-inheritance of loci involved in increased γ-globin gene expression such as the *Xmn*I-^{G}γ site (T at *rs7482144*).[3] Four university students of northern Thai descent were found to be HbE/β^{O}-thalassemia following routine blood tests. However, they all had a mild form of this disease as they required either no or occasional blood transfusion which was categorized as Non-Transfusion Dependent Thalassemia.[4] This study was therefore aimed to analyze involvement of these three genetic determinants in mild clinical symptoms observed in these propositi. This finding would be useful in predicting the clinical symptoms of *in-utero* fetuses prenatally diagnosed to be HbE/β^{O}-thalassemia.

Materials and methods

Subjects and blood samples

Four university students of northern Thai descent and their family members were collectively analyzed. EDTA blood samples were collected from all subjects after signing the informed consents. The protocol of this study was reviewed and approved by the Ethic Committee of Faculty of Medicine, Chiang Mai University, Thailand with the approval number 205/2012.

Blood analysis and Hb typing

Automated complete blood count (Sysmex KX-21 Hematology Analyzer, Sysmex Corporation, Kobe, Japan) with blood smear examination was performed. Hb typing was determined by cation exchange high-performance liquid chromatography (HPLC) using the Primus Variant System 99 (Primus Corporation, Kansas City) and VARIANT II Hemoglobin Testing System (Bio-Rad Laboratories Ltd., Hercules, California).

Molecular analysis

The α- and β-globin gene mutations were identified by multiplex allele-specific PCR routinely performed in our laboratory.[5,6] The *Xmn*I-^{G}γ site was identified by PCR-RFLP analysis also routinely performed in our laboratory.[7,8] The β-globin gene RFLP haplotypes (*Hind*II-ε , *Hind*III-^{G}γ, *Hind*II-^{A}γ , *Hind*II-5'ψβ, *Hind*II-3'ψβ, *Ava*II-β and *Hinf*I-β) were determined following the procedure described elsewhere.[9,10]

Results

Family 1

Family 1 consisted of a father, mother, brother, and patient (Supplementary Figure 1). Father was β-thalassemia carrier with Hb type of A_2A (Supplementary Figure 2), not anemic with mild change of RBC morphology (Figure 1). He was heterozygous for β^{17} mutations (HBB:c.52A>T) (β^{17}/β^{N}) and had no co-existence of α thalassemia, Hb Constant Spring (Cs) and *Xmn*I-^{G}γ site (T at *rs7482144*). The mother was HbE carrier with Hb type of AE (Supplementary Figure 2). She was mildly anemic with mild change of RBC morphology (Figure 1). She was heterozygous for β^{E} mutation (HBB:c.79G>A) (β^{E}/β^{N}) with compound heterozygote of 3.7kb-α thalassemia 2 (NG_000006.1:g.34164_37967del3804) and Hb Constant Spring (ABA2:c.427T>C) (-α$^{3.7}$/α ^{Cs}α). The *Xmn*I-^{G}γ site was in homozygous state (*Xmn*I-^{G}γ : +/+). Brother was HbE carrier with Hb type of AECs (Supplementary Figure 2). He was not anemic with normal RBC morphology (Figure 1). He was heterozygous for β^{E} mutation, Hb Constant Spring and for *Xmn*I-^{G}γ site, having genotypes as β^{E}/β^{N}, α^{Cs}α/αα, *Xmn*I-^{G}γ:+/-, respectively. The patient had HbE/β^{O} thalassemia with Hb type of EF (Supplementary Figure 2). She was mildly anemic with a thalassemia blood picture (Figure 1). She had Mahidol score for clinical severity of zero [11]. She was compound heterozygous for β^{17} and β^{E} mutations (β^{17}/β^{E}), inherited from her father and mother, respectively. She was also heterozygous for Hb Constant Spring (α^{Cs}α/αα) and *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/-), inherited from her mother. Details of the hematologic parameters of this family are shown in Table 1.

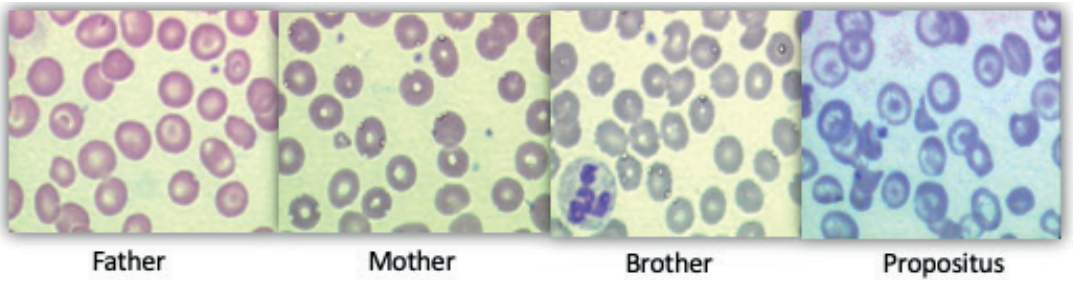

Figure 1. *RBC morphology of Family 1 members. Father and Mother had mild changes in RBC morphology. Brother had normal RBC morphology. The patient had thalassemic RBC morphology.*

Table 1. Hematological parameters, Hb typing results, globin gene genotype, and *Xmn*I-^{G}γ genotype of members in Family 1.

	Father	Mother	Brother	Patient
RBC(x10^{6}/μL)	5.72	5.09	5.55	5.17
Hb (g/dL)	12.0	10.8	13.8	8.9
Hct (%)	39.9	36.6	43.5	29.9
MCV (fL)	69.8	71.9	78.4	57.8
MCH (pg)	21	21.2	24.9	17.2
MCHC (g/dL)	30.1	29.5	31.7	29.8
RDW (%)	14.8	13.8	14.4	27.3
RBC morphology	Sl. change	Sl. Change	Normal	Thalassemia
Cation-exchange HPLC	A_2A [A_0 65.6%, A_2 5.4%]	EA [A_0 68.8%, E 19.0%]	EA [A_0 64.8%, E 23.6%]	EF [E 61.3%, F22.1%]
α-globin gene	αα/αα	-α$^{3.7}$/α^{Cs}α	α^{Cs}α/αα	α^{Cs}α/αα
β-globin gene	β^{17}/β^{N*}	β^{E}/β^{N*}	β^{E}/β^{N*}	β^{E}/β^{17}
*Xmn*I-^{G}γ site	-/-	+/+	+/-	+/-

Note: β^{N} is normal β-globin gene.

Family 2

Family 2 consisted of a father, mother, brother, and patient (Supplementary Figure 1). The father was HbE carrier with Hb type of AE (Supplementary Figure 3), not anemic, and had normal RBC morphology (Figure 4). He was heterozygous for Hb Constant Spring (α^{Cs}α/αα) and homozygous for the *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/+). The mother was β-thalassemia carrier with Hb type of A_2A (Supplementary Figure 3). She was mildly anemic with moderate change of RBC morphology (Figure 2). She was heterozygous for β$^{41/42}$ mutation (β$^{41/42}$/β^{N}) without co-existence α thalassemia, Hb Constant Spring, and the *Xmn*I-^{G}γ site. Brother was normal with Hb type of A_2A (Supplementary Figure 3). He was not anemic with normal RBC morphology (Figure 2).. He was heterozygous for Hb Constant Spring (α^{Cs}α/αα) and *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/+). The patient had HbE/β^{O} thalassemia with Hb type of EF (Supplementary Figure 3). He was mildly anemic with thalassemia blood picture (Figure 2). His Mahidol score for clinical severity was zero.[11] He was compound heterozygous for β^{E} and β$^{41/42}$ mutations (HBB:c.126_129delCTTT) (β$^{41/42}$/β^{E}), inherited from his father and mother, respectively. He was also heterozygous for Hb Constant Spring (α^{Cs}α/αα) and *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/+), inherited from his father. Details of the hematologic parameters of this family are shown in Table 2.

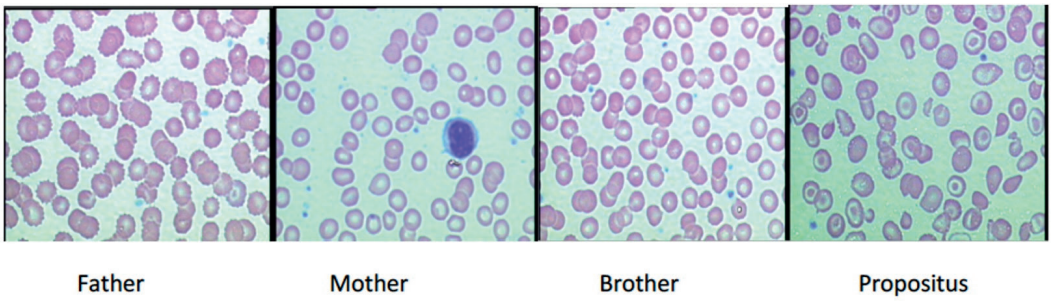

Figure 2*. RBC morphology of Family 2 members. Mother had a moderate change in RBC morphology. Father and Brother had normal RBC morphology. The creation seen in Father RBC was RBC shrinkage due to prolonged storage in EDTA. The patient had thalassemic RBC morphology.*

Table 2. Hematological parameters, Hb typing results, globin gene genotype, and *Xmn*I-^{G}γ genotype of members in Family 2.

	Father	Mother	Brother	Patient
RBC(x10^{6}/μL)	4.81	4.12	5.6	5.54
Hb (g/dL)	14.3	8.2*	14	11.2
Hct (%)	44.2	27.4	43.8	36.4
MCV (fL)	91.9	66.5	78.2	56.9
MCH (pg)	32.4	19.9	25	17.3
MCHC (g/dL)	32.4	29.9	32	30.5
RDW (%)	13.6	15.9	12.9	27.5
RBC morphology	Normal	Mod Change	Normal	Thalassemia
Cation-exchange HPLC	AE [A_0 65.2%, E 25.2%]	A_2A [A_0 71.4%, A_2 4.8%]	A_2A [A_0 86.8%, A_2 2.5%]	EF [E 66.6%, F20.9%]
α-globin gene	$\alpha^{Cs}\alpha/\alpha\alpha$	αα/αα	$\alpha^{Cs}\alpha/\alpha\alpha$	$\alpha^{Cs}\alpha/\alpha\alpha$
β-globin gene	β^{E}/β^{N**}	$\beta^{41/42}/\beta^{N*}$	β^{N}/β^{N*}	$\beta^{E}/\beta^{41/42}$
*Xmn*I-^{G}γ site	+/+	-/-	+/-	+/-

*Note: Co-occurrence of iron deficiency anemia was suspected, **β^{N} is normal β-globin gene.*

Family 3

Family 3 consisted of a father, mother, and patient (Supplementary Figure 1). The father was HbE carrier with Hb type of AE (Supplementary Figure 4), not anemic, and normal RBC morphology (Figure 3). He was heterozygous for β^{E}-mutation (β^{E}/β^{N}) and *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/-), without co-existence of α-thalassemia and Hb Constant Spring. The mother was β-thalassemia carrier with Hb type of A_2A (Supplementary Figure 4). She was mildly anemic with moderate change of RBC morphology (Figure 3). She was heterozygous for β^{IVS1-1} mutation (HBB:c.92+1G>T) (β^{IVS1-1}/β^{N}) with co-existence of SEA-α^{O} thalassemia (- -^{SEA}/αα). The patient had HbE/β^{O} thalassemia with Hb type of EE (Supplementary Figure 4). She was mildly anemic with thalassemia blood picture (Figure 3). Her Mahidol score for clinical severity was zero.[11] She was compound heterozygous for β^{E} and β^{IVS1-1} mutations (β^{E}/β^{IVS1-1}), inherited from her father and mother, respectively, and typical for HbE/β^{O}-thalassemia (Supplementary Figure 5). She was also heterozygous for the SEA-α^{O} thalassemia (- -^{SEA}/αα) and the *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/-), inherited from her mother and father, respectively. As no HbF peak was seen in HPLC, the Acid-Urea-Triton X (AUT)-Polyacrylamide Gel Electrophoresis (PAGE) of globin chain analysis was performed and found no γ-globin chain band, confirming the Hb typing result of EE without HbF peak (Supplementary Figure 6). Analysis of RFLP haplotype of β-globin gene cluster found that the patient carried haplotypes III and I, inherited from her father and mother, respectively. Details of hematologic parameters of this family are shown in Table 3.

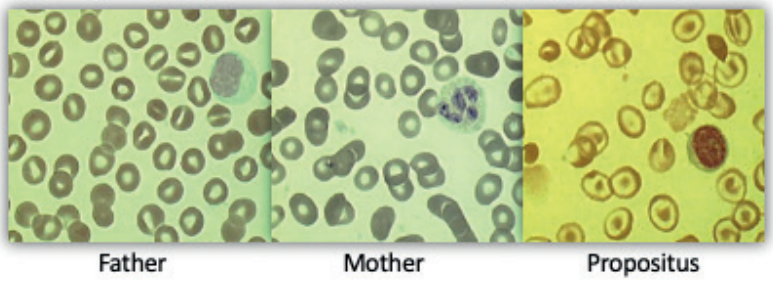

Figure 3. *RBC morphology of Family 3 members. Father and Mother had moderate changes in RBC morphology. The patient had thalassemic RBC morphology.*

Table 3. Hematological parameters, Hb typing results, globin gene genotype, and *Xmn*I-^{G}γ genotype of members in Family 3.

	Father	Mother	Patient
RBC(x10^{6}/μL)	4.95	4.3	5.3
Hb (g/dL)	13.0	10.3	8.5
Hct (%)	40.7	33.9	29.4
MCV (fL)	82.2	78.8	55.5
MCH (pg)	26.3	24.0	16.0
MCHC (g/dL)	31.9	30.4	28.9
RDW (%)	13.8	17	22.7
RBC morphology	Mild change	Mild change	Severely change
Cation-exchange HPLC	AE [A_0 62.2%, E 25.3%]	A_2A [A_0 83.5%, A_2 4.4%]	EE [E 89.9%]
α-globin gene	αα/αα	--^{SEA}/αα	--^{SEA}/αα
β-globin gene	β^{E}/β^{N*}	β$^{IVS1-1(G>T)}$/β^{N*}	β$^{IVS1-1(G>T)}$/β^{E}
*Xmn*I-^{G}γ site	+/-	-/-	+/-
β-globin gene haplotype	I/III	I/VII	I/III

β^{N} is normal β-globin gene.

Family 4

Family 4 consisted of mother, brother, and patient (Supplementary Figure 1). Mother was HbE carrier with Hb type of AE (Supplementary Figure 7), not anemic and with normal RBC morphology (Figure 4). She was heterozygous for β^{E}-mutation (β^{E}/β^{N}) and the *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/-). Brother was otherwise normal. The patient was HbE/β^{O} thalassemia with Hb type of EF (Supplementary Figure 7). She was mildly anemic with thalassemia blood picture (Figure 4). Her Mahidol score for clinical severity was 3.5, indicating moderate degree of clinical severity.[11] She was compound heterozygous for β^{17} and β^{E} mutations (β^{E}/β^{17}), inherited from her deceased father and mother, respectively. She had no co-inherited α-thalassemia and was only heterozygous for *Xmn*I-^{G}γ site (*Xmn*I-^{G}γ:+/-). Details of hematologic parameters of this family are shown in Table 4.

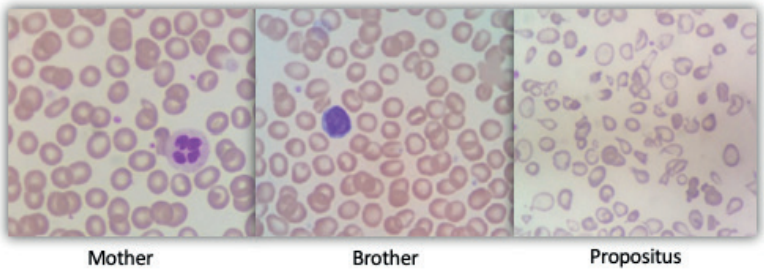

Figure 4. *RBC morphology of Family 4 members. Mother and Brother had mild changes in RBC morphology. The patient had thalassemic RBC morphology.*

Table 4. Hematological parameters, Hb typing results, globin gene genotype and *Xmn*I-^{G}γ genotype of members in Family 4.

	Mother	Brother	Patient
RBC(x10^6/μL)	5.24	6.37	4.66
Hb (g/dL)	12.7	16.3	6.7
Hct (%)	40.4	51.7	22.9
MCV (fL)	77.1	81.2	49.1
MCH (pg)	24.2	25.6	14.4
MCHC (g/dL)	31.4	31.5	29.3
RDW (%)	12.0	14.6	26.4
RBC morphology	Normal	Normal	Thalassemia
Cation-exchange HPLC	AE [A_0 63.4%, E 26.5%]	A_2A [A_0 86.7%, A_2 3.2%]	EF [E 62.8%, F30.4%]
α-globin gene	αα/αα	αα/αα	αα/αα
β-globin gene	β^E/β^{N*}	β^N/β^{N*}	β^E/β^{17}
*Xmn*I-^{G}γ site	+/-	-/-	+/-

Note: β^N is normal β-globin gene.

Discussion

HbE/β-thalassemia is genotypically a compound heterozygote of β^E and $\beta^{Thalassemia}$ alleles. There are broadly two groups of $\beta^{Thalassemia}$ genes, including the severe β^0-and mild β^+-genes. The β^{17}, $\beta^{41/42}$, and β^{IVS1-1} mutations observed in the propositi of this study were all β^0-mutations, in which no functional β-globin chain is produced. The β^{17} is a nonsense mutation (A>T) at codon 17, causing premature stopping of β-globin mRNA translation at this codon. In addition, the $\beta^{41/42}$ is a frameshift mutation caused by a 4 base pair (bp) deletion at codons $^{41/42}$ of the β-globin gene. This 4-bp deletion causes a shift of triplet codons that leads to the formation of a premature stop codon at codon 59. In contrast, the IVS1-1 is the G>T mutation at the first nucleotide of intron or intervening sequence (IVS)#1 of the β-globin gene. This mutation abolishes normal splicing of the β-globin mRNA and, as a result, no normal β-globin mRNA is formed, causing the β^0-type of thalassemia.[1] Compound heterozygote of these β^0 alleles with the β^E allele can lead to disorder of HbE/β^0-thalassemia.[2]

The clinical phenotype of HbE/β^0-thalassemia varies widely with steady-state hemoglobin levels ranging from 3.0 g/dL to 11.0 g/dL.[2] Types of β-globin mutations, co-existence of α-thalassemia and co-inheritance of loci linked to increased γ-globin gene expression were shown to ameliorate clinical symptoms of β-thalassemia and HbE/β-thalassemia. [2,3,12-14] The patients in this report were compound heterozygote for β^E allele and β^{17} or $\beta^{41/42}$ or β^{IVS1-1}, all of which were β^0-thalassemia genes. Based on the β-globin mutations, all these propositi should be clinically severe with thalassemia major or transfusion-dependent types.[15-19] However, the clinical symptoms of these propositi were only of intermedia types, requiring no or only occasional blood transfusion.

For patients 1 and 2, Hb Constant Spring combined with the *Xmn*I-^{G}γ site could account for their mild clinical symptoms. The reduction of free α-globin chain in Hb Constant Spring and the increased formation of HbF in the presence of the *Xmn*I-^{G}γ site lessened the chance of α-globin aggregation which can destroy red blood cells. Hb Constant Spring is an α-structural variant (α-Constant Spring) that originated from a point mutation (T>C) at a stop codon of α2-globin chain, leading to instability of the α-Constant Spring mRNA.[20,21] In addition, expression of the downstream α1-globin gene is reduced due to the α-Constant Spring mutation in the α2-globin gene. [22] The combination of these two mechanisms leads to the α-globin chain being markedly decreased in Hb Constant Spring, causing severe α^+-thalassemia. The severe characteristics of Hb Constant Spring were observed in clinical analysis of HbH disease in Thailand, which clearly showed that HbH-Constant Spring disease (- -/$\alpha^{Cs}\alpha$) was more severe than deletional HbH disease (- -/-α).[23,24]

The *Xmn*I-^{G}γ polymorphism, co-inherited in patients 1, and 2, was shown to be the major *cis*-acting factor for re-activating γ-globin gene expression and HbF/F cells in human adults.[25-28] It has also been found to reduce clinical symptoms in sickle cell disease, β-thalassemia, and HbE/β thalassemia.[18,19,29,30] Therefore, a co-occurrence of the *Xmn*I-^{G}γ site could contribute to reducing even more the clinical symptoms in these two propositi.

However, patient 4, who only had *Xmn*I-^{G}γ site in the heterozygous form, had more severe clinical symptoms than patients 1, and 2. It was clear from the results that the patient 4 was a compound heterozygote for β^E and β^{17} alleles. The β^{17} is nonsense mutation (A-T), resulting in the complete absence of β-globin chain synthesis.[19] The β^E is missense mutation (glutamic – lysine) that also causes reduction of β^E globin chain due to random usage of cryptic splice site at codon 25 of β-globin gene during β^E-mRNA production.[33] As a result, synthesis of β-globin chain in patient 4 was markedly decreased, leading to

an overwhelmingly increased quantity of free α-globin chain and α/β-globin chain synthetic ratio. However, It has been known that the γ-globin gene reactivation efficiency of the *Xmn*I-^{G}γ site alone is limited and that the resulting γ-globin chain produced cannot assemble all the free α-globin chain to produce HbF. As a result, the free α-globin chain remaining can aggregate, precipitate, and damage red blood cells.[3] In fact, previous surveys in Thailand had reported minimal impact of the heterozygous *Xmn*I-^{G}γ site in reducing the clinical severity of HbE/β-thalassemia.[7,18,19,30,32]

Patient 3, who also had very mild HbE/β^{O}-thalassemia with an atypical pattern of Hb typing of EE instead of EF as determined by cation-exchange HPLC (Supplementary Figure 5) had both SEA-α^{O}-thalassemia and *Xmn*I-^{G}γ site. Her γ-globin chain was not reactivated as shown by AUT-PAGE (Supplementary Figure 8). Therefore, this atypical Hb pattern could be explained by non-upregulated γ-globin gene expression and mild clinical symptoms of this patient would be attributed to the SEA-α^{O}-thalassemia alone. In fact, it has been established that the SEA-α^{O}-thalassemia is a severe form of α-thalassemia producing no α-globin chain.[22,34,35] Thus, reduced chance of α-globin aggregation and precipitation occurred in this patient and her clinical symptoms improved. Further studies on the exact mechanism controlling the expression of the γ-globin gene should clarify the low γ-globin gene expression in this HbE/β^{O}-thalassemia patient.

Analysis of 925 Thai HbE/β-thalassemia patients in 2008 showed that the patients having co-existent SEA-α thalassemia and Hb Constant Spring had later disease onset, less frequent requirement for blood transfusion, fewer episodes of hepatosplenomegaly than those having no α-thalassemia. The hematological parameters were better in the HbE/β-thalassemia patients having SEA-α^{O}-thalassemia and Hb Constant Spring than those having normal α-globin gene.[36] In addition, the study of 240 Asian Indian HbE/β-thalassemia patients by Sharma V and colleagues demonstrated ameliorating effect of α-thalassemia, including Hb Constant Spring, on clinical severity.[37] They concluded that the α-thalassemia was the major clinical modifying factor of their HbE/β-thalassemia patients. The findings in this study supported those raised by these two studies.

In conclusion, these family studies proved the findings of previous cohort studies that SEA-α^{O} thalassemia and Hb Constant Spring had the potential in alleviating the clinical symptoms of HbE/β^{O}-thalassemia. The minimal effect of the *Xmn*I-^{G}γ site on clinical severity of HbE/β^{O}-thalassemia was evident. This information may be useful for family counseling during prenatal diagnosis of β-thalassemia syndrome.

Conflict of interest

None

Acknowledgements

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References

[1] Weatherall DJ, Clegg JB. The Thalassaemia Syndromes. 4th Ed. Oxford: Blackwell Scientific, 2001. doi:10.1002/9780470696705

[2] Fucharoen S, Weatherall DJ (2012) The hemoglobin E thalassemias. Cold Spring Harb Perspect Med. 2012; 2(8): 1-15. doi: 10.1101/cshperspect.a011734.

[3] Thein SL. Genetic modifiers of β-thalassemia. Haematologica 2005; 90(5): 649-60. PMID: 15921380

[4] Musallam KM, Cappellini MD, Viprakasit V, *et al*. Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassemia classification 10 years later. Am J Hematol. 2021; 96(2): E54-E56. doi: 10.1002/ajh.26056.

[5] Tatu T, Kiewkarnkha T, Khuntarak S, *et al*. Screening for co-existence of α-thalassemia in β-thalassemia and in HbE heterozygotes via an enzyme-linked immunosorbent assay for Hb Bart's and embryonic ζ-globin chain. Int J Hematol. 2012; 95(4): 386-93. doi: 10.1007/s12185-012-1039-4.

[6] Srisuwan W, Tatu T. Diagnosis of thalassemia carriers commonly found in northern Thailand via a combination of MCV or MCH and PCR-based methods. Bull Chiang Mai Assoc Med Sci. 2013; 46(1): 22-32.

[7] Tatu T, Sritong W, Sa-Nguansermsri T. The associations of SEA-α thalassemia 1, *Xmn*I-Gγ polymorphism and β-globin gene mutations with the clinical severity of β-thalassemia syndrome in northern Thailand. J Med Assoc Thai. 2014; 97(3): 300-7. PMID: 25123009

[8] Kerdpoo S, Limweeraprajak E, Tatu T. Effect of Swiss-type heterocellular HPFH from *Xmn*I-Gγ and *HBBP1* polymorphisms on HbF, HbE, MCV and MCH levels in Thai HbE carriers. Int J Hematol. 2014; 99(3): 338-44. doi: 10.1007/s12185-014-1516-z.

[9] Dos Santos Silva W,de Nazare Klautau-Guimaraes M, Grisolia CK. β-globin haplotypes in normal and hemoglobinopathic individuals from Reconcavo Baiano, State of Bahia, Brazil. Genet Mol Biol. 2010; 33(3): 411-7. doi: 10.1590/S1415-47572010005000042.

[10] Sutton M, Bouhassira EE, Nagel RL (1989) Polymerase chain reaction amplification applied to the determination of β-like globin gene cluster haplotypes. Am J Hematol. 1989; 32(1): 66-9. doi: 10.1002/ajh.2830320113.

[11] Sripichai O, Makarasara W, Munkongdee T, *et al*. A scoring system for the classification of β-thalassemia/Hb E disease severity. Am J Hematol. 2008; 83(6): 482-4. doi: 10.1002/ajh.21130.

[12] Galanello R, Cao A. Relationship between genotype

and phenotype. Thalassemia intermedia. Ann NY Acad Sci. 1998; 850: 325-33. doi: 10.1111/j.1749-6632.1998.tb10489.x.

[13] Cao A, Galanello R, Rosatelli MC. Genotype-phenotype correlations in β-thalassemias. Blood Rev. 1994; 8(1): 1-12. doi: 10.1016/0268-960x(94)90002-7.

[14] Fucharoen S, Winichagoon P. Clinical and hematologic aspects of hemoglobin E beta-thalassemia. Curr Opin Hematol. 2000; 7(2): 106-12. doi: 10.1097/00062752-200003000-00006.

[15] Traivaree C, Monsereenusorn C, Rujkijyanont P, *et al*. Genotype-phenotype correlation among β-thalassemia and β-thalassemia/HbE disease in Thai children: predictable clinical spectrum using genotypic analysis. J Blood Med. 2018; 9: 35-41. doi: 10.2147/JBM.S159295. eCollection 2018.

[16] Viprakasit V, Ekwattanakit S. Clinical Classification, Screening and Diagnosis for Thalassemia. Hematol Oncol Clin North Am. 2018; 32(2): 193-211. doi: 10.1016/j.hoc.2017.11.006.

[17] Laboratories of Computer Science & Engineering and Biochemistry & Molecular Biology at the Pennsylvania State University. Globin Gene Server. 1999. https://globin.bx.psu.edu/. Accessed 25 Sept 2021.

[18] Laosombat V, Wongchanchailert M, Sattayasevana B, *et al*. Clinical and hematologic features of β^{O}-thalassemia (frameshift 41/42 mutation) in Thai patients. Haematologica. 2001; 86(2): 138-41. PMID: 11224481

[19] Laosombat V, Wongchanchailert M, Sattayasevana B, *et al* (2001) Clinical and hematological features of codon 17, A-T mutation of β-thalassemia in Thai patients. Eur J Haematol. 2001; 66(2): 126-9. D doi: 10.1034/j.1600-0609.2001.00305.x.

[20] Hunt DM, Higgs DR, Winichagoon, P, *et al*. Haemoglobin Constant Spring has an unstable α chain messenger RNA. Br J Haematol. 1982; 51(3): 405-13. doi: 10.1111/j.1365-2141.1982.tb02796.x.

[21] Waggoner SA, Liebhaber SA. Regulation of α-globin mRNA stability. Exp Biol Med. (Maywood) 2003; 228(4): 387-95. doi: 10.1177/153537020322800409.

[22] Bernini LF, Harteveld CL (1998) Alpha-Thalassemia. In: Rodgers GP, editor. Bailliere's Clinical Haematology, International Practice and Research, Sickle cell disease and Thalassaemia. London: Bailliere Tindall; 1998. pp. 53-90.

[23] Charoenkwan P, Taweephon R, Sae-Tung R, *et al*. Molecular and clinical features of Hb H disease in northern Thailand. Hemoglobin. 2005; 29(2): 133-40. PMID: 15921165

[24] Fucharoen S, Viprakasit V. Hb H disease: clinical course and disease modifiers. Hematology Am Soc Hematol Educ Program. 2009: 26-34. doi: 10.1182/asheducation-2009.1.26.

[25] Garner C, Tatu T, Reittie JE, *et al*. Genetic influences on F cells and other hematologic variables: a twin heritability study. Blood. 2000; 95(1): 342-6. PMID: 10607722

[26] Thein SL, Craig JE. Genetics of Hb F/F cell variance in adults and heterocellular hereditary persistence of fetal hemoglobin. Hemoglobin. 1998; 22(5-6): 401-14. doi: 10.3109/03630269809071538.

[27] Thein SL, Menzel S. Discovering the genetics underlying foetal haemoglobin production in adults. Br J Haematol. 2009; 145(4): 455-67. doi:10.1111/j.1365-2141.2009.07650.x.

[28] Thein SL, Menzel S, Lathrop M, *et al*. Control of fetal hemoglobin: new insights emerging from genomics and clinical implications. Hum Mol Genet. 2009; 18(R2): R216-23. doi: 10.1093/hmg/ddp401.

[29] Alaoui-Ismaili FZ, Laghmich A, Ghailani-Nourouti N, *et al*. *Xmn*I polymorphism in sickle cell disease in north Morocco. Hemoglobin. 2020; 44(3): 190-4. doi: 10.1080/03630269.2020.1772284.

[30] Laosombat V, Wongchanchailert M, Sattayasevana B, *et al*. Clinical and hematological features of β^{+}-thalassemia (IVS-1 nt 5, G-C mutation) in Thai patients. Eur J Haematol 2001; 67(2): 100-4. doi: 10.1034/j.1600-0609.2001.t01-1-00431.x.

[31] Winichagoon P, Fucharoen S, Chen P, *et al*. Genetic factors affecting clinical severity in beta-thalassemia syndromes. J Pediatr Hematol Oncol. 2000; 22(6): 573-80. doi: 10.1097/00043426-200011000-00026.

[32] Charoenkwan P, Teerachaimahit P, Sanguansermsri T. The correlation of α-globin gene mutations and the *Xmn*I polymorphism with clinical severity of Hb E/β-thalassemia. Hemoglobin. 2014; 38(5): 335-8. doi: 10.3109/03630269.2014.952744.

[33] Winichagoon P, Fucharoen S, Wilairat P, Chihara K, Fukumaki Y. Role of alternatively spliced β^{E}-globin mRNA on clinical severity of β-thalassemia/hemoglobin E disease. Southeast Asian J Trop Med Public Health. 1995;26(Suppl 1):241-5. PMID: 8629114

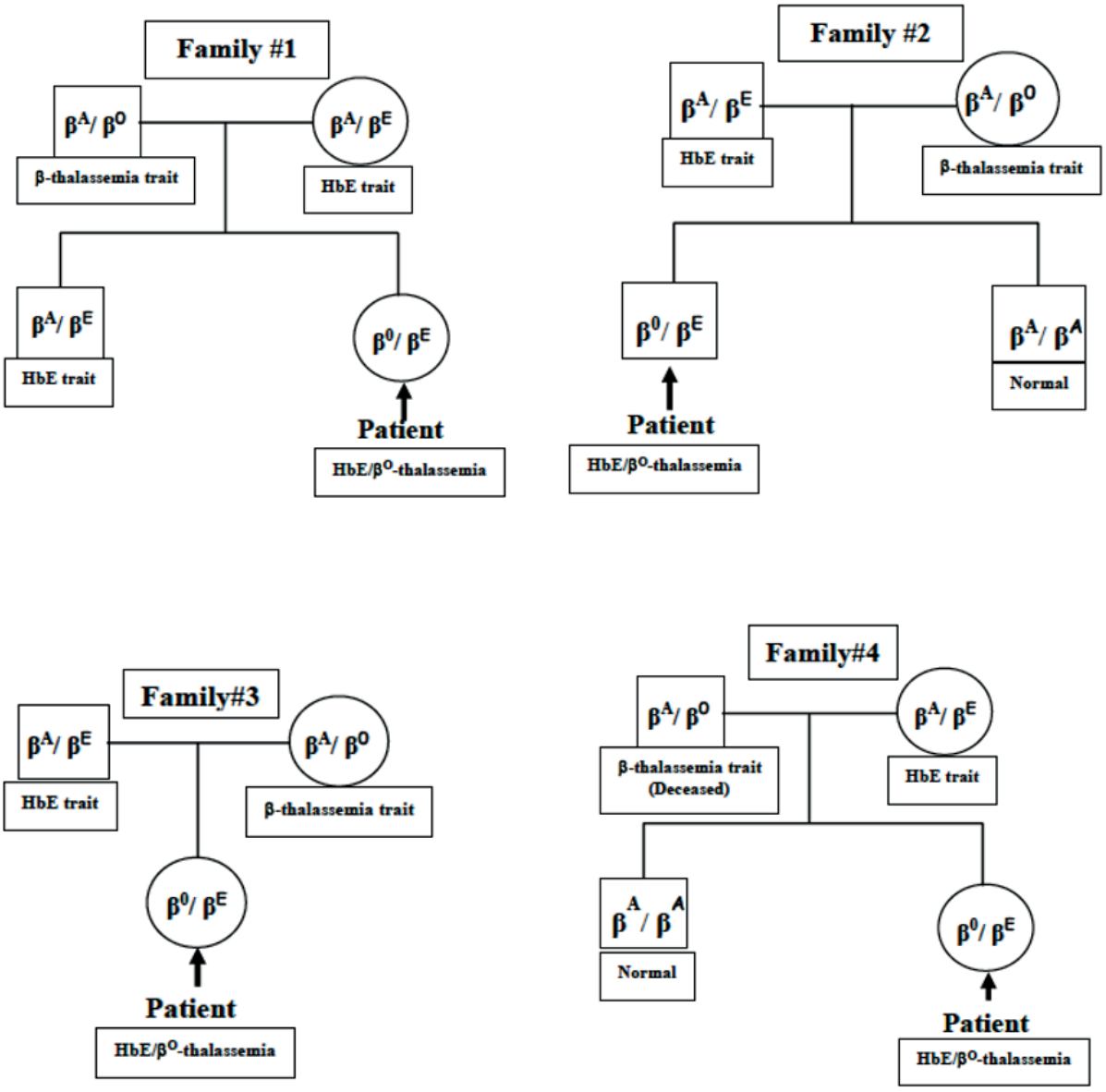
[34] Harteveld CL, Higgs DR. Alpha-thalassaemia. Orphanet J Rare Dis. 2010; 5: 13. doi: 10.1186/1750-1172-5-13.

[35] Higgs DR, Weatherall DJ. The Alpha Thalassaemias. Cell Mol Life Sci. 2009; 66(7): 1154-62. doi: 10.1007/s00018-008-8529-9.

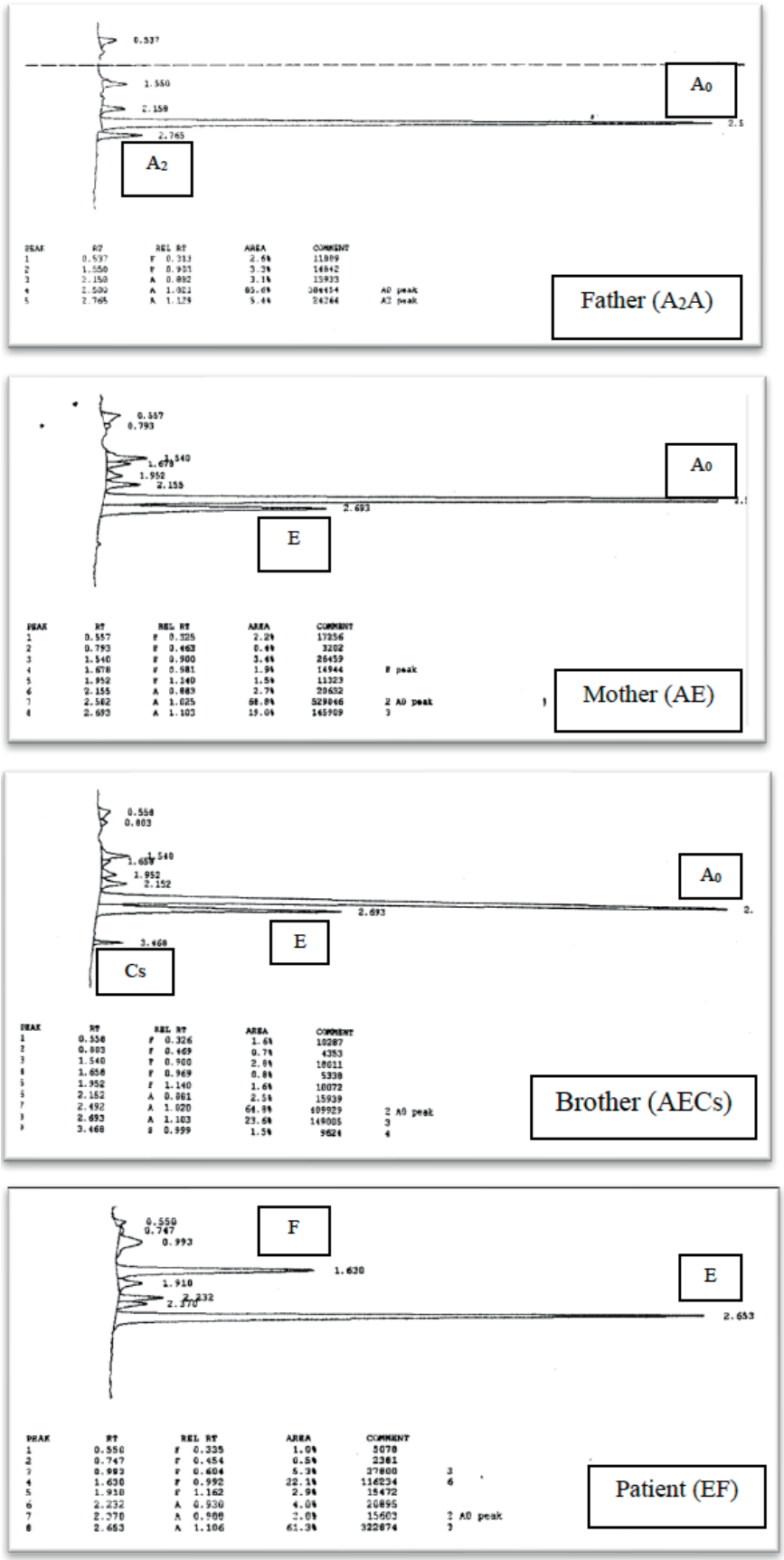
[36] Sripichai O, Munkongdee T, Kumkhaek C, *et al*. Coinheritance of the different copy numbers of α-globin gene modifies severity of β-thalassemia/Hb E disease. Ann Hematol. 2008; 87(5): 375-9. doi: 10.1007/s00277-007-0407-2.

[37] Sharma V, Kumar B, Kumar G, *et al*. Alpha globin gene numbers: an important modifier of HbE/β thalassemia. Hematology. 2009; 14(5): 297-300. doi: 10.1179/102453309X446126.

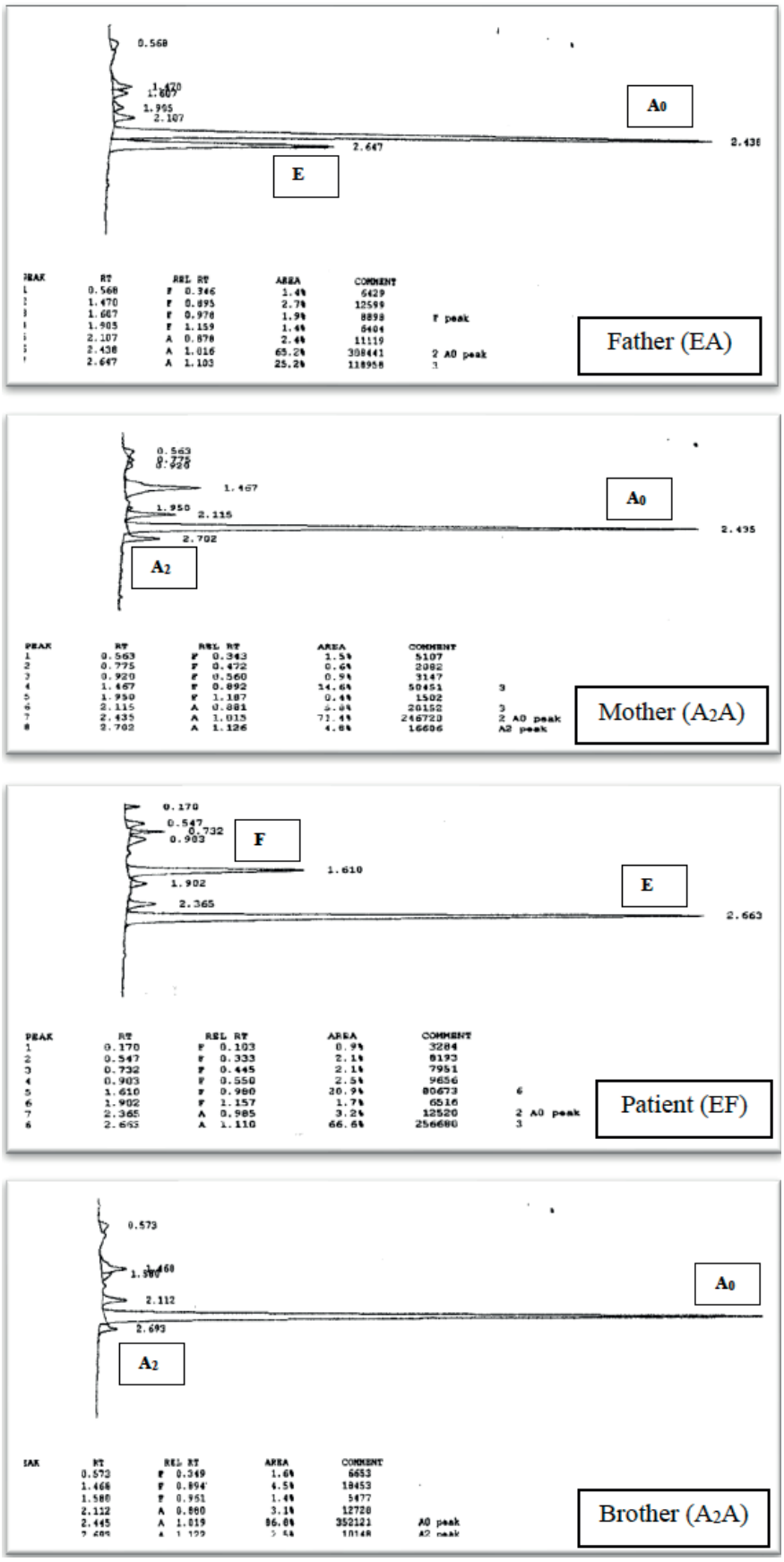
Supplementary Figures



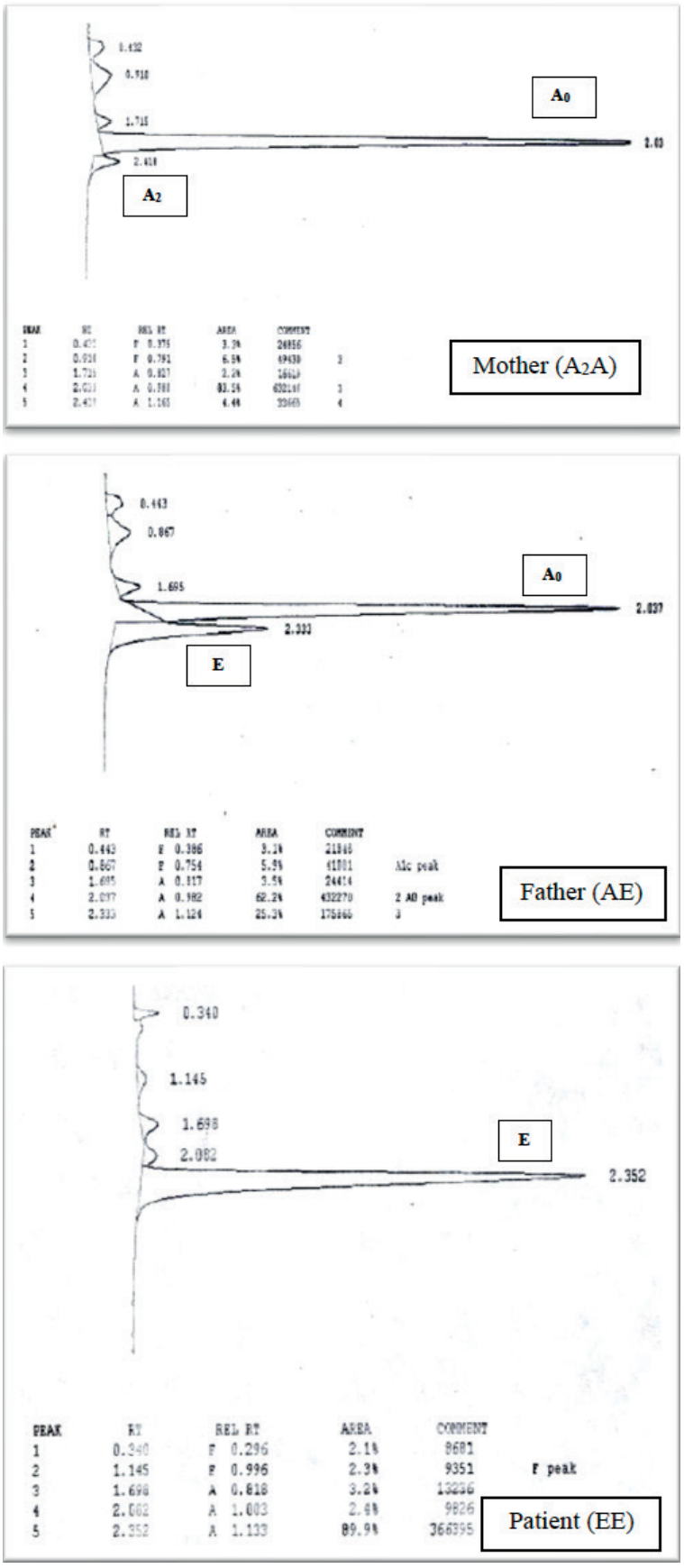
Supplementary Figure 1. *Pedigree of 4 families analyzed in this family study.*



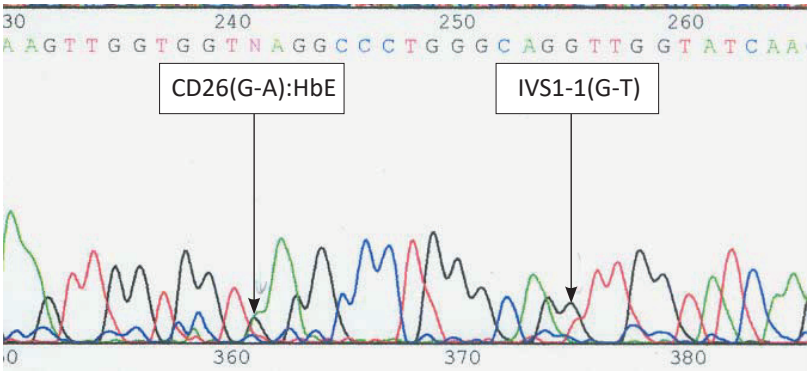
Supplementary Figure 2. *HPLC chromatogram (Primus Variant System 99) of hemoglobins of Family 1. The father was β-thalassemia heterozygote with HbsA$_2$A. Mother and brother were HbE heterozygote with Hbs EA. The patient was HbE/β^0-thalassemia with Hbs EF. The major HbA is HbA$_0$. The number above each peak is the retention time.*



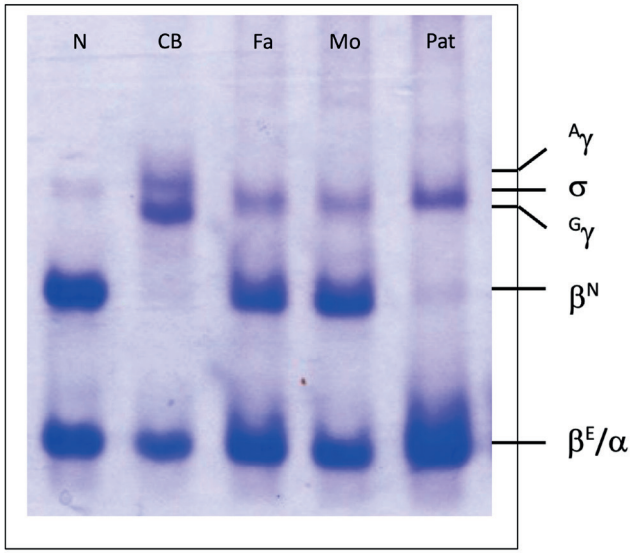
Supplementary Figure 3. *HPLC chromatogram (Primus Variant System 99) of hemoglobin in Family 2. The father was HbE heterozygote with Hbs EA. Mother was β-thalassemia heterozygote with Hbs A_2A. Brother was normal with Hbs A_2A. Patient was HbE/β^0-thalassemia with Hbs EF. The major HbA is HbA_0. The number above each peak is the retention time.*



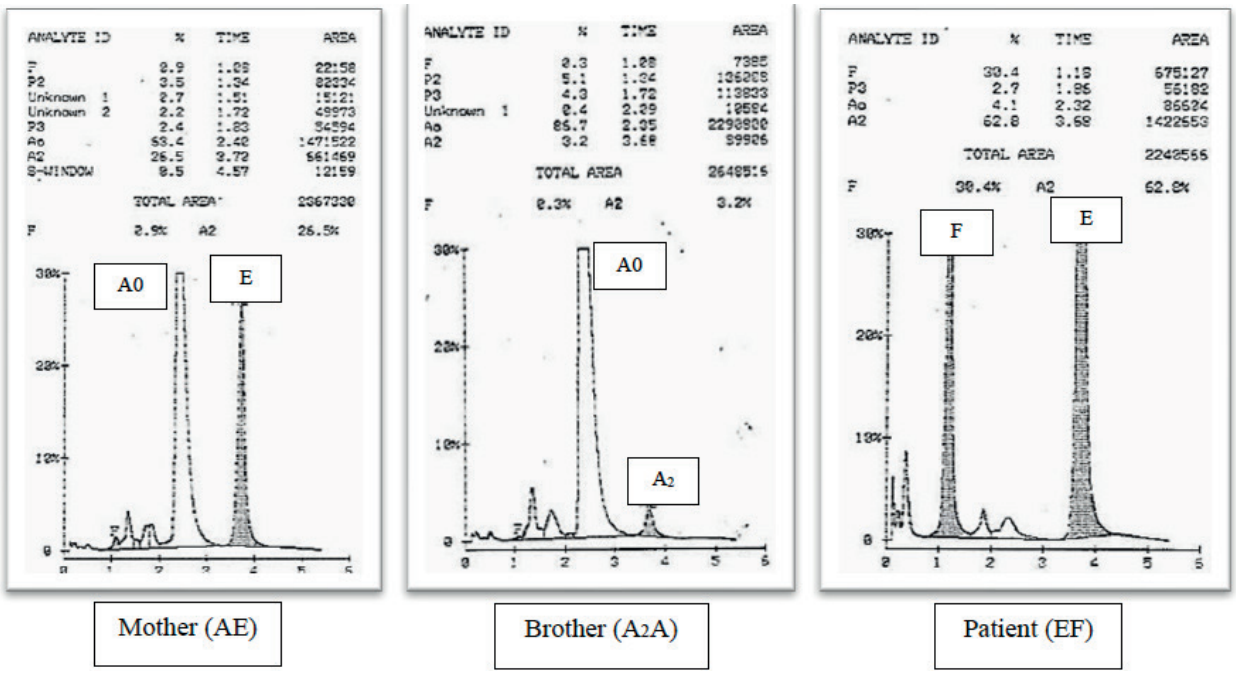
Supplementary Figure 4. *HPLC chromatogram (Primus Variant System 99) of hemoglobin in Family 3. The father was HbE heterozygote with Hbs EA. Mother was β-thalassemia heterozygote with Hbs A_2A. Patient was HbE/$β^0$-thalassemia with atypical Hb pattern of EE. The major HbA is HbA_0. Number above each peak is the retention time.*



Supplementary Figure 5. *Nucleotide sequencing of β-globin gene in patient of Family 3. Overlapped peaks are seen at codon 26 and IVS1-nucleotide 1, indicating heterozygous state of these base substitutions.*



Supplementary Figure 6. *AUT-PAGE for globin chain separation in Family 3. Note: No γ globin bands are seen in the patient. (N: normal, CB: cord blood, Fa: father, Mo: mother, Pat: patient)*



Supplementary Figure 7. *HPLC chromatogram (BioRad VARIANT II Hemoglobin Testing System) of hemoglobin in Family 4. The mother was HbE heterozygote with Hbs EA. Brother was normal with Hbs A_2A. The patient was HbE/β^0-thalassemia with Hbs EF. The major HbA is HbA_0.*

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The Development of manual for speech and language treatment for parents of children with cleft palate ages 0-3 years old

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ABSTRACT

Background: Cleft palate is a congenital disability affecting the palate's structure and function, which may lead to delayed speech and language development and communication disorders. There is a shortage of speech therapists in Thailand. Therefore, developing a manual for speech and language treatment for the parents of children with a cleft palate would be an essential early intervention that parents could perform.

Objectives: This research aimed to develop a speech and language treatment manual for parents of children with cleft palate ages 0-3 years old and evaluate the manual's effectiveness, satisfaction, and feedback.

Materials and methods: This research was divided into Phase 1, which involved developing a speech and language treatment manual for parents of children with cleft palate ages 0-3 years old and assessing its validity and reliability. Phase 2 tested the manual's effectiveness by conducting a three-month of 16 participants, who were divided into two groups: A control group without the manual and an experimental group with the manual and phase 3 data analysis.

Results: The content validity of the manual and satisfaction of the questionnaires were found to have a content validity index of 0.87 and 0.92, respectively. The total language and speech development scores before and after participating between groups found a statistically significant difference. After participating, the number of the experimental group who passed the 90th percentile of language development was higher than the control group. The language between the children and parents showed that the experimental group displayed more communication skills than the control group.

Conclusion: The speech and language treatment manual for parents of children with cleft palate ages 0-3 years old could stimulate the language and speech development of the experimental group more effectively than the control group that did not receive the manual.

Introduction

Cleft lip and palate (CLP) are a congenital anomaly of the facial structure of the lips and palate caused by the failure of fetal facial development during incomplete fusion of the fetus. As a result, the upper lip and palate have separated.[1]

Children with a congenital anomaly with CLP represent a worldwide average prevalence of about one to two per 1,000 live births, while in Thailand, the prevalence is approximately 0.58-2.49 per 1,000 live births. The highest

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incidence is found in the Northeastern region of Thailand, with approximately 2.49 per 1,000 live births or around 700-800 cases per year.[2]

Although the surgical treatment could help with oral consumption and external appearance, some issues remain and impact speech mechanisms, such as respiration (some people have short breathing and/or hoarseness), phonation, articulation, and resonance.[3] In some cases, people could also experience hearing problems. Consequently, these impacts often lead to speech and language development delays, especially in children under three years old. In most cases, playing complex sounds is challenging, which results in a delay in combining sounds to form meaningful words and combining words, thus eventually leading to limitations in vocabulary development and speech use.[4,5] Moreover, receptive language and expressive language abilities could be delayed.[6,7] Furthermore, there may be a limitation in the lexical vocabulary bank, a limited ability to play consonant sounds, or a lack of diversity compared to typical children.[4] Problems with articulation disorder, resonance, mispronunciation of sounds or rhythms, and compensatory articulation disorders could also occur.[3,4] Accordingly, it could be concluded that a cleft palate would increase the risk of communication difficulties, resulting in delayed language development and speech problems if not diagnosed and treated.[8] Additionally, this could increase the risk of language and learning disabilities when the child enters school.[8] Language and speech are important tools for communication, learning, and development of various skills, as well as for everyday life. As such, communication difficulties could lead to emotional and social difficulties that could impact communication and learning in daily life, as well as other areas of development. Therefore, children with a cleft palate would need to receive language and speech therapy, as children with this condition would be more likely to have delayed language and speech development or speech impairments. Hence, early intervention would be a critical process providing the necessary counseling and therapy to the parents and children[9] to prepare them to develop their full potential during the early learning stage, which would vary according to each child and family.[10,11] The objective of language and speech therapy for children with a cleft palate with and without a cleft lip would be to stimulate sound production, increase the variety of consonant sounds, improve control of the airflow in the mouth, and expand the vocabulary bank.[12] As such, early intervention should begin before the child starts producing consonant sounds or speaking[13,14] to prevent a delay in language and speech development and reduce the severity of speech impairments.[14]

Language and speech therapy is an ongoing yet long-term process requiring speech therapists' involvement. However, there is a need for more speech therapists in many areas of Thailand, which has limited access to continual therapy for children in need. Therefore, this study recognized the importance of early intervention in language and speech therapy, which would require the active participation of the parents to stimulate or train their children and reduce the risk of delayed language and speech development. Consequently, parents need to be educated about language and speech development and techniques and strategies to guide and support their children during the therapy sessions.[15]

The researchers, thus, realized the significance of developing a manual for speech and language treatment for parents of children with a cleft palate aged 0-3 years old. The manual would be provided to parents who would be the primary trainers at home so they could understand and effectively apply the training methods at home. This would allow children with a cleft palate to access appropriate language and speech stimulation and improve their language and speech development, which could reduce the severity of language and speech impairment and enhance their efficiency in communication. Thus, this research aimed to develop a manual for speech and language treatment for parents of children with a cleft palate aged 0-3 years old and to evaluate the content validity of the manual before being employed in further assessment of its effectiveness.

Material and methods

This study aimed to not only develop and test the effectiveness of a parental manual for speech and language in children with a cleft palate aged 0-3 years old but also to assess the satisfaction of the parents in using the manual and to inquire into the problems and suggestions from using the manual for further development. The Ethics Committee for Human Research, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, approved this research project with project number COA No. 1133/2022.

Participants

This study involved 16 children with a cleft palate with or without a cleft lip. They were divided into a control group of eight participants whose parents did not receive the parental manual and an experimental group of eight participants whose parents received such a manual. All participants were aged 0-3 years.

1. The experimental group of eight participants comprised one child aged 0-12 months, five children aged 13-24 months, and two children aged 25-36 months.
2. The control group of eight participants consisted of one child aged 0-12 months, five children aged 13-24 months, and two children aged 25-36 months.

Procedures

Phase 1: *Develop a speech and language treatment manual for parents of children with cleft palate ages 0-3 years old.*

After collecting all the relevant documents about the concepts, rehabilitation theories, language, and speech development in children with a cleft palate, including researching parental involvement in language and speech development and the methods for developing the manual, the development process of this manual was as follows:

1.1 The researchers determined the size of the

book, including the cover, introduction, table of contents, objectives of the manual, instructions for using the manual, language and speech treatment for children aged 0-3 years, information about cleft lip and palate, speech and language problems in children with a cleft palate, stimulation of language and speech development, which consisted of pictures, objectives of the activities, samples of the activities descriptions, expected results, activity record forms, problems or questions that could arise, and topics to be used in the satisfaction assessment form.

1.2 The researchers drafted the manual's content by selecting only relevant information and using simple and basic vocabulary to suit the user group's role and educational level. The content was divided into sections on the methods or strategies used for stimulating language and speech development, along with incorporating illustrations and captions that were corresponsive to the objectives, plans, or strategies used during the training in a precise sequence. A record form for activities was also designed. After completing the manual, the researchers created a satisfaction assessment form by drafting the instructions and questions aligned with the manual by referring to the principles of creating an assessment form.

1.3 The accuracy of the language used in the manual and the satisfaction assessment form was tested content validity by five speech-language pathologist experts.

1.4 Finding the congruence between the manual and satisfaction assessment form was verified.

The researchers presented a training and development manual for speech and language treatment for the parents of children with a cleft palate with and without a cleft lip aged 0-3 years and an evaluation form of satisfaction with the manual. The manual was presented to five qualified experts who met the criteria of being a speech therapist with at least a Master's degree and a minimum of 10 years of experience working with children with a cleft palate with and without a cleft lip. Subsequently, the researchers analyzed the index of item objective congruence (IOC) of the manual, including the appearance and content of the introduction, table of contents, objectives, methods of use, training methods, expected results, and images. Additionally, the researchers analyzed the IOC value of the satisfaction evaluation form and gathered the experts' feedback to improve the manual's content and evaluation form. This ensured that they were aligned with the research objectives and definitions of the terminology. Once the experts reviewed and approved the manual, it was produced as the final version.

Phase 2: *Trial of the manual*

The researchers evaluated language and speech development using the Thai Speech and Language Test (TSLT)[17] assessment tool for children aged 0-4 years. They recorded a 30-minute video of the parents and children playing together freely. After recording, the research participants, including the parents, received training and guidance on language stimulation techniques. This was the first time the experimental group had received the manual and guidance, while the control group did not receive any. The data were collected over three months. The researchers scheduled the participants to attend sessions for data collection once a month as follows: Session 2 in week 4, Session 3 in week 8, and Session 4 in week 12. For the participants to receive initial language and speech therapy by the speech therapist, during Session 4, the researchers assessed the language and speech development using the same evaluation form.[17] A 30-minute video was recorded while the participants played freely with their parents. The parents from the experimental group assessed their satisfaction and provided suggestions for improving or developing the manual, which the control group received at the end of the study. The researchers provided the pre and post-participated evaluation results video recordings by TSLT and language use between children and parents while playing independently for 30 minutes to another speech-language pathologist to score and transcribe to assess the participants' language and speech development to compare language and speech development progress, including differences in language use before and after each person participating in the research and between the control group and the experimental group after participating in this project.

Phase 3: *Data analysis*

1.1 Analysis of the data on the content validity was determined by considering the opinions of the five experts and calculating the value of the IOC, which had to be at least 0.5, according to the experts' assessment. It was found that the manual and satisfaction assessment forms have the content validity index for the entire set at 0.87 and 0.92, respectively.

1.2 Analysis of language comprehension and expression development were assessed before and after participating in this project using the Mann-Whitney U test and comparing it with the percentile rank[17] between the control and experimental groups to measure the effectiveness of the manual.

1.3 Analysis and comparison of the pre and post-language development were conducted using video and audio recordings of each participant, which were recorded before and after participating in the research project. For other another speech-language pathologist to evaluate and transcribe audio. This is to prevent bias and help to see differences in language use between parents and children.

Results

The study aimed to develop a speech and language

treatment manual for parents of children with cleft palate ages 0-3 years old. The results of this study were as follows:

1. The level of consistency between the objectives and the format and content (IOC value) of the manual was appropriate. The appearance of the manual's components, such as font and image size, was clear and suitable for usage. The instructions on using the manual were understandable and appropriate for the objectives of each age group. The methods and strategies were correct and consistent with the relevant purposes. This manual was also easy to understand, appropriate, and straightforward. From a total of 66 content items, five experts expressed their opinions within the range of 0.6-1, and the overall consistency value from evaluating the effectiveness (IOC value) was 0.87.
2. The level of consistency between the objectives and the format and content (IOC value) of the evaluation form for satisfaction after using the manual in terms of its appearance, accessibility, instructions, font size, and use of the questions or content was correct, appropriate, complete, and clear. Five experts evaluated all 16 items; their comments were between 0.6-1. The consistency score from the IOC assessment was 0.92.
3. The results from assessing the development in receptive and expression of language between the control and the experimental groups are shown in Table 1.

Table 1 Comparative speech and language assessment analysis between the experimental and control groups.

Participants		n	Mean	SD	Median (Lo; up)	Mean Difference	Sum Rank	Mann-Whitney U Value	P Value	95 % CI	
										Low	Upper
Pre	Control Group	8	9.00	5.65	6.50 (4;15)	0.25	64.50	28.50	0.71	- 5.00	4.00
	Experimental group	8	9.00	4.37	7.50 (5; 18)		71.50				
Post	Control group	8	20.87	5.69	20.50 (15; 29)	8	48.00	12.00	0.03	3.50	16.00
	Experimental group	8	29.75	7.38	30.00 (19; 39)		88.00				

From Table 1**,** The results of the language development and speech assessment between the control and experimental groups that were done before participating in the research showed that the average scores of both groups were nine points. When comparing the total scores of the speech and language development between both groups before participating in this study using the Mann-Whitney U Test, it was found that there was no statistically significant difference (p<0.05).

On the other hand, the post-participating speech and language development assessment results between the control and experimental groups showed that the control group had an average score of 20.87 points. In contrast, the experimental group had an average score of 29.75 points. When comparing the total scores of speeches and language development between the control and experimental groups after participating in the research project using the Mann-Whitney U Test, it was found that there was a statistically significant difference between the two groups (p<0.05).

The difference in the total scores between the pre- and post-speech and language development among the two groups revealed that the control group had a mean score difference of 11.87 points, and the experimental group had a mean score difference of 20.75 points. Moreover, after comparing the scores between the two groups using the Mann-Whitney U Test, it was found that there was a statistically significant difference (p<0.05).

Table 2 depicts the total speech and language performance of the control group after participating in this study; only two participants passed the 90% percentile benchmark. Simultaneously, five participants from the experimental group passed the 90% percentile criteria.

From Table 3, The language use data of the research participants were evaluated through a 30-minute video recording of free play between the children and their parents. Comparing the pre- and post-participation, it was found that the speech and language development of the participants in both the control and experimental groups improved. The area that showed the greatest improvement was their ability to express themselves in language, as seen in the age range of 0-12 months, where there was no sound or communication through speech before joining this study. However, after the post-participation, they were able to communicate with meaningful words. In the age range of 25-36 months, the experimental group developed from one to two-word utterances to communicate in complete sentences. The clear difference between the two groups was the diversity and number of vocabulary used, thus noting that the experimental group appeared to have a more extensive and diverse vocabulary.

Table 2 Total speech and language score comprehension of the control and the experimental groups. Pre and Post-participation in the study compared to the percentile.[17]

Age (Months)	Participants	Receptive Language				Expressive Language				Total Speech and Language Score			
		Result		Percentile		Result		Percentile		Result		Percentile	
		Pre	Post	75	90	Pre	Post	75	90	Pre	Post	75	90
12	Control group	41.66	141.66	91.67	100.00	41.66	100.00	112.50	116.67	50.00	120.83	100.00	104.17
	Experimental group	58.33	141.66			41.66	150.00			50.00	145.58		
15	Control group	0.00	80.00	80.00	80.00	33.33	80.00	43.34	60.00	16.67	80.00	53.33	65.67
		0.00	40.00			26.66	60.00			13.33	50.00		
	Experimental group	80.00	140.00			40.00	120.00			60.0	130.00		
		26.66	86.66			13.33	86.66			20.00	86.66		
18	Control group	72.22	94.44	77.78	77.78	33.33	50.00	75.00	88.89	52.76	72.22	75.00	81.95
		50.00	83.33			33.33	55.55			41.67	69.44		
	Experimental group	44.44	83.33			22.22	122.22			16.67	102.78		
		33.33	88.88			22.22	38.88			27.78	63.89		
24	Control group	12.50	33.33	70.83	75.00	4.16	37.50	56.25	60.42	8.33	35.42	61.46	64.58
	Experimental group	4.16	33.33			33.33	108.33			18.75	70.58		
30	Control group	6.66	20.00	73.33	73.33	13.33	30.00	10.00	13.33	10.00	25.00	83.33	86.67
	Experimental group	16.66	56.66			0.00	26.66			8.33	41.67		
36	Control group	8.33	22.22	25.00	27.78	11. 11	22.22	22.22	25.00	9.72	22.22	44.44	50.00
	Experimental group	8.33	25.00			8.33	27.77			8.33	26.39		

Table 3 Comparison of the language used between the control and experimental groups for pre and post-participation.

Participants		Pre	Post
0-12 months	Control group	1. No consonants and vocal play. 2. Not saying meaningful words. 3. Crying was the main form of communication.	1. Spoke in one-syllable words for repetition. 2. Mainly used gestures to communicate. 3. The types of words were nouns and verbs.
	Experimental group	1. No consonants and vocal play. 2. Not saying meaningful words. 3. Crying was the main form of communication.	1. Spoke in one-syllable words meaning to request, answer, or respond to parental communication. 2. Used common gestures to communicate. 3. The types of words were nouns and verbs.
13-24 months	Control group	1. Spoke in one syllable, meaning to request, refuse, and repeat the last syllable. 2. The types of words were nouns and verbs.	1. Spoke in one syllable, meaning to request, refuse, and answer with common gestures to communicate. 2. The types of words were nouns, verbs, and adverbs.
	Experimental group	1. Spoke in one syllable, meaning to express a wish. 2. Rarely followed or communicated during playtime with the parents. 3. The types of words were nouns and verbs.	1. Spoke one to two syllables to indicate a rejection, answer, request, persuade, and/or respond more quickly to parental communication. 2. There were more varieties of words. 3. The types of words were nouns, verbs, adverbs, and prepositions.
25-36 months	Control group	1. Spoke two syllables, short phrases, and sentences to request, give information, or perform actions while playing pretend. 2. The types of words were nouns, verbs, and adverbs.	1. Spoke two syllables at the level of a phrase or sentence (subject + action + object). 2. Rearranged words in sentences 3. There was a limited vocabulary in communicating using words instead. 4. The types of words were nouns, verbs, and adverbs.
	Experimental group	1. Used own language and gestures. 2. Spoke one-two syllables to indicate, request, answer, question, and refuse.	1. Spoke two to three syllables at the level of a phrase or sentence (Subject + verb + object) 2. The types of words were nouns, verbs, adverbs, prepositions, and conjunctions.

Discussion

Regarding developing a parental manual for speech and language in children with a cleft palate aged 0-3 years, the experts provided feedback on the manual's appearance, content, and practicality and that it was found to be usable and in line with the acceptable standards. After receiving the manual, both the control and experimental groups showed an improvement in language development and speaking abilities, especially the experimental group, which made significant progress in language development and speaking skills comparable to those of older children (Tables 1 and 2).

In addition, the results from transcribing the video and audio recordings revealed that the experimental group increased their vocabulary bank, could use a broader range of words, and had longer utterances. Consequently, the manual effectively stimulated language development and improved language expression, especially in approaching age-appropriate language development and speaking skills. As demonstrated in Table 3, the experimental group showed more significant progress in language expression than the control group. This suggested that the ability to produce consonant sounds was found to lead to vocabulary development. Therefore, stimulating pronunciation and vocabulary development in young children with a cleft palate could be achieved through daily interactions with the parents via activities that would foster continuous communication, which would require involvement from the parents in developing language and speaking abilities.[5,14]

Therefore, early intervention with parental involvement in speech and language development would be considered a primary rehabilitation approach. The goals would be to use daily activities to promote language learning for everyday usage, enhance pronunciation, increase speech length, develop vocabulary, improve the ability to use specific words, and make it easier for parents to model speech.[14,18-21]

Limitations

This study was limited by the small sample size, which could have led to statistical inaccuracies. Additionally, due to the time constraints in the data collection, this research lacked adequate measures to reduce the severity of speech problems, such as compensatory articulation or nasal resonance.

Conclusion

The parental manual for speech and language development in children with a cleft palate aged 0-3 years could improve the language and speech development of children in the experimental group better than the control group, whose parents did not receive the manual. Furthermore, the experimental group showed trends toward age-appropriate development. Linguistically, it was revealed that this manual could increase vocabulary and communication in daily life and could be used as a guide for speech and language treatment at home based on the advice of speech therapists. The manual could also be used for early language and speech intervention in children who were at risk or tended to delay language and speech development, apart from the group of children with a cleft palate.

Conflict of interest

The authors have declared that no competing interests existed at the time of publication. The authors have declared that no competing interests existed at the time of publication.

References

[1] Kummer AW. Cleft palate and craniofacial anomalies: effects on speech and resonance. 3rd Ed. Clifton Park, Ny: Delmar, Cengage Learning; 2014.

[2] Prathanee B, editor. Cleft lip and cleft palate Speech problems and multidisciplinary treatment. Vol. 1. Khon Kaen: Institute of Learning and Teaching Innovation Center Khon Kaen University; 2014. 3-26.

[3] Nagarajan R, Savitha VH, Subramaniyan B. Communication disorders in individuals with cleft lip and palate: An overview. Indian J Plast Surg. 2009; 42(1): 137-43. doi: 10.4103/0970-0358.57199.

[4] Scherer N, D'Antonio L, McGahey H. Early intervention for speech impairments in children with cleft palate. Cleft Palate Craniofac J. 2008; 45: 18-31. doi: 10.1597 /06-085.1.

[5] Groenewald H, Kritzinger A, Viviers M. Age-specific communication functioning of young children with cleft lip and palate in a South African database. Cleft Palate Craniofac J. 2013; 50(6): 717-29. doi: 10.1597 /12-146.

[6] Kennard H. Cleft palate and early intervention: beginning the treatment process. [Master of Science]. Communication Disorders and Sciences: Southern Illinois University Carbondale; 2013.

[7] Hattee C, Farrow K, Harland K, Sommerlad B, Walsh M. Are we ready to predict speech development from babble in cleft lip and palate children? Int J Lang Commun Disord. 2001; 36(1): 115-20. doi: 10.3109/13682820109177869.

[8] Snyder LE, Scherer N. The development of symbolic play and language in toddlers with cleft palate. Am J Speech Lang. 2004; 13(1): 66-80. doi: 10.1044/1058-0360(2004/008).

[9] Smith C, Williams E, Bryan K. A systematic scoping review of speech and language therapists' public health practice for early language development. Int J Lang Commun Disord. 2016; 52(4): 407-25. doi: 10.1111/1460-6984.1229.

[10] Melvin K, Meyer C, Scarinci N. What does "engagement" mean in early speech pathology intervention? A qualitative systematised review. Disability and rehabilitation. Taylor & Francis, 2020; 27;42(18):2665-78. doi: 10.1080/09638288.2018.1563640

[11] Pushpavathi M. Efficacy of focused stimulation in early language intervention program for toddlers with repaired cleft palate. Glob J Oto. 2017; 18; 9(1). doi: 10.19080/GJO.2017.09.555752.

[12] Hardin-Jones M, Chapman K, Scherer NJ. Early

intervention in children with cleft palate. The ASHA Leader. 2006; 11(8): 8-32. doi: 10.1044/leader.FTR3. 11082006.8.

[13] Hardin-Jones M, Chapman KL. The impact of early intervention on speech and lexical development for toddlers with cleft palate: A retrospective look at outcome. Lang Speech Hear Serv Sch. 2008; 39(1): 89-96. doi: 10.1044/0161-1461(2008/009).

[14] Zajac DJ, Vallino LD. Evaluation and management of cleft lip and palate: a developmental perspective. San Diego: Plural Publishing, Inc; 2016: 177-193.

[15] Kaiser AP, Hancock TB. Teaching parents new skills to support their young children's development. Infants & Young Children. 2003 Jan 1; 16(1): 9-21.

[16] Phaophung K. The development of manual on enhancing the reading, thinking, analyzing and writing activities for Thai language teachers [Master of Education]. Curriculum and Instruction Graduate School: Silpakorn University; 2005

[17] Prathanee B, Angsupakorn N, Pumnum T, Seepuaham C, Jaiyong P. Standardization of Speech and Language Test for Thai Children Aged 0 - 4 Years. Health Systems Research Institute (HSRI). 2011

[18] Kaiser AP, Scherer NJ, Frey JR, Roberts MY. The effects of enhanced milieu teaching with phonological emphasis on the speech and language skills of young children with cleft palate: A pilot study. Am J Speech Lang Patho. 2017; 26(3): 806-18. doi: 10.1044/2016_ AJSLP-16-0008.

[19] Meinusch M, Romonath R. Early language intervention for children with cleft lip and/or palate: A systematic review.Evid Based Commun Assess Interv. 2011; 5(4): 197-215. doi: 10.1080/17489539.2012.678093.

[20] Scherer NJ. Update on early speech and language intervention for children with craniofacial conditions. Perspect ASHA Spec Interest Groups. 2016; 1(5): 50-8. doi: 10.1044/persp1.SIG5.50.EDGTHYUJI9OP

[21] Whitehill TL, Chau CH -F. Single-word intelligibility in speakers with repaired cleft palate. Clin Linguist Phon. 2004; 18(4-5): 341-55. doi: 10.1080/02699200 410001663344.

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Development of prognostic model and multivariate analysis for breast cancer survival patients using SEER database

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ABSTRACT

Background: Many studies employed machine learning (ML) to forecast the prognosis of breast cancer (BC) patients and discovered that the ML model showed high individualized forecasting ability. Breast cancer is the most frequent kind of carcinoma in women globally and ranks as the leading cause of death in women.

Objectives: This study intends to use the Surveillance, Epidemiology, and End Results dataset to categorize breast carcinoma cases' alive and dead conditions. Deep learning and machine learning have been extensively utilized in clinical studies to address various categorization problems due to their ability to manage massive data sets in an organized manner. Pre-processing the data allows it to be visualized and analyzed for making critical choices. This study describes a realistic machine learning-based strategy for categorizing the SEER breast cancer dataset.

Materials and methods: We employed classification and machine learning algorithms to classify breast cancer mortality. Four well-known classification ML algorithms were employed in this study. To identify risk factors, we employed multivariate analysis using the data set.

Results: The decision tree performed the best accuracy (0.914) among all the models. T4 stage (β=1.4, p<0.001, OR=4.22, 95% CI (2.06-8.64), N2 stage (β=0.39, p=0.008, OR= 1.49, 95% CI (1.111-1.997) found to be major risk factors for breast cancer mortality using multivariate analysis.

Conclusion: The significant prognostic variables affecting the breast carcinoma survival rates reported in the current research are relevant and might be turned into decision support systems in the medical realm.

Introduction

Breast cancer is widely recognized as the prevailing type of carcinoma affecting women globally. It has emerged as the primary cause of cancer. Epidemiological research has discovered that the global incidence of breast cancer is estimated to surpass nearly 2 million cases by the year 2030.[1] According to recent data from GlobeCan in 2020, in India, breast cancer accounted for 13.5% of all cancer cases and 10.6%of all cancer-related mortalities in India. Breast cancer is categorized as a form of tissue cancer primarily affecting the inner layer of milk glands or lobules as well as the ducts, which are small tubes responsible for transporting milk. Several crucial factors contribute

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to the development of breast cancer, such as age, race, a deficit of iodine in the diet, and elevated hormone levels[2,3] Breast cancer is characterized by the combined effect of genetic predisposition and environmental influence. This interaction gives rise to the gradual accumulation of genetic and epigenetic alterations within breast cancer cells, ultimately contributing to ailment development.[2]

In the past, various methods have been employed for the detection of breast cancer, such as biopsies, ultrasonography, and mammography. These diagnostic approaches have proven valuable in facilitating the initial identification of breast cancer. However, it is important to note that these detection methods do not always provide precise results and can be financially burdensome. Chemotherapy serves as a treatment approach for breast cancer. Still, it exerts a significant impact on the quality of life of patients, profoundly affecting their physical and mental social being.[4] In contemporary times, advanced detection and treatment methods have emerged, offering a promising solution to overcome the drawbacks associated with conventional methods. Machine learning, the branch of artificial intelligence, has gained considerable attention to significantly enhance accuracy and precision detection, as well as providing innovative treatment strategies, thereby addressing the drawbacks previously encountered.[5]

Materials and methods

The data used for this research were obtained from SEER database, which was extracted from Kaggle website.[31] The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Programme https://seer.cancer.gov/about/overview.html was an authoritative source of information on cancer incidence and survival in the United States, covering roughly 48.0% of the US population. This dataset of breast cancer patients was taken from the NCI's SEER Program's November 2017 update, which offers information on population-based cancer statistics.

Statistical analysis

All the statistical analysis was done by R programming language(version 4.3.1) and JASP Statistical software. We summarized the categorical variables using frequencies and percentages. The association between breast cancer survival and other predictors was evaluated by using a chi-square test or Fisher exact test. Similarly, in continuous variables, data were summarized in terms of mean (sd) or median (iqr). Comparison between continuous variables concerning breast cancer survival status was done by students t-test or man Whitney test. The multivariate logistic regression model observed the adjusted association between survival status and other predictors.

Data processing

In the context of machine learning, processing information refers to the modification, transformation, and preparation of raw data to prepare it for analysis and training machine learning algorithms.[6,7]It entails several strategies and processes to clean, organize, and prepare work data before it is fed into a machine learning algorithm. The SEER Data was collected and checked for missing values and outliers using various methods. Once the data were clean and ready for analysis, we built various machine-learning algorithms to predict breast cancer mortality.

Machine learning

Machine learning techniques may be utilized for forecasting and diagnosing carcinoma of the breast by analyzing data sources such as medical imaging, patient demographics, and clinical records.[8,9]Keep in mind that establishing a trustworthy breast cancer prediction model needs access to high-quality data, working with healthcare experts, and thorough testing to assure its safety and efficacy. Naive Bayes (NB), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), k-nearest neighbor (KNN), SVM, classification and regression trees (CART), Random forest(RF), multivariate adaptive regression splines (MARS), and logistic regression are examples of common ML methods.(LR) and extreme gradient boosting (XGBoost) can be used to predict breast cancer.

The Random Forest (RF) technique is frequently employed in clinical investigations because of its higher performance than other methodologies. [10,11] It has been proven to attain excellent accuracy and to be especially successful in dealing with extremely non-linear data and datasets with many characteristics. Furthermore, RF is more adaptable to data noise and easier to tune than other ensemble learning techniques. The technique includes a method for predicting missing values, the usage of a Weighted Random Forest (WRF) for balancing mistakes in unbalanced data, and the capacity to assess the relevance of the parameters used for classification.

In the current investigation, logistic regression describes the connection between the independent variables (the 23 factors influencing survival status) and the dependent variable (survival status). The odds ratio is determined using the Gaussian distribution. Because logistic regression is best suited for binary outcomes, patients' condition for survival (alive/dead) is recorded as 1/0 in the dataset. Because the dependent variable is a binary survival status, logistic regression was chosen as one of the evaluation techniques to measure accuracy using binary values.[12-14]

The logistic equation is given by

$$(Y) = \frac{1}{1+e^{-(\beta_0+\beta_1 x_1)}} = \frac{e^{(\beta_0+\beta_1 x_1)}}{1+e^{(\beta_0+\beta_1 x_1)}} \quad ....(1)$$

When there are several predictors in the data, the equation becomes:

$$P(y) = \frac{1}{1+e^{-(\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+...+\beta_i x_i)}} \quad ...(2)$$

$$= \frac{e^{(\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+...+\beta_i x_i)}}{1+e^{(\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+...+\beta_i x_i)}} \quad ...(3)$$

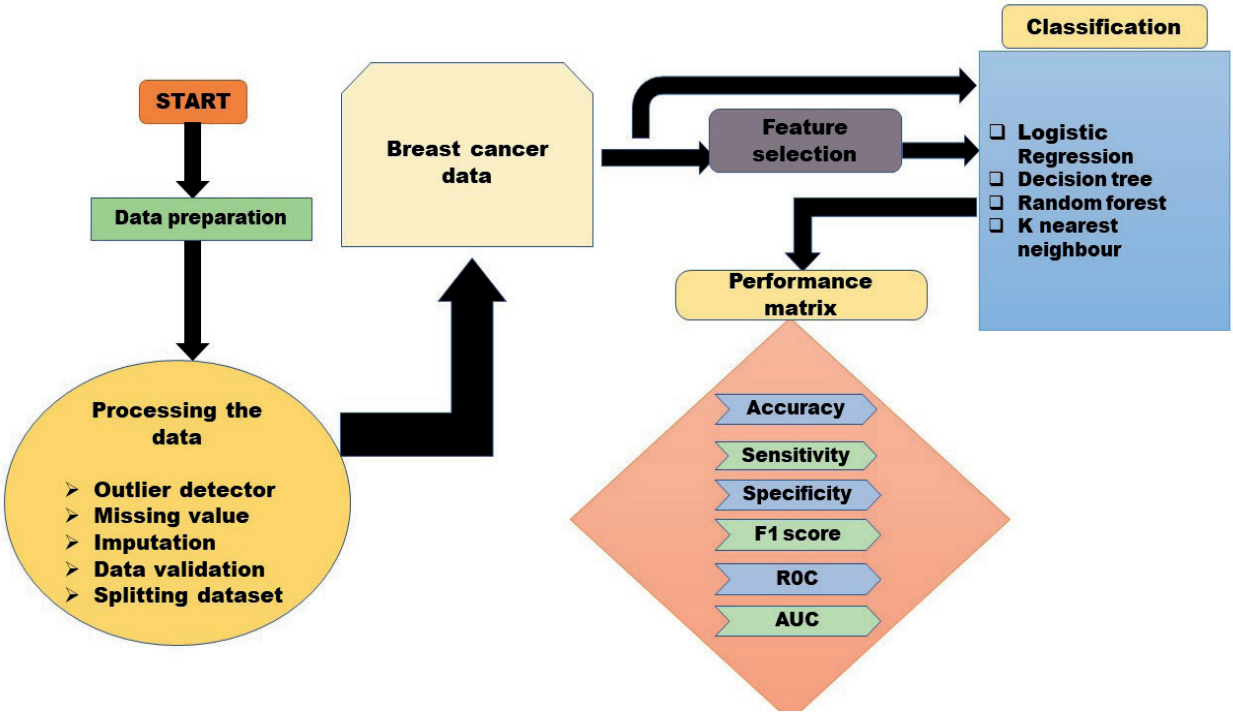

Figure 1. *Road map from data processing to model building.*

K-Nearest Neighbours (KNN) is a well-known machine learning technique that may be used for both classification and regression applications.[15 16] It represents a straightforward yet effective method that is frequently used as a starting point for comparison with more complicated models. KNN may be employed for carrying out tasks related to classification, such as assigning a label from a class to a specified input data point. It operates by locating the k nearest neighbours in the feature space and giving a majority class to the query point among them. KNN may also be employed to solve regression problems when the aim is to forecast a continuous value. rather than using the overwhelming vote in regression, KNN computes the average (or weighted average) of the target values of the k nearest neighbors as the forecast value for the query point. KNN utilizes a non-parametric method, which means it makes no assumptions about the data distribution. It can deal with complicated and nonlinear interactions between characteristics and target variables without requiring a specialized model structure.[17,18]

Decision trees represent a clear and understandable depiction of the decision-making procedure.[19-21] The learned tree framework may be visualized and comprehended, making the logic behind the predictions easier to convey. Developing predictions for new cases is quick when a Decision Tree has been trained. The method traverses the tree structure by assessing the feature conditions at each internal node until it reaches the expected outcome at a leaf node. Decision Overfitting is common in trees, especially when the tree depth is not restricted.[22] They can build too complicated trees that remember noise in the training data, resulting in poor generalization on unseen data. Pruning and establishing maximum depth or minimum samples per leaf might assist in reducing fitting.[23,24]

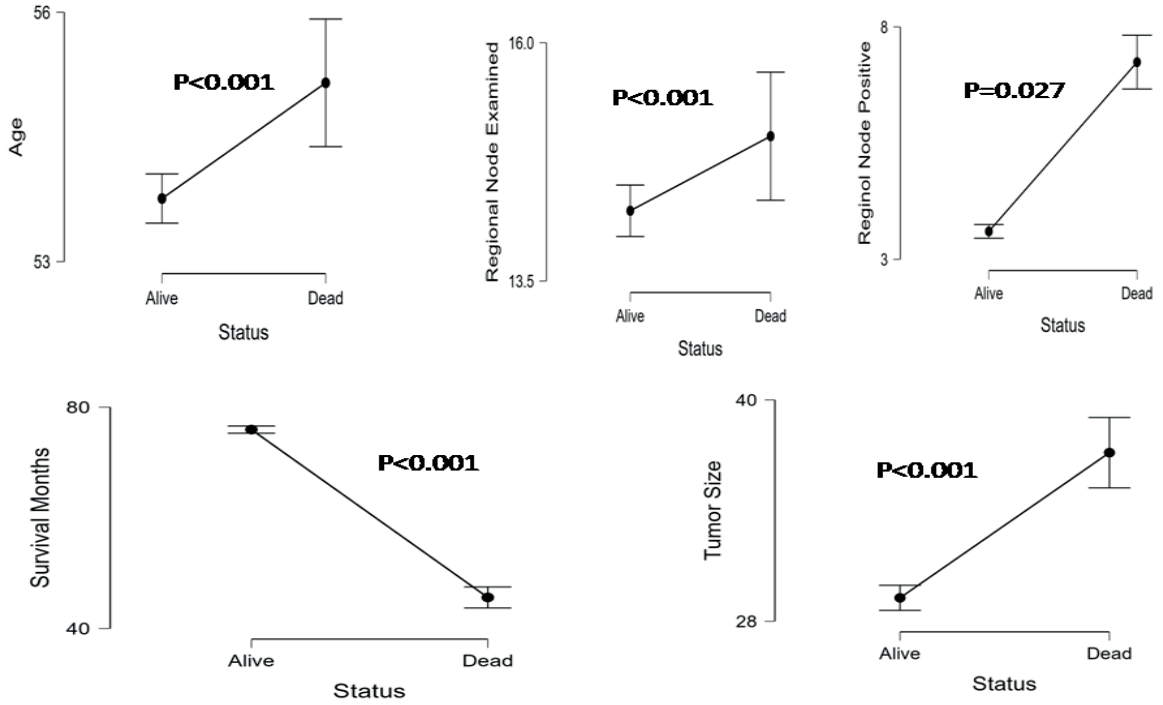

Figure 2. *Mean plot showing the difference between alive and dead breast cancer patients.*

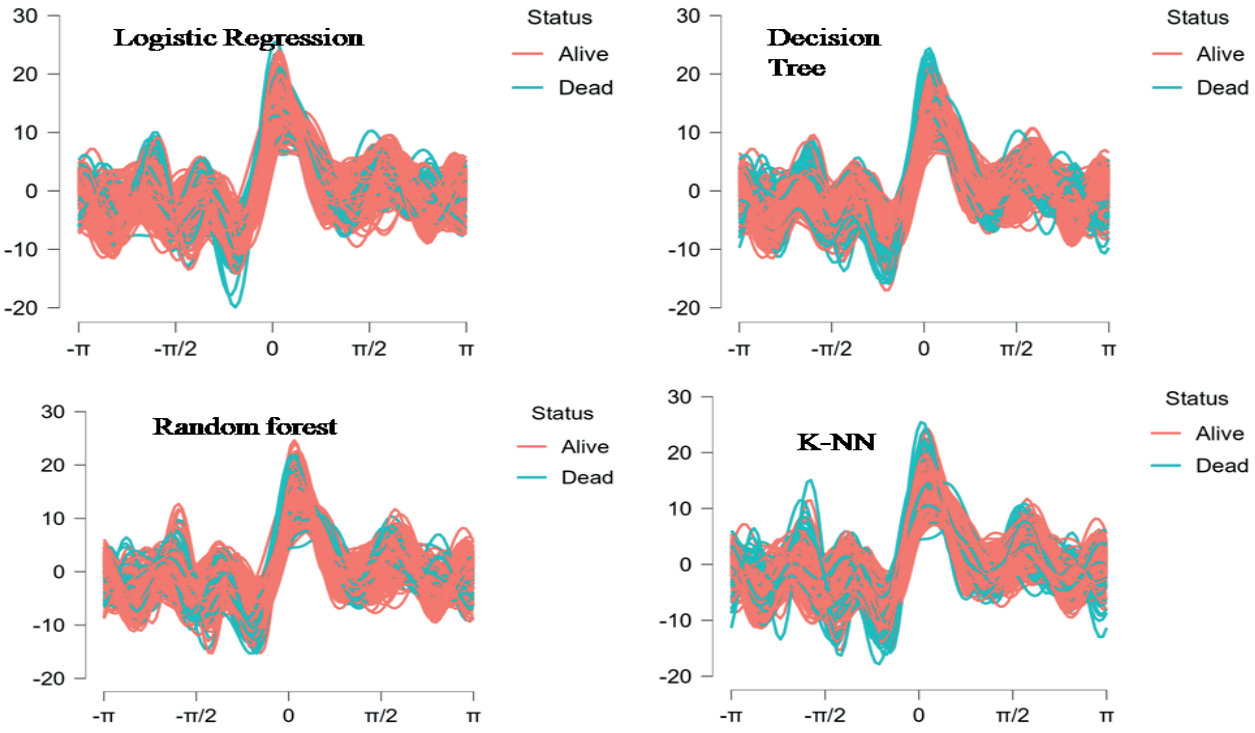

Figure 3. *Andrews plot describing the classification of breast cancer using machine learning.*

Results

Table 1 represents the demographic and clinical features of breast cancer variables. The data were separated into two groups for comparing the number and percentage. A total of 4024 patients' data were analyzed. The mortality percentage was 15.3. Table 2 describes the factors associated with breast cancer mortality. The analysis observed that age (β=٠,٠٢٧, p<0.001, OR=1.028,95% CI (1.015-1.041), T4 stage (β=1.4, p<0.001, OR=4.22,95% CI (2.06-8.64), N2 stage (β=0.39, p=0.008, OR=1.49,95% CI (1.111-1.997), regional node examined (β=-0.03, p<0.001, OR=0.96,95% CI (0.954-0.985), regional node positive (β=0.078, p<0.001, OR=1.08,95% CI (1.04-1.12), survival month (β=-0.06, p<0.001, OR=0.94,95% CI (0.935-0.946), Grade 1 (β=-2.272, p=0.03, OR=0.18,95% CI (0.03-0.86), progesterone status (β=-0.5174, p<0.001, OR=0.596,95% CI (0.442-0.803) were significantly associated with breast cancer mortality.

Table 1. Demographic and clinical parameters for breast cancer patients.

Variables		Dead (N=616)	Alive (N=3408)
Race	Black	73 (11.8)	218 (6.3)
	Other	33 (5.3)	284 (8.4)
	White	510 (82.7)	2,903 (85.1)
Marital Status	Divorced	90 (14.6)	396 (11.6)
	Married	358 (58.11)	2,285 (67.04)
	Separated	15 (2.43)	30 (0.8)
	Single	104 (16.88)	511 (14.9)
	Widowed	49 (7.95)	186 (5.45)
T Stage	T1	157 (25.48)	1,446 (42.43)
	T2	303 (49.18)	1,483 (43.51)
	T3	116 (18.83)	417 (12.23)
	T4	40 (6.49)	62 (1.81)
N Stage	N1	270 (43.83)	2,462 (72.24)
	N2	165 (26.78)	655 (19.21)
	N3	181 (29.38)	291 (8.53)
6th Stage	IIA	96 (15.88)	1,209 (35.47)
	IIB	135 (21.91)	995 (29.19)
	IIIA	184 (29.87)	866 (25.41)
	IIIB	20 (3.24)	47 (1.39)
	IIIC	181 (29.38)	291 (8.53)
Differentiate	Moderately differentiated	305 (49.1)	2,046 (60.03)
	Poorly differentiated	263 (42.69)	848 (24.88)
	Undifferentiated	9 (1.46)	10 (0.29)
	Well differentiated	39 (6.3)	504 (14.78)
Grade	1	39 (6.33)	504 (14.78)
	2	305 (49.55)	2,046 (60.03)
	3	263 (42.69)	848 (24.88)
	4	9 (1.46)	10 (0.29)
A Stage	Distant	35 (5.68)	57 (1.67)
	Regional	581 (94.31)	3,351 (58.32)
Estrogen status	Negative	108 (17.53)	161 (4.72)
	Positive	508 (82.46)	3,247 (95.27)
Progesterone status	Negative	204 (33.11)	449 (14.49)
	Positive	412 (66.68)	2,914 (85.50)
	Age	55.15±9.6	53.75±8.8
	Tumor size	37.14±24.11	29.26±20.3
	Regional node examined	15.01±8.4	14.23±8.02
	Regional node positive	7.24±7.3	3.6±2.3
	Survival months	45.61±23.96	75.94±19.8

Table 2 Multivariate analysis for independent risk factors associated with breast cancer.

						95% Confidence Interval	
Predictor	**Estimate**	**SE**	**Z**	***p***	**Odds ratio**	**Lower**	**Upper**
Intercept	2.94326	0.97116	3.031	0.002	18.978	2.8287	127.32
Age	0.02763	0.0065	4.253	**<0.001**	**1.028**	**1.015**	**1.041**
Race:	Black Ref						
Other	-0.90096	0.29066	-3.1	0.002	0.406	0.2298	0.718
White	-0.45649	0.18989	-2.404	0.016	0.634	0.4366	0.919
Marital Status:	DivRef						
Married	-0.16482	0.1663	-0.991	0.322	0.848	0.6122	1.175
Separated	0.4864	0.48814	0.996	0.319	1.626	0.6248	4.234
Single	-0.08819	0.20602	-0.428	0.669	0.916	0.6114	1.371
Widowed	0.04388	0.26033	0.169	0.866	1.045	0.6273	1.74
T Stage:	T1 Ref						
T2	0.38822	0.15162	2.56	0.01	1.474	1.0953	1.985
T3	0.66963	0.3111	2.152	0.031	1.954	1.0617	3.594
T4	1.43979	0.36581	3.936	**<0.001**	**4.22**	**2.0602**	**8.643**
N Stage:	N1 Ref						
N2	0.39872	0.14944	2.668	**0.008**	**1.49**	**1.1116**	**1.997**
N3	0.52133	0.2793	1.867	0.062	1.684	0.9742	2.912
Regional Node Examined	-0.03108	0.00804	-3.866	<0.001	0.969	0.9542	0.985
Reginal Node Positive	0.07811	0.01788	4.37	**<0.001**	**1.081**	**1.044**	**1.12**
Survival Months	-0.06141	0.00276	-22.272	<0.001	0.94	0.9354	0.946
Tumor Size	-0.00271	0.00469	-0.578	0.563	0.997	0.9882	1.007
Grade:	Ref						
1-anaplastic; Grade IV	-2.27296	0.80448	-2.825	0.005	0.103	0.0213	0.498
2-anaplastic; Grade IV	-1.68538	0.78332	-2.152	0.031	0.185	0.0399	0.861
3-anaplastic; Grade IV	-1.2529	0.78415	-1.598	0.11	0.286	0.0614	1.328
A Stage:	Ref						
Regional-Distant	0.17649	0.32326	0.546	0.585	1.193	0.6331	2.248
Estrogen Status:	Ref						
Positive-Negative	-0.38257	0.22699	-1.685	0.092	0.682	0.4372	1.064
Progesterone Status:	Ref						
Positive-Negative	-0.51744	0.15216	-3.401	<0.001	0.596	0.4423	0.803

Table 3 demonstrates the results of the prediction model from the SEER database after splitting the data set into a training set and testing set. Four classification models were developed and executed to the training data set, whereas the model's performance was checked using testing data. The performance of the various models was checked using various parameters like accuracy, sensitivity, specificity, and F1 score area under the curve (AUC).

$$\text{Accuracy} = \frac{TP+TN}{FP+FN+TP+TN}$$

Sensitivity is the proportion of accurately categorized high-risk samples to the total number of high-risk samples in the collection. The classifier's sensitivity is also known as the true-positive Rate (TPR), and it may be calculated using a formula as follows:

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

The true-negative rate (TNR) is the proportion of low-risk samples accurately identified as low-risk. The following formula may be used to calculate the specificity score:

$$\text{Specificity} = \frac{TN}{TN+FP}$$

From the classification machine learning techniques, we evaluate the model performances using a test data set. The accuracy sensitivity specificity F1 score and AUC were calculated for each model. The accuracy of the decision tree was the best among all classification models.

Table 3. Performance matrix of classification models.

	Accuracy	Sensitivity	Specificity	F1 score	AUC
Random forest	0.898	0.892	0.856	0.877	0.866
KNN	0.891	0.883	0.769	0.876	0.766
Decision tree	0.914	0.908	0.916	0.907	0.858
Logistic regression	0.904	0.898	0.793	0.896	0.833

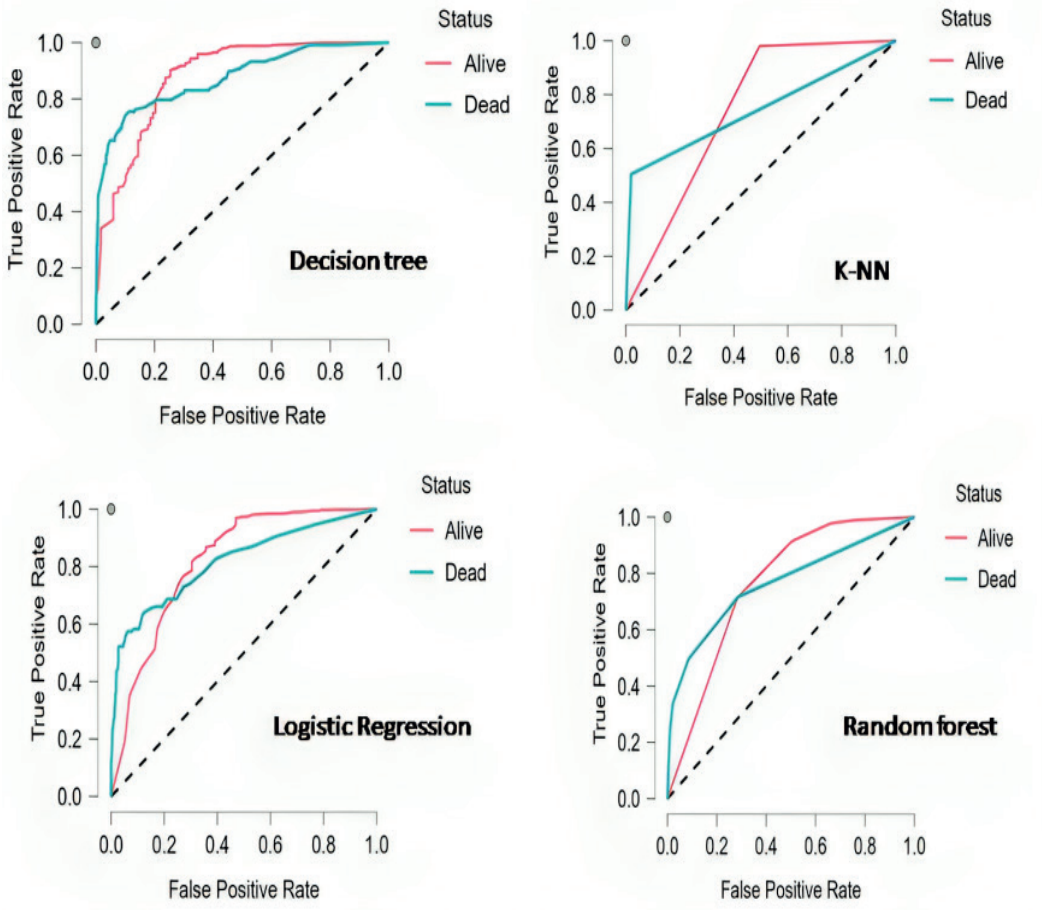

Figure 4. *Roc curve of four classification models for breast cancer mortality prediction.*

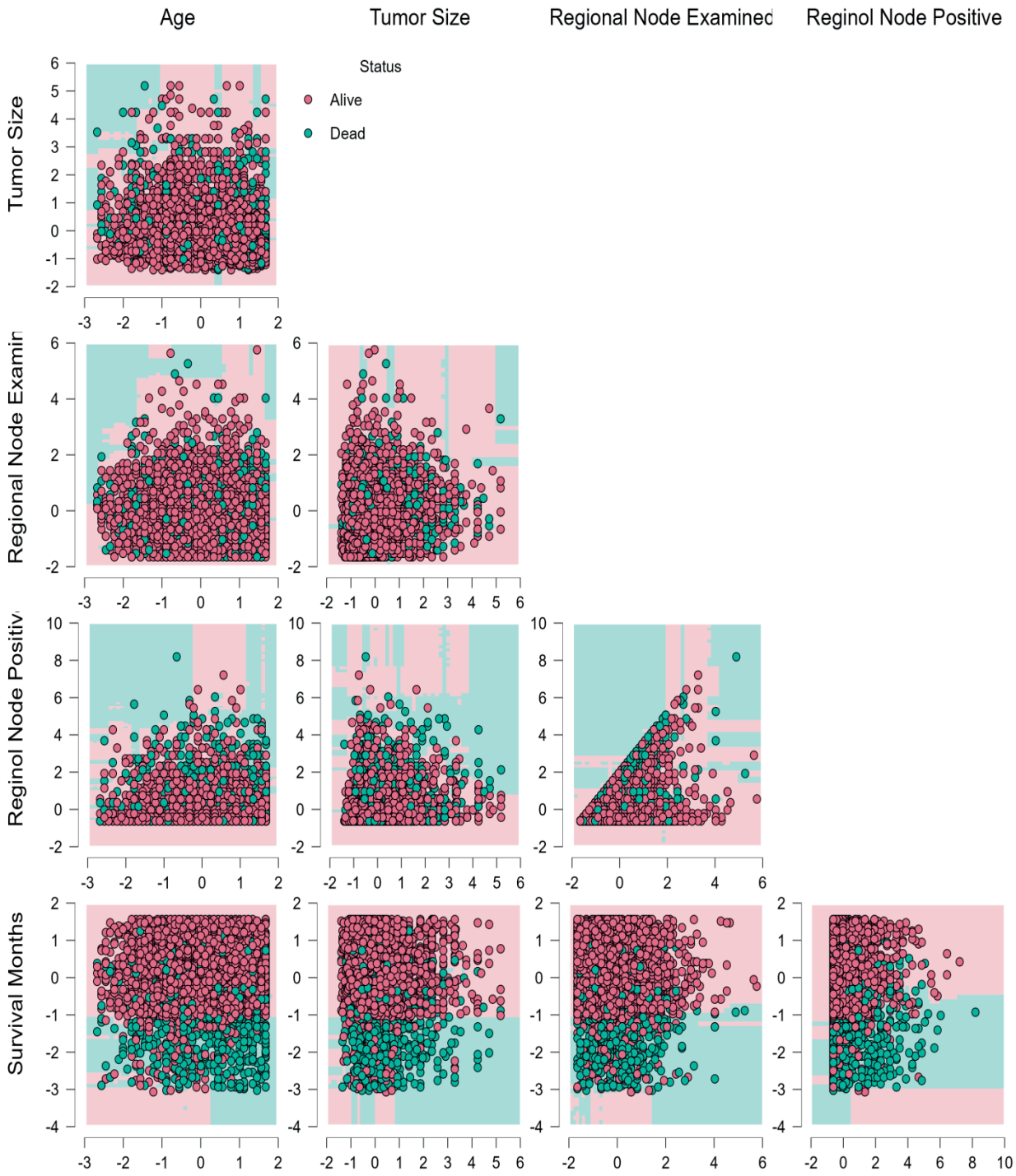

Figure 5. *Decision boundary matrix of a classification model.*

Discussion

To identify relevant characteristics from Wisconsin breast cancer data, methods for selecting features such as Recursive Feature Elimination, Forward Feature Selection, f-test, and correlation are utilized.[25] The Random Forest, KNN (k-Nearest-Neighbor), and Naive Bayes models are also employed to classify the Wisconsin dataset.[26] An evaluation of recent literature for categorizing breast cancer datasets was also conducted. To categorize breast cancer datasets, artificial intelligence approaches such as machine learning techniques and Deep Learning techniques were used.[27]Fuzzy-based techniques are used for forecasting micro-RNA-regulated protein interaction networks and for evaluating Arabidopsis Thaliana.[28]

Huang *et al.* created their prognostic models using Taiwan Cancer Registry (TCR) data and a multivariate Cox proportional hazard model.[29] For internal validation, a data-splitting mechanism was applied, and for prognostic continuous variables, a multivariable fractional polynomial approach was used. External validation was performed on subjects who were Asian, black, or white in the Surveillance, Epidemiology, and End Results (SEER)

database in the United States. Both internal and external datasets were used to assess model discrimination and calibration.

Ganggayah *et al.* and co-authors retrieved a large breast cancer data set. They applied machine learning algorithms to predict breast cancer survival.[30]Prediction algorithms have been developed utilizing decision trees, random forests, neural networks, extreme boost, logistic regression, and support vector machines to determine the main prognostic features of breast cancer survival rate.

Conclusion

From the current research and investigation, we may conclude that prediction models play a vital role in predicting the survival status of breast cancer patients. Prognostic models can be developed and validated using different feature selection methods. We developed and validated four machine learning algorithms and compared each other with different performance matrices. ML approaches have demonstrated an extraordinary capacity to enhance prediction and classification accuracy.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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References

[1] DeSantis C, Siegel R, Bandi P, Jemal A. Breast cancer statistics, 2011. CA Cancer J Clin. 2011; 61(6): 408-18. doi: 10.3322/caac.20134.

[2] Ataollahi MR, Sharifi J, Paknahad MR, Paknahad A. Breast cancer and associated factors: a review. J Med Life. 2015; 8(Spec Iss 4): 6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5319297

[3] Venturi S. Is there a role for iodine in breast diseases?. The Breast. 2001; 10(5): 379-82. doi.org/10.1054/brst.2000.0267

[4] Heravi Karimovi M, Pourdehqan M, Jadid Milani M, Foroutan SK, Aieen F. Study of the effects of group counseling on quality of sexual life of patients with breast cancer under chemotherapy at Imam Khomeini Hospital. J Maz Univ Med. 2006; 16(54): 43-51. http://jmums.mazums.ac.ir/article-1-133-en.html

[5] Wu R, Luo J, Wan H, Zhang H, Yuan Y, Hu H, Feng J, Wen J, Wang Y, Li J, Liang Q. Evaluation of machine learning algorithms for the prognosis of breast cancer from the Surveillance, Epidemiology, and End Results database. PlosOne. 2023; 18(1): e0280340. doi.org/10.1371/journal.pone.0280340

[6] Qiu J, Wu Q, Ding G, Xu Y, Feng S. A survey of machine learning for big data processing. Eurasip J Adv. 2016; 2016: 1-6.

[7] Sarker IH. Machine learning: Algorithms, real-world applications and research directions. SN Comput Sci. 2021; 2(3): 160. doi.org/10.1007/s42979-021-00592-x

[8] Gardezi SJ, Elazab A, Lei B, Wang T. Breast cancer detection and diagnosis using mammographic data: Systematic review. J Med Internet Res. 2019; 21(7): e14464. doi.org/10.2196/14464

[9] Kourou K, Exarchos TP, Exarchos KP, Karamouzis MV, Fotiadis DI. Machine learning applications in cancer prognosis and prediction. Comput Struct Biotechnol J. 2015; 13: 8-17. doi.org/10.1016/j.csbj.2014.11.005

[10] Toth R, Schiffmann H, Hube-Magg C, Büscheck F, Höflmayer D, Weidemann S, Lebok P, Fraune C, Minner S, Schlomm T, Sauter G. Random forest-based modelling to detect biomarkers for prostate cancer progression. Clin Epigenetics. 2019; 11: 1-5. doi.org/10.1186/s13148-019-0736-8

[11] Statnikov A, Wang L, Aliferis CF. A comprehensive comparison of random forests and support vector machines for microarray-based cancer classification. BMC Bioinformatics. 2008; 9(1): 1-0. doi.org/10.1186/1471-2105-9-319

[12] Panda NR. A review on logistic regression in medical research. Natl J community Med. 2022; 13(04): 265-70. doi.org/10.55489/njcm.134202222

[13] Zhou X, Liu KY, Wong ST. Cancer classification and prediction using logistic regression with Bayesian gene selection. J Biomed Inform. 2004; 37(4): 249-59. doi.org/10.1016/j.jbi.2004.07.009

[14] Liu L. Research on logistic regression algorithm of breast cancer diagnose data by machine learning. In: 2018 International Conference on Robots & Intelligent System (ICRIS) 2018 May 26 (pp. 157-160). doi.org/10.1109/ICRIS.2018.00049

[15] Sharma M, Singh SK, Agrawal P, Madaan V. Classification of clinical dataset of cervical cancer using KNN. Indian J Sci Technol. 2016; 9(28): 1-5. doi: 10.17485/ijst/2016/v9i28/98380

[16] Odajima K, Pawlovsky AP. A detailed description of the use of the kNN method for breast cancer diagnosis. In: 2014 7th International Conference on Biomedical Engineering and Informatics 2014 Oct 14 (pp. 688-692). doi.org/10.1109/BMEI.2014.7002861

[17] Pawlovsky AP, Nagahashi M. A method to select a good setting for the kNN algorithm when using it for breast cancer prognosis. In: IEEE-EMBS International conference on biomedical and health informatics (BHI) 2014 Jun 1 (pp. 189-192). doi.org/10.1109/BHI.2014.6864336

[18] Chaudhari P, Agarwal H, Bhateja V. Data augmentation for cancer classification in oncogenomics: an improved KNN based approach. Evol Intell. 2021; 14: 489-98. doi.org/10.1007/s12065-019-00283-w

[19] Panda NR, Mahanta KL Pati JK, Varanasi PR, Bhuyan R. Comparison of Some Prediction Models and their Relevance in the Clinical Research. Int. J. Stat. Med. 2023; 12: 12-19. doi.org/10.6000/1929-6029.2023.12.02

[20] Venkatesan EV, Velmurugan T. Performance analysis of decision tree algorithms for breast cancer classification. Indian J Sci Technol. 2015; 8(29): 1-8.

[21] Hamsagayathri P, Sampath P. Performance analysis

of breast cancer classification using decision tree classifiers. Int J Curr Pharm Res. 2017; 9(2): 19-25. doi.org/10.22159/ijcpr.2017v9i2.17383

[22] Hazra R, Banerjee M, Badia L. Machine learning for breast cancer classification with Ann and decision tree. In: 2020 11th IEEE Annual Information Technology, Electronics and Mobile Communication Conference (IEMCON) 2020 Nov 4 (pp. 0522-0527). doi.org/10.1109/IEMCON51383.2020.9284936

[23] Ghiasi MM, Zendehboudi S. Application of decision tree-based ensemble learning in the classification of breast cancer. Comput Biol Med.2021; 128: 104089. doi.org/10.1016/j.compbiomed.2020.104089

[24] Tarawneh O, Otair M, Husni M, Abuaddous HY, Tarawneh M, Almomani MA. Breast cancer classification using decision tree algorithms. Int J Adv Comput Sci Appl. 2022; 13(4): 676-80

[25] Dhanya R, Paul IR, Akula SS, Sivakumar M, Nair JJ. A comparative study for breast cancer prediction using machine learning and feature selection. In: 2019 International conference on intelligent computing and control systems (ICCS) 2019 May 15 (pp.1049-1055). doi.org/10.1109/ICCS45141.2019. 9065563

[26] Sharma S, Aggarwal A, Choudhury T. Breast cancer detection using machine learning algorithms. In: 2018 International conference on computational techniques, electronics and mechanical systems (CTEMS) 2018 Dec 21 (pp. 114-118). doi.org/10.1109/CTEMS.2018.8769187

[27] Abdulla SH, Sagheer AM, Veisi H. Breast cancer classification using machine learning techniques: A review. TURCOMAT. 2021; 12(14): 1970-9.

[28] Manikandan P, Ramyachitra D, Nandhini R. Fuzzy based algorithms to predict MicroRNA regulated protein interaction pathways and ranking estimation in Arabidopsis thaliana. Gene. 2019; 692: 170-5. doi.org/10.1016/j.gene.2018.12.066

[29] Huang CC, Chan SY, Lee WC, Chiang CJ, Lu TP, Cheng SH. Development of a prediction model for breast cancer based on the national cancer registry in Taiwan. Breast Cancer Res. 2019; 21(1): 1-9. doi.org/10.1186/s13058-019-1172-6

[30] Ganggayah MD, Taib NA, Har YC, Lio P, Dhillon SK. Predicting factors for survival of breast cancer patients using machine learning techniques. BMC Med Inform Decis Mak. 2019; 19: 1-7. doi.org/10.1186/s12911-019-0801-4

[31] Chen MT, Sun HF, Zhao Y, Fu WY, Yang LP, Gao SP, Li LD, Jiang HL, Jin W. Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: a SEER population-based analysis. Sci Rep. 2017; 7(1): 9254. doi.org/10.1038/s41598-017-10166-8

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Effect of movement-based priming combined with task specific training on upper limb recovery in patients after stroke

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ABSTRACT

Background: Rehabilitation of upper limb impairments and functional deficits is a top goal in stroke rehabilitation. Alternative therapeutic methods may be developed to facilitate upper limb recovery. Priming prepares the brain for better action. When some therapies accompany Priming, it results in a change in behaviour at the performance level by improving the effect of Neuro-Rehabilitation Therapies and enhancing change in the neural process.

Objectives: To investigate the efficacy of movement-based priming combined with task-specific training on upper limb recovery in patients after stroke.

Materials and methods: Twenty-four subjects in the early phase of stroke, attending the Department of Neurology in a tertiary care hospital of Bhubaneswar, Odisha participated in a single-blind randomized controlled trial. 24 subjects after stroke were recruited to the study and randomly allocated to a control group receiving task-specific training only (TST) and an experimental group receiving Movement-Based Priming with task-specific training (MBP+TST). The control group received only task-specific training for 45 minutes per session three days a week for six weeks, while the experimental group received 15 minutes of priming and 30 minutes of task-specific training. Fugl-Mayer Assessment of upper extremity (FMA-UE)was used to measure upper extremity motor recovery, and the Motor Activity Log (MAL) was used to measure the use of arm and hand during activities of daily living at baseline and after six weeks of therapy.

Results: Both the TST group and the MBP+TST group had significantly improved their capacity to move and use their upper limbs functionally (p<0.001). FMA-UE and MAL scores improved more favorably in the MBP+TST group than in the TST group (p<0.001).

Conclusion: Priming in combination with task-specific training results in better upper limb recovery than task-specific training alone.

Introduction

Normal functioning of the upper limb is the most important prerequisite for performing activities of daily living independently. One major factor associated with poor quality of life and reduced independence in doing daily activities is upper extremity motor deficit after stroke.[1] In stroke, recovery of motor impairment and daily function is mostly attributed to neuroplasticity. Therapy administered early with increased intensity and involving more challenge can enhance functional outcomes and

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influence neuroplasticity.[2-5] Recent evidence suggests using motor priming to facilitate motor learning for upper limb recovery. In priming, learning occurs indirectly; the brain, primed by a prior activation method, is generally more responsive to the accompanying training. Priming is used to rehabilitate post-stroke patients to maximize motor gains of the upper extremity. Methods of priming the motor cortex that is most relevant to rehabilitation include (1) stimulation-based priming, (2) motor imagery and action observation, (3) manipulation of sensory input, (4) movement-based priming, and (5) pharmacology-based priming.[6] The Brain's ability to react to motor rehabilitation is enhanced by regulating the excitability of cortical structures, and priming creates an appropriate environment before therapy begins.[7,8] The manipulation of sensory input using a sensory stimulation or a sensory deprivation paradigm is known as sensory-based priming. Techniques for sensory-based priming can change how the somatosensory cortex is organized and improve motor outcomes.[9,10] The basic sensory cortex and motor cortex have strong connections, which makes this conceivable.[11] Transient functional deafferentation (TFD), somatosensory electrical stimulation (SES), peripheral nerve stimulation (PNS), and vibration are all examples of sensory stimulation. Movement-based priming refers to any kind of repetitive or continuous movement carried out to enhance the effects of concurrent therapy. Bilateral or unilateral movements, mirror symmetric active or passive movements, or any kind of exercise, such as isometric, aerobic, and balance exercises, are common components of movement-based priming. Repetitive movements can be symmetrical bilateral movements of both limbs, such as bilateral wrist flexion-extension, or single-joint movements, such as repetitive unilateral wrist or elbow flexion and extension. Movement-based priming typically refers to movements that are repetitive but not skill-based.[6] In Active Passive Bilateral Therapy, bilateral wrist flexion extension modifies the inhibitory effect within and between the hemispheres by acting as a neuromodulator that facilitates motor recovery.[12] With continuous, repetitive movements known as "motor priming" or "movement-based priming," the effectiveness of the primary therapy that comes next may be enhanced.[6] Movement-based priming may improve the effect of associated neuro-rehabilitation therapies.[13] Compared to stimulation-based priming, research on movement-based strategies to prime the motor system is minimal, and movement-based priming is easier to use in clinical setups like ours. Task-specific training, constraint-induced movement therapy (CIMT), robotic therapy, etc., have all been used in stroke upper limb rehabilitation alongside priming, with improved results in the recovery of motor deficits and functional usage.[14-17]

There is growing evidence that task-specific training can be used therapeutically as a neuromotor intervention in neurological rehabilitation, improving upper limb motor and functional recovery.[18] Goal-directed practice and repetition have been used as the principle of task-specific training that focuses on functional improvement[15] The usefulness of task-specific training in reducing upper limb motor deficits and enhancing daily function after stroke has been demonstrated in numerous trials.[19,20] A comparison between stroke education and task-specific training with bilateral motor priming was made in a study by Stoykov *et al.* in 2020.[21] Up until follow-up, the priming group outperformed the stroke education group on the FMA (UE) scale regarding progress. In another study on upper limb recovery in patients severely affected after stroke, sensory-based priming was combined with TST, and a comparison was carried out with only TST.[22] Their study resulted in significant improvement in the upper limb motor function when repetitive task-oriented training was provided in combination with peripheral nerve stimulation. The contralaterally Controlled passive movement performed can produce a priming effect.[23] Active Passive Bilateral Therapy (APBT) was used on subacute stroke patients, and the result of their study concluded that APBT is a feasible priming mechanism to be used to improve upper limb recovery.[24] In another study, chronic stroke patients were provided passive movements and then progressed towards performing active movements.[12] The results showed improved and sustained upper extremity function of the affected hand. Another study found that Bi-manual motor priming can enhance upper extremity function in the initial stroke period after four weeks of intervention.[25]

These studies were done mostly in chronic stroke patients using either movement-based or sensory-based priming. In the present study, movement-based priming is provided by using a simple device called the wrist roller (Figure 2) that assists symmetrical wrist flexion and extension, and it was combined with skill-based training (TST) aimed at improving recovery of upper limb in post-stroke patients. Movement-based priming is any type of continuous movement that may augment the effect of the subsequent primary therapy.[6] Movement-based priming used simultaneous bilateral wrist movements that are mass, non-skill based, and repetitive, similar to the previous studies.[23-25] The device used in this study provided mirror-symmetric, bilateral, wrist flexion, and extension movements. The control group received only task-specific training. As the potential of therapeutic recovery is high within weeks to months after stroke, it is necessary to facilitate the neuroplastic changes more during the early days of the stroke to have a better outcome. Recovery often reaches its plateau after three months.[26] Our sample included patients from 4-12 weeks after stroke to determine the maximum benefit of priming the brain. Evidence of task-specific training in improving upper limb recovery has been studied earlier. Priming the brain before task- specific training in the early phase of stroke is less in number in the literature. Thus, it is necessary to find a better therapeutic method for upper limb rehabilitation in the early stage of stroke recovery. Previous studies showed that adding a priming session before a motor-learning task improved motor learning. The expected outcome of this study will be better upper limb recovery compared to other studies of acute phase priming where patients with severe paretic upper limb were included. In this study, priming will be followed by task-specific training, further facilitating task performance.

We hypothesize that movement-based priming, when provided with task-specific training, will derive better improvement of the upper limb than task-specific training alone. The result of this study may be used to address the upper limb problems in the early phase of stroke for better upper limb recovery.

Methods

The study was a randomized controlled clinical trial having two groups. The study included baseline and post-intervention data collection. The study was conducted at the Neurology Department of Institute of Medical Sciences and SUM Hospital, Odisha, India, and The Institute Ethical Committee approved the study protocol. Participants: It has been estimated that the middle cerebral artery (MCA) was the most commonly affected vessel, and upper extremity is more affected than the lower extremity in MCA involvement.[27] Keeping upper limb stroke rehabilitation in view, this study included participants fulfilling the following inclusion criteria. Screening was conducted for 169 stroke patients who participated in the present study. Eligibility criteria included were: (a) 18 years of age and above; (b) at least four weeks to 12 weeks post-stroke (c) able to sit independently for 45 minutes; (d) unilateral stroke with Middle Cerebral Artery involvement; (e) can lift the affected hand from their lap to a wall mounted device. (f) A score of 24 and above in MMSE. Participants were excluded if they had the following criteria. (a) 4/5 points on Modified Ashworth Scale, (b) >5 points on the Visual Analog Scale for Upper Limb pain at rest, (c) Uncontrolled hypertension or cardiovascular disease, (d) other neurodegenerative conditions, such as Parkinson's or Alzheimer's (e) deformities or a fracture of the wrist, (f) Aphasia, and (g) severe vision impairment. Out of 169 participants, 86 patients were excluded per the exclusion criteria. Of the rest, 83 patients who completed baseline evaluation, 59 could not meet the inclusion criteria.[24] patients after stroke were finally found eligible as per the inclusion criteria who completed the intervention and post-intervention assessment.

The primary investigator enrolled the study participants. The study's participants were chosen based on the criteria for inclusion. Participants recruited to the study were randomly allocated to movement based priming plus task-specific training (experimental=MBP+TST) group and Task Specific Training only (control=TST) group by another Occupational Therapist not otherwise involved in the study through block randomization (in a block of 4). Random numbers were generated by using the random numbers table. An opaque, sealed envelope held the allocation. The sample size required for this study was calculated and estimated based on previous studies.[28] Based on the smallest sample size needed for achieving a statistical power of 0.80 with a one-sided type I error of 0.05, a total sample size of at least 12 subjects per group was deemed sufficient. Twenty-four patients, consisting of 17 men and seven women with stroke, were finally recruited for the study. Figure No. 1 shows a flow diagram of the study procedure. The recruitment of participants was done over 13 months. Post-treatment measurement was done immediately after six weeks of intervention in both groups.

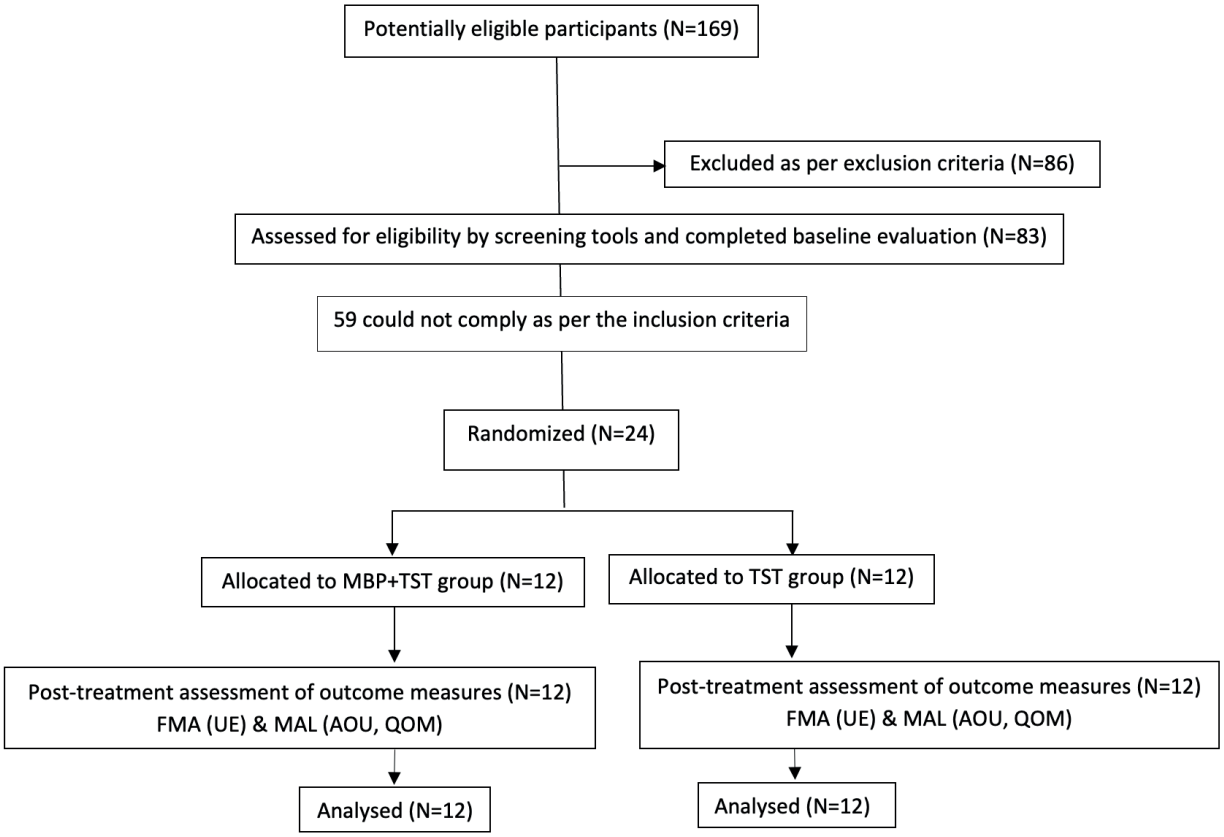

Figure 1. *Study flow diagram.*

Outcome measures

Motor recovery

The Fugl-Meyer Assessment (FMA) is a stroke-specific, performance-based impairment index. It is designed to assess motor functioning, sensation, balance, joint range of motion, and joint pain in patients with post-stroke hemiplegia. Scale items are scored based on ability to complete the item using a 3-point ordinal scale where 0=cannot perform, 1=performs partially, and 2=performs fully. The total possible scale score is 226. The motor section is divided into two subsections: 66 for the upper extremities (upper arm and wrist and hand) and 34 for the lower extremities. In this study, motor recovery of the upper limb was assessed by Fugl-Mayer Assessment (U/E section). The motor section of FMA-UE evaluates aspects of movement, reflexes, coordination, and speed. FMA-UE is scored out of 66, with sub-scores of 36 for the upper arm and 30 for the wrist and hand. The total motor score ranges from 0 to 66 points, and the higher the score, the less the impairment. The FMA-UE had a 0.97 intra-rater and inter-rater reliability.[29-31]

Motor Activity Log

The Motor Activity Log (MAL) is a semi-structured interview for hemiparetic stroke patients to assess the use of their paretic arm and hand during ADL. In this study, the motor activity log (MAL) version was used to measure how well the affected upper limb performed functionally in the past seven days**.** It measures 30 daily activities on a six-point rating scale. Patients are scored in a range where 0 is no use, and 5 is normal use of the extremity. It comprises two subscales: the Quality of Movement (QOM) scale and the Amount of Use (AOU) scale. The total mean MAL score (ranging from 0 to 5) is calculated for both subscales (AOU and QOM) by adding the rating scores for each item and dividing by the number of items that responded. MAL has a 0.94 score of reliability (test-retest). Internal consistency ranges from 0.88 to 0.95, while 0.90 is the inter-rater reliability.[32]

Intervention

Before taking part in the trial, each subject provided written informed consent. A blinded assessor, another study's author, recorded all the measurements. Participants were instructed to observe active movement of the upper limb that was less affected and passive movement of the upper limb that was affected. Bilateral priming activity progressed from passive to active assistive movement, and then the active movement of the wrist of the side affected. In this study, instead of a priming device (Rocker) used originally, a wall-mounted wrist roller (Figure 2) was used for wrist movements.[30] The height of the device was 28 inches from the floor.

It was a cylindrical gripping object attached with a base to be fixed on the wall. Patients were instructed to grasp and hold the cylindrical gripping tool using the less affected and affected hand. Patients were guided by the therapist on how to perform repetitive wrist flexion-extension (Figure 3). Initially, therapist support was provided to hold the affected wrist on the roller. Later on, the patient was encouraged to do the movement independently. Movement-based priming uses simultaneous bilateral wrist movements that are mass, non-skill based, and repetitive, similar to the previous studies.[33] The device used in this study provided mirror-symmetric, bilateral, wrist flexion, and extension movements. While performing wrist movements by the less affected side, the affected side wrist automatically moved with the assistance of the device's motion, resulting in simultaneous bilateral symmetric wrist flexion and extension.

Figure 2. *Wrist roller used for MBP.*

Figure 3. *Different phases of wrist movements during MBP. A: passive movement, B: active assistive movement, C: active movement.*

Task-specific training

Task-specific training, or functional task training, involves performing tasks relevant to daily life, including part- and whole-task practice. Occupational therapists frequently use task-specific training, a form of repetitive task training, as a component of neuromotor therapies, particularly in managing post-stroke upper limbs. Reach-to-grasp exercise is a form of task-specific training, as reach-to-grasp is a common functional task performed by the upper limb.[34] In this study, functional tasks representing essential reaching, grasping, releasing, pointing, and manipulating were included in the task-specific training. To make the training protocol the same for all participants, Task Specific Training used in this study comprised a specific number of common tasks to be practiced by all patients. The guidelines followed for selecting the tasks are; (1) training should be relevant to the patient, (2) should be repeated, (3) provided in a randomized order, (4) contain tasks that are part of a whole task, (reconstruction) and (5) feedback should be provided (Reinforcement).[34] Patients in the control group had undergone task-specific training for 45 minutes. Patients in the experimental group underwent 30 minutes of task-specific training after 15 minutes of priming activity. In both groups, therapy was provided for six weeks and three days a week. Evaluations using all the outcome measures were done after six weeks of intervention. Task-specific training included the repetitive practice of selected tasks like reaching a shelf height, lifting and carrying a glass of water to mouth, grasping pegs and putting those into peg holes, manipulating a small ball within the hand, holding a water bottle and opening the lid of it, manipulating a lock and key. Activities were selected based on the patients' abilities and impairments and the tasks that could be performed in an institutional clinical setup. After completing the above tasks for 15 minutes, patients were given real-life tasks based on individual preferences/choices for the rest 15 minutes. Purposeful, real-life tasks include combing hair, drinking tea, opening and closing a door, grasping a tennis ball and releasing, manipulating coins within the hand, and manipulating the cards etc. While performing the tasks, active movements were encouraged, and compensatory movements were avoided. Wherever required therapist's assistance was provided in guiding the movements required for completing the task. All patients were encouraged to perform the task at a faster speed they can. During task performance, intermittent rest periods of one minute were provided.

Statistical analysis

Data analysis was carried out by using IBM SPSS Statistics for Windows, Version 23.0"(IBM Corp., Armonk, N.Y., USA)'.[35]To compare the demographic and clinical characteristics of the two groups at baseline t-tests and Chi square test were used for continuous and categorical data respectively. Data for both groups were homogeneous at baseline. After six weeks of the intervention, all outcome variables (FMA-UE MAL AOU and QOM) were compared within each group using Paired t-tests and between groups using independent t-tests. The level of significance was established at $p<0.05$.

Results

The present study screened 169 patients after stroke over 13 months. At the end of the screening process, only 24 subjects could be recruited for the study. Random selection was used to assign all participants to one of two groups of 12 patients. Table 1 lists the clinical and demographic characteristics of all the patients at baseline. The baseline clinical and demographic characteristics were comparable in the control and experimental groups.

All patients in both the control (TST) group and experimental (MBP+TST) group completed six weeks of intervention successfully. Upon within-group comparison, (MBP+TST) group showed statistically significant ($p<0.05$) improvement on FMA-UE and MAL subscale of AOU and QOM scores. A statistically significant ($p<0.05$) improvement in the FMA-UE and AOU QOM scores was also seen in the TST group. On between-group analyses, in comparison to the TST group, the MBP+TST group's post-intervention mean scores on both of the outcome measures FMA-UE and MAL demonstrated a statistically significant improvement ($p<0.05$). Both within-group and between-group analyses showed a positive change in all outcome measures post-intervention.

Table 1 Clinical and demographic Characteristics of the subject population.

Sl No.	Characteristics	Control group (TST)	Experimental group (MBP+TST)	*p* value
1	No of participant (N=24)	12	12	
2	Mean age (SD, years)	53.2±1.95	51.7±2.5	0.675
3	Sex (Male:Female)	8:4	9:3	0.653
4	Side affected (Rt:Lt)	5:7	8:4	0.219
5	Weeks since stroke (Mean±SD)	9.33±2.10	9.58±2.31	0.784
6	MMSE	5.36±4.78	24.89±4.92	0.443
7	FMA (UE)	14.50±2.23	14.08±2.87	0.696
8	MAL (AOU)	0.98±0.39	1.01±0.36	0.819
9	MAL (QOM)	0.45±0.33	0.63±0.33	0.855

Both groups experienced a significant increase in FMA-UE score(MBP+TST: from 14·08±2.87 to 25·83±3.48 points (p<0·001); TST only: from 14.50±2.23 to 21.91±04.54 points (p<0.05). The mean increase in the FMA-UE score was 11.750.61± points in the MBP+TST group compared to 7.412.31± points in the TST only group. Table 2 shows there is an increase in the AOU score (MBP+TST: from 1.01±0.36 to 1.930.39±; TST only: from 0.98±0.39 to 1.42±0.47 (p<0.001).QOM scale of MAL had an increase in score (MBP+TST: from 0.630.32± to 1.920.43±; TST only: from 0.450.33± to 0.820.28± (p<0.001) in both the groups.

Table 2 Descriptive statistics showing pretest and posttest comparison of outcome measures of the control and experimental group.

Outcome measures	Control group (TST)		Mean Difference	t value	p value	95% Confidence Interval of the Difference		Experimental group (MBP+TST)		Mean Difference	t value	p value	95% Confidence Interval of the Difference	
	Pretest Mean (SD)	Posttest Mean (SD)				Lower	Upper	Pretest Mean (SD)	Post test Mean (SD)				Lower	Upper
Fugl-Mayer (U/E)	14.50 (2.23)	21.91 (4.54)	7.42 (2.31)	-7.178	0.001*	-9.690	-7.178	14.08 (2.87)	25.83 (3.48)	11.75 0.61	-20.769	0.001*	-12.995	-10.504
MAL (AOU)	0.98 (.39)	1.42 (0.47)	0.44 (0.08)	-7.233	0.001*	-0.563	-0.300	1.01 (0.36)	1.93 (0.39)	0.92 0.03	8.908	0.001*	-1.141	-0.688
MAL (QOM)	0.45 (0.33)	0.82 (0.28)	0.37 (0.05)	-2.970	0.001*	-0.631	-0.093	0.63 (0.32)	1.92 (0.43)	1.29 0.11	-8.961	0.001*	-1.611	-0.975

*Note *statistically significant, FMA (UE): Fugl-Meyer Assessment (Upper Extremity), AOU: motor activity log (amount of use), QOM: motor activity log (quality of movement).*

On between-group comparison of the FMA-UE, AOU, and QOM scores, a statistically significant difference between the MBP+TST group and the TST only group was found. The MBP+TST group improved more than the TST only group regarding the change in the mean difference score (p<0.05), as shown in Table 3.

Table 3. Between-group analyses (MBP+TST and TST only group).

Outcome measure	TST Only Mean	MBP+TST Mean	Mean difference	t value	*p* value	95% Confidence Interval of the Difference	
						Lower	Upper
FMA (U/E)	21.91	25.83	3.92	2.370	0.027*	0.489	7.344
MAL (AOU)	1.42	1.93	0.51	2.859	0.012*	0.140	0.882
MAL (QOM)	0.82	1.92	1.1	7.318	0.001*	0.791	1.417

*Note *statistically significant, FMA (UE): Fugl-Meyer Assessment (upper extremity), AOU: motor activity log (amount of use), QOM: motor activity log (quality of movement).*

Discussion

The study showed that, compared to task-specific training alone, movement-based priming in combination with task-specific training was more helpful in enhancing UE recovery in the initial days after stroke. There was an improvement seen in both the intervention groups in all the outcome measures measuring motor recovery and functional use. All the outcome measures, including the FMA-UE and both subscales of MAL significantly improved within each group. The findings of this study demonstrate that combining movement-based priming with task-specific training improved post-stroke patients' motor recovery and quality and quantity of upper limb use in day-to-day activities.

The study hypothesized that additional movement-based priming with task-specific training would result in better upper limb recovery. This study found that the movement-based priming group considerably outperformed the Task-specific training only group in terms of improvements on the Fugl-Mayer assessment of the Upper Extremity scale. A significant difference in FMA-UE score was found between group analyses (p<0.05). Additional movement-based priming may have resulted in better motor improvement in the primed group due to repetitive practice of bilateral movements, thus inducing better force generation in the muscles of the upper limb.[36-38] Bilateral symmetrical wrist movements provided movement-based priming, which helped the brain respond more favorably to the following training. Normalization of cortical inhibition is the suggested neurobiological mechanism of bilateral priming. Both neuroplasticity and the effectiveness of therapies were improved by bilateral priming.[12] The brain is more receptive to subsequent instruction if primed by earlier activity. This might have contributed to the primed group's faster recovery. The priming theory is based on the inter-hemispheric imbalance alteration in cortico-motor excitability after stroke, which has been linked to a slower rate of motor recovery and/or a decrease in neural activation of Ipsi-lesional motor cortex (M1).[36]

The score of FMA-UE increased within and between the two groups, which was statistically significant. The study's findings were likewise significant regarding the FMA-UE's minimal clinically meaningful difference (MCID). In stroke patients who have experienced a sub-acute stroke, a mean difference score of 9 to 10 on the FMA-UE is likely to be interpreted as clinically significant.[39] Those with sub-acute stroke are more likely to experience a considerable and clinically meaningful decline in their level of disability than those who do not earn a score of 9 to 10 on the FM-UE.[39] In this study, the MBP+TST group had a mean difference of 11.75, above the MCID value of 9 to 10. This finding suggests MBP+TST group had a clinically important improvement post-intervention. Compared to the mean difference found in the FMA-UE in the MBP+TST group, the TST only group had a mean difference of 7.41, which is smaller. The study's findings suggest that, despite improvements in motor recovery in both groups, the MBP+TST group showed greater statistical and clinical improvement than the TST only group. The results of this study are in line with another study in which Fugl-Meyer Assessment showed clinically significant improvements in both the primed group and the non-primed group.[36] Another finding of the study demonstrated significant improvement in the AOU scale when comparing the pre (1.01±0.36) to post-test mean (1.93±0.39) score in MBP+TST and from (0.98±.39) to (1.42±0.47) in TST groups on the within-group comparison. Use of the upper limb in day-to-day activities might have improved through the practice of tasks that target functional use. The improvement was better in the MBP+TST group than the TST group, according to the statistically significant difference in the AOU subscale of MAL between the two groups (p<0.001).QOM scale of MAL had an increase in score in both the groups. This result indicates improvement in quality of movement was there in both groups post-intervention. The experimental and control groups improved significantly in AOU and QOM scores of the motor activity log. Evidence of Task-specific training in improving upper limb motor and functional recovery has already been established in the literature. The use of task-specific training might have resulted in improvement in AOU and QOM in both controls as well as in the experimental group. In a study with moderate to severe

upper limb hemiparesis, it was found that when bilateral motor priming was used as additional training with task-specific training, there was a greater improvement in the FMA-UE score in the experimental group than in the control group. However there was no difference found in the score of Chedoke Arm & Hand Activity Index 9.[16] The result of this study says that priming resulted in better motor recovery in the experimental group than functional recovery.

Between-group analyses showed a significant difference in the quality of movement subscale between the two groups. Comparing the MBP+TST group to the TST group, a significant improvement was seen in the QOM sub-scale of MAL (p<0.001). The findings may be attributed to the additional movement during the priming activity. The repetitive, continuous wrist flexion and extension movement improved the motor power of the flexor and extensor muscles of the wrist. Gripping the device to perform the repetitive movements might have increased the grip strength of both hands. This might have derived a change not only at the muscular level but also at the neuronal level, resulting in better neural plasticity and better improvement in the quality of movement. Many studies have been conducted to determine the outcome of various priming methods on task-oriented training in chronic stroke patients.[12,17,25] The evidence of movement priming has a better effect size, whereas other priming methods remain inconclusive.[40] In this study, movement-based priming was provided to stroke patients in the acute stage of their recovery. It resulted in better improvement in motor as well as in functional recovery. In a study with severely impaired stroke patients in the acute phase, visual and movement-based priming resulted in significant improvements in motor recovery as measured by the Action Research Arm Test (ARAT) and the Fugl Meyer Assessment of Upper Extremity (FMA-UE) at six months post-training. As the patients were in the early phase of their recovery, the tasks provided in the task-specific training were kept simple and adapted wherever required. The study is limited in many aspects like no follow-up data could be collected. It could not be established whether the motor and functional recovery improved post-intervention due to the lack of follow-up study. It is recommended that future studies use follow-up data to examine the long-term retention impact of priming with task-specific training. The MAL is subject to experimenter bias and the patient's ability to recall upper limb use accurately.[41] The improvement shown in AOU and QOM may be verified in future studies employing additional functional performance metrics to examine the additional impact of priming on recovery of upper limb functions following stroke. It is advised that additional research be conducted by enlisting a large sample to confirm the conclusion of this study.

Conclusion

This study shows that movement-based priming combined with task-specific training results in better motor recovery and improved upper limb function in stroke than only task-specific training. Movement-based priming can be provided in hospital-based and institutional-based clinical stroke rehabilitation setups more easily than other priming methods. It is an effective and safer method of priming and can be used as restorative occupational therapy in the rehabilitation of the upper limb after a stroke. To maximize the benefits of task-specific training after stroke, movement-based priming can be utilized as a supplementary therapy.

References

[1] Dobkin BH. Rehabilitation after stroke. N Engl J Med. 2005; 352(16): 1677-84.

[2] Horn SD, DeJong G, Smout RJ, Gassaway J, James R, Conroy B. Stroke rehabilitation patients, practice, and outcomes: is earlier and more aggressive therapy better? Arch Phys Med Rehabil. 2005; 86(12): 101-14. doi: 10.1016/j.apmr.2005.09.016.

[3] Maulden SA, Gassaway J, Horn SD, Smout RJ, DeJong G. Timing of initiation of rehabilitation after stroke. Arch Phys Med Rehabil. 2005; 86(12): 34-40. doi: 10.1016/j.apmr.2005.08.119.

[4] Rodgers H, Mackintosh J, Price C, Wood R, McNamee P, Fearon T, Marritt A, Curless R. Does an early increased-intensity interdisciplinary upper limb therapy programme following acute stroke improve outcome? Clin Rehabil. 2003; 17(6): 579-89. doi: 10.1191/0269 215503cr652oa.

[5] Carey LM, Abbott DF, Egan GF, O'Keefe GJ, Jackson GD, Bernhardt J, Donnan GA. Evolution of brain activation with good and poor motor recovery after stroke. Neurorehabil Neural Repair. 2006; 20(1): 24-41. doi: 10.1177/1545968305283053.

[6] Stoykov ME, Madhavan S. Motor priming in neurorehabilitation. J Neurol Phys Ther. 2015; 39(1): 33-42. doi: 10.1097/NPT.0000000000000065.

[7] Nowak DA, Grefkes C, Ameli M, Fink GR. Interhemispheric competition after stroke: brain stimulation to enhance recovery of function of the affected hand. Neurorehabil Neural Repair. 2009; 23(7): 641-56. doi: 10.1177/1545968309336661.

[8] Stinear CM, Byblow WD. Modulation of corticospinal excitability and intracortical inhibition during motor imagery is task-dependent. Experimental brain research. Clin Rehabil. 2004; 157: 351-8. doi: 10.1007 /s00221-004-1851-z

[9] Ridding MC, McKay DR, Thompson PD, Miles TS. Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neuro Physiol. 2001; 112(8): 1461-9.

[10] Conforto, A. B., et al. Effects of somatosensory stimulation on motor function after subacute stroke. Neurorehabili Neural Repair. 2010; 24(3); 263-72. doi: 10.1016/s1388-2457(01)00592-2.

[11] Catani M. A little man of some importance. Brain. 2017; 140(11): 3055-61. doi: 10.1093/brain/awx270.

[12] Stinear CM, Barber PA, Coxon JP, Fleming MK, Byblow WD. Priming the motor system enhances the effects of upper limb therapy in chronic stroke. Brain. 2008;

131(5): 1381-90. doi: 10.1093/brain/awn051.

[13] Stoykov ME, Corcos DM, Madhavan S. Movement-based priming: clinical applications and neural mechanisms. J Mot Behav. 2017; 49(1): 88-97. doi: 10.1080/00222895.2016.1250716

[14] Takebayashi T, Takahashi K, Moriwaki M, Sakamoto T, Domen K. Improvement of upper extremity deficit after constraint-induced movement therapy combined with and without preconditioning stimulation using dual-hemisphere transcranial direct current stimulation and peripheral neuromuscular stimulation in chronic stroke patients: A pilot randomized controlled trial. Front Neurol. 2017; 8: 568. doi: 10.3389/fneur.2017.00568

[15] Shiner CT, Byblow WD, McNulty PA. Bilateral priming before wii-based movement therapy enhances upper limb rehabilitation and its retention after stroke: a case-controlled study. Neurorehabil Neural Repair. 2014; 28(9): 828-38. doi: 10.1177/1545968314523679.

[16] Stoykov ME, King E, David FJ, Vatinno A, Fogg L, Corcos DM. Bilateral motor priming for post stroke upper extremity hemiparesis: A randomized pilot study. Restor Neurol Neurosci. 2020; 38(1): 11-22. doi: 10.3233/RNN-190943. PMID: 31609714; PMCID: PMC7205167.

[17] Stoykov ME, Biller OM, Wax A, King E, Schauer JM, Fogg LF, Corcos DM. Bilateral upper extremity motor priming (BUMP) plus task-specific training for severe, chronic upper limb hemiparesis: study protocol for a randomized clinical trial. Trials. 2022; 23(1): 1-4.

[18] Hubbard IJ, Vyslysel G, Parsons MW. Interprofessional, Practice-driven Research: Reflections of One "Community of Inquiry" Based in Acute Stroke. J Allied Health. 2009; 38(2): E69.

[19] Waddell KJ, Birkenmeier RL, Moore JL, Hornby TG, Lang CE. Feasibility of high-repetition, task-specific training for individuals with upper-extremity paresis. The Am J Occup Ther. 2014; 68(4): 444-53. doi: 10.5014/ajot.2014.011619

[20] Birkenmeier RL, Prager EM, Lang CE. Translating animal doses of task-specific training to people with chronic stroke in 1-hour therapy sessions: a proof-of-concept study. Neuro Rehabil Neural Repair. 2010; 24(7): 620-35. doi: 10.1177/1545968310361957

[21] Stoykov ME, King E, David FJ, Vatinno A, Fogg L, Corcos DM. Bilateral motor priming for post stroke upper extremity hemiparesis: a randomized pilot study. Restor Neurol Neurosci. 2020; 38(1): 11-22. doi: 10.3233/RNN-190943

[22] Carrico C, Chelette KC, Westgate PM, Powell E, Nichols L, Fleischer A, Sawaki L. Nerve stimulation enhances task-oriented training in chronic, severe motor deficit after stroke: a randomized trial. Stroke. 2016; 47(7): 1879-84. doi: 10.1161/STROKEAHA.116.012671.

[23] Patel J, Qiu Q, Yarossi M, Merians A, Massood S, Tunik E, Adamovich S, Fluet G. Exploring the impact of visual and movement based priming on a motor intervention in the acute phase post-stroke in persons with severe hemiparesis of the upper extremity. Disabil Rehabil. 2017; 39(15): 1515-23. doi: 10.1080/09638288.2016.1226419.

[24] Stoykov ME, Stinear JW. Active-passive bilateral therapy as a priming mechanism for individuals in the subacute phase of post-stroke recovery: a feasibility study. Am J Phys Med Rehabil. 2010; 89(11): 873-8. doi: 10.1097/PHM.0b013e3181f1c31c

[25] Stinear CM, Petoe MA, Anwar S, Barber PA, Byblow WD. Bilateral priming accelerates recovery of upper limb function after stroke: a randomized controlled trial. Stroke. 2014; 45(1): 205-10. doi: 10.1161/STROKEAHA.113.003537

[26] Kwakkel G, Kollen BJ, Krebs HI. Effects of robot-assisted therapy on upper limb recovery after stroke: a systematic review. Neurorehabil neural repair. 2008; 22(2): 111-21. doi: 10.1177/1545968307305457.

[27] T.E. Nogles, M.A. Galuska. Middle cerebral artery stroke. StatPearls [Internet]. Treasure Island (FL), StatPearls Publishing (2021). Available from:https://www.ncbi.nlm.nih.gov/books/NBK556132/

[28] Li YC, Lin KC, Chen CL, Yao G, Chang YJ, Lee YY, Liu CT. A Comparative Efficacy Study of Robotic Priming of Bilateral Approach in Stroke Rehabilitation. Front Neurol. 2021; 12: 658567. doi: 10.3389/fneur.2021.658567.

[29] Duncan PW, Propst M, Nelson SG. Reliability of the Fugl-Meyer assessment of sensorimotor recovery following cerebrovascular accident. Phys Ther. 1983; 63(10): 1606-10. doi: 10.1093/ptj/63.10.1606.

[30] Gladstone DJ, Danells CJ, Black SE. The Fugl-Meyer assessment of motor recovery after stroke: A critical review of its measurement properties. Neurorehabil Neural Repair. 2002; 16(3): 232-40. doi: 10.1177/154596802401105171.

[31] Fugl-Meyer AR, Jääskö L, Leyman I, Olsson S, Steglind S. A method for evaluation of physical performance. Scand. J Rehabil Med. 1975; 7(1): 13-31.

[32] Taub E, Miller NE, Novack TA, Cook EW, Fleming WC, Nepomuceno CS, Connell JS, Crago JE. Technique to improve chronic motor deficit after stroke. Arch Phys Med Rehabil. 1993; 74(4): 347-54. PMID: 8466415

[33] Stoykov ME, Stinear JW. Active-passive bilateral therapy as a priming mechanism for individuals in the subacute phase of post-stroke recovery: a feasibility study. Am J Phys Med Rehabil. 2010; 89(11): 873. doi: 10.1097/PHM.0b013e3181f1c31c

[34] Hubbard IJ, Parsons MW, Neilson C, Carey LM. Task-specific training: evidence for and translation to clinical practice. OccupTher Int. 2009; 16(3-4): 175-89. doi: 10.1002/oti.275. PMID: 19504501.

[35] IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

[36] Yang CL, Lin KC, Chen HC, Wu CY, Chen CL. Pilot comparative study of unilateral and bilateral robot-assisted training on upper-extremity performance in patients with stroke. Am J OccupTher. 2012; 66(2): 198-206. doi: 10.5014/ajot.2012.003103

[37] Hsieh YW, Liing RJ, Lin KC, Wu CY, Liou TH, Lin JC, Hung JW. Sequencing bilateral robot-assisted arm therapy and constraint-induced therapy improves reach to press and trunk kinematics in patients with stroke. J. NeuroEng Rehabil. 2016; 13(1): 1-9. doi: 10.1186/s12984-016-0138-5.

[38] Di Lazzaro V, Profice P, Pilato F, Capone F, Ranieri F, Pasqualetti P, Colosimo C, Pravatà E, Cianfoni A, Dileone M. Motor cortex plasticity predicts recovery in acute stroke. Cereb Cortex. 2010; 20(7): 1523-8. doi: 10.1093/cercor/bhp216

[39] Narayan Arya K, Verma R, Garg RK. Estimating the minimal clinically important difference of an upper extremity recovery measure in subacute stroke patients. Top Stroke Rehabil. 2011; 18(sup1): 599-610. doi: 10.1310/tsr18s01-599.

[40] da Silva ESM, Ocamoto GN, Santos-Maia GLD, de FátimaCarreira Moreira Padovez R, Trevisan C, de Noronha MA, Pereira ND, Borstad A, Russo TL. The Effect of Priming on Outcomes of Task-Oriented Training for the Upper Extremity in Chronic Stroke: A Systematic Review and Meta-analysis. Neurorehabil Neural Repair. 2020; 34(6): 479-504. doi: 10.1177/1545968320912760.

[41] Uswatte G, Taub E, Morris D, VignoloM, McCulloch K. Reliability and validity of the upper extremity motor activity log-14 for measuring real worldarm use. Stroke. 2005; 36:2493-6. doi: 10.1161/01.STR.0000185928.90848.2e.

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Toxicity effects of Cannabidiol (CBD) on immune cells

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ABSTRACT

Background: Cannabis extract has a long history of being used in the treatment and prevention of several medical conditions. The utilization of cannabis extracts, whether for medical or localized purposes, is widely observed. In cannabis extract, cannabidiol (CBD) is one of the most important non-psychoactive compounds. Several studies have demonstrated that CBD has several benefits in the treatment of various medical conditions. Nevertheless, CBD has also been demonstrated to suppress both innate and adaptive immune responses. Despite CBD has claimed to have many benefits, the toxicity of CBD is often pointed out and discussed. Nonetheless, the data on the toxicity effects of CBD on immune cells are limited.

Objectives: In this study, we aimed to investigate the toxicity effects of various concentrations of CBD on immune cells, including CD4 T cells, CD8 T cells, B cells, NK cells, and monocytes.

Materials and methods: Various concentrations of peripheral blood mononuclear cells (PBMCs) were treated with various concentrations of CBD or relative concentrations of methanol as a diluent control for 12, 24, and 48 hrs. Cell morphology was observed using flow cytometry. The percentage of cell death in the treated cells was determined by cell viability assay. In addition, the toxic effects of CBD on PBMC sub-populations were determined by staining with fluorochrome-conjugated zombie viability dye and fluorochrome-conjugated monoclonal antibodies specific to each cell sub-population. Then, the percentage of cell death in each sub-population was assessed using flow cytometry.

Results: CBD at concentrations of 40 and 80 µM showed toxicity effects on PBMCs. At these concentrations, CBD induced both cell morphological changes and cell death. While 20 µM CBD induced different effects, ranging from none to mild and high toxicity. The toxicity of CBD at 20 µM concentration depends on the individual. In contrast, CBD at ten µM and below showed no toxicity to PBMCs. The observed toxic effects of CBD occurred in all sub-populations of PBMCs, including CD4 T cells, CD8 T cells, B cells, NK cells, and monocytes.

Conclusion: CBD has toxicity effects on immune cells. These effects depend on CBD concentrations, PBMC concentrations, and the duration of CBD exposure. Our findings emphasize the importance of awareness for CBD users when consuming CBD.

Introduction

Recently, several natural plant extracts have been getting attention for their potential in treating various medical conditions. Cannabis extract has become popular in the treatment of various conditions, e.g., epilepsy, anxiety, depression, and cancer.[1] Plant cannabis, or phytocannabinoids, contains many components, possibly up to 500 by some estimates.[2] Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are two major components

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present in cannabis extract.[3] THC is widely recognized as a psychoactive compound and is often associated with several adverse effects.[4] Nevertheless, THC is still used in the medical field. The FDAs of several countries have approved THC for the reduction of chemotherapy-induced nausea and vomiting, as well as for appetite stimulation.[5] CBD was also demonstrated to have several important positive impacts on many diseases, such as diabetes, Parkinson's disease, Alzheimer's disease, and cancer, but without psychotropic effects.[6]

CBD is a naturally occurring cannabinoid. It contains 21 carbon atoms, with the formula $C_{21}H_{30}O_2$, and a molecular weight of 314.464 g/mol.[7] CBD has a wide range of biological effects.[6] The effects of CBD are mediated through several receptors expressed throughout the body, i.e., cannabinoid receptors of the endocannabinoid system, 5-HT1A serotonin receptors, and TRPV1 channels.[8] The effect of CBD is predominantly observed within two major systems, including the nervous system and the immune system.[9-11] In the nervous system, CBD has demonstrated significant benefits in experimental models of various neurological disorders, such as seizures, epilepsy, Parkinson's disease, and Alzheimer's disease.[12,13] In the immune system, the induction of immunosuppression by CBD has been reported.[11] CBD inhibited both innate and adaptive responses. It had a strong suppressive effect on the release of innate cytokines (IL-1 and TNF) and could induce apoptosis in primary human monocytic cells.[14,15] In adaptive immune cells, CBD inhibited IFN-γ production and induced apoptosis in T cells.[16,17] As a result, CBD is often used as an anti-inflammatory drug to treat a variety of inflammatory conditions.[18,19]

Even though CBD has exhibited a range of advantageous effects, research in the clinical trial phase of CBD is still limited and controversial. There is only one CBD product (Epidiolex®, GW Pharmaceuticals) approved by the FDAs in 2018 for the treatment of rare pediatric seizure disorders.[20] The toxicity of CBD has also been focused on and highlighted as the significance of dosage considerations. At high doses, CBD exhibits various toxicities on multiple systems within the body, including reduced fertility, hormonal alteration, severe hypotension, and the potential to induce damage to liver cells (hepatocellular damage).[21-23] However, data on the toxic effect of CBD on immune cells is limited.

In this study, we investigated the toxicity effects of CBD on immune cells. The examinations were composed of various factors, including PBMC concentrations, CBD concentrations, and CBD exposure time. Our study indicated the toxicity effects of CBD on immune cells. These results might raise caution to the CBD user.

Materials and methods

Reagents, antibodies, and cells

Cannabidiol (CBD) solution of 1 mg/mL concentration in methanol (catalog number C-045-1ML) was purchased from Cerilliant (Round Rock, Texas, USA). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, New York, USA). Ficoll-Hypaque solution was purchased from Robbins Scientific Corporation (Sunnyvale, California, USA). 7-AAD solution, BV510 conjugated Zombie dye, FITC-conjugated anti-CD3 mAb, PerCP-conjugated anti-CD4 mAb, BV785-conjugated anti-CD8 mAb, PE-conjugated anti-CD14 mAb, FITC-conjugated anti-CD19 mAb, and PECy5-conjugated anti-CD56 mAb were purchased from BioLegend (San Diego, California, USA).

PBMCs were isolated from the buffy coat of healthy donors obtained from the Thai Red Cross Society (Regional Blood Center X, Chiang Mai, Thailand) or heparinized blood of healthy blood donors using Ficoll-Hypaque gradient centrifugation.

Toxicity effects of CBD on PBMCs

The PBMCs were adjusted into a concentration of $2x10^6$ and $8x10^6$ cells/mL in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 40 mg/mL gentamicin, and 2.5 mg/mL amphotericin B (10% FBS-RPMI). The PBMCs were plated in a 96-well V-bottom plate, and various concentrations of CBD (0, 10, 20, 40, and 80 µM) or a relative methanol concentration as a diluent control were added. The cells, in total volume of 100 µL, were incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 12, 24, and 48 hrs. After incubation, the cells were harvested and monitored for cell morphological changes by flow cytometry. Cells were also stained with 7-AAD 0.5 µg/mL. The percentage of dead cells (7-AAD+ cells) was analyzed by BD Accuri C6 plus flow cytometry (BD Bioscience, San Jose, California, USA).

Toxicity effects of CBD on sub-populations of PBMCs

The PBMCs at a concentration of $2x10^6$ cells/mL were incubated with 20 µM CBD or a relative concentration of methanol in total volume of 100 µL in a 96-well V-bottom plate in a 5% CO_2 incubator at 37 °C for 24 hrs. After cultivation, the cells were harvested and stained with the BV510-conjugated zombie dye for 15 min at room temperature in a dark box. Then, the cells were washed twice with PBS containing 1% BSA and 0.1% NaN_3 (FACS) buffer. Next, the cells were fixed with 4% paraformaldehyde for 15 min at room temperature. The fixed cells were permeabilized with 0.1% saponin, 5% FCS, and 0.1% NaN_3 in PBS to allow the antibodies to access the intracellular targets. After that, the cells were blocked by incubation with 10% AB serum in 0.1% saponin, 5% FCS, and 0.1% NaN_3 in PBS for 30 min at 4 °C. Blocked cells were stained with fluorochrome conjugated antibodies against CD3, CD4, CD8, CD14, CD19, or CD56 (for identifying the cell sub-populations) for 30 minutes at 4 °C. Then, the cells were washed twice with 0.01% saponin, 5% FCS, and 0.1% NaN_3 in PBS. After washing, the cells were fixed with PBS containing 1% paraformaldehyde. The stained cells were then analyzed by BD FACSCelesta flow cytometry (BD Bioscience, San Jose, California, USA) to assess the percentage of dead cells (zombie dye+ cells).

Statistical analysis

Data were analyzed using GraphPad Prism 9 software. An unpaired t-test was used to compare data.

Results

Toxicity effects of CBD on PBMCs

To investigate the toxicity effects of CBD on PBMCs, various concentrations of PBMCs ($2x10^6$, and $8x10^6$ cells/mL) were treated with various concentrations of CBD (0, 10, 20, 40, and 80 µM) or relative concentrations of methanol as a diluent control. The treated PBMCs were incubated for 12, 24, and 48 hrs and monitored for cell morphological changes and cell death by flow cytometry.

Upon treatment with CBD at 40 and 80 µM, PBMCs at all concentrations showed morphological change after being incubated for all incubation times (Figure 1A and 1B). The morphological change was not observed in the control group, which was treated with relative concentrations of methanol diluent (Figure 1A and 1B). These findings indicated that 40 and 80 µM of CBD exhibited cytotoxic effects on PBMCs. We subsequently performed a viability assay to investigate cell death in each condition. As predicted, 40 and 80 µM of CBD could induce cell death in treated PBMCs compared with diluent controls (Figure 1C). It is worth noting that the methanol control did not induce cell death compared with the medium control. The toxicity effect of CBD was dose-dependent. CBD at 80 µM showed a higher toxicity than at 40 µM. Longer exposure time induced more cell death. Taken together, our findings suggest that CBD at concentrations of 40 and 80 µM have cytotoxic effects on PBMCs.

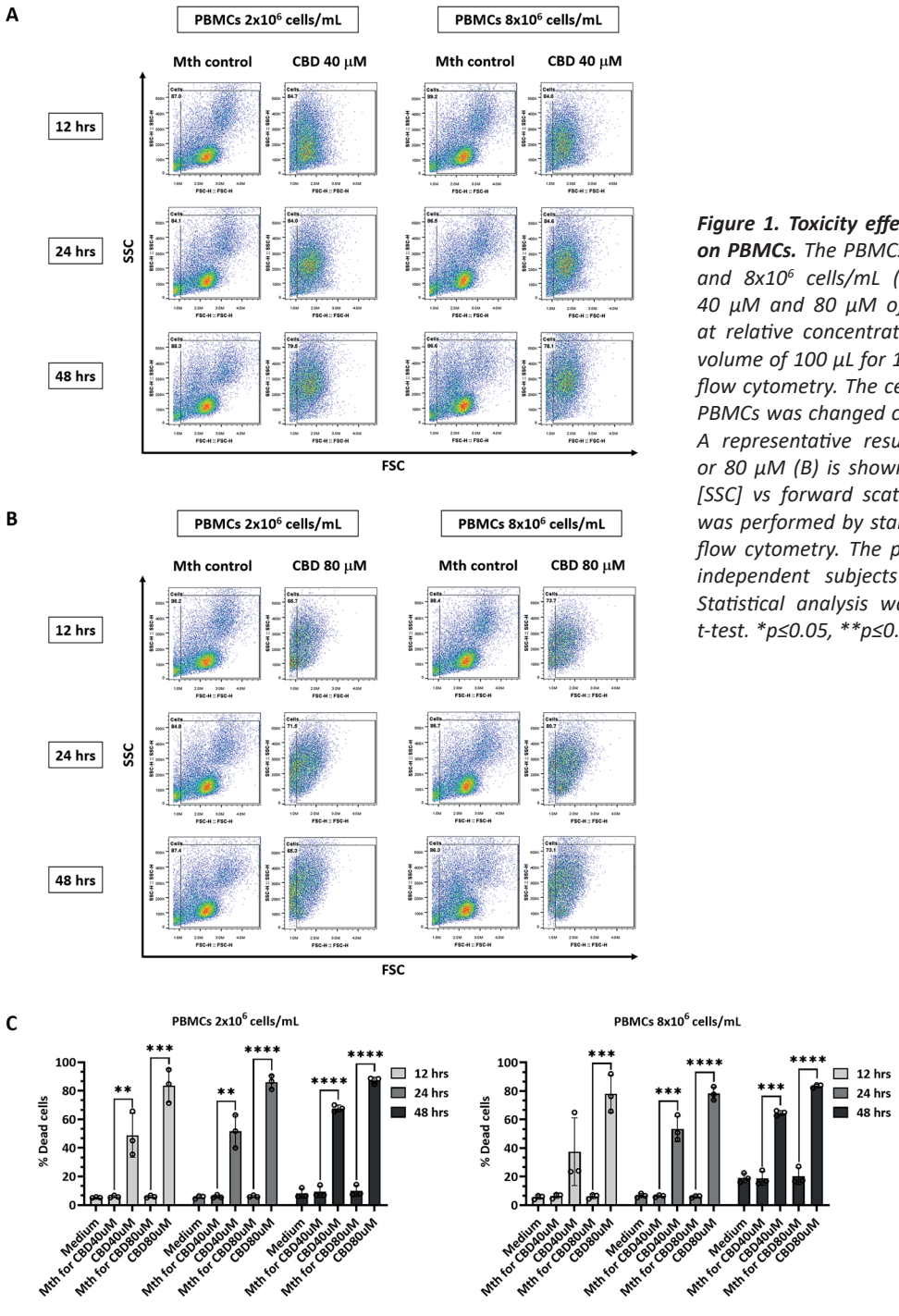

Figure 1. Toxicity effects of CBD at 40 µM and 80 µM on PBMCs. *The PBMCs (N=3) at concentrations of $2x10^6$ and $8x10^6$ cells/mL (as indicated) were treated with 40 µM and 80 µM of CBD or methanol (Mth control) at relative concentrations as a diluent control in total volume of 100 µL for 12, 24, and 48 hrs and analyzed by flow cytometry. The cell morphology of all three treated PBMCs was changed compared to the methanol control. A representative result treated with CBD 40 µM (A) or 80 µM (B) is shown in the scatter plots (side scatter [SSC] vs forward scatter [FSC]). (C) The viability assay was performed by staining with 7-AAD and analyzed by flow cytometry. The percentages of dead cells of three independent subjects in each condition are shown. Statistical analysis was performed using an unpaired t-test. *p≤0.05, **p≤0.01, ***p<0.0005, ****p<0.0001.*

The PBMCs treated with 20 µM of CBD did not show cell morphological change after being incubated for 12 hrs (Figure 2A). However, the morphological change was observed after 24 and 48 hrs of incubation, compared with the diluent controls (Figure 2A). The 48-hr incubation time showed higher morphological changes than the 24-hr incubation period. Subjects treated with 20 µM CBD at the 24-hr incubation period, however, exhibited a different CBD effect on cell morphology. The morphological changes were found in nine independent subjects (called CBD-sensitive subjects) out of sixteen tested subjects, ranging from mild to high alterations (Figure 2B). However, seven out of sixteen tested subjects have no morphological change when compared with the diluent controls. These seven subjects were called CBD-resistant subjects (Figure 2B). Therefore, upon treatment with 20 µM CBD, the tested subjects could be categorized as CBD-sensitive (56.3%) and CBD-resistant individuals (43.7%).

We further confirmed CBD-induced cell death using a 7-AAD cell viability assay. 20 µM CBD induced PBMC death was observed at 24- and 48-hr incubation times, but not at 12-hr incubation, in comparison with diluent control (Figure 2C). Our findings suggested that 20 µM of CBD might have different effects on PBMCs individually. Using CBD at this concentration is cautionary due to its cytotoxic effect in some subjects.

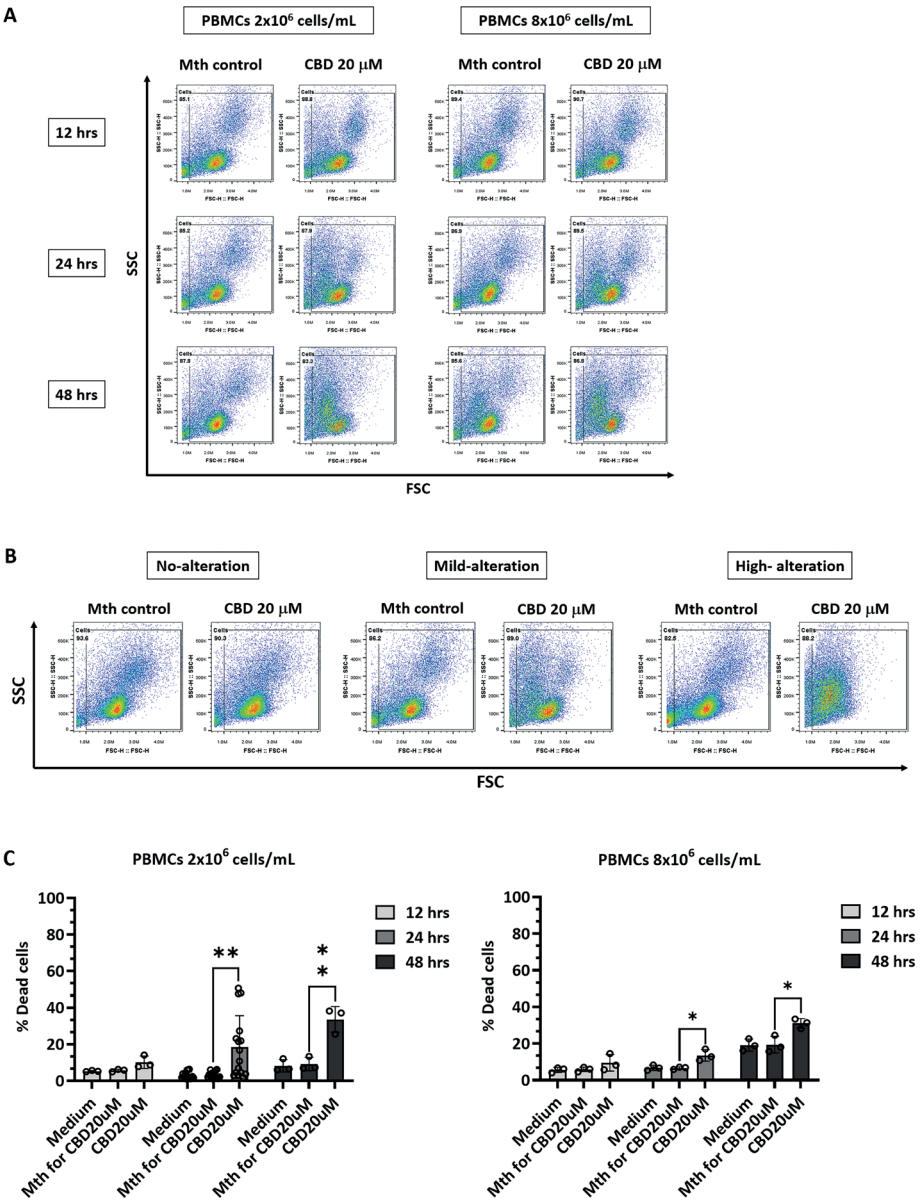

Figure 2. Toxicity effects of CBD at 20 µM on PBMCs. *The PBMCs (N=3) at concentrations of 2x10⁶ and 8x10⁶ cells/mL (as indicated) were treated with 20 µM CBD or methanol (Mth control) at relative concentrations as a diluent control in 100 µL total volume for 12, 24, and 48 hrs. The 24-hr PBMCs treatment at a concentration of 2x10⁶ cells/mL was performed with sixteen independent subjects. The cell morphology was investigated using flow cytometry. (A) The morphological change appeared after incubation for 24 and 48 hrs, but not for 12 hrs. (B) The patterns of morphological change in CBD-treated PBMCs at 2x10⁶ cells/mL incubated for 24 hrs are displayed. The extent of morphological change, ranging from no alteration to mild alteration and high alteration of a representative result, is shown. (C) The cell viability was determined using 7-AAD viability assay and analyzed by flow cytometry. The percentages of dead cells in each condition are shown. Statistical analysis was performed using an unpaired t-test. *p≤0.05, **p≤0.01.*

The cytotoxic effects of CBD at 10 µM concentration were also determined. The PBMCs at all concentrations tested upon treatment with 10 µM CBD did not show any morphological changes after being incubated for 12, 24, and 48 hrs, in comparison with the diluent controls (Figure 3A). The cytotoxic effects of CBD at 10 µM were determined by cell viability assay. The percentage of dead cells in the treated PBMCs showed no statistically significant differences compared with the relative concentrations of methanol (Figure 3B). Therefore, these results indicated that 10 µM of CBD did not have a cytotoxic effect on PBMCs.

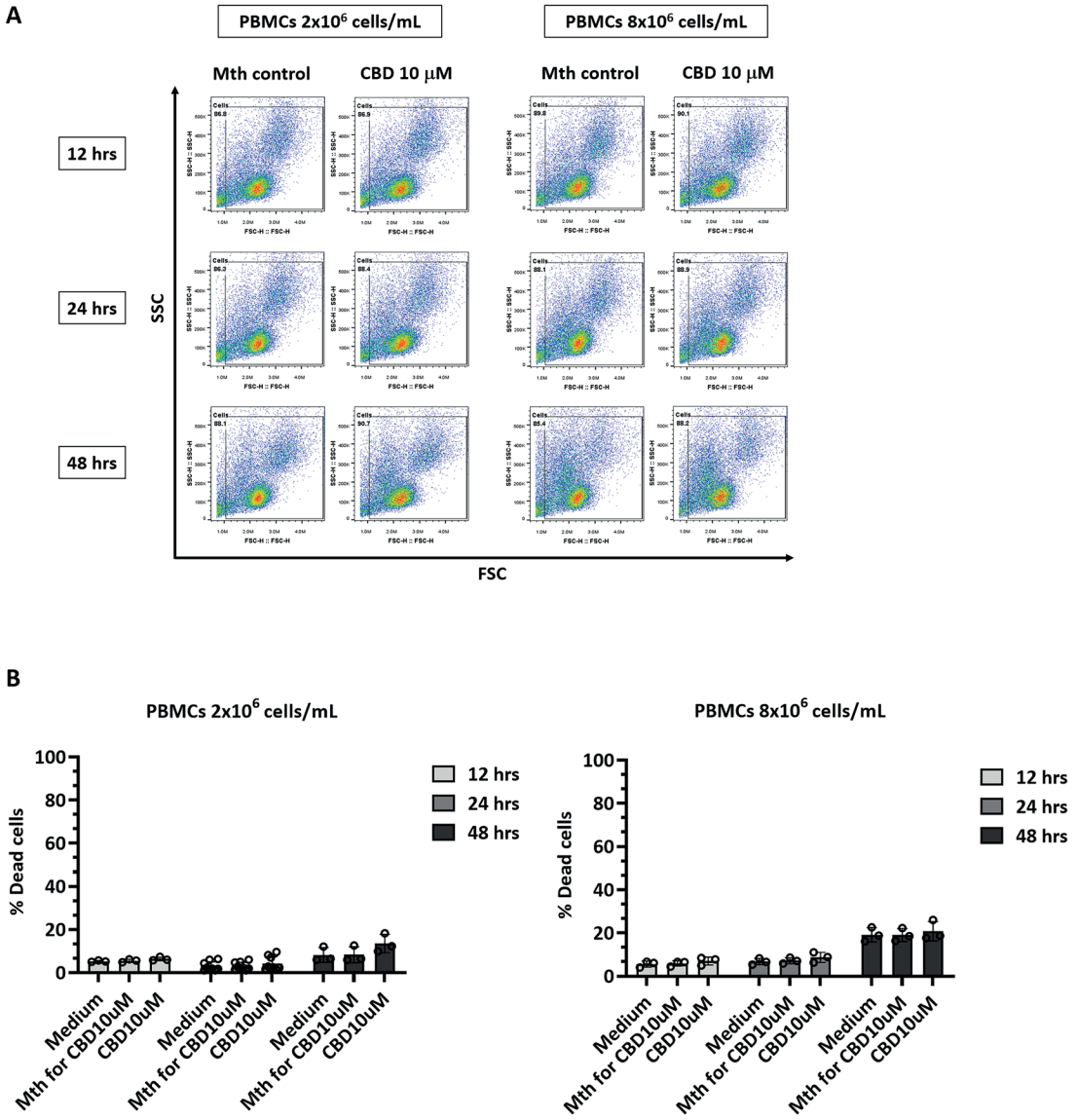

Figure 3. Toxicity effects of CBD at 10 µM on PBMCs. *The PBMCs (N=3) at concentration of 2x10^6 and 8x10^6 cells/mL (as indicated) were treated with 10 µM CBD or methanol (Mth control) in total volume of 100 µL for 12, 24, and 48 hrs. The 24-hr CBD treatment of PBMCs at a concentration of 2x10^6 cells/mL was performed with nine independent subjects. Then, the cells were analyzed by flow cytometry. (A) A representative result treated with 10 µM CBD is shown in the scatter plots (side scatter [SSC] vs forward scatter [FSC]). The morphology of all treated PBMCs did not show morphological changes compared with methanol control. (B) The cell viability was investigated using 7-AAD viability assay and analyzed by flow cytometry. The percentages of dead cells of all independent subjects in each condition are shown. Statistical analysis was performed using an unpaired t-test. No statistically significant differences were observed.*

As CBD at 10 µM was not toxic to the cells, we investigated the toxicity effects of CBD at concentrations of 1, 2, and 5 µM. In this study, PBMCs at a concentration of $2x10^6$ cells/mL with 24 hrs of incubation time were selected as a representative condition. As predicted, CBD at these concentrations was not toxic to the PBMCs of any study subjects (Figure 4). This confirms the non-toxic effects of CBD at concentrations below 10 µM.

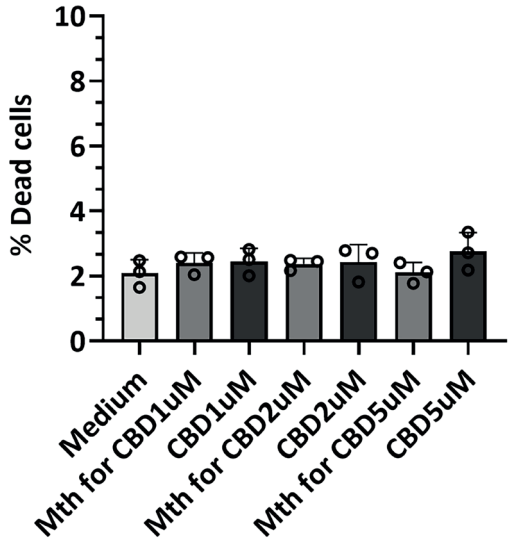

Figure 4. Toxicity effects of CBD at 1, 2, and 5 µM on PBMCs. *The PBMCs (N=3) at a concentration of $2x10^6$ cells/mL were treated with 1, 2, and 5 µM of CBD, or methanol (Mth) or medium alone with total volume of 100 µL for 24 hrs. The treated cells were stained with 7-AAD and analyzed by flow cytometry. The percentage of dead cells in each condition is shown. Statistical analysis was performed using an unpaired t-test. No statistically significant differences were observed.*

Toxicity effects of CBD on PBMC sub-populations

To get insight into CBD's cytotoxic effects on PBMCs, the toxicity of CBD in specific sub-populations of PBMCs, including CD4 T cells, CD8 T cells, B cells, NK cells, and monocytes, was studied. The PBMCs at a concentration of $2x10^6$ cells/mL were incubated for 24 hrs with 20 µM CBD or relative concentrations of methanol (diluent control). After cultivation, the cells were stained with fluorochrome-conjugated zombie dye, which indicated cell death. They were intracellular immunofluorescence stained with fluorochrome-conjugated monoclonal antibodies specific to each cell sub-population. The percentage of dead cells in each PBMC sup-populations was measured.

In this experiment, four independent subjects were performed. The observed morphological changes were significant in two independent subjects (N3, N4), and the other two independent subjects (N1, N2) were modulate-change observed (Figure 5A). The study on cell viability in the specific sub-populations of PBMCs showed that CBD could induce cell death in all PBMC sub-populations, i.e., CD4 T cells, CD8 T cells, B cells, NK cells, and monocytes. Surprisingly, CD4 T cells were less affected, with almost statistically significant difference compared with diluent control (Figure 5B). Our findings suggest that the cytotoxic effect of CBD affects all PBMCs sub-populations.

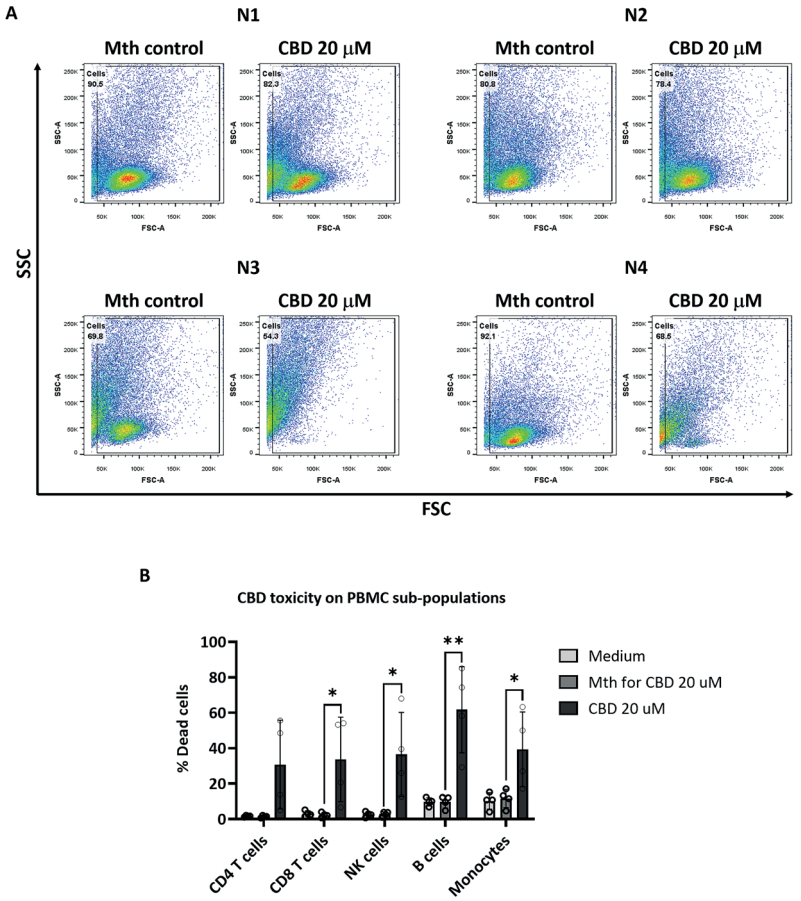

Figure 5. CBD cytotoxic effects on PBMC sub-populations. *The PBMCs (N=4) at a concentration of 2x10^6 cells/mL were treated with 20 µM CBD or methanol (Mth control) as diluent control in 100 µL total volume for 24 hrs. The cells were analyzed by flow cytometry. (A) The morphological change was observed in all test subjects. The cell morphology is shown in the scatter plots (side scatter [SSC] vs forward scatter [FSC]). (B) The treated cells were stained with BV510-conjugated zombie dye to assess cell viability. The percentage of dead cells of PBMC sub-populations, including CD4 T cells (CD3$^+$/CD4$^+$), CD8 T cells (CD3$^+$/CD8$^+$), NK cells (CD3$^-$/CD56$^+$), B cells (CD19$^+$), and monocytes (CD14$^+$), are shown. Statistical analysis was performed using an unpaired t-test. *p≤0.05, **p≤0.01.*

Discussion

In several countries, including Thailand, cannabis extracts and CBD were suggested to be used in the treatment of various diseases, including central nervous system diseases, inflammatory diseases, cardiovascular disorders, metabolic syndrome-related disorders, and cancer.[1,12,18,19,24,25] The use of cannabis extracts in treatment, however, usually comes without control of CBD concentration or an understanding of CBD toxicity effect. In a previous study, various CBD concentrations, ranging from 0.03 to 100 µM, were used to evaluate its impact on immune cells.[11] The majority of published articles suggest that CBD has immunosuppressive effects. [14-17] Nevertheless, some of these studies did not investigate the direct cytotoxicity of CBD on immune cells.[18,26] The data of the toxicity effect of CBD on immune cells is limited. We speculated that CBD may have a toxic effect on immune cells, suppressing immune cell functions. In the present study, we investigated whether CBD was toxic to immune cells. To investigate the toxicity effects of CBD, various concentrations of PBMCs were treated with various concentrations of CBD over various exposure times. Our study would answer the toxicity effect of CBD.

We found that CBD at 40 and 80 µM showed strong toxicity in PBMCs. These concentrations induced cell morphological changes of PBMCs in all tested subjects. The results indicated that CBD at these concentrations might disrupt the cell membrane, causing a change in cell morphology.[27] CBD at 40 and 80 µM concentrations could induce PBMC death, particularly at low concentrations of PBMCs and long exposure time to CBD. A low PBMC:CBD ratio induced a higher percentage of cell death, indicating a higher concentration of CBD per cell led to higher cell toxicity.

Moreover, the toxicity effect of CBD followed a dose-dependent manner. This suggested that CBD at 40 µM and higher was toxic to immune cells; when using CBD for medical treatment, the dosage of CBD needs to be carefully considered. Several in-house cannabis extracts, which had an unknown concentration of CBD, were discovered to be widely in use in several countries. This, therefore, poses a danger to the users.

CBD at 20 µM, nevertheless, showed a different effect. At 20 µM, CBD toxicity appeared in some subjects, called CBD-sensitive persons. We found that nine out of sixteen subjects (56.3%) were sensitive to CBD. Cell

morphological change and cell death could be seen in these sensitive subjects. However, 43.7% of tested subjects were not affected by CBD at this concentration. The different observed effects may be because of the levels of CBD receptors, and the membrane fragility of each subject may not be the same.[27] Furthermore, it might be influenced by other biological factors, such as age, sex, blood group, genetics, etc. However, this study did not clarify these influences due to limitations restricted by human ethics. According to the results, we suspected that CBD at 20 µM might be the cytotoxic threshold dosage. Using CBD at 20 µM, therefore, needed to be individually considered.

In contrast, CBD at concentrations of 10 µM and lower did not show any cytotoxic effect on PBMCs in all tested subjects. Neither cell morphological change nor cell death was seen at all concentrations of PBMC:CBD ratio and any exposure times at these CBD concentrations. Importantly, the subjects tested with CBD at ten µM were also included in the 20 µM treatment group. Whether classified as CBD-sensitive or CBD-resistant based on the effects of CBD at 20 µM, no morphological changes and cell death were observed when these subjects were exposed to CBD at ten µM. Our results align with the previous study, which demonstrated that the toxicity of CBD was approximately in the range of 20-22 µM, depending on the cell type. The highest concentration of CBD that did not cause cell death was 13 µM.[28] In our study, we demonstrated that CBD concentrations at ten µM or lower would be an appropriate condition for any future studies involving CBD effect.

As mentioned, CBD at a high dose has a cytotoxic effect on PBMCs. We asked whether this effect was on all PBMC sub-populations or a specific cell sub-population. We, therefore, investigated the toxicity of CBD on PBMC sub-populations, including T cells, B cells, NK cells, and monocytes. In this study, 4 CBD-sensitive subjects with varied responses to 20 µM CBD were chosen.

We found that in strong 20 µM CBD-sensitive persons, the high toxicity of CBD was observed in all PBMC sub-populations, including CD4 T cells, CD8 T cells, B cells, NK cells, and monocytes. While, in individuals who are less sensitive to CBD, low cytotoxicity of CBD was observed in all PBMC sub-population. The mechanism of CBD cell toxicity is still unproven in this study.

To the best of our knowledge, this is the first time the toxicity effect of CBD on PBMCs has been demonstrated. Our results of the toxicity of CBD are in line with the previous studies, which reported that CBD could induce immune suppression, both in innate and adaptive immune responses.[14-17] The effects of CBD on the suppression of immune cell function may be due to the toxicity of CBD that affects both innate immune cells, monocytes and NK cells, and adaptive immune cells, T and B lymphocytes if the high concentration of CBD were used in the study. The researchers who perform experiments on the function of CBD need to monitor the toxicity of CBD on the tested cells concurrently in their research.

Conclusion

In this study, we investigated the toxic effects of CBD on each PBMC sub-population. Our study indicated that CBD had toxicity effects on immune cells in a dose-dependent manner. The toxicity effect of CBD was influenced by CBD concentrations, PBMC concentration, and exposure time. Specifically, the percentage of dead cells increased with a high CBD:cell concentration ratio and an extended exposure time. The obtained results are a caution for CBD users to be aware of the concentration of CBD being consumed.

Conflict of interests

The authors declare no conflict of interest.

Ethics approval

The human ethics of this study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (study code; AMSEC-66EX-013).

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References

[1] Pattnaik F, Nanda S, Mohanty S, Dalai AK, Kumar V, Ponnusamy SK, et al. Cannabis: Chemistry, extraction and therapeutic applications. Chemosphere. 2022; 289: 133012. doi: 10.1016/j.chemosphere.2021.

[2] Radwan MM, Chandra S, Gul S, ElSohly MA. Cannabinoids, Phenolics, Terpenes and Alkaloids of Cannabis. Molecules. 2021; 26(9): 2774. doi: 10.3390/molecules26092774.

[3] Pagano C, Navarra G, Coppola L, Avilia G, Bifulco M, Laezza C. Cannabinoids: Therapeutic Use in Clinical Practice. Int J Mol Sci. 2022; 23(6): 3344. doi: 10.90/ijms23063344.

[4] D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, et al. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacol. 2004; 29(8): 1558-72. doi: 10.038/sj.npp.1300496.

[5] Bajtel A, Kiss T, Toth B, Kiss S, Hegyi P, Vorhendi N, *et al*. The Safety of Dronabinol and Nabilone: A Systematic Review and Meta-Analysis of Clinical Trials. Pharmaceuticals (Basel). 2022; 15(1): 100. doi: 10.3390/ph15010100.

[6] Zuardi AW. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. Braz J Psychiatry. 2008; 30(3): 271-80. doi: 10.1590/s16-44462008000300015.

[7] Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants (Basel). 2019; 9(1): 21. doi: 10.3390/antiox9010021.

[8] de Almeida DL, Devi LA. Diversity of molecular

targets and signaling pathways for CBD. Pharmacol Res Perspect. 2020; 8(6): e00682. doi: 10.1002/prp2.682.

[9] Britch SC, Babalonis S, Walsh SL. Cannabidiol: pharmacology and therapeutic targets. Psychopharmacology (Berl). 2021; 238(1): 9-28. doi: 10.1007/s00213-020-5712-8.

[10] Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia. 2014; 55(6): 791-802. doi: 10.1111/epi.12631.

[11] Nichols JM, Kaplan BLF. Immune Responses Regulated by Cannabidiol. Cannabis Cannabinoid Res. 2020; 5(1): 12-31. doi: 10.1089/can.2018.0073.

[12] Hill AJ, Williams CM, Whalley BJ, Stephens GJ. Phytocannabinoids as novel therapeutic agents in CNS disorders. Pharmacol Ther. 2012; 133(1): 79-97. doi: 10.1016/j.pharmthera.2011.09.002.

[13] Bhunia S, Kolishetti N, Arias AY, Vashist A, Nair M. Cannabidiol for neurodegenerative disorders: A comprehensive review. Front Pharmacol. 2022; 13: 989717. doi: 10.3389/fphar.2022.

[14] Wu HY, Huang CH, Lin YH, Wang CC, Jan TR. Cannabidiol induced apoptosis in human monocytes through mitochondrial permeability transition pore-mediated ROS production. Free Radic Biol Med. 2018; 124: 311-8. doi: 10.1016/j.freeradbiomed.2018.06.023.

[15] Watzl B, Scuderi P, Watson RR. Marijuana components stimulate human peripheral blood mononuclear cell secretion of interferon-gamma and suppress interleukin-1 alpha in vitro. Int J Immunopharmacol. 1991; 13(8): 1091-7. doi: 10.16/0192-561(91)90160-9.

[16] Watzl B, Scuderi P, Watson RR. Influence of marijuana components (THC and CBD) on human mononuclear cell cytokine secretion in vitro. Adv Exp Med Biol. 1991; 288: 63-70. doi: 10.1007/978-1-4684-5925-8_7.

[17] McKallip RJ, Jia W, Schlomer J, Warren JW, Nagarkatti PS, Nagarkatti M. Cannabidiol-induced apoptosis in human leukemia cells: A novel role of cannabidiol in the regulation of p22phox and Nox4 expression. Mol Pharmacol. 2006; 70(3): 897-908. doi: 10.1124/mol.106.023937.

[18] Couch DG, Tasker C, Theophilidou E, Lund JN, O'Sullivan SE. Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. Clin Sci (Lond). 2017; 131(21): 2611-26. doi: 10.1042/cs20171288.

[19] Peyravian N, Deo S, Daunert S, Jimenez JJ. The Anti-Inflammatory Effects of Cannabidiol (CBD) on Acne. J Inflamm Res. 2022; 15: 2795-801. doi: 10.147/jir.S355489.

[20] Sholler DJ, Schoene L, Spindle TR. Therapeutic Efficacy of Cannabidiol (CBD): A Review of the Evidence from Clinical Trials and Human Laboratory Studies. Curr Addict Rep. 2020; 7(3): 405-12. doi: 10.1007/s40429-020-00326-8.

[21] Rosenkrantz H, Fleischman RW, Grant RJ. Toxicity of short-term administration of cannabinoids to rhesus monkeys. Toxicol Appl Pharmacol. 1981; 58(1): 118-31. doi: 10.1016/0041-008x(81)90122-8.

[22] Chesney E, Oliver D, Green A, Sovi S, Wilson J, Englund A, et al. Adverse effects of cannabidiol: a systematic review and meta-analysis of randomized clinical trials. Neuropsychopharmacol. 2020; 45(11): 1799-806. doi: 10.038/s41386-020-0667-2.

[23] Garberg HT, Solberg R, Barlinn J, Martinez-Orgado J, Loberg EM, Saugstad OD. High-Dose Cannabidiol Induced Hypotension after Global Hypoxia-Ischemia in Piglets. Neonatology. 2017; 112(2): 143-9. doi: 10.1159/000471786.

[24] Kogan NM, Mechoulam R. Cannabinoids in health and disease. Dialogues Clin Neurosci. 2007; 9(4): 413-30. doi: 10.31887/DCNS.2007.9.4/nkogan.

[25] Assanangkornchai S, Thaikla K, Talek M, Saingam D. Medical cannabis use in Thailand after its legalization: a respondent-driven sample survey. PeerJ. 2022; 10: e12809. doi: 10.7717/peerj.

[26] Huang Y, Wan T, Pang N, Zhou Y, Jiang X, Li B, *et al*. Cannabidiol protects livers against nonalcoholic steatohepatitis induced by high-fat high cholesterol diet via regulating NF-κB and NLRP3 inflammasome pathway. J Cell Physiol. 2019; 234(11): 21224-34.

[27] Guard SE, Chapnick DA, Poss ZC, Ebmeier CC, Jacobsen J, Nemkov T, *et al*. Multiomic Analysis Reveals Disruption of Cholesterol Homeostasis by Cannabidiol in Human Cell Lines. Mol Cell Proteomics. 2022; 21(10): 100262. doi: 10.1016/j.mcpro.2022.

[28] Holland ML, Panetta JA, Hoskins JM, Bebawy M, Roufogalis BD, Allen JD, *et al*. The effects of cannabinoids on P-glycoprotein transport and expression in multidrug resistant cells. Biochem Pharmacol. 2006; 71(8): 1146-54. doi: 10.016/j.bcp.2005.12.033.

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Effect of music therapy on language skills in children with specific language impairment: A systematic review

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ABSTRACT

Background: Specific language impairment (SLI) delays language development without any neurological damage or disease. This impairment extends to non-linguistic tasks, such as music perception skills. In recent years, speech-language pathologists (SLPs) and music therapists (MTs) have collaborated to develop and improve approaches for children with communication disorders (CDs), including global developmental delay (GDD), SLI, mild developmental delay (DD), and the risk of developing reading difficulties, by integrating music therapy (MT) and speech therapy (ST). MT could be considered as one of the alternative methods offered to children with SLI to enhance their language skills.

Objectives: The purpose of this study was to investigate the effectiveness of MT interventions on language skills in children with SLI and to investigate the characteristics of other intervention features in these studies, such as interventionists, intervention, settings, session type, and music methods.

Materials and methods: The study was a systematic review conducted within the framework of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The quality of the research results was assessed using the critical appraisal tools provided by the Joanna Briggs Institute (JBI).

Results: Two studies met the inclusion criteria and were included in this systematic review. The two main types of MT employed were song cues and creative music therapy based on the Nordoff-Robbins approach. Current evidence suggests that music therapy improves components of language, including phonology, syntax, morphology, and other aspects of speech development, such as understanding sentences and memory for sentences, in children with SLI.

Conclusion: MT can be a valuable and effective intervention for children with SLI. The introduction of transdisciplinary programs that integrate MT and ST could be recommended. However, MT training courses are required for SLPs.

Introduction

Specific language impairment (SLI) refers to a delay in language development in the absence of any neurological damage or disease. The term "SLI" can be used interchangeably with developmental language disorder (DLD).[2] Regardless of the emphasis on language difficulties in speech comprehension and production, SLI can affect one or several components of language, including syntax (grammar), phonology, morphology, semantics, and pragmatics to varying degrees.[3] Specifically, the main language problems observed often pertain to syntax and morphology components.[4-6]

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Interestingly, this impairment extends more broadly to non-linguistic tasks. For instance, children with SLI tend to exhibit poorer music perception skills compared to typically developing children.[7] This is noteworthy because music and language share numerous commonalities and consist of perceptually unique components arranged in hierarchically structured sequences. Both are highly structured systems that we are constantly exposed to daily.[8-9] According to Levitin[10] and Schmidt-Jones,[11] the fundamental components of music encompass rhythm (duration, tempo, and meter), dynamics, melody (pitch and scales), harmony, timbre, texture, and form/structure. Music and speech share several characteristics: they utilize frequency (pitch and timbre) and temporal (rhythm) cues to convey information at an acoustic level.[12] Additionally, both music and speech involve similar cognitive skills such as learning, memory, and attention at a cognitive level.[13]

Furthermore, studies have attempted to provide deeper insights into the connection between overlapping brain regions for music and language,[14-16] some of which are located in overlapping brain areas such as the lateral parts of the inferior frontal gyrus and the anterior superior temporal gyrus.[14-15] According to Leonard *et al.,* the superior temporal gyrus, precentral gyrus, postcentral gyrus, and inferior frontal gyrus have been associated with language. Some of the regions in the temporal, parietal, and frontal lobes also seem to be involved in playing music. [16]

Music therapy is the clinical and evidence-based use of music treatments in a therapeutic relationship by a certified practitioner who has completed an accredited music therapy curriculum. MT aims to use music to reach non-musical goals, including improving communication, social, emotional, physical, and cognitive domains. Research in the field of MT supports its effectiveness in a wide range of clinical and educational settings.[17-18] According to Geist *et al*., SLPs and MTs have collaborated to develop and enhance approaches for children with severe CDs such as GDD by integrating MT and ST. Positive outcomes of MT/ST collaboration have been observed in a relatively short period.[19]

Studies have examined the effect of using songs for language learning in individuals with language disorders (LDs), including SLI and mild DD,[20] as well as the risk of developing reading problems in individuals with a history of speech and language delay and weak phonological awareness skills.[21] Both studies utilized audio-recorded, live-sung, and spoken-script presentations. These studies have demonstrated a positive effect of songs on incidental word learning,[20] and narratives exhibited a richer vocabulary.[21] Mari *et al*. investigated music understanding and specific linguistic abilities in children with SLI and found that they struggled with music perception, specifically melody and song identification tasks. The authors suggested that training programs incorporating both language and music to stimulate children with SLI might be beneficial in their rehabilitation.[7] Based on the evidence cited above, we hypothesize that MT could serve as an alternative approach for enhancing the language skills of children with SLI.

Currently, there is no systematic review on how music therapy affects language skills, especially in children with SLI. Therefore, the primary objective of this systematic review was to investigate the effectiveness of MT interventions on language skills in children with SLI. Also, the second objective of this study was to investigate the characteristics of the other intervention features of these studies (i.e., interventionists, intervention, setting, session type, and music methods). This method offers a comprehensive perspective on current knowledge, which will help guide future research and clinical practice. The authors aimed to answer the following research questions:

(1) What music therapy interventions have affected language skills for children with specific language impairments?

(2) What are the characteristics of the other intervention features of these studies? (i.e., interventionists, intervention, setting, session type, and music methods)?

Materials and methods

The study was a systematic review based on the PRISMA framework.[22] Relevant articles were retrieved using four electronic databases: PubMed, ERIC, APA PsycInfo, and CINAHL Complete. The publications included in the review were published between 1994 and 2023.

The basic query developed for the literature search was: ("music" OR "music therapy") AND ("specific language impairment" OR "developmental language disorder" OR "delayed speech" OR "expressive language disorder" OR "receptive language disorder") AND (language OR speech OR receptive OR expressive OR phonology OR semantic OR syntax OR morphology OR pragmatic) AND child*. The boolean operators in this query were modified to accommodate database settings.

Inclusion and exclusion criteria

The inclusion criteria included studies with quantitative and qualitative descriptive research. Published articles and grey literature, including theses and dissertations, were also considered. The literature included in this review focused on children aged birth to six years with a documented SLI, regardless of etiology. MT must be used in the interventions outlined to address language skills. The search was also limited to English-language journal articles.

The exclusion criteria were pre-publication, unpublished articles, and other types of grey literature. Clinical outcomes in other treatment areas (physical/motor, cognitive, and socio-emotional) were also not considered. Participants included children older than six years old and diagnosed with other communication disorders (e.g., autistic spectrum disorder (ASD), hearing loss (HL), intellectual disability (ID), visual impairment, learning disorder (LD), and DD) were excluded. Studies were only included if they were written in English.

Data collection and analysis

Following the screening of publications for inclusion,

the two researchers (N.W. and V.B.) independently extracted the data relevant to the research questions. They analyzed the quality of the research results using the JBI critical appraisal tools according to research type. The researchers then addressed any mistakes, discrepancies, or clarifications needed for the coding document. In cases of disagreement, a consensus approach was adopted that included the third author. However, a third opinion was not required because there was no disagreement between reviewers.

Figure 1 illustrates the methodology flowchart for each stage of the search process, as explained in this methodology.[22] Based on the PRISMA, the researchers also generated a coding document and collected inter-rater reliability (IRR) to limit the risk of bias during data extraction.

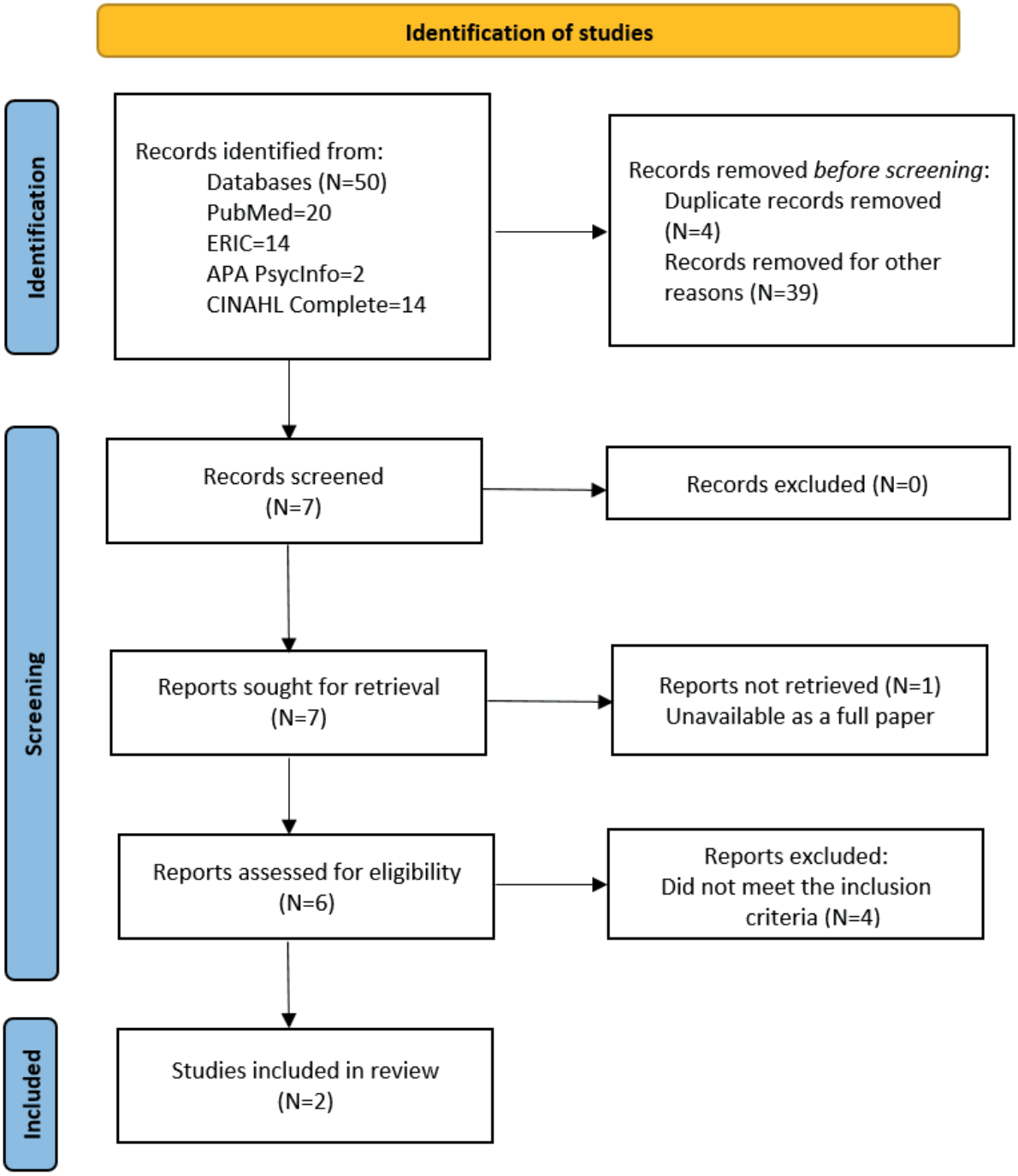

Figure 1. *Methodology flowchart.*

Data extraction

During data extraction, the researchers randomly selected 50% of the data set to estimate the IRR on the inclusion and exclusion of studies, which was obtained for 1 of the 2 studies. We calculated the IRR on all 12 variables by taking the total number of exact agreements divided by agreements plus disagreements and multiplying by 100%. Once the IRR process was complete, the researchers continued to code the remaining documents in the data set independently.

The extracted information included document details (database source, publication origin, publication age, and document type), participant information (diagnoses, age), information about the interventionists (professional occupation), characteristics of the intervention (setting, session type, musical methods), and outcomes of the intervention (developmental domain(s) addressed, types of language skills, and outcomes).

The researchers provided a narrative description of the literature in the dataset, including the type, age, and source of publication. Then, the researchers identified and analyzed variations in diagnosis, age groups, interventionist profession, types of music experiences, and language goals covered in the literature as part of this evaluation. Furthermore, the researchers discovered gaps in the literature and described limitations or disagreements found in the literature. Finally, the researchers might obtain future research directions from this analysis.

Results

The searches identified 50 articles published between 1994 and 2023. After removing records before screening, seven studies remained. One study was excluded because it was unavailable as a full paper, and four studies still needed to meet the inclusion criteria. Finally, two studies met the inclusion criteria and were included in this systematic review (Figure 1). The two studies identified for this systematic review included one case study (50%) and one observational pilot study (50%). These studies were generally of low scientific quality since they consisted of case studies (level IV evidence) and observational pilot studies (level III evidence). Additionally, one study was conducted in Australia, while the other was conducted in Germany (Table 1).

In all, these studies included 20 participants with SLI who met the inclusion criteria for this review. This included 13 males (65.0%), and seven females (25.0%). Children ranged in age from 3 to 6. Race or ethnicity was not reported for 20 children (100%), of whom 2 spoke English and 18 spoke German. Participants in one study had moderate-to-severe SLI,[23] while participants in the other did not mention it.[24] Children receiving direct language intervention from an SLP were excluded.[23] On the other hand, children receive speech therapy and early intervention programs continuously due to ethical considerations.[24]

Two studies provided MT exclusively through song cues[23] and creative music therapy based on the Nordoff-Robbins approach.[24] The treatment intensity ranged between six bi-weekly individual sessions of up to 30 minutes[23] and eight weekly individual sessions of up to 25 minutes.[24] One study (50.0%) took place in school-based settings[23], and the other (50.0%) in hospital-based settings[24. The] intervention was delivered individually.[23,24] One study (50.0%) delivered the intervention by a speech-language pathologist,[23] and the other (50.0%) delivered it by a music therapist.[24]

All studies (100%) reported formal or standardized language measures. Tan and Shoemark used the Renfrew Action Picture Test (RAPT) to evaluate the grammatical structures used and the Language Assessment and Remediation Procedure (LARSP) to evaluate a more in-depth analysis of the language structures used, which provided information about both the syntax and morphological structures.[23] Whereas Groß *et al*. used the speech development test for children aged three to five years (SETK 3-5) as the first standardized German language test to evaluate speech abilities. This test instrument was divided into three areas of speech development with five subtests: understanding sentences (VS), generation of morphological rules (MR), phonological memory for non-words (PGN), memory for sentences (SG), and memory for word sequences (GW).[24]

Table 1. Included studies.

Study	Design	Participants	Music therapy	Control	Treatment intensity	Setting	Interventionist	Measures	Language outcomes	Results
Tan & Shoemark, *2017* (Australia)	Case study (feasibility study)	Two English speaking children with moderate-to-severe SLI (one male and one female, 6.3 and 6.8 years, respectively)	Song cues	Traditional speech cues	Six bi-weekly individual sessions of up to 30 minutes	School-based settings	Speech-language pathologist	1. The Renfrew Action Picture Test (RAPT) 2. Language Assessment and Remediation Procedure (LARSP)	1. Syntax (sentence structure) 2. Morphology (word structure)	• There was an increase in syntax learning, but no increase in morphology learning after receiving song cues. • Song and speech cues in morphology intervention could not be compared since neither participant achieved a morphology goal.
Groß *et al.*, 2010 (Germany)	Observational pilot study	Eighteen German speaking children with SLI, (twelve male and six females, aged 3.5 to 6 years	Creative music therapy based on the Nordoff-Robbins approach	No control group	Eight weeks individual sessions of up to 25 minutes	Hospital-based settings	Music therapist	1. Speech development test for children aged three to five years (SETK 3-5) 2. The SON-R 2 1/2 - 7 (individual intelligence test) 3. The Nordoff-Robbins scales	1. Understanding sentences (VS) 2. Generation of morphological rules (MR) 3. Phonological memory for non-words (PGN) 4. Memory for sentences (SG) 5. Memory for word sequences (GW)	• Creative music therapy had a significant improvement kn phonological memory and the children's understanding of sentences. • A positive shift in the memory of sentences and generation of morphological rules was observed.

Discussion

The primary objective of this systematic review was to investigate the effectiveness of MT interventions on language skills in children with SLI. There have been a few studies that have investigated the effect of MT on language skills for individuals with SLI.[23,24] Current evidence suggests that music therapy, including song cues and creative music therapy based on the Nordoff-Robbins approach, improves components of language such as phonology, syntax, morphology, and other areas of speech development in children with SLI.

Phonology

Groß *et al*. found that phonological memory for non-words showed statistically significant outcomes, which supports the possibility that MT intervention may influence the development of this parameter, which grew more noticeably during periods with MT. Phonological memory is undoubtedly associated with prosodic abilities.[24] PGN is the ability to store and recall speech sounds in short-term memory. Groß *et al*. believed that the gains occur because MT targets listening, perception, processing, and memorizing sounds and musical structures.[24] According to Anvari *et al*., music perception involves auditory systems that partially overlap with those engaged in phonological awareness.[25] Auditory-temporal processing is relevant in speech and music. Children must discriminate, and process sounds extracted from perceived acoustic signals that change over time in both areas. The ability to discriminate between similar-sounding consonants is essential to phonological awareness.[26] Thus, creative music therapy based on the Nordoff-Robbins approach, which included singing and making music with percussion instruments, can support the development of PA.

Morphology

Tan and Shoemark found an increase in syntax learning after receiving song cues. One participant achieved the first three syntax goals (e.g., subject-(verb)ing, subject-(verb)ing-object, and subject-(verb)ing-location), and the other participant reached the fourth syntax goal but did not achieve it (subject-verb-complement).[23] The natural speech inflection patterns of target sentences in the syntax intervention were transformed into musical prosody during the intervention by singing a tune that was accompanied by the guitar to provide rhythmic emphasis.[27] Rhythmic attendance aids in the learning and recall of text in songs. The rhythm inherent in song provides a temporal-metrical framework that enables perceptual grouping and chunking of information into more manageable units to aid recall.[27] In song, that combines speech, melody, and rhythm, the prosodic aspects of speech are modified more than usual. The additional cues offered by rhythm and melody will likely reduce the cognitive demands required for auditory signal processing and allow children to concentrate on language learning.[28] These results suggest that adding syntax intervention benefits children with SLI, and song cues could be similarly effective—or even better—than speech cues in syntactic intervention. However, it should be noted that both participants continued to fall below the 1st percentile for their age groups.[23]

Other areas of speech development

Furthermore, Groß *et al*. found that understanding sentences showed statistically significant outcomes, supporting the notion that MT intervention may influence the development of this parameter, which expanded more significantly during periods with MT. The improvement in the understanding of sentences on the subscale might be due to the ability to relate to another person. Children started to communicate more frequently and have more social contacts during MT sessions.[24] In creative music therapy based on the Nordoff-Robbins approach, the therapist traditionally interacts directly with the child, promoting musical participation and engagement; therefore, it encourages relatedness, communication, and socializing.[29]

Groß *et al.* also found that memory for sentences, which had previously been at a very low level, improved after MT intervention. It could be that when making music, it is constantly practiced and worked on to comprehend the form and general structure of a sentence in order to comprehend its meaning fully. Additionally, they can modify the music according to his or her abilities by having it gradually shortened or enlarged.[24]

The second objective of this study was to investigate the characteristics of the other intervention features of these studies (i.e., interventionists, intervention, setting, session type, and music methods).

Setting and interventionists

Tan and Shoemark conducted MT in school by a registered SLP, a master's equivalency student, who was enrolled in a music therapy equivalency program. Her experience and confidence as a SLP may have resulted in differences in how the two cueing techniques were executed.[23] These concurred with previous studies by Kouri and Winn,[20] and Kouri and Telander.[21] A graduate student in speech-language pathology with a strong musical background provided interventions. On the other hand, Groß *et al*. conducted MT in outpatients in hospitals by two MTs with a master's degree in music therapy and sufficient clinical experience (a minimum of two years) in their field. [24] Therefore, it is important to note that MT training courses are required for SLPs to understand and learn aspects of MT and develop new approaches to practice within school or clinical settings.

Intervention, session type, and music methods

Tan and Shoemark used song cues delivered individually, which were divided, with the first half focused on syntax and the second half on morphology.[23] In the syntax intervention, the natural speech inflection patterns of target sentences were turned into musical prosody[27] by singing a tune that was accompanied on the guitar to create rhythmic emphasis. In the morphology intervention, the natural speech inflection patterns of target sentences were turned into musical prosody[27] by using aspects of

melodic intonation therapy.

The structure of a song cue session includes listening, unison production, partially supported production, and independent production. In this single therapy session, the SLP introduced the target sentence structure by displaying a picture and modeling the sentence response in the specified modality (song or speech). Then, the SLP and the child sang or said the target sentence together. The SLP and the child then sang or said the first half of the sentence. The SLP faded out while the child independently completed the second half of the sentence. Lastly, the child was presented with 14 picture stimuli and sang or said the target sentences independently.[23]

On the other hand, Groß *et al*. used creative music therapy based on the Nordoff-Robbins approach, which included singing and making music with percussion instruments (i.e., bells, drums, pentatonic tone bars, shakers, reed horns, and lyres) and piano. Interventions were delivered on an individual basis.[24]

Songs were chosen for playtime and dealt with the child's interests, such as hide-and-seek or animal songs. Individual themes and musical developments arose for each child; some desired to sing and dance, others desired to be sung to, and still others desired to play an instrument on their own. According to this single therapy session, the improvised music was oriented toward the musical and vocal expressions of the child and therefore played a central role in the therapy.[24]

The findings of this study indicated that music delivery methods were in the form of using a song. These concurred with previous studies conducted by Kouri and Winn,[20] and Kouri and Telander.[21] However, it should be noted that they provided different MT interventions, including song cues,[23] the Nordoff-Robbins approach,[24] and audio-recorded, live-sung, and spoken-script presentations.[20,21]

Our findings support the idea that using a song can be used as a form of therapy by professionals such as SLPs and MTs. Also, both professionals can establish language and speech goals for children with SLI. The introduction of transdisciplinary programs in therapies that integrate MT and ST could be recommended. Tan and Shoemark suggested that it did not require the use of expensive instruments or resources to be successful.[23] This may be attractive to service providers, especially where resources and monetary funding are frequently restricted.[23]

Limitations and future research

Some limitations to this study must be considered. The first was a potential bias in this systematic review process because small sample sizes were observed in individual studies. Further research with a randomized controlled trial and larger sample sizes must be explored. It might increase the potential for results that capture the MT effects on language skills in children with SLI. Also, these two studies included participants with SLI who spoke English and German, which might differ along the opacity-transparency dimension. These two languages, however, are both intonation languages that indicate communication intentions through prosodic variations at the sentence level. More research is needed to investigate the transparency in language and lexical tone between English and German speakers about music. Additionally, there is no study focusing on each level of severity of individuals with SLI and its effect on MT. Therefore, participant characteristics should be considered.

Conclusions

The current systematic review represents a summary of the existing evidence of the effectiveness of music therapy in improving language skills in children with specific language impairments. Despite the lack of a wide range of music methods, current evidence suggests that music therapy, including song cues and creative music therapy based on the Nordoff-Robbins approach, improves components of language such as phonology, syntax, morphology, and other areas of speech development, including understanding sentences and memory for sentences in children with SLI. Language and speech development progress over time as a result of the intervention. Especially, using a song can be used as a form of therapy by professionals who work with children with SLI. In practice, music is increasingly being used in speech and language therapy. MT can be a valuable and effective intervention for children with SLI. Transdisciplinary programs that integrate MT and ST could be recommended. However, training courses are required for SLPs.

Conflict of interest

The authors declare no conflict of interest, and the review did not require full board ethics approval because it was a systematic review. The study was approved for exemption review by the Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University (CMU), Thailand (AMSEC-66EM-009).

References

[1] Leonard LB. Specific language impairment across languages. Child Dev Perspect. 2014; 8(1): 1-5. doi:10.1111/cdep.12053.

[2] Bishop DV. Why is it so hard to reach agreement on terminology? The case of developmental language disorder (DLD). Int J Lang Comm Dis. 2017; 52(6): 671-80. doi:10.1111/1460-6984.1233.

[3] Bishop DV, Leonard LB, editors. Speech and language impairments in children: Causes, characteristics, intervention and outcome. Psychology Press; 2000.

[4] Fey ME, Long SH, Finestack LH. Ten principles of grammar facilitation for children with specific language impairments. Am J Speech Lang Pathol. 2003; 12(1): 3-15. doi:10.1044/1058-0360(2003/048).

[5] Gillam SL, Kamhi AG. Specific language impairment. The handbook of language and speech disorders. 2010: 210-26. doi:10.1002/9781444318975.

[6] Goffman L, Leonard J. Growth of language skills in preschool children with specific language impairment: Implications for assessment and intervention Am J

Speech Lang Pathol. 2000; 9(2): 151-61. doi:10.1044/1058-0360.0902.151.

[7] Mari G, Scorpecci A, Reali L, D'Alatri L. Music identification skills of children with specific language impairment. Int J Lang Commun Disord. 2016; 51(2): 203-11. doi:10.1111/1460-6984.12200

[8] Lerdahl F. Tonal pitch space. Oxford University Press, USA; 2001.

[9] Deutsch D. The processing of pitch combinations. In-The psychology of music 1999 Jan 1 (pp. 349-411). Academic Press. doi:10.1016/B978-0122135644/50011-1.

[10] Levitin DJ. This is your brain on music: The science of a human obsession. Penguin; 2006 Aug 3.

[11] Schmidt-Jones C. The basic elements of music. Connexions; 2012.

[12] Kraus N, Skoe E, Parbery-Clark A, Ashley R. Experience-induced malleability in neural encoding of pitch, timbre, and timing: Implications for language and music. Ann NY Acad Sci. 2009; 1169(1): 543-57. doi:10.1111/j.1749-6632.2009.04549.x.

[13] Patel AD. A neurobiological strategy for exploring links between emotion recognition in music and speech. Behav Brain Sci. 2008; 31(5): 589-90. doi:10.1017/S0140525X0800544X.

[14] Koelsch S. Neural substrates of processing syntax and semantics in music. Springer Vienna; 2009.

[15] Patel AD. Language, music, syntax and the brain. Nat Neurosci. 2003; 6(7): 674-81. doi:10.1038/nn1082.

[16] Leonard MK, Cai R, Babiak MC, Ren A, Chang EF. The peri-Sylvian cortical network underlying single word repetition revealed by electrocortical stimulation and direct neural recordings. Brain Lang. 2019; 93: 58-72. doi:10.1016/j.bandl.2016.06.001.

[17] American Music Therapy Association. Available at http://www.musictherapy.org. Accessed Aug 2, 2023.

[18] Adamek M, Darrow AA. Music in special education. Silver Spring, MD: American Music Therapy Association; 2005.

[19] Geist K, McCarthy J, Rodgers-Smith A, Porter J. Integrating Music Therapy Services and Speech-Language Therapy Services for Children with Severe Communication Impairments: A Co-Treatment Model. J Instr Psychol. 2008; 35(4): 311-6.

[20] Kouri TA, Winn J. Lexical learning in sung and spoken story script contexts. Child Lang Teach Ther. 2006 Oct;22(3):293-313 doi:10.1191/0265659006ct309xx.

[21] Kouri T, Telander K. Children's reading comprehension and narrative recall in sung and spoken story contexts. Child Lang Teach Ther. 2008; 24(3): 329-49. doi:10.1177/0265659008096296.

[22] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Int Surg J. 2021; 88: 105906.

[23] Tan EY, Shoemark H. Case study: The feasibility of using song to cue expressive language in children with specific language impairment. Music Ther Perspect.. 2017; 35(1): 63-70. doi:10.1093/mtp/miv039.

[24] Groß W, Linden U, Ostermann T. Effects of music therapy in the treatment of children with delayed speech development-results of a pilot study. BMC Complement Altern Med. 2010; 10: 1-0. doi:10.1186/1472-6882-10-39.

[25] Anvari SH, Trainor LJ, Woodside J, Levy BA. Relations among musical skills, phonological processing, and early reading ability in preschool children. J Exp Child Psychol. 2002; 83(2): 111-30. doi:10.1016/S0022-0965(02)00124-8.

[26] Lathroum LM. The role of music perception in predicting phonological awareness in five-and six-year-old children. University of Miami; 2011.

[27] Thaut, M. Rhythm, music, and the brain: Scientific foundations and clinical applications. Taylor & Francis; 2013.

[28] Hazelett M, Wagner B. Specific language impairment (SLI). Muncie, IN: Ball State University; 2002.

[29] Nordoff P, Robbins C. Therapy in Music for Handicapped Children. Barcelona Publishers; 2004.

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COVID-19 transmission due to interplay between $PM_{2.5}$ and weather conditions

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ABSTRACT

Background: The association of air pollution with the COVID-19 pandemic majorly caused respiratory diseases among the major outcomes of COVID-19 infection. In addition, meteorological factors play an important role in spreading COVID-19 infection in humans who have been exposed to air pollutants.

Objectives: This study aims to estimate and comprehend the linkages between the contribution of $PM_{2.5}$ concentrations and meteorological parameters to the spreading coronavirus infection in Gurugram, a badly affected city in India due to the COVID-19 pandemic.

Materials and methods: We employed some statistical analysis on daily average data of $PM_{2.5}$ concentrations and meteorological conditions with daily COVID-19 cases from March 2020 to February 2022. To optimize $PM_{2.5}$ concentrations linked with COVID-19 instances, a time series analysis was performed. The Pearson correlation test investigated the relationships between $PM_{2.5}$ levels, meteorological data, and COVID-19 instances. The PCA was applied to reveal the most significant factor attributable to affecting the rate of COVID-19 transmission in Gurugram.

Results: The highest cases of COVID-19 (250,000) were observed in February 2022 when $PM_{2.5}$ concentration was 286.6µg/m^3, 12.64 °C temperature, 73.81% RH, and 68.265 km/h wind speed while minimum cases (3125) were found in March 2020 with the 18.18µg/m^3 $PM_{2.5}$ concentration, 10.62 °C temperature, 50.05% RH, and 83.295km/h wind speed.

Conclusion: The principal component analysis helped conclude the results, which revealed that the daily COVID-19 cases were significantly positively correlated with $PM_{2.5}$ concentrations, RH, and temperature. However, daily COVID-19 cases were negatively or poorly correlated with wind speed. COVID-19 pandemic is prominently affected by $PM_{2.5,}$ while RH and temperature were found to be important meteorological factors significantly affecting its human-to-human transmission. This study may provide useful indications to regulatory bodies to modify environmental health policies.

Introduction

Recently, a novel coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) appeared in December 2019 and spread rapidly across the world.[1] Later, the World Health Organization (WHO) confirmed the disease as a pandemic.[2] The HCoVs (human coronaviruses) are coined in rodents and bats which are transmitted to human beings through zoonotic interactions. The people infected with SARS-CoV-2 were found with a similarity of 79.5% genetic sequence to SARS-CoV.[3]

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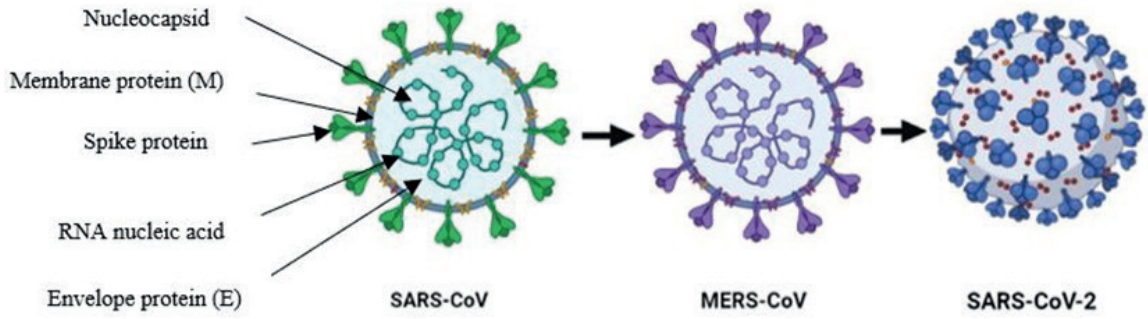

Figure 1. *Evolution of coronavirus.*

The SARS-CoV-2 is structured with long RNA polymers surrounded by nucleocapsid proteins- membrane protein, spike protein, and envelope protein (Figure 1). The Coronavirus is transmitted through contaminated droplets from an infected person, enters cells through spike protein, and interacts with extracellular domains of the transmembrane angiotensin converting enzyme2 (ACE2) proteins for cell surface binding and internalization and subsequently to downregulation of surface ACE2 expression.[4,5] ACE2, a genetic risk factor, acts as a host receptor for SARS-CoV-2 infection and is also responsible for post-infection regulation, such as immune response, cytokine secretion, and viral genome replication.[6] The symptoms of COVID-19 infection include fever, dry cough, tiredness, loss of smell and taste, and mild to severe respiratory illness.

When COVID-19 infection is combined with comorbid conditions, it may cause blood clots or failure of the respiratory system, hypoxemia, renal failure, septic shock, multiple organ failure, and cardiogenic shock, consequent to mortality.[7,8] Gurugram, a highly polluted city in India, was significantly impacted by the COVID-19 pandemic. Previous studies have highlighted the association between $PM_{2.5}$ and respiratory illnesses.[9-11] The air quality index (AQI) across the National Capital Region (NCR) cities of Gurugram stayed mostly between 321 and 426 $\mu g/m^3$ levels in October 2020. Several hospitals were witnessing a spike in recovered COVID patients reporting respiratory complications caused by pollution, such as chronic obstructive pulmonary disease (COPD), recurrence of cough, and breathlessness. With their lungs weakened by COVID-19 infection, the poor air quality has solitary compounded the problem.[5] Hence, we studied the variability of COVID-19 and its relationship between temperature, $PM_{2.5}$ concentrations, relative humidity, and wind speed to evaluate the transmission of coronavirus infection in humans.

Methodology

Site description

Gurugram is a northern Indian city of Haryana state situated at 28.4595° N, 77.0266° E. This satellite city is part of the Delhi NCR of India (Figure 2) and has an area of 232 km^2 with 1,726,452 estimated population.[12] The hot of Gurugram contains distinct seasons, namely pre-monsoon (March-May) with approx. 45% humidity & 27 °C temperature; Monsoon (June-August) is a hot and humid season with approx. 67% humidity & 31 °C temperature; Post-monsoon (September-November) season is pleasant and mild seasons have 36% humidity & 33 °C temperature; and winter (December-February) has 74% humidity and average temperature 22 °C are foggy and cool with few sunny days. Thunderstorms are common during monsoons, with an average annual rainfall of approximately 714 mm.[13] We have considered $PM_{2.5}$ concentrations of the location of sector 51 in Gurugram for our study. One of the main reasons behind the high air pollution level in Gurugram's Sector-51 is dust comes in from various sources, mainly from the intensive construction activities as the city is developing rapidly.

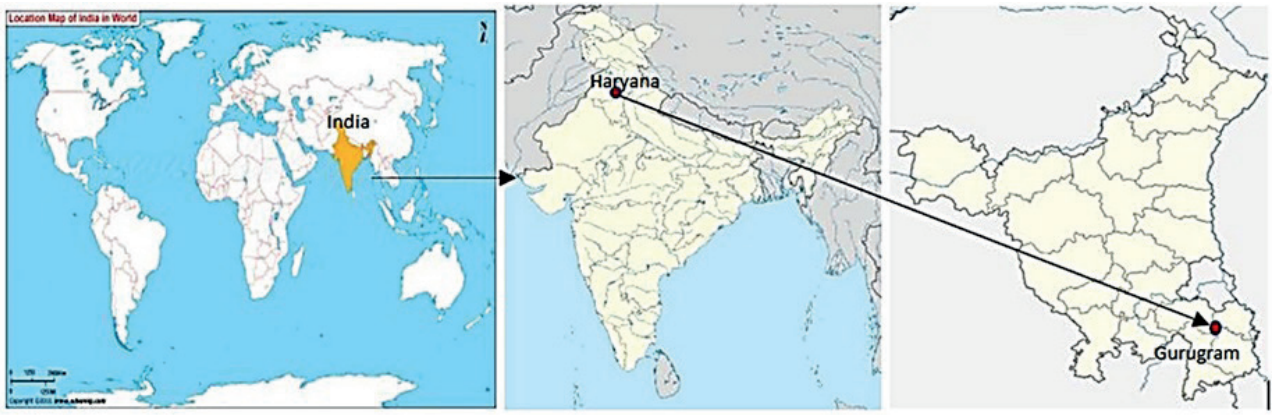

Figure 2. *World map showing India followed by Gurugram and Sector-51.*

Nationwide lockdown is a major factor in distinguishing the impact of $PM_{2.5}$ and weather conditions on COVID-19 cases. The nationwide lockdown was forced four times in the year 2020 to stop the transmission of COVID-19 infection. The phase-wise lockdown periods were based on varied restrictions (Table 1).

Table 1. Different phases of lockdown periods and COVID-19 cases (%) in Gurugram.

Phase	Duration	COVID-19 cases (%) of total population in Gurugram
Phase 1	25 March 2020 - 14 April 2020 (21 days)	1%
Phase 2	15 April 2020 - 03 May 2020 (19 days)	2%
Phase 3	04 May 2020 - 17 May 2020 (14 days)	5%
Phase 4	18 May 2020 - 31 May 2020 (14 days)	11%

Data availability, collection, and processing

Daily average $PM_{2.5}$ data for Sector-51, Gurugram, was obtained from the website of the Central Pollution Control Board from 01 March 2020 to 28 February 2022.[14] Meteorological data in the metropolitan region, viz. temperature, relative humidity, and wind speed were also downloaded from the website of the central pollution control board for the same period. Daily COVID-19 cases in Gurugram from 01 March 2020 to 28 February 2022 were obtained from the Health Bulletin of Govt. of India.[15] Unlock 1.0 began on 01 June 2020, while Unlock2.0 on 01 March 2022 in India. Both lockdown and normal periods were analyzed separately to compare the effects.

Time series analysis was conducted to examine the variability of COVID-19 confirmed cases and $PM_{2.5}$ concentrations, along with their monthly linkages, i.e., the meteorological parameters. The variability was observed in four seasons; namely, pre-monsoon includes months of March-May (MAM); monsoon=June-August (JJA); post-monsoon=September-November (SON); winter= December-February (DJF). The relationship between $PM_{2.5}$ and COVID-19 cases were determined by analyzing the daily mean $PM_{2.5}$ concentrations and daily COVID-19 confirmed cases using the Pearson correlation test. The statistical analyses and Pearson correlation tests were performed using SPSS 22.0 (IBM SPSS Statistics 22.0). The significance threshold was set at $p<0.05$ and $p<0.01$. Further, to understand the effect of meteorological variables on COVID-19 transmission in Gurugram, which is surrounded by diverse geographical situations, we have conducted Principal Component Analysis (PCA) by using R-software. PCA accentuates the variation and carries out the significant patterns of the data concerned. PCA is mostly employed to lessen the dimensions of the dataset, which contains interrelated variables, by transforming it into a new set of independent variables called Principal Components (PCs). The PCs are further rotated by PCs varimax rotation to obtain a better relationship between the variables and the original dataset. When varimax rotation is performed, it ensures that each variable is maximally associated with one single component and has zero correlation with other components.[16]

Results and Discussion

Time series analysis of $PM_{2.5}$ concentrations, COVID-19 cases, and meteorological variables

Time series of COVID-19 cases, relative humidity, temperature, and correlated $PM_{2.5}$ pollutants are shown in Figure 3 to better comprehend the variability in COVID-19 cases due to daily $PM_{2.5}$ concentrations and meteorological variables across the region over the study period. At the onset of the COVID-19 pandemic in March 2020, the number of cases was lower than in September. However, in the last month of 2020, the average rise in COVID-19 cases was noted. A linear increase in COVID-19 cases was observed over the year. Additionally, the $PM_{2.5}$ variability is influenced, and a modest decrease in $PM_{2.5}$ is noticed till August 2020 due to the obvious reason of lockdown. However, there was a steady increase in $PM_{2.5}$ concentrations during the winter- December, January, and February (DJF) months. The linear trend of $PM_{2.5}$ for 2020 indicates that $PM_{2.5}$ levels in the region continue to

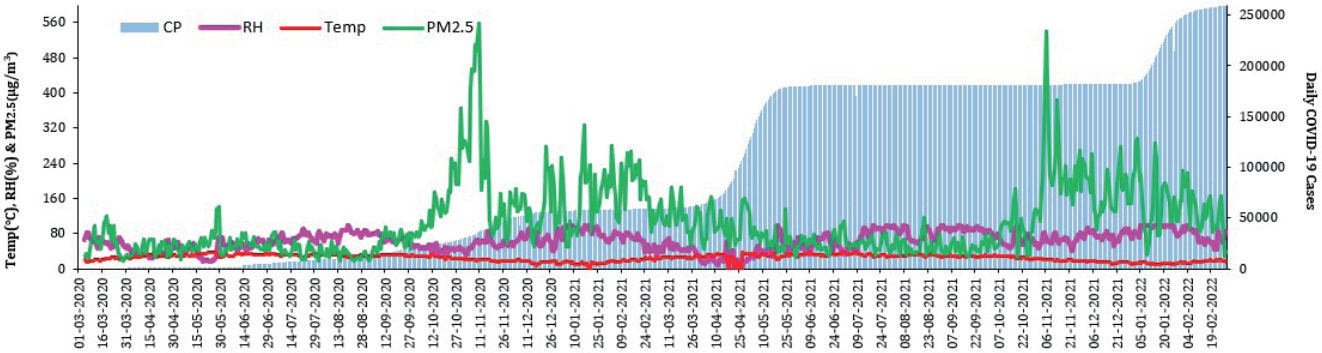

Figure 3. *Time series analysis of $PM_{2.5}$, COVID-19 cases, and meteorological variables in Gurugram.*

rise in the peak months. In the second wave of COVID-19 in 2021, the meteorological parameters also exhibited seasonal variability throughout the year, whereas $PM_{2.5}$ showed sharp peaks in October and November for 2020 and 2021. In 2020, there may be a statistically significant link between the COVID-19 number of cases and $PM_{2.5}$ concentrations for the study region. From April to May, due to lockdown, the cases were diminished. Most of the cases were reported in November and December 2020. In August, the cases crossed 10,000 trademarks. There is a sharp increase in $PM_{2.5}$ concentration (559.31 µg/m^3), lowering the temperature (20.23 °C) and RH (33.41), indicating the correlation with rising cases of COVID-19 (39933) on 27 October 2020. A similar sharp increase can also be seen on 06 November 2021 with (539.34 µg/m^3) level of $PM_{2.5}$, temperature (19.41 °C), and RH (55.13). With increasing $PM_{2.5}$ concentration in Gurugram, due to dust coming from different sources, coronavirus cases have also been rising. Pati also reported the average air quality index (AQI) of Gurugram for November was higher this year (2021) compared to the previous two-357 (upper levels of very poor) this time, up from 303 (very poor threshold level) in November 2020 and 273 (poor) in November 2019.[17] Wind direction and local sources also play an important part in spreading the virus. A recent study has found that the virus can remain viable in the air for multiple hours. Thus, the spread of the virus can be affected by wind conditions such as wind speed and direction. Ambient wind will enhance the complexity of the secondary flows with recirculation between the two virtual humans. Microdroplets follow the airflow streamlines well and deposit on human bodies and head regions, even with the 3.05 m (10 ft) separation distance.[18] The rest of the microdroplets can transport in the air farther than 3.05 m (10 ft) due to wind convection, causing a potential health risk to nearby people.[16] According to the Haryana State Pollution Control Board (HSPCB), the city was witnessing the worst AQI level in the past five years.[15]

Seasonal variations between $PM_{2.5}$ concentrations, COVID-19 cases, and meteorological variables

Further, we investigated the association between COVID-19 cases and the variability of $PM_{2.5}$ concentrations during all seasons for Gurugram. To understand the seasonal contribution towards $PM_{2.5}$ concentrations and associated COVID-19 cases, the cumulative seasonal mean was computed and plotted (Figure 4). During the monsoon (JJA) season, a smaller number of COVID-19 cases (619) was observed whereas, post-monsoon and winter seasons are dominated by the increased number of COVID-19 cases (250000) with elevated (559.31 µg/m^3) $PM_{2.5}$ concentrations. The analysis was compared with another location of Gurugram, i.e., Teri Gram, where no linear variability was noted in the COVID-19 cases at both locations. Although the concentration of $PM_{2.5}$ differs in post-monsoon and winter seasons, the highest $PM_{2.5}$ (467.28 µg/m^3) concentrations were observed at the Teri gram location compared with Sec 51 (figure not shown). Prime Minister of India, Mr. Narendra Modi announced the Janta curfew in March 2020. Due to this, the $PM_{2.5}$ reduction was 8% on the curfew day but declined to 34% the next day, owing to negligible combustion activities in March 2020 in and around the city, resulting in a decline in COVID cases. The COVID positivity rate rages when Unlock 1.0 begins in June 2020. High humidity accelerates the release of hazardous substances into the atmosphere. Rising temperatures also contribute to increased ground-level ozone smog, which worsens air pollution.[3,19]

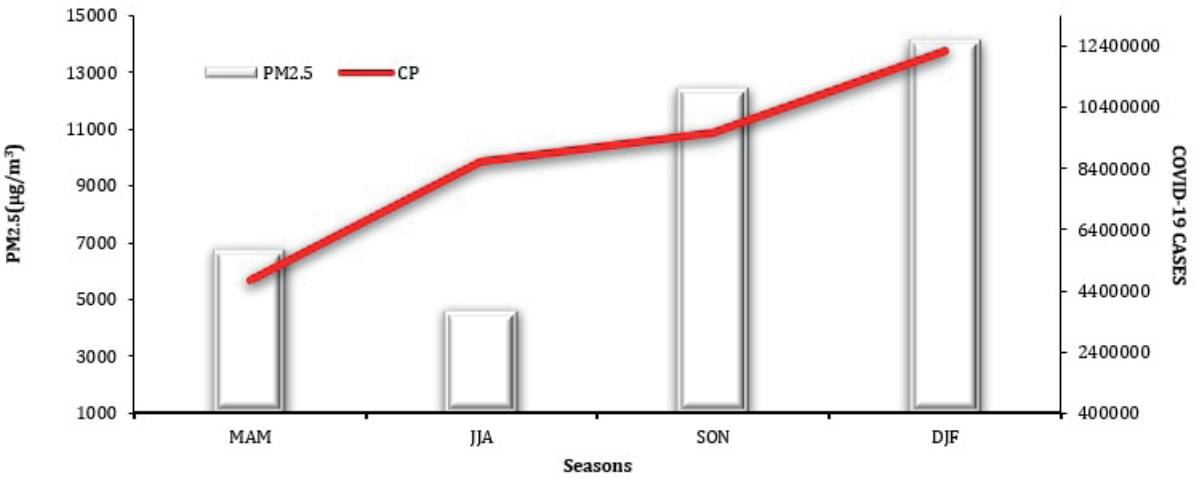

Figure 4. *Seasonal variations of $PM_{2.5}$ concentrations and COVID-19 cases.*

Table 2. Seasonal regression values of COVID-19 patients and $PM_{2.5}$ concentrations.

Seasons	R-values
March, April, May (MAM2020)	0.215
June, July, August (JJA2020)	-0.536**
September October November (SON2020)	0.131
December January February (DJF2020-21)	0.233*
March, April, May (MAM2021)	-0.558**
June, July, August (JJA2021)	0.013
September, October, November (SON2021)	0.716**
December, January, February (DJF2021-22)	-0.355**

*** Significant at 0.01 level * Significant at 0.05 level*

In the year 2021, the number of COVID-19 cases decreased in the first months of the year due to several government-imposed restrictions, and as the second wave of COVID-19 was seen in the pre-monsoon (MAM) season and consistent daily cumulative values throughout the year. In JJA, there was a decrease in $PM_{2.5}$ concentrations and COVID-19 instances. During the summer, meteorological conditions such as increased temperature, higher wind speeds, and improved atmospheric dispersion can contribute to lower PM levels, resulting in lower COVID-19 cases. A slight peak of COVID-19 cases and $PM_{2.5}$ levels is observed in SON 2021 (r=0.716**), followed by an exponential rise in DJF (r=-0.355**), as mentioned in Table 2. COVID-19 cases have risen in winter because colder weather often drives people indoors, with less ventilation and increased proximity to others. Indoor settings, especially in poorly ventilated spaces, can facilitate the spread of the virus through close contact and respiratory droplets.

$PM_{2.5}$ levels increased slightly in the winter and pre-monsoon seasons of 2020 and 2021, which could be owing to a relaxation in the strict lockdown. $PM_{2.5}$ seasonality was seen in both years, with less $PM_{2.5}$ concentration (19.97 µg/m^3) in the season of monsoon and more (559.31 µg/m^3) in the post-monsoon and in winter seasons (326.28 µg/m^3).

Correlations between $PM_{2.5}$ concentrations, COVID-19 cases, and meteorological variables

We employed the Pearson correlation technique for the study area to identify the relation between daily cases of COVID-19 and $PM_{2.5}$ concentrations. Further, we divided the number of COVID-19 cases into different seasons to know the seasonal variability and their association with $PM_{2.5}$. As COVID-19 hit India in March 2020, we obtained the data of COVID-19 cases from March 2020 to February 2022. Correlations were tested at 0.01 and 0.05 levels of significance.

A negative correlation (r=-0.536) between the number of COVID-19 cases and $PM_{2.5}$ was noted in the monsoon season (JJA) of the year 2020 for Gurugram, which could be due to the reduction of $PM_{2.5}$ level by precipitation and subsequent removal of the pollutants. On the other hand, $PM_{2.5}$ and COVID-19 cases were correlated as positively significant (r=0.233) in the winter season (DJF) of the year 2020 (Table 2). During the winter season, a lot of biomass burning is practiced in the North-western parts of the state, increasing pollutant levels over the neighboring regions. This could be the major factor in finding a positive association between COVID-19 cases and $PM_{2.5}$ concentrations. However, Zoran *et al*. observed the strong influence of daily average particulate matter concentrations with the positive association of average surface air temperature and inversely related to air relative humidity on the COVID-19 cases outbreak in Milan. Being a novel pandemic coronavirus version, COVID-19 might be ongoing during summer conditions associated with higher temperatures and low humidity levels.[11,20] In the second wave of the COVID-19 pandemic in the pre-monsoon (MAM) season in 2021, we noticed a seasonal shift of relation in comparison with the year 2020. A significant negative relation (r=-0.558) was observed in the pre-monsoon season. In contrast, the post-monsoon season showed strong positive relations (r=0.716) between COVID-19 cases and $PM_{2.5}$ concentrations, and surprisingly, negative correlations were noted in the winter season of 2021. An early lockdown was associated with a lower-case count in this season.

Recent evidence corroborates our results, such as the daily cases of COVID-19 infection were significantly positively correlated (r=0.46) with absolute humidity in Delhi, Mumbai, and Pune. At the same time, a strong negative correlation was found with the minimum temperature in Ahmedabad (r=0.38).[21] Thus, lower temperatures and high humidity were responsible for the increased rate of COVID-19 spread throughout the city. Particulate matter pollution is positively correlated with a rise in cases of COVID-19 and increased mortality rates.[20] Additionally, our study also reveals that $PM_{2.5}$ concentrations are significantly associated with COVID-19 cases in Gurugram. Cole et al. also observed that a 1 µg/m^3 rise in $PM_{2.5}$ concentrations is 9.4 times more COVID-19 cases and 2.3 times more deaths.[21] Chinese people living in a highly polluted zone were more prone to die from SARS as compared to someone living in a region with cleaner air.[22] Researchers analyzed that several viruses, including influenza and adenovirus, can be loaded on air particles.[16] Zhao *et al.* concluded that particulate matter was a reason behind the spread of 2015 avian influenza.[23] Particulate pollution can accelerate the spread of respiratory infections and elevate the mortality risk.[12] Higher PM

concentrations of air pollution may favor the SARS-CoV-2 spread. Untangling the role of particulate matter in air contamination in the spread of the virus is thus crucial and urgent.[23]

Principal component analysis (PCA)

We have determined the Pearson correlations between COVID-19 cases and $PM_{2.5}$ concentrations and meteorological parameters. Still, a robust and critical analysis is required to understand year-to-year seasonal variability and accountable attributes. Therefore, we applied principal component analysis (PCA) and visualized the patterns and correlations between the daily COVID-19 cases, $PM_{2.5}$ concentrations, and meteorological variables (temperature, RH, wind speed). The PCA was performed on a correlation matrix after measuring the data sets on varying scales, and variables were standardized before the PCA was applied.[22] Eigenvalues of all principal components (PCs) were determined for both years. Eigenvalues assist in establishing the number of PCs carried out for interpretation. As per Kaiser's Rule, PCs having an eigenvalue less than one cannot be interpreted due to carrying insignificant information.[23] Therefore, out of 5 PCs, only two having eigenvalues of more than one was selected for further processing. The Eigenvalues and accumulated variance of selected PCs for the years 2020 and 2021 are mentioned in Table 3. Further, the PCs were imperiled to varimax rotation and generated factor loadings matrix for interpretation. The rotation of PCs ensures equal spreading of the significance between the factors (Figure 5).

We have visualized the patterns and correlations between the daily COVID-19 cases, $PM_{2.5}$ concentrations, and meteorological variables- temperature, RH, and wind speed. The summary plots show the collective variance of the PCs through loading factors for the years 2020 and 2021 (Figure 5). In 2020, the eigenvalue (2.59) of PC1 with 51.78% variance indicates strong positive factor loadings for $PM_{2.5}$, RH, and daily COVID-19 cases. However, PC1, having a 2.08 eigenvalue with 41.5% variance for the year 2021, shows obvious positive factor loadings for $PM_{2.5}$, RH, and daily cases of COVID-19 but slight positive factor loadings for wind speed also observed (Figure 6). A significant correlation was seen between relative humidity, temperature, and daily cases of COVID-19 in November and December 2020. Figure 5a and Figure 5b depicts the strongly correlated variables with each other. It could be interpreted that the daily COVID-19 cases were

Table 3. Eigenvalue and variability of Principal components (PC) reflecting $PM_{2.5}$ concentrations, meteorological parameters, and daily cases of COVID-19 infection.

Year	2020		2021	
	PC1	PC2	PC1	PC2
Eigenvalue	2.5892	1.0809	2.0774	1.3515
Variability (%)	51.784	21.619	41.547	27.03
Cumulative variability %	51.784	73.403	41.547	68.577

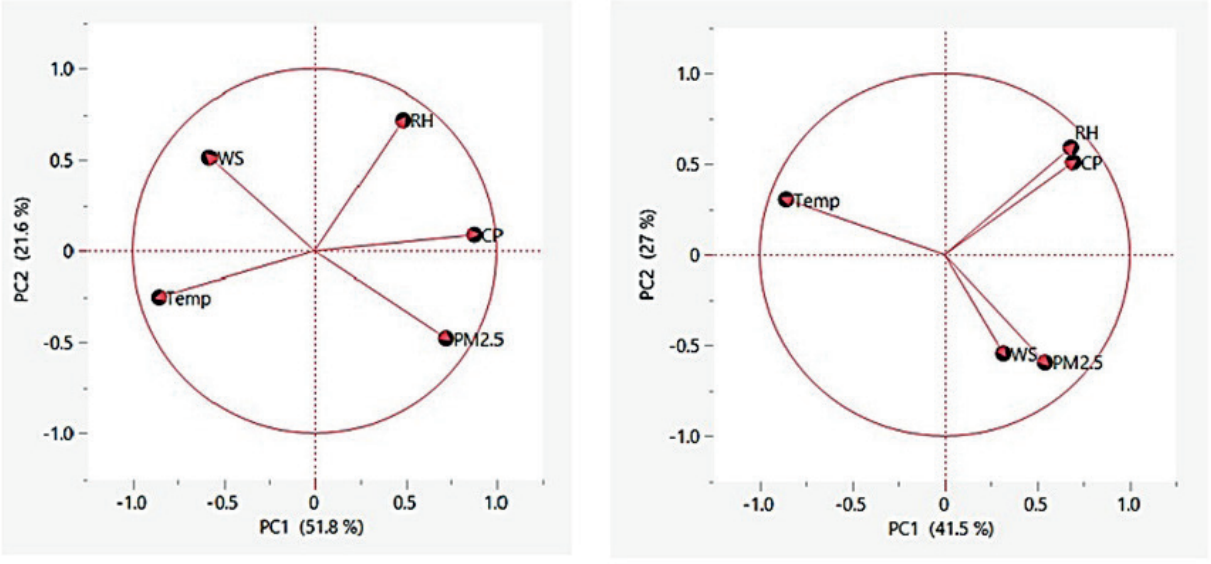

Figure 5. *Summary plots showing projections of $PM_{2.5}$ concentrations, meteorological parameters, and COVID-19 cases based on PC1 and PC2 for the (a) year 2020 and (b) year 2021.*

significantly correlated with $PM_{2.5}$ concentrations, RH, in both years and poorly correlated with wind speed in the year 2021. However, a negative correlation was noticed between daily cases of COVID-19 and wind speed. In an ambient environment, wind speed plays an important role in diluting and removing the droplets that may reduce the viral load in the air, consequently reducing COVID-19 transmission.[24] Zoran et al. also reported that $PM_{2.5}$ and wind speed show a weak association with respiratory diseases but a strong correlation with increasing temperature and decreasing humidity.[11] The temperature in 2021 was positively correlated with COVID-19 cases, but there was a negative correlation observed in 2020, which implies that lower temperature enhanced transmission. Anand et al. also suggested that the meteorological variables, such as relative humidity and absolute humidity, showed a moderate positive correlation with the daily COVID-19 cases in three cities.[12] PCA analysis revealed that COVID-19 cases are closely correlated with humidity.[25] The same pattern was seen in the year 2021. Thus, PCA concluded that daily cases of COVID-19 were strongly correlated with the $PM_{2.5}$ concentrations, temperature, and relative humidity in the years 2020 and 2021.

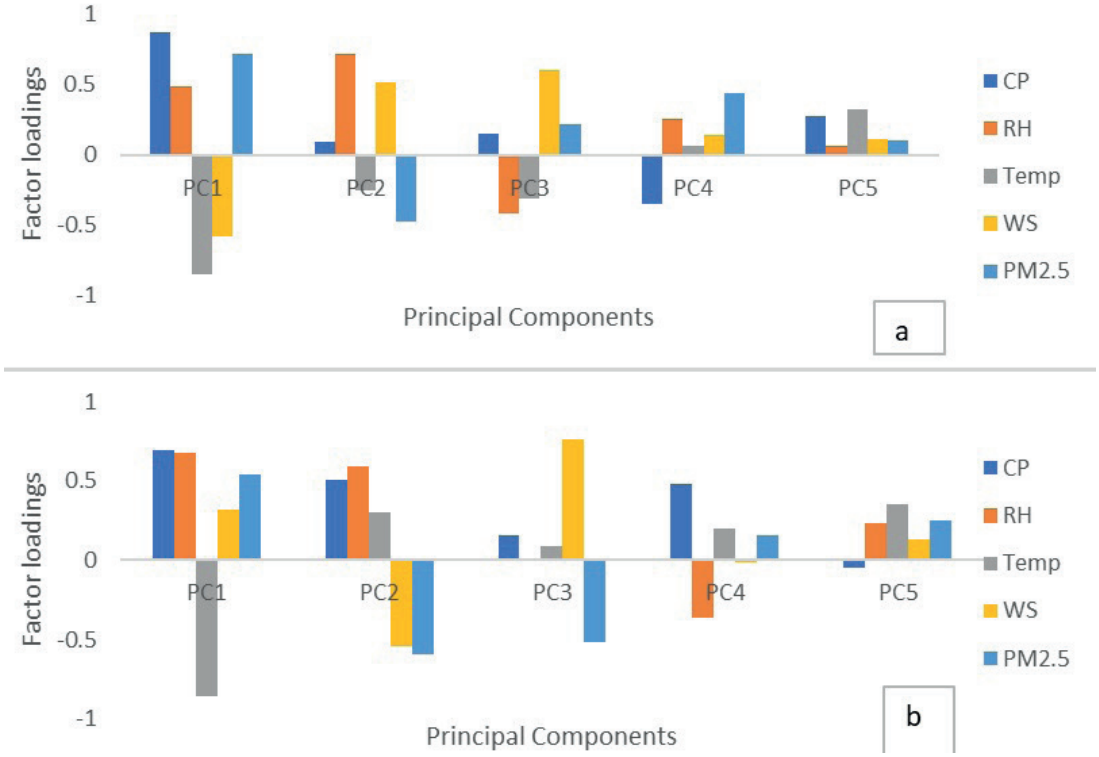

Figure 6. *Factor loadings after varimax rotation for the years (a) 2020 and (b)2021.*

Conclusion

Our results indicate that exposure to high levels of $PM_{2.5}$ pollutants in association with meteorological factors may enhance vulnerability. Consequently, people may be affected by the COVID-19 infection. The maximum COVID-19 cases (33%) were observed with 286.6 µg/m^3 $PM_{2.5}$ concentration, 68.265 km/h wind speed, 73.81% RH, and 12.64 °C temperature in the winter season. Minimum COVID-19 cases (1.39%) were reported in the pre-monsoon season with 18.18µg/m^3 $PM_{2.5}$ concentration, 83.295 km/h wind speed, 50.05% RH, and 10.62 °C Temperature. Hence, this study reveals the positive significant association between high $PM_{2.5}$ concentrations, meteorological factors, and COVID-19 cases in Gurugram.

The consequences of $PM_{2.5}$ indicate that the limited $PM_{2.5}$ exposure will contribute to defeating the COVID-19 pandemic. Green environmental strategies should be encouraged as these would safeguard human beings who are vulnerable to the COVID-19 pandemic. Also, it is suggested that further analyses with subsequent clinical studies should be conducted in other parts of India, applying robust techniques to identify the contribution of other pollutants in spreading the COVID-19 pandemic.

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Conflict of interest

The authors have no conflict of interest relevant to this article.

References

[1] Satarker S, Nampoothiri M. Structural proteins in severe acute respiratory syndrome coronavirus 2. Arch Med Res. 2020; 51(3): 482-91. doi: 10.1016/j.

arcmed.2020.05.012.

[2] World Health Organization (WHO). Daily cases and deaths by date reported to WHO. 2023 [cited 2023 January 03]. Available from: https://covid19.who.int.

[3] Malik YA. Properties of coronavirus and SARS-CoV-2. Malays J Pathol. 2020; 42(3): 5-11. PMID: 32342926.

[4] Guruprasad L. Human coronavirus spike protein-host receptor recognition. Prog Biophys Mol Biol. 2021; 161(9): 39-53. doi: 10.1016/j.pbiomolbio.2020.10.006.

[5] Choudhary A, Sreenivasulu K, Mitra P, Misra S, Sharma P. Role of genetic variants and gene expression in the susceptibility and severity of COVID-19. Ann Lab Med. 2021; 41(2): 129-38. doi:10.3343/alm.2021.41.2.129.

[6] Upadhyay E, Mohammad AA, Dasgupta N, Rahman S, Kim J, Datta M. Assessment of occupational health hazards due to particulate matter originated from spices. Int J Environ Res Public Health. 2019; 16(6): 15-9. doi: 10.3390/ijerph16091519.

[7] Biswas, K. Chatterjee, A. Chakraborty, J. Comparison of air pollutants between Kolkata and Siliguri, India, and its relationship to temperature change. J Geovis Spat Anal. 2020; 4(7): 1-15. doi:10.1007/s41651-020-00065-4.

[8] Kumar A, Rana S. Population abundance of Greater Flamingo Phoenicopterus roseus (Aves: Phoenicopteridae) in district Gurugram of Haryana, India. J Threat Taxa. 2022; 14(7): 20821-7. doi:10.11609/jott.2785.8.7.8953-8969.

[9] Travaglio MY, Popovic R, Selley L, Leal NS, Martins LM. Links between air pollution and COVID-19 in England. Environ Poll. 2021; 268(2): 115855-9. doi: 10.1016/j.envpol.2020.115859.

[10] Upadhyay E. An Introduction to Air Quality Index and Health Concerns. Bio Evolution. 2014; 1(4): 51-4. ISBN 978-81-925781-3-2.

[11] Zoran MA, Savastru RS, Savastru DM, Tautan MN. Assessing the relationship between surface levels of $PM_{2.5}$ and PM_{10} particulate matter impact on COVID-19 in Milan, Italy. Sci Total Environ. 2020; 738: 139825. doi: 10.1016/j.scitotenv.2020.139825.

[12] Anand V, Korhale N, Tikle S, Rawat MS, Beig G. Is Meteorology a factor to COVID-19 Spread in a Tropical Climate? Earth Syst Environ. 2021; 5(2): 939-48. doi:10.1007/s41748-021-00253-2.

[13] Climate & weather averages in Gurgaon, Haryana, India. [cited 2023 April 29]. Available from: www.timeanddate.com/weather/india/gurgaon/climate.

[14] CPCB. Air quality monitoring, emission inventory and source apportionment study for Indian cities, New Delhi. 2022: 1-658. [cited 2022 December 12]. Available from: http://cpcb.nic.in/Delhi.pdf.

[15] Health Bulletin District Gurugram for COVID-19. National Informatics Centre, Ministry of Electronics & Information Technology, Government of India. 2022. [cited 2022 April 29]: 1-702. Available from: https://gurugram.gov.in/health-bulletin.

[16] Wang B, Li R, Lu Z, Huang Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. Aging. 2020; 5(12): 60-49. doi: 10.18632/aging.103000.

[17] Pati. City's average AQI in November this year worse than last two years. 2022. [cited 2022 September 21]. Available from: http://timesofindia.indiatimes.com/articleshow/88017474.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst.

[18] Feng Y, Marchal T, Sperry T, Yi H. Influence of wind and relative humidity on the social distancing effectiveness to prevent COVID-19 airborne transmission: A numerical study. J Aerosol Sci. 2020; 147: 105585. doi: 10.1016/j.jaerosci.2020.105585.

[19] Kim KH, Kabir E, Ara JS. A review of the consequences of global climate change on human health. J Environ Sci Hlth, Part C. 2014; 32(3): 299-318. doi:10.1080/10590501.2014.941279.

[20] Liu Y, Gayle A, Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med. 2020; 115(3): 55-80. doi: 10.1093/jtm/taaa021.

[21] Cole MA, Ozgen C, Strobl E. Air pollution exposure and COVID-19 in Dutch municipalities. Environ Resour Econ. 2020; 76(2): 581-610. doi: 10.1007/s10640-020-00491-4.

[22] Ortiz A, Fernández L, Sawalha AH. Genetic variability in the expression of the SARS-CoV-2 host cell entry factors across populations. Genes Immun. 2020; 21(3): 269-72. doi: 10.1038/s41435-020-0107-7.

[23] Zhao P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(2): 270-3. doi:10.1038/s41586-020-2012-7.

[24] Chen PM, Hemmen TM. Evolving healthcare delivery in neurology during the coronavirus disease 2019 (COVID-19) pandemic. Front Neurol. 2020; 28(11): 571-8. doi: 10.3389/fneur.2020.00578.

[25] Daraei H, Toolabian K, Kazempour M, Javanbakht M. The role of the environment and its pollution in the prevalence of COVID-19. J Infect. 2020; 8(2): 168-9. doi: 10.1016/j.jinf.2020.06.019.

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Working memory program for improving language skills in older adults with mild neurocognitive disorders: A pilot study

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ABSTRACT

Background: Older adults with mild neurocognitive disorders (mild NCD) have a higher risk of major NCD. Cognitive deterioration can cause a deficit in working memory and language. Recent studies have shown some involvement overlapping the brain structure of working memory and language skills. Therefore, working memory intervention effectively enhances language skills in mild NCD. However, more research on this topic in Thailand is required.

Objectives: This study aimed to develop and implement a pilot study on a working memory program for improving language skills in older adults with mild NCD.

Materials and methods: This study was a developmental research design with two phases. Phase one involved the development and examination of the content validity of the working memory program for improving language skills by five experts. Phase two involved piloting the program with three older adults with mild NCD. The participants were asked to provide suggestions about the clarity of content and images, language usage, font size, the comprehensibility of instructions in each activity, and the quality of audio files used to complement the program activities through semi-structured interviews. Descriptive statistics were employed to analyze the collected data.

Results: The program comprised nine activities associated with the phonological loop, visual-spatial sketchpad, episodic buffer, and central executive tasks. The program's content validity was evaluated by five experts, resulting in a content validity index of 0.94, meeting the established criteria. Among the pilot users, three participants meeting the specified criteria indicated their ability to use and practice the program at home effectively. While most participants agreed that the images were clear and the font size was appropriate, there were concerns regarding the clarity of the training process steps and instructions. Additionally, some participants encountered challenges in accessing audio files through quick response (QR) codes.

Conclusion: In summary, the pilot study of the working memory program for improving language skills in older adults with mild NCD passed the content validity test and underwent revisions based on suggestions from the pilot users. Consequently, the program could enhance the language abilities of older adults with mild NCD. The next phase will investigate its effectiveness in improving language skills in older adults with mild NCD.

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Introduction

Mild neurocognitive disorders (mild NCD) is a term recently introduced to replace mild cognitive impairment (MCI) in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) published by the American Psychiatric Association (APA) in 2013.[1] Mild NCD is a progressive neurocognitive condition that can progress into major NCD. During the transitional phase, this condition may affect one or more cognitive functions, including complex attention, executive function, learning and memory, language, perceptual-motor skills, and social cognition.[2] Global studies conducted in 2012 and 2015 indicated a wide range of MCI prevalence rates, varying from 3% to 42% and 5% to 36.7%, respectively-prevalence of MCI in older individuals at 71.4%, which increased with age.[4]

In 2019, McCullough et al.[5] investigated language impairments in older adults with mild NCD. They found impairments in receptive language skills, such as comprehension from reading and listening, repetition, and language expression skills, including fluent speech, word recall, and concept definition. They also proposed a language skill rehabilitation approach involving working memory training to enhance neuroplasticity based on the underlying structure and functioning of working memory and overlapping language skills.[6] Additionally, Huntley and Howard's[7] study revealed that working memory deficits occur in the early stages of mild NCD, suggesting that working memory performance can indicate the progression of cognitive decline.

Working memory (WM) is the ability to hold and manipulate information while performing various daily tasks involving short-term storage and information processing. As a multi-component system, WM has a limited capacity to store information and allocate attention to different processes temporarily. It is closely linked to executive functioning, encompassing attention allocation, information selection, inhibition, and updating.[8] In 2000, Baddeley proposed a multi-component model of WM consisting of four components: the central executive, phonological loop, visual-spatial sketchpad, and episodic buffer.[9] The central executive is an attentional control system, consciously processing, manipulating, and storing information from other subsystems while regulating actions. The phonological loop temporarily stores and manipulates verbal information through articulatory rehearsal.

Similarly, the visual-spatial sketchpad operates but with different types of information and mechanisms, including visual, spatial, kinesthetic, and image decoding. Finally, the episodic buffer facilitates interaction among WM subsystems, their connection with long-term memory, and perception. Moreover, WM is also involved in retrieving information from long-term memory and language skills, especially comprehension from listening, reading, and verbal communication. For instance, during a conversation, individuals need to remember spoken words, organize the information, and retrieve data from long-term memory to process responses and engage in dialogue.[10] Deficiencies in working memory can lead to language and communication impairments in various aspects, such as listening, reading, language processing, understanding meaning, and complex syntax. This is because there are deficits in manipulating and processing incoming data.[11,12] Borella *et al*.'s study explored WM capacity in individuals aged 20 to 86 years and found that it declines with age.[13] Furthermore, they observed that lower WM capacity is associated with shorter, more straightforward phrases, less complex grammatical structures, and more error word selection and meaning.

As mentioned above, studies have applied the concept of WM to design activities for training healthy older adults and older adults with cognitive disorders. These activities, presented in formats like paper-pencil tasks and computer programs, were based on Baddeley's WM model. The applied activities involve listening and reading comprehension, verbal fluency, and engaging with images and texts. The study by Lee and Kim[14] investigated the effectiveness of WM training programs on language skills in older adults with mild to moderate cognitive impairment. They applied activities based on Baddeley's WM model and found that the WM training program had positively affected language skills. The participants demonstrated improved abilities in fluent speech, naming objects, defining words, and describing images compared to their performance before undergoing the training program. Likewise, Payne and Stine-Morrow[15] investigated the effectiveness of a WM training program called iTrain in enhancing language comprehension in older adults. This program involved computer-based training conducted at home, with 30-min sessions five days a week for three weeks (15 sessions). The findings revealed that the training's effectiveness extended to untrained language tasks, improving sentence comprehension and understanding of complex grammar.

There has been limited research on using WM training programs to promote language skills in Thailand's older adults with mild NCD. We designed a program based on Baddeley's WM model to address this research gap. It includes an exercise workbook and an answer key book, providing flexibility for older adults to self-train at home. The program aims to empower older adults with mild NCD to enhance their language skills and integrate them into their daily lives.

Materials and method

This study was divided into two phases: 1) To develop the program and examine content validity, and 2) to implement a pilot study of the program. Researchers developed and assessed it with three pilot users from May to October 2022 at a speech therapy clinic, Faculty of Associated Medical Sciences, Chiang Mai University.

Study design

This research was developmental research. The study aimed to develop a working memory program for improving language skills and investigate its feasibility for use with older adults with mild NCD.

Phase 1: Development of a working memory program for improving language skills

The researchers studied the theories and principles of speech training in older adults with mild NCD, knowledge about WM, and the development of WM programs to enhance language skills in older adults with mild NCD. This research was conducted by reviewing relevant literature and receiving guidance from experts specializing in cognitive rehabilitation in older adults with mild NCD. The activities are categorized according to the complexity of components based on Baddeley's WM model, activities based on Lee and Kim's study and Zimmermann *et al*.'s study.[14,16] The activities are divided into groups: Group 1, the phonological loop and visuospatial sketchpad, and Group 2, the central executive and episodic buffer. The program uses vocabulary from primary school word lists (grades 1-6) and adapted passages from a youth-oriented Thai encyclopedia for older adults with mild NCD. This selection ensured that the vocabulary consisted of familiar words used in daily life and was easily understandable. Accordingly, language and speech development in children at the primary education level occurs when they have an adequate vocabulary for communication and a grammatical structure similar to that of adults.[17] Hence, the designated content comprised uncomplicated vocabulary and everyday life scenarios. Moreover, the content of the activities was chosen based on Thai culture, language, and environment-activities and detail (Table 1).

Table 1. Description of activities on the working memory program on language skills[14,16]

Component of working memory	Activity	Description
Visuospatial sketchpad/ Phonological loop	Word list	Say and write the list of words presented.
	Picture list	Remember the presented picture and recall it by writing.
	Verbal fluency	Write the words of specific categories.
Episodic buffer/ Central executive	Missing item	Nine figures were shown in the 3*3 table. Next page, all figures remained in the same position, including the missing figure. Participants were asked to identify the missing figures.
	Day task/months task	At least three days or three months were presented in different sequences in a calendar on the page. Participants should order them mentally in calendar orders and write on the next page.
	Odd or even numbers of syllables in the word	Words were presented. Participants should mentally count the number of syllables in each and decide that word odd or even number
	Category span task	Present with a semantic category. Choose words that match the topic.
	Reading and recognition	Read a story and answer questions.
	Images and text	Read and remember text-related pictures, then fill in the text on the next page

We designed the program with paper-pencil activities for easy access, and participants could practice at their homes. The program consisted of two books:

1) Exercise Workbook: Part 1) provided an outline of the program. The training duration is six weeks, and each activity consists of six sets of exercises arranged in order of increasing difficulty, except for verbal fluency, which includes 18 exercise sets. This allows participants to recall vocabulary at the end of each training activity. Part 2) gave detailed instructions for each activity and training methods according to the program with sample activities. Part 3) Worksheets for participants to record activity performance.
2) Answer key book containing Part 4) the answer keys for the exercises.

Content validity

After The WM program for improving language skills had been developed, five experts (who had at least five years of specialization in cognitive-language rehabilitation) examined the WM program i.e., one geriatric psychiatrist, one lecturer in the field of language impairment in the older adults from The Faculty of Medicine Ramathibodi Hospital, Mahidol University. Additionally, one lecturer in occupational therapy for cognitive impairment from The Faculty of Associated Medical Sciences, Chiang Mai University, and two speech and language pathologists. The item-objective congruence (IOC) was IOC >0.5 and passed the criteria. The flowchart of Phase 1 can be seen in Figure 1.

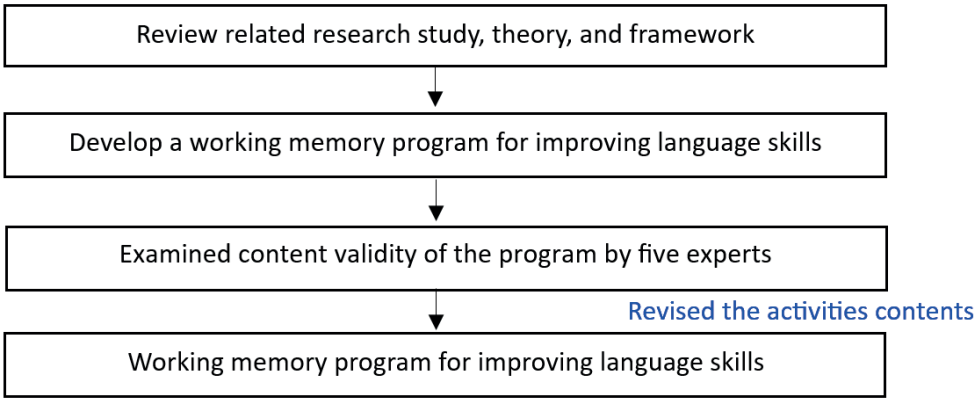

Figure 1. *Flow chart of the development process of working memory for improving language skills.*

Phase 2: trial of the working memory program for improving language skills with three pilot users

This phase aimed to investigate the usage feasibility of a working memory program for improving language skills in older adults with mild NCD. We revised the program contents following the experts' advice. Before the one-week pilot in October 2022 at a speech therapy clinic, Faculty of Associated Medical Sciences, Chiang Mai University, with three older adults with mild NCD. The inclusion criteria were (1) aged 60-79 years, (2) mild NCD diagnosis based on DSM-5 criteria and determined by a neurologist or geriatric psychiatrist, (3) no dementia, according to the Thai version of Mini-Cog,[18] 4) a cut-off score of 24 for mild NCD according to the Montreal Cognitive Assessment-Basic Thai version (MoCA-B-Thai),[19] 5) being independent when performing the Thai version of the Barthel Activities of Daily Living Index (Barthel ADL Index)[20], scale scores of ≥12, (6) not suffering from depression according to a cut-off score <6 on the Thai version of the 15-item Geriatric Depression Scale (TGDS-15),[21,22] (all of the instruments were screened by speech and language pathologists with more than five years of experience) and (7) spoke the Thai language and literate. The exclusion criteria were (1) a history of psychiatric disorders, (2) cerebrovascular accidents and brain injury, (3) vision and hearing problems that spectacles or hearing aids cannot fix, (4) substance or medication abuse, (5) used cognitive enhancers, and (6) participation in a cognitive training program during this study. Participants were selected based on the order in which they volunteered and met the inclusion criteria, including three older adults with mild NCD. The researcher described the details of the research project, demonstrated the program in various activities for one hour per individual, allowed them to practice independently, and obtained signed consent before the participants started the program at home for one week. Practicing by themselves in each activity for 25-30 minutes per session. The purpose was to assess the clarity of content and images, language usage, clarity of instructions in each activity, and the quality of audio files used in the program. We collected feedback and suggestions from three older adults with mild NCD through semi-structured interviews for the final minor revision of the WM program for improving language skills.

Instrumental for screening participant

1) The Thai version of Mini-Cog, a brief cognitive screening test, includes a three-item recall and a clock drawing test (CDT).[18] The three-item recall is scored up to three points, one for each correctly recalled word. The CDT requires drawing a circular clock displaying a specific time (11:10). Mini-Cog scores are categorized as (i) possible abnormal cognitive function (scores 0-2) and (ii) normal cognitive function (scores 3-5). This test showed good interrater reliability (K=0.80, p<0.001, 95% CI 0.50-1.00) and positive concurrent validity (r=0.47, p=0.007, 95% CI 0.37, 0.55) compared to the Mini-Mental State Examination Thai 2002.
2) The MoCA-B-Thai version was specifically developed to identify mild cognitive impairments in participants with limited educational attainment.[19] The scoring system includes assigning one point to individuals with ≤4 years of education and an additional point for low educational attainment. The optimal cut-off score is 24 out of 25. This test exhibited a high test-retest reliability of 0.91 (p<0.001) and had an internal consistency of 0.82.
3) The Thai version of the Barthel Activities of Daily Living Index (Barthel ADL Index) assesses the self-care and independence of geriatric individuals in a community setting.[20] The maximum score is 20. Participants with a score of ≥12 are considered independent. This assessment displayed Kappa inter-rater reliability coefficients and repeatability tests at 0.79 and 0.68, respectively.
4) The Thai version of the 15-item Geriatric Depression Scale (TGDS-15) serves as an effective screening tool for major depressive disorder in the Thai older adult population. The maximum score is 15.[21,22] Participants with a score of ≥6 (indicative of depression) were excluded from the study. This instrument demonstrated a sensitivity of 0.92 and a specificity of 0.87 in Geriatric Outpatients (cut-off score of ≥5) and a sensitivity of 100% and a specificity of 49% in the Thai Long-Term Care Home group (cut-off score of ≥8) when considering cognitively intact subjects.

Interview form for explicit problems that pilot users found during the practice of the WM program for improving language skills.

It is a semi-structured Interview form for gathering the opinions and problems found when using the WM program to improve language skills. The interview form focused on five topics: font size, size and clarity of images, accessibility and clarity of audio files, clarity of instructions, and overall usability and convenience. The opinion of the pilot users was recorded during the interviews.

Statistical analysis

The content validity and satisfaction of the pilot users were analyzed using descriptive statistics.

Results

Phase 1: Development of a working memory program for improving language skills.

The working memory (WM) program for improving language skills comprised nine activities, categorized according to the components of Baddeley's WM model. The activities were divided into two groups:

Group 1: Involving the phonological loop and the visuospatial sketchpad. The first activity was the 'word list' task, in which participants were asked to memorize words from audio files (e.g., mango and flower), then recall and write them in order in an exercise book. The second activity was the 'picture list' task, which required participants to remember presented pictures (e.g., lion and zebra) and then recall and write them in an exercise book. The third activity was verbal fluency, where participants wrote words related to specific categories (e.g., food, animal, sports).

Group 2: Involved the central executive and the episodic buffer. These activities included the 'missing item' task, where participants identify missing items on the second page (e.g., shirt, hat, shoes) after viewing the first page (e.g., shirt, hat, shoes, glove). The 'day task/months task' required participants to mentally arrange days or months in calendar order and write them on the next page. The 'odd or even numbers of syllables in the word' task involved mentally counting the syllables in each word and determining whether the count was odd or even. In the 'category span task,' participants selected words matching a given topic. The 'reading and recognition' activities involved reading a story (e.g., shopping in a market) and answering questions. In the last activity, 'Images and text,' participants read and remembered text-related pictures, then filled in the missing images and completed the text on the following page.

The researchers organized the program for each activity based on complexity levels within the WM model, progressing from easy to difficult. The activities gradually increase complexity by incorporating more words, phrases, texts, and images, corresponding to higher difficulty levels—this arrangement aimed to provide a challenging yet motivating experience for the participants.

According to the examined IOC, each item of the content validity test passed the criteria. (Table 2)

Table 2. Content validity of the working memory program for improving language skills.

Content validity			
Part	**Content**	**IOC**	**Conclusion**
1	Outline	0.90	Passed
2	Manual	0.94	Passed
3	Activity	0.95	Passed
4	Answer	0.98	Passed
total	All four parts	0.94	Passed

Phase 2: trial of the* working memory program for improving language skills *with three pilot users.

When applied in the pilot group of older adults who met the inclusion criteria, consisting of three individuals, the demographic characteristics can be seen in Table 3. It was found that the pilot group could utilize the program for self-training at home. Overall, there was a consensus that clear images and font sizes were beneficial. However, there was some confusion regarding the training steps and instructions, and a few participants encountered difficulties opening the audio files from the QR codes. The researchers summarized feedback and revised the program based on suggestions. All suggestions are shown in Table 4

The researchers incorporated these adjustments according to the recommendations until the working memory program was complete. Furthermore, the researchers developed guidelines to explain the training processes, allowing the pilot group participants to understand and apply the training activities at home.

Table 3. General information of the pilot users.

N	Gender	Age	Years of education	Occupation	Instrumental for screening			
					The Thai version of Mini-Cog	Barthel ADL index	TGDS-15	The MoCA-B Thai version
1	Male	75	18	Retire	4	20	2	23
2	Female	77	6	Agriculturist	4	19	1	21
3	Female	71	12	Freelancer	5	20	1	22

Table 4. Satisfaction and suggestions from the pilot users.

Topic	Percentage (%)	Suggestion	Implementation
Font size	100	The font is large, clearly visible	-
Size and clarity of images	100	Appropriate image size. Clear images with beautiful colors that make one feel motivated to do the practice exercises. Older adults can recognize all images used in the training program and won't be confused when interpreting them.	-
Accessibility and clarity of audio files	67	The audio files have an adequately balanced volume, clear speech, and appropriate pauses between each word. However, the pilot users feel unfamiliar with accessing audio files via scanning QR codes.	Teach older adults to access audio files by scanning QR codes before proceeding with independent practice, and use the Line application to deliver audio files.
Clarity of instructions	67	The instructions are quite lengthy, causing confusion in some activities.	Revise the instructions for conciseness, and underline vocabulary words in the instructions. Allow older adults to try a sample activity before proceeding with independent practice.
Overall usability and convenience	100	At first, the experimental group was not familiar with using the practice workbook and the answer key. However, after trying the exercises, they found that practicing and checking their answers independently became much more convenient	-

Discussion

In the discussion section, we separate into two parts: The working memory program for improving language in older adults with mild NCD, and pilot users' satisfaction and suggestions.

Phase 1: The working memory program for improving language skills.

In content validity, experts recommended adjustments to the program's content. For example, in Part 1, the outline of the program's content, experts advised removing redundant content from the accompanying training manual, making the content more concise, and considering the difficulty level of certain activities. Additionally, since this program was designed for older adults to practice independently at home, it was essential to ensure a clear understanding of the training process, specifying the practice dates should be clear and providing guidance on self-assessment. It was suggested to make the content distinction between the exercise workbook and the answer key book to make sure everything is clear among older adults. In Part 2, the manual should use easily understandable categories for the exercises, with more examples and clear explanations for multi-step activities. In Part 3, Activity Worksheets, working memory activities involving listening, the experts suggested considerations regarding word selection. For example, words with similar sounds, the number of words, and the number of syllables can all affect memory in older adults, aligning with the "phonological similarity effect".[23] This concept is directly related to the auditory data storage unit. In other words, if letters or words with similar pronunciation are used, it can reduce the ability to retain information.

Additionally, word length is considered a significant

variable in hindering the process of word rehearsal. When multiple spoken words need to be memorized but cannot be repeated mentally, it can lead to rapid forgetting of those words. Furthermore, this program was designed to be accessible to older adults with mild NCD, without limitations based on educational levels. Therefore, the words in the program should be familiar words with a common name for better comprehension and recall. In activities related to images, experts have recommended using real images instead of cartoons. The images should be in color, appropriately sized, and proportioned realistically to allow the older adults to see them clearly and interpret the images accurately. This recommendation aligns with Myers' memory encoding process[24], which involves the initial process of receiving information from the environment, and transmitting data to visual and auditory receptors until it reaches the sensory areas in the cerebral cortex, resulting in sensory memory. Therefore, the stimuli must be clear and unambiguous. In Part 4 Answer keys, the researchers addressed discrepancies in Part 4 based on the experts' recommendations.

Moreover, for the WM program, the researchers used a single paradigm, emphasizing working memory training by utilizing various components within the working memory model. These components included a phonological loop, visual-spatial sketchpad, central executive, and episodic buffer. These activities were adapted from previous studies which adopted similar guidelines for training.[14,16] Alongside this, the study by von Bastian and Oberauer suggested that training focused on a single paradigm or specific skill might be more effective than multiple paradigms or diverse skills.[25] Furthermore, the program's activities were designed to gradually increase complexity, including expanding vocabulary, longer sentences, and complex tasks to enhance the WM capacity. This approach aligned with Lövden *et al*.'s idea that adaptive adjustments are necessary for survival when there is a 'mismatch' between living beings and environmental requirements.[26] This perspective is relevant to the studies conducted by Lee and Kim, Carretti *et al*., and Zimmermann *et al*.[14,27,16.] Their study adjusted the activity levels according to the participants' abilities and gradually increased the task difficulty. The findings indicated improvements in working memory capacity during usage and language skills.

Phase 2: pilot users' satisfaction and suggestions

In the pilot user phase, we demonstrated the feasibility of using the working memory program to improve language skills in older adults with mild NCD. Regarding this, most participants in the study expressed their positive satisfaction with the program. All of them (100%) agreed that the images were clear and the font size was appropriate. However, there were some concerns regarding the instructions' clarity. Additionally, some participants encountered challenges accessing audio files through QR codes (33%). We addressed this by teaching older adults how to access audio files by scanning QR codes before proceeding with independent practice. Furthermore, we utilized the Line application to deliver audio files and allowed older adults to try a sample activity before proceeding with independent practice. Based on their satisfaction and suggestions, as revealed by McGee, it is evident that older adults often experience impairments in sensory systems, including vision and hearing, and a decline in cognitive abilities.[28] Therefore, the development of the exercise workbook must consider usability factors. This includes providing clear explanations, relevant examples with accompanying images, using large font sizes, and incorporating real images, all enhancing visibility for older adults.

After revising the program based on the suggestions from pilot users, we developed the WM program for improving language skills. The program consists of two books: an Exercise Workbook, which contains an outline of the program, detailed instructions for each activity, training methods and protocol aligned with the program, as well as Worksheets for participants to record their activity performance, and an Answer Key book. The program comprises nine activities, each featuring different difficulty levels, ranging from easy to hard. These levels include an increasing number of words, phrases, texts, and images as the difficulty progresses. Inside the Exercise Workbook, you'll find clearly legible fonts and high-quality images with vibrant colors. The researcher has revised the instructions for conciseness and underlined vocabulary words for emphasis. Additionally, older adults are taught to access audio files by scanning QR codes before proceeding with independent practice. This program is designed for older adults with Mild NCD, who have decreased cognitive abilities compared to healthy older adults with the same educational level. Therefore, the researcher should be concerned about the content of the program, whether the words and images are appropriate for the Thai cultural context, making it easy for older adults with Mild NCD. The researcher should also consider the level of difficulty, accessibility, and comprehension limitations.

In addition, the pilot user group also provided feedback that home-based training allows for greater accessibility to training. This aligns with a previous study by Payne and Stine-Morrow[15] that investigated the effectiveness of a WM training program in enhancing language comprehension in older. The home-based training sessions lasted 30 minutes each and were conducted five times a week for three weeks, totaling 15 sessions. The study's results showed a statistically significant improvement in working memory capacity and language skills within the experimental group compared to the control group. Furthermore, the researchers suggested that home-based training could offer benefits such as increased convenience for older adults, reduced resource requirements, and greater flexibility in managing training schedules. These findings are consistent with a study conducted in Thailand by Pratoomtan *et al*., which explored the effects of home-based cognitive training on older adults with MCI.[29] In their study of 11 participants who underwent 18 training sessions over six weeks, it was discovered that older adults with MCI could engage in self-training activities and relied only partially on caregiver

assistance. Therefore, home-based training may be suitable for older adults with mild NCD.

Limitation

In this study, the limited duration of the second phase and the small sample size might impact program improvements. Nevertheless, the suggestion is valuable for future research investigating the program's effectiveness.

Conclusion

The working memory program for improving language skills consisted of nine activities, each containing different difficulty levels ranging from easy to hard. These levels included an increasing number of words, phrases, texts, and images according to higher difficulty levels. The program underwent content validity assessment by five experts and revisions based on suggestions from pilot users. Consequently, the program could enhance the language abilities of older adults with mild NCD. The next phase will investigate its effectiveness in improving language skills in older adults with mild NCD.

Ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University (Approval ID: AMSEC-65EX-018) and the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University Study code: None-2565-08999 Research ID:8999. All participants received all necessary information related to the research and informed written consent was gathered before enrolling.

Conflicts of interests

The authors declare that they have no conflict of interest.

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References

[1] Sachs-Ericsson N, Blazer DG. The new DSM-5 diagnosis of mild neurocognitive disorder and its relation to research in mild cognitive impairment. Aging Ment Health. 2015; 19: 2-12. doi: 10.1080/13607863.2014.920303

[2] Stokin GB, Krell-Roesch J, Petersen RC, Geda YE. Mild neurocognitive disorder: an old wine in a new bottle. Harv Rev Psychiatry. 2015; 23: 368-76. doi: 10.1097/HRP.0000000000000084

[3] Sachdev PS, Lipnicki DM, Kochan NA, Crawford JD, Thalamuthu A, Andrews G, et al. The Prevalence of Mild Cognitive Impairment in Diverse Geographical and Ethnocultural Regions: The COSMIC Collaboration. PLoS One. 2015; 10(11). doi: 10.1371/journal.pone.0142388

[4] Griffiths J, Thaikruea L, Wongpakaran N, Munkhetvit P. Prevalence of Mild Cognitive Impairment in Rural Thai Older People, Associated Risk Factors and their Cognitive Characteristics. Dement Geriatr Cogn Dis Extra. 2020; 10(1): 38-45. doi: 10.1159/000506279

[5] McCullough KC, Bayles KA, Bouldin ED. Language performance of individuals at risk for mild cognitive impairment. J Speech Lang Hear Res. 2019; 62: 706-22. doi: 10.1044/2018_JSLHR-L-18-0232

[6] Chai WJ, Abd Hamid AI, Abdullah JM. Working memory from the psychological and neurosciences perspectives: a review. Front Psychol. 2018; 9: 1-16. doi: 10.3389/fpsyg.2018.00401

[7] Huntley JD, Howard RJ. Working memory in early Alzheimer's disease: a neuropsychological review. Int J Geriatr Psychiatry. 2010; 5: 121-32. doi: 10.1002/gps.2314

[8] Peach RK, Shapiro LP, Shapiro LP. Cognition and acquired language disorders: an information processing approach. St. Louis: Elsevier Mosby; 2012.

[9] Baddeley AD. The episodic buffer: a new component of working memory?. Trends Cogn Sci. 2000; 4: 417-23. doi: 10.1016/s1364-6613(00)01538-2

[10] Rudner M, Rönnberg J. The role of the episodic buffer in working memory for language processing. Cogn Process. 2008; 9: 19-28. doi: 10.1007/s10339-007-0183-x

[11] Waters GS, Caplan D. Age, working memory, and online syntactic processing in sentence comprehension. Psychol Aging. 2001; 16: 128-44. doi: 10.1037/0882-7974.16.1.128

[12] Lee MS, Kim BS. Characteristics of word fluency in healthy aging and Alzheimer's disease. Audiol Speech Res. 2019; 15: 135-43. doi: 10.21848/asr.2019.15.2.135

[13] Borella E, Carretti B, De Beni R. Working memory and inhibition across the adult life-span. Acta Psychologica. 2008; 128: 33-44. doi: 10.1016/j.actpsy.2007.09.008

[14] Lee MS, Kim BS. Effects of working memory intervention on language production by individuals with dementia. Neuropsychol Rehabil. 2020; 31: 1-25. doi: 10.1080/09602011.2020.1789479

[15] Payne BR, Stine-Morrow EAL. The effects of home-based cognitive training on verbal working memory and language comprehension in older adulthood. Front Aging Neurosci. 2017; 9: 1-14. doi: 10.3389/fnagi.2017.00256

[16] Zimmermann N, Netto TM, Amodeo MT, Ska B, Fonseca RP. Working memory training and poetry-based stimulation programs: are there differences in cognitive outcome in healthy older adults?. NeuroRehabilitation. 2014; 35: 159-70. doi: 10.3233/NRE-141104

[17] Feldman HM. How Young Children Learn Language and Speech. Pediatr Rev. 2019; 40(8): 398-411. doi: 10.1542/pir.2017-0325

[18] Trongsakul S, Lambert R, Clark A, Wongpakaran N, Cross J. Development of the Thai version of Mini-Cog, a brief cognitive screening test. Geriatr Gerontol Int. 2015; 15: 594-600. doi: 10.1111/ggi.12318

[19] Julayanont P, Tangwongchai S, Hemrungrojn S, Tunvirachaisakul C, Phanthumchinda K, Hongsawat J, *et al*. The Montreal cognitive assessment-basic: a screening tool for mild cognitive impairment in illiterate and low-educated elderly adults. J Am Geriatr Soc. 2015; 63: 2550-4. doi: 10.1111/jgs.13820

[20] Jitapunkul S, Kamolratanakul P, Ebrahim S. The meaning of activities of daily living in a Thai elderly population: development of a new index. Age Ageing. 1994; 23: 97-101. doi: 10.1093/ageing/23.2.97

[21] Sheikh JI, Yesavage JA. Geriatric depression scale (GDS): recent evidence and development of a shorter version. Clinical Gerontologist. 1986; 5: 165-73. doi.org/10.1300/J018v05n01_09

[22] Wongpakaran N, Wongpakaran T. Prevalence of major depressive disorders and suicide in long-term care facilities: a report from northern Thailand. Psychogeriatrics. 2012; 12: 11-7. doi: 10.1111/j.1479-8301.2011.00383.x

[23] Chow M, Macnamara BN, Conway AR. Phonological similarity in working memory span tasks. Mem Cognit. 2016; 44(6): 937-49. doi: 10.3758/s13421-016-0609-8

[24] Myers DG. Exploring psychology. 6th Ed. New York: Worth Publishers; 2000.

[25] Von Bastian CC, Oberauer K. Effects and mechanisms of working memory training: a review. Psychol Res. 2014; 78(6): 803-20. doi: 10.1007/s00426-013-0524-6

[26] Lövdén M, Bäckman L, Lindenberger U, Schaefer S, Schmiedek F. A theoretical framework for the study of adult cognitive plasticity. Psychol Bull. 2010; 136: 659-76. doi: 10.1037/a0020080

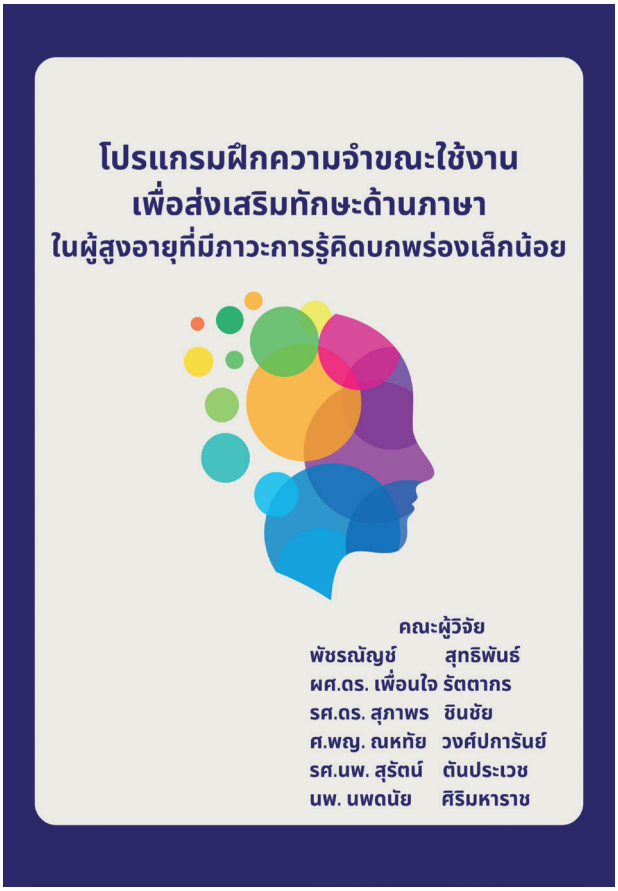
[27] Carretti B, Borella E, Fostinelli S, Zavagnin M. Benefits of training working memory in amnestic mild cognitive impairment: specific and transfer effects. Int Psychogeriatr. 2013; 25: 617-26. doi: 10.1017/S1041610212002177

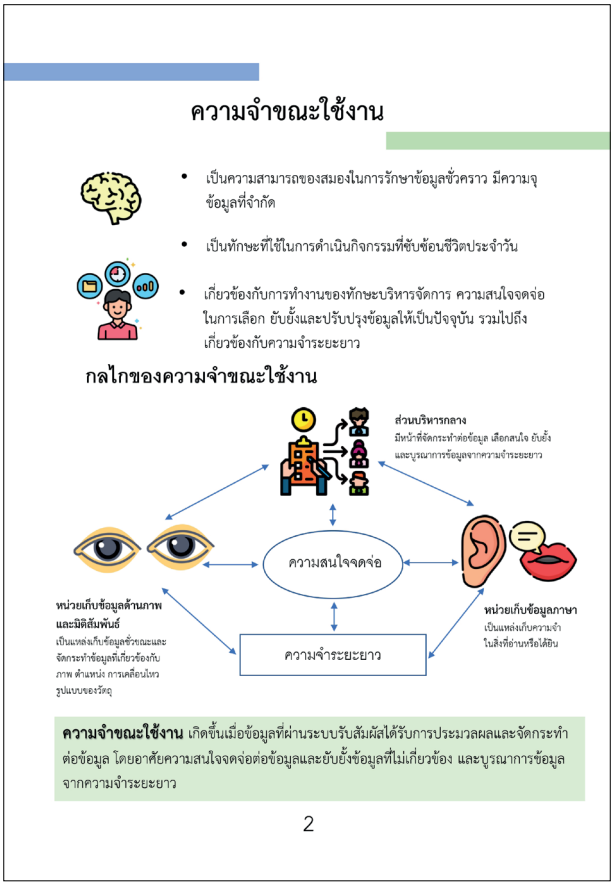
[28] McGee J. Toolkit for making written material clear and effective: Things to know if your written material is for older adults [Internet]. 2020 [cited 2 Nov 2020]. Available from:https://www.cms.gov/Outreach-and Education/Outreach/WrittenMaterialsToolkit.

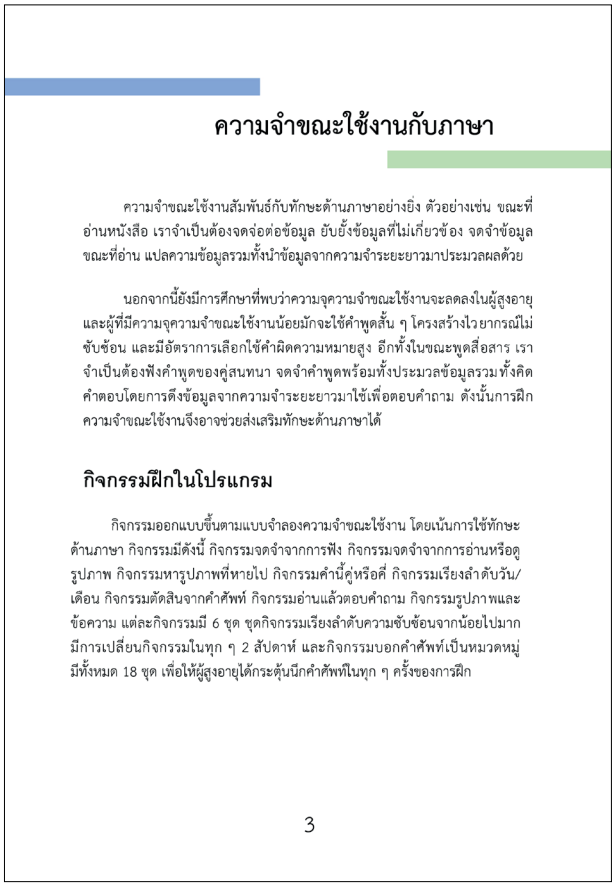
[29] Pratoomtan D. Effect of home-based cognitive training kit and satisfaction of elderly with mild cognitive impairment [Dissertation]. Faculty of Associated Medical Sciences: Chiang Mai University; 2013 [in Thai].

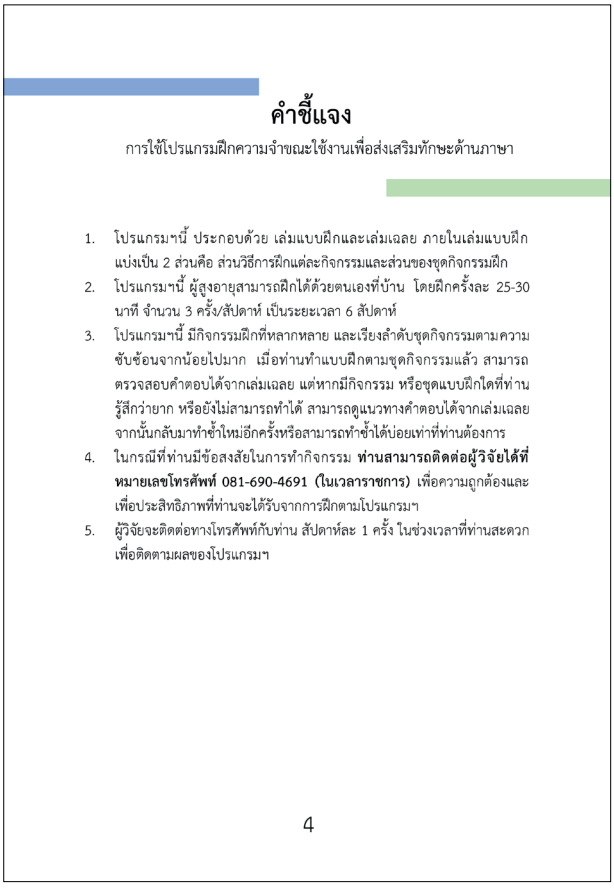
Appendix

Appendix 1. Example of a manual for using the working memory program for improving language skills.









Appendix 2. Example of activities in the working memory program for improving language skills.

วันที่ 1

กิจกรรมจดจำจากการฟัง **ชุดที่ 1**

คำชี้แจง สแกน QR code ฟังคำศัพท์จากไฟล์เสียง แล้วพูดทวนคำศัพท์ทั้งหมดหลังจากที่ได้ยิน จากนั้นเขียนคำศัพท์ที่ได้ยินลงในช่องว่างตามลำดับ

ข้อที่ 1 1. ..... 2. .....

ข้อที่ 2 1. ..... 2. .....

19

วันที่ 1

กิจกรรมบอกคำศัพท์เป็นหมวดหมู่ **ชุดที่ 1**

คำชี้แจง เขียนคำศัพท์หมวด **ของใช้ในห้องครัว** ให้ได้มากที่สุด ภายในเวลา 5 นาที ลงในตาราง

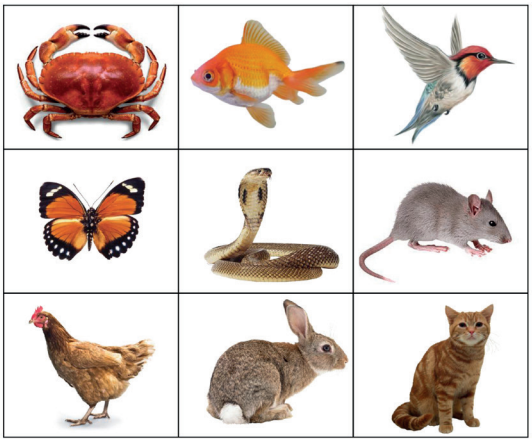
คำศัพท์	คำศัพท์
1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

22

วันที่ 7

กิจกรรมหารูปภาพที่หายไป **ชุดที่ 1**

คำชี้แจง จดจำชื่อรูปภาพสัตว์ในตารางจนจำได้ทั้งหมด จากนั้นเปิดหน้าถัดไป เขียนชื่อรูปภาพสัตว์ที่หายไปในตำแหน่งเดียวกันลงในตาราง โดยไม่กลับมาเปิดหน้านี้ซ้ำอีก



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วันที่ 7

กิจกรรมคำนี้คู่หรือคี่ **ชุดที่ 1**

คำชี้แจง อ่านและตัดสินว่าคำศัพท์นั้น มีจำนวนพยางค์เป็นจำนวนคู่หรือคี่ โดยทำเครื่องหมาย ✓ ในช่องคำตอบที่เลือก

คำศัพท์	จำนวนพยางค์	
	จำนวนคี่	จำนวนคู่
1. ฉุกเฉิน		
2. เฉพาะกิจ		
3. ออกกำลังกาย		
4. ปกปักรักษา		
5. บุรุษไปรษณีย์		
6. อนุญาต		
7. ดวงอาทิตย์		
8. อาณาเขต		
9. ประชาธิปไตย		
10. สนุกสนาน		

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Appendix 3. Protocol of activities in the working memory program for improving language skills.

Week	Day 1	Day 2	Day 3
1	Word list	Word list	Word list
	Picture list	Picture list	Picture list
	Verbal fluency	Verbal fluency	Verbal fluency
2	**Day 4**	**Day 5**	**Day 6**
	Word list	Word list	Word list
	Picture list	Picture list	Picture list
	Verbal fluency	Verbal fluency	Verbal fluency
3	**Day 7**	**Day 8**	**Day 9**
	Missing item	Missing item	Missing item
	Day task/months task	Day task/months task	Day task/months task
	Odd or even numbers of syllables in the word	Odd or even numbers of syllables in the word	Odd or even numbers of syllables in the word
	Verbal fluency	Verbal fluency	Verbal fluency
4	**Day 10**	**Day 11**	**Day 12**
	Missing item	Missing item	Missing item
	Day task/months task	Day task/months task	Day task/months task
	Odd or even numbers of syllables in the word	Odd or even numbers of syllables in the word	Odd or even numbers of syllables in the word
	Verbal fluency	Verbal fluency	Verbal fluency
5	**Day 13**	**Day 14**	**Day 15**
	Category span task	Category span task	Category span task
	Reading and recognition	Reading and recognition	Reading and recognition
	Images and text	Images and text	Images and text
	Verbal fluency	Verbal fluency	Verbal fluency
6	**Day 16**	**Day 17**	**Day 18**
	Category span task	Category span task	Category span task
	Reading and recognition	Reading and recognition	Reading and recognition
	Images and text	Images and text	Images and text
	Verbal fluency	Verbal fluency	Verbal fluency

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Emerging updates on tracking new landscapes in nanotechnology for the diagnosis and ovarian cancer therapy

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ABSTRACT

The sixth most common recurrent malignancy worldwide is ovarian cancer in women, and it causes more women to die compared to any other issue impacting the female reproductive system. Ovarian cancer has several histological subgroups differing in clinical traits, risk factors, cell sources, molecular makeups, and treatment possibilities. There is no effective screening procedure, and it is typically discovered at a late stage. Newly found cancer is currently treated with platinum-based chemotherapy and cytoreductive surgery. Due to its recurrence and late diagnosis, ovarian cancer has the highest fatality rates in contrast to all gynecological cancers. The discipline of medical nanotechnology has made great strides in recent years in resolving issues and enhancing the detection and treatment of various illnesses, including cancer. However, most studies and recent reviews on nanotechnology are devoted to how it might be utilized to treat other tumors or disorders. This review's main objective was the precise diagnosis and treatment of ovarian cancer using nanoscale drug delivery systems. Various nanocarrier systems, such as dendrimers, nanoparticles, liposomes, nanocapsules, and nano micelles, have been discussed. Additionally, we explore how the potency of the combination of immunotherapy and nanotechnology may help to overcome the current therapeutic constraints connected with each application and reveal a novel paradigm in cancer therapy. The unique nanotherapeutic approaches that have demonstrated promising outcomes in preclinical in vivo research are highlighted, along with new nanoformulations actively advancing into clinical trials. Additionally, the possible use of nanomaterials in diagnostic imaging methods and the capacity to use nanotechnology for early ovarian cancer detection are also highlighted.

Introduction

Ovarian cancer, which only becomes apparent in an advanced stage, is the foremost reason for transience for women universally. During the initial stages of the malady, patients exhibit a few basic symptoms because there aren't any reliable ways to diagnose the condition. Contrary to stromal or germ cells, ovarian epithelium frequently contributes to significant occurrences of ovarian cancer.[1] There are numerous morphological and symptomatic changes present in these ovarian cancer subtypes.[2,3] Modern approaches for ovarian cancer diagnosis include CT, CA-125 (cancer antigen 125) levels in the serum, transvaginal ultrasonography, MRI, etc. Years of research have consistently shown how dynamic the disease is, and despite better treatment options, there are still serious side effects from aggressive chemotherapy.[4,5] Patients suffer when more severe therapy is required, especially

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when aggressive tumors lie dormant and subsequently reappear.[6-8]

The prevalence of resistance mechanisms is one of the major obstacles to the creation of effective cancer treatments. Resistance mechanisms are engaged in concurrent signaling pathways and reroute to promote cancer progression after the key oncogenic pathways are shut down.[9] Due to the wide range of cancer cells, patient tumors, genetic abnormalities, and epigenetic patterns, drug resistance might develop and limit the efficacy of therapeutic measures.[10] Most anticancer medications also have low bioavailability due to their poor physicochemical stability, low water solubility, or overall electronegative surface charge, which inhibits the medications from penetrating the cells due to the negative charge of the cytomembrane. The natural negative charge of cell membranes repels these medications, resulting in poor cell adhesion and low bioavailability.[11] This incites doctors to prescribe more medication than is required to preserve diffusion-controlled phenomena. The existing methods of diagnosis and therapy are insufficiently sensitive and effective to detect and treat ovarian cancer (OC) at an early stage. Furthermore, a delayed diagnosis is brought on by the absence of a distinct detection point and significant costs.

Integrating immunotherapy and chemotherapy could enhance the remedial outcome because immunotherapy may prevent immunological harm and balance the acute immunosuppression induced by chemotherapy. In contrast, chemotherapy might activate antitumor immunity and create antigenic molecules. However, there are several issues with immunotherapeutic drugs that chemotherapy suffers from as well, including immune-related adverse effects, instability, and ineffective administration.[12] There are ways to get over the limitations mentioned above, involving the use of nanoparticulate drug delivery techniques to deliver two or more medicines. Controlling medication ratios and ensuring co-localization of the combination treatments at the tumor site is made possible by a delivery method that simultaneously combines several therapeutic agents.[13]

In order to produce aqueous dispersions that make it easier to administer hydrophobic therapeutic agents like paclitaxel (PTX), or to accommodate hydrophilic therapeutic agents like RNA that make it easier for them to enter cells, nanoparticulate drug delivery devices can encapsulate these agents.[14,15] To enhance the accumulation of delivered cargoes in the tumor by passive targeting or the enhanced permeability and retention (EPR) effect, nanoparticle-based therapies frequently use nanosized particles having a diameter in a spectrum of 10 to 200 nm. (Figure 1).[16,17] Targeted substances that can precisely bind to receptors overexpressed by tumor cells, such as antibodies, growth factors, peptides, or fragments of antibodies, can also be included in nanoparticles. By boosting their internalization by the targeted cells, active targeting improves the selectivity and therapeutic responsiveness of drug-loaded nanoparticles.[18]

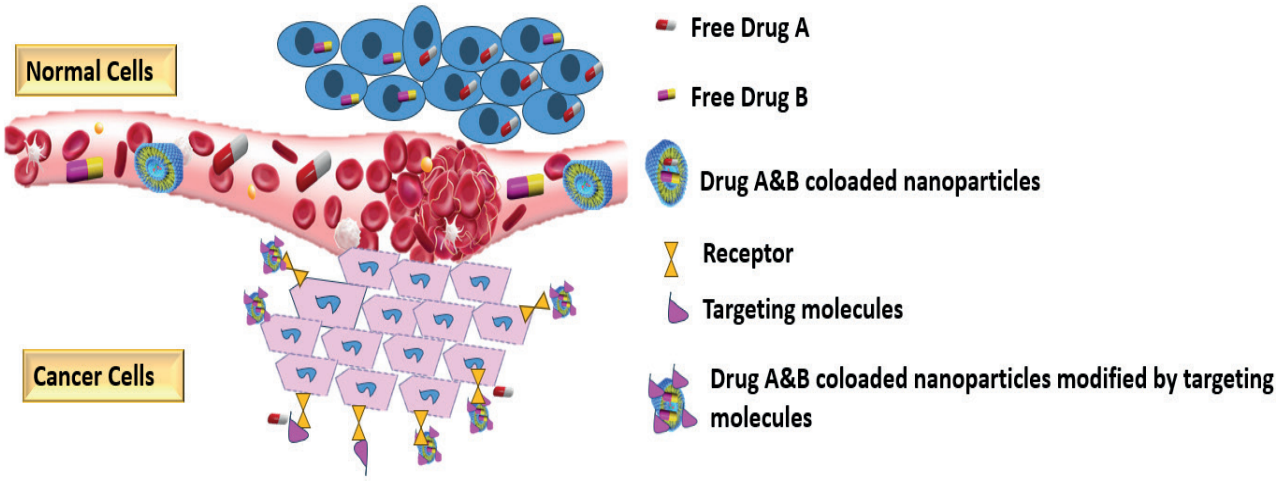

Figure 1. *Benefits of co-delivery of 2 treatments employing nanoparticles in contrast with free pharmaceuticals are illustrated schematically. These benefits include better water solubility, regulated ratios of the drug, and guarantee of the same drug disposition behavior at the tumor site. Other benefits include increased drug co-accumulation at tumors via the EPR effect and/or receptor-mediated endocytosis, commonly known as active or passive targeting.*

Additionally, by modifying the kind and features of the nanoparticle-forming materials, such as their molecular weight, compositions, and architectures, nanoparticles can be altered to enhance their cargo-loaded features, involving circulation time, In-vivo stability, retention of the drug, and renal clearance. Numerous reports have explored and depicted how well different types of nanoparticles can be used to encapsulate different medicinal ingredients, including liposomes, polymeric micelles, dendrimers, and nanoparticles related to lipids (Shown in Figure 2), for several cancer therapies, particularly ovarian cancer.[19-21] Additionally, we explore how the power of combining immunotherapy and nanotechnology may help overcome the current therapeutic limits connected to each application and reveal a novel paradigm in the treatment of this cancer. Nanotechnology-based therapies support the controlled delivery of chemotherapeutic drug(s) in a targeted manner that acts for an extended period directly on the cancer site with low or non-toxicity to normal organs. Designing nanotechnology formulations for the treatment of ovarian cancer will thus be the main emphasis of this review.

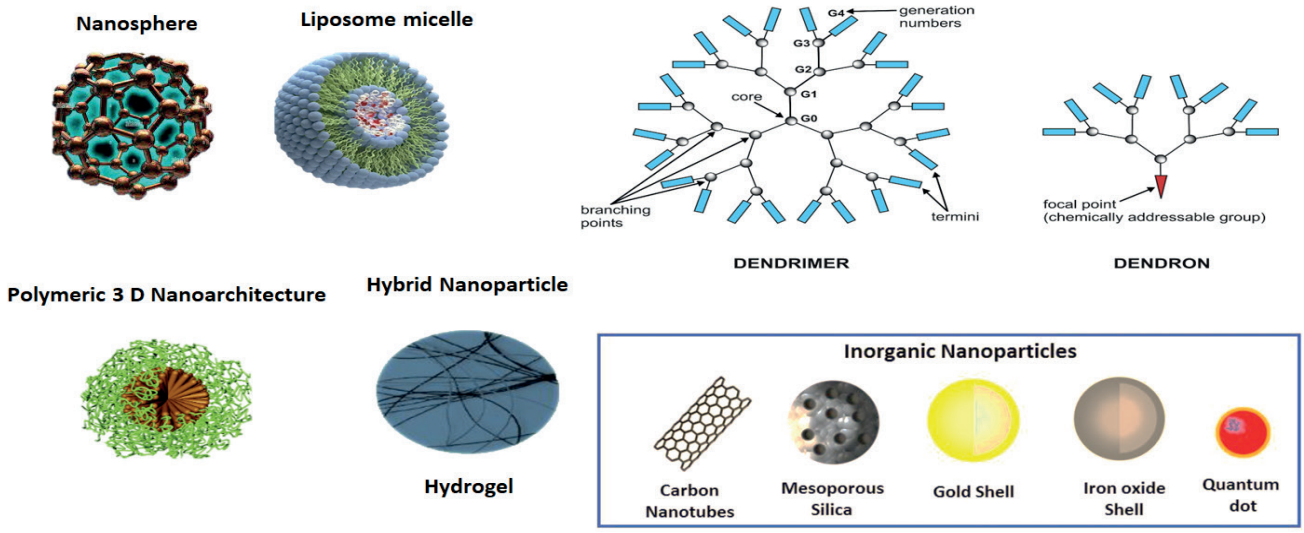

Figure 2. *Different kinds of nanoparticles are employed for drug delivery depending on the cancer treatment medication.*

Impediments to OC perception and intervention

Surgery to reduce the size of a tumor

To ascertain the stage and prognosis of the cancer, an ovarian cancer patient must have tumor debulking surgery.[22] The 5-year survival probability for Stages I, II, III, and IV is predicted to be between 90% and 10% [23,24] based on the surgical pathologic degrees used in the International Federation of Gynecology and Obstetrics (FIGO's) staging evaluation system. The tumor in the patient and the therapy measures can all be categorized as prognostic variables for ovarian cancer.[25] By the Gynecologic Oncology Group, a residual tumor less than 1 cm after surgery is considered to have undergone appropriate cytoreduction. In contrast, one more than that is considered to have undergone poor cytoreduction.[26-28] Complete cytoreduction is frequently not attainable for tumors in their advanced stages (stages III and IV). Three cycles of neoadjuvant chemotherapy are administered to patients who are too sick or have lesions that cannot be removed surgically. If the chemotherapy is effective, there will be six rounds given, followed by an interval debulking surgery.[29] The removal of all residual diseases, whether treated as a primary or secondary disease, is the ultimate goal of tumor debulking surgery. Despite an initial response, recurrence occurs in 75% of individuals. Finding a different strategy to treat ovarian cancer is the final goal.

Chemotherapeutic drugs

Chemotherapeutic medications are used to treat patients after surgery. Carboplatin and cisplatin are the two medications used the most frequently to treat OC.[30] Due to its resemblance to cisplatin in terms of response rate and survival statistics, carboplatin was launched at the beginning of the 1980s as an alternative. However, carboplatin is preferable over cisplatin due to the risk of ototoxicity, nephrotoxicity, and nausea or vomiting found with cisplatin. [31-33] Platinum is inserted into DNA by the alkylating chemical carboplatin, creating crosslinks. Apoptosis is brought on by a signaling cascade that is set off by the ensuing structural deformation of the DNA.[34-36]

Frontline treatment with PARPi for ovarian cancer

For several years, researchers have been exploring drug resistance and tailored treatment approaches for ovarian cancer patients. Many patients experience recurrence, even though there is frequently no sign of the disease after the initial surgery and chemotherapy.[37] The recurrence following the initial therapy and knowledge and analysis of the issue from a molecular perspective led to the creation of poly-ADP-ribose polymerase (PARP) inhibitors. Rucaparib Niraparib, or Olaparib, [38] medications in a recent study revealed less DNA repair in tumors with a BRCA gene mutation, which led to the

demise of cancer cells. Numerous clinical trials have been undertaken to encourage progression-free survival (PFS) because empirical data points to an anti-tumorigenic role (NCT04573933). According to the phase-2 trial of rucaparib,[39] patients with platinum-sensitive, high-grade ovarian cancer who also had a BRCA mutation (germline or somatic), a high level of chromosomal loss of heterozygosity, and several other variables had increased PFS. This medication has been given FDA approval to treat advanced OC. Olaparib monotherapy, which is given to OC patients who have had chemotherapy and may have a germline BRCA mutation, received another groundbreaking FDA approval. The European Medicines Agency (EMA) further endorsed olaparib for high-grade fallopian tubes, primary peritoneal cancer, serous epithelial ovarian, or with a somatic mutation or germline responsive to platinum treatment.

Nanotechnology-based drug carriers

As shown in Figure 3, when administered orally or intravenously, chemotherapeutic drugs in solution or pharmacokinetics of polymer solutions are unsatisfactory, with limited beneficial properties. These substances immediately influence the highest concentration that may be tolerated before being removed from circulation. For maximal patient advantages, a medication formulation should be released over time at the lowest possible effective concentration. As a medication delivery carrier, nanotechnology has the potential to significantly contribute to meeting these requirements. Drug delivery systems based on nanotechnologies, such as carbon nanotubes, polymer micelles, dendrimers, polymer nanoparticles, and lipid/solid nanoparticles, have many advantages over traditional approaches. Nanotechnology-based therapies have been shown to improve therapeutic efficacy, reduce toxicity in healthy tissue, and increase patient compliance. The therapy of cancer currently makes use of several of these nanoparticles.[40]

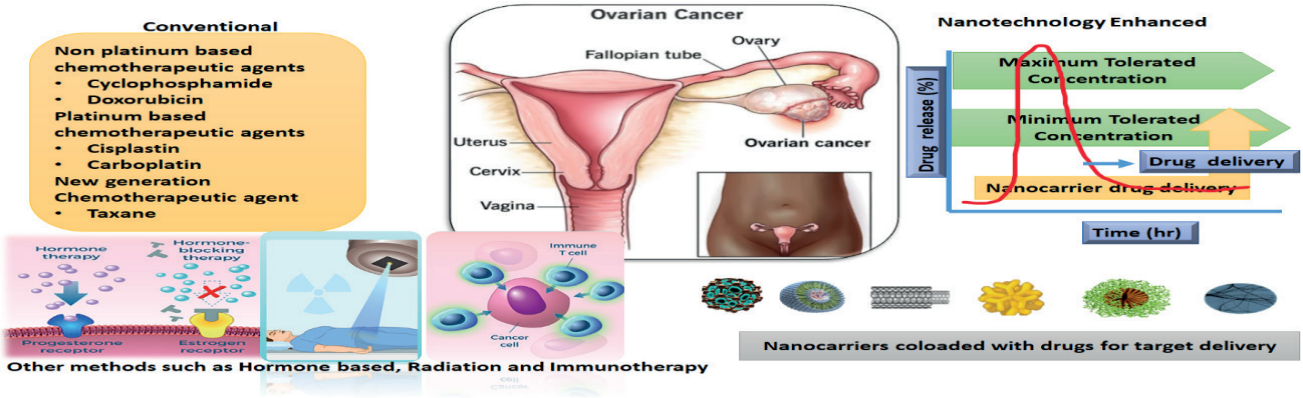

Figure 3. *Application of nanotechnology to increase efficacy via toxicity reduction in contrast to conventional techniques and provide effective treatment against ovarian cancer.*

Nano micelle-based ovarian cancer diagnosis

Due to the relative paucity of timely detection and exploratory capabilities in the early stages, ovarian cancer is frequently discovered in the late stages.[41,42] Both the administration and regulated release of medications for site-specific targeted treatment and imaging for early cancer identification are crucial.[43] Imaging can be used to monitor the progression of OC illness, assess the effectiveness of therapy and how medications are distributed throughout the tumor, or find potential molecular biomarkers.[44] Utilizing modern medical visualization techniques, such as anatomical probes (Computed Tomography scanning), basic radiography, magnetic resonance imaging (MRI), ultrasound, disease inspection, and therapy efficacy monitoring can be accomplished.[45] Currently, nanotherapeutic applications such as non-invasive cancer molecular imaging offer prospects in early prognosis for enhancing the accuracy efficiency of chemotherapeutics and enabling enhanced infection detection. When imaging malignancies with image modalities, contrast nanocarrier devices internalize increased tumor intensity. Nanoparticles can deliver drugs to specific molecular targets, encapsulate drugs, or improve pathological areal imaging. PEG-b-poly(Lysine) copolymers, among other polymeric nanoparticles, show enormous potential for analytical molecular imaging and the tracking of cancer progression or remission. Gold-plated and coated metallic quantum molecules are the most frequently utilized nanometer-sized particles. Still, other nanoparticles and biomarkers are also showing promise as efficient tools for prospective transmission development and therapeutic administration in the diagnosis of infected locations. By enhancing the targeting capabilities of existing medicines, increasing the effectiveness of locally administered medications, lowering systemic toxicity, enhancing imaging, and minimizing the requirement for radiation therapy, nanotechnology may hold the secret to the cure.[46,47]

Nanotechnology: A scientific viewpoint on diagnostic vs. therapeutic applications

According to reports from the World Health Organization (WHO) [48] and the European Federation of Pharmaceutical Industries and Associations,[49] cancer is one of the most common diseases, with an increase of more than 14 million new cases and 8.8 million fatalities worldwide each year. Thankfully, in many cases, the origins of cancer have been discovered (at least from a genomic perspective), and new therapeutic approaches have been created. No other illness is anticipated to advance as quickly as cancer despite these developments. By 2040, the incidence and mortality rates will be 70%.[50] This startling forecast is based on several coexisting elements, including population aging, environmental conditions, gender, lifestyle, food, gut microbiota, and molecular heterogeneity. Creating innovative technologies for the early detection of cancer-specific molecular aberrations before tumor formation is the only other way to stop the current trend. A promising replacement for conventional therapies and treatments is offered by recent applications of nano-biotechnology for the early diagnosis and treatment of cancer.[51] Nanotechnology may provide novel ways to target chemotherapies to cancer cells selectively, enabling speedier and more accurate tumor removal during surgery and improving the efficacy of radiation-based therapies.[52] Some of the world's most advanced nanoscience laboratories have been built over the past ten years thanks to significant government funding for this field of study, and most of them are currently working to develop cutting-edge cancer treatments.

Biosynthesis of natural products with nanoparticles for cancer therapy

Most of the chemical processes used to create nanoparticles (NPs) are excessively expensive and also involve the use of dangerous, poisonous chemicals that pose several biological concerns. This increases the rising demand to create environmentally friendly procedures using biological techniques and green synthesis. The "green synthesis" of NPs uses chemicals that are safe for the environment and are not harmful. Utilizing diverse plant resources to biosynthesize metallic NP'S is known as green nanotechnology. Numerous bioactive substances, including proteins, alkaloids, flavonoids, phenols, reducing sugars, steroids, carbohydrates, and tannins, were discovered in natural products. In addition to the most recent cutting-edge technologies, natural ingredients from many different sources are also studied for cancer treatment. Low toxicity and good tolerability are characteristics of natural compounds utilized in cancer therapy.[53-56] Through various modes of action, they demonstrate anti-cancer effects (Table 1).

Table 1. Biological processes by which natural chemicals fight OC cells.

Bioactive natural product	Type of cell	Mode of action
Thymoquinone	SKOV-3 cells	reduces the expression of Bcl-2 and increases the expression of Bax to cause apoptosis.[57]
Piperine	OVCAR-3 cells	suppresses the Akt / PI3K /GSK3 signaling alleyway, causes G_2/M stage in the arrest of the cell cycle, is caspase-activated, and prevents migration of cells.[58]
Quercetin	Epithelial OC cell line	reduced surviving, produced cell cycle arrest, inflicted apoptosis, and inhibited proliferation
Zeylenone	SKOV-3 cells	Signal transducer and activator of transcription (p-STAT) and the Janus family of tyrosine kinases (p-JAK) countenance levels were both lowered in separate ways. Zeylenone induced MMP, apoptosis-inducing factor (AIF), and cytochrome c depletion in a dose-dependent fashion. Additionally, it enhanced the production of caspase-3, Fas, Fasl, and Bax in both mRNA and protein forms while reducing the expression of Bcl-2.[60]
Curcumin	Cisplatin-resistant OC cells	promotes apoptosis and phosphorylation of p53, induces G_2/M cell-cycle arrest.[61]
Flavonoid	PA-1 Cells	lowers viability, causes apoptosis, upsurges caspase-3, 9, Bad, Bid, Bax, & cytochrome-c while dwindling Bcl-2 & Bcl-xL.[62]
Sideroxylin	ES2, OV-90	ERK1/2 signalling mechanism Increased expression of miR-27a and FBXW7 results in increased DNA and ROS damage and depolarizes the mitochondrial membrane.[63]

Ovarian cancer prevention via Cassia auriculata (Avartaki) plant-based nanoformulations

The impact of natural remedies on ovarian cancer has recently attracted the attention of numerous studies. The mechanisms through which bioactive substances prevent ovarian cancer have been clarified through several experimental experiments. The results showed that quercetin induced apoptosis via intrinsic and caspase-dependent mitochondrial pathways. In addition, quercetin produced ER stress in ovarian cancer cells, which led to mitochondria-mediated death. Among several natural products, we primarily focused on *Cassia auriculata* (*Avartaki*). An annual or biennial plant known as Tanner's Cassia (Ceasalpinaceae) or Avartaki in Ayurveda, *Cassia auriculata Linn.* is found in open woods all over India.

A protective function of autophagy in ovarian cancer cells was also induced by *Cassia auriculata*. Overall, this research showed that plant-based products activated the p-STAT3/Bcl-2 axis to cause ER stress, apoptosis, and autophagy (Figure 4). According to a different study, quercetin reduces viability and triggers apoptosis in cells harboring metastatic ovarian cancer. Several anti-apoptotic molecules, including Bcl-2 and Bcl-xL, are decreased by bioactive compounds, whereas pro-apoptotic molecules, such as caspase-3, caspase-9, Bid, Bax, Bad, and cytochrome c, are increased. Additionally, natural products induce mitochondrial-mediated apoptosis, which stops the spread of ovarian cancer cells that have metastasized. A recent study investigated the anti-cancer capabilities of quercetin's nano-formulation.[64] In vitro and in animals that had received ovarian cancer xenografts, this type of quercetin greatly slowed the growth of ovarian cancer cells. By upregulating caspase-3, caspase-9, and Bax while down-regulating MCL-1 and Bcl-2, quercetin in nanoformulations also induced apoptosis.[65]

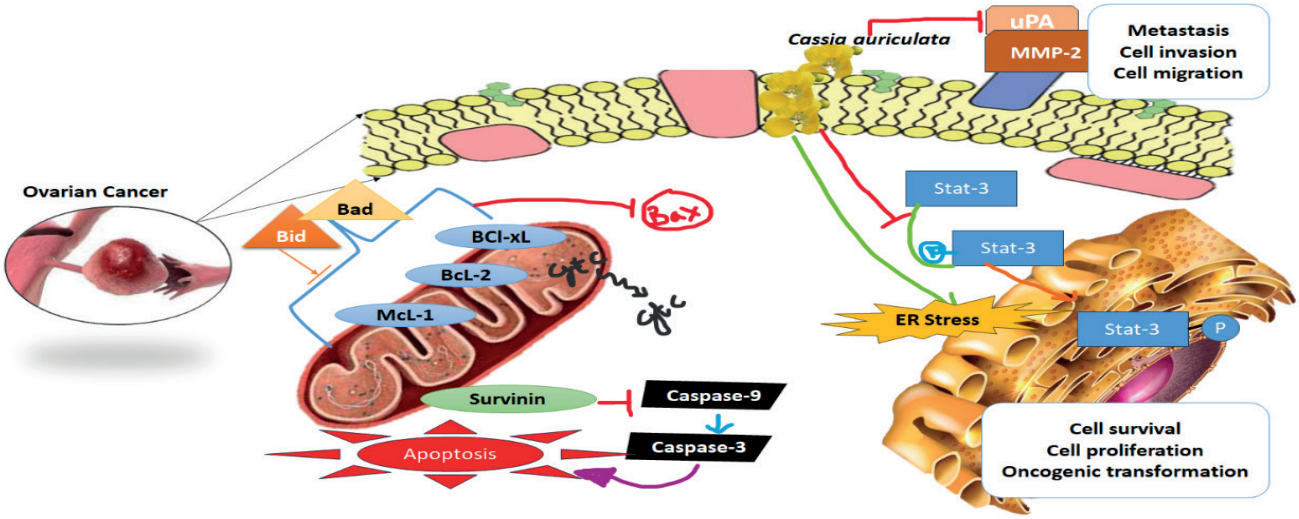

Figure 4. *Schematic representation of a potential therapeutic strategy for treating ovarian cancer that targets multiple signaling pathways with plant-based products.*

Nano medication delivery issues

The utilization of various nanomaterials with desired properties and significant obstacles to the management and treatment of cancer have been brought to light by recent developments in the drug delivery sector. It is anticipated that the usage of nanoparticles would radically revolutionize the healthcare industry, based on the tremendous breakthroughs made in the pharmaceutical delivery sector over the past few decades. Only a few nanoformulations have entered clinical trials, and it is still difficult to create effective cancer nanotherapeutics. Figure 5 shows a schematic illustration of the principal difficulties in delivering cancer nanotherapeutics.

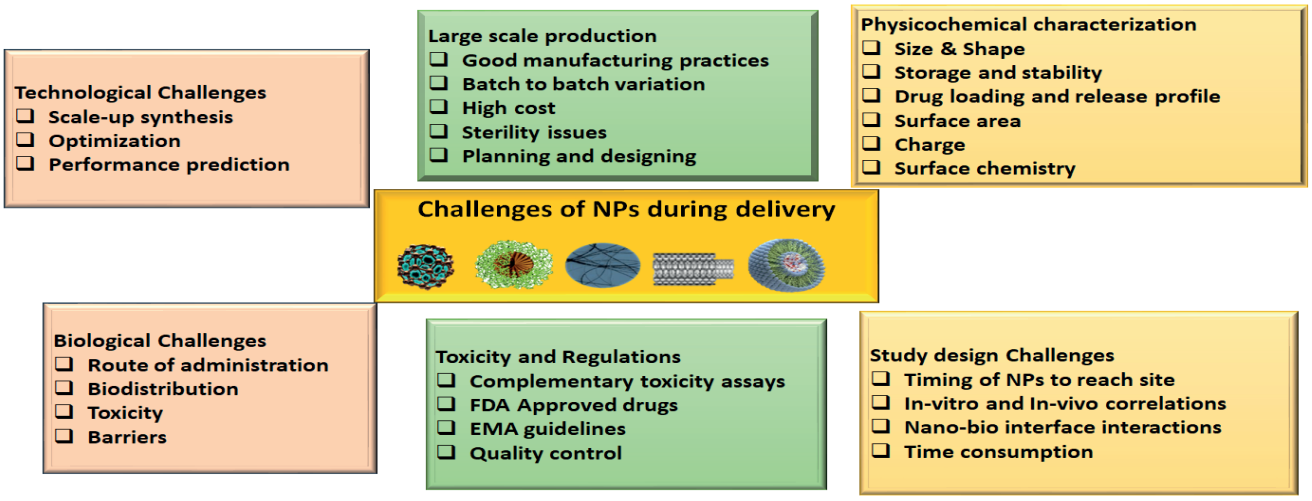

Figure 5. *Illustrates the grand challenges and perspectives for nanotechnology in healthcare systems.*

The advancement of nanotechnology has significantly expanded our understanding of and research into nanoparticles. Only a small portion of them, nevertheless, participate in the in-vivo and in-vitro clinical studies to reach their conclusion. Every nano-formulation has unique difficulties in the clinical setting. Even though most NPs deal with broad issues that fall into three categories-biological, technological and study-design-related, the majority of them are dealt with by these three categories. Lack of administration routes, biodistribution regulation, NPs' ability to overcome biological barriers, degradation, and toxicity are a few biological concerns.[66,67] Since NPs are frequently injected intravenously into the bloodstream, they are frequently removed from the body and find it challenging to interact with the target region. Some biological issues are lack of administration routes, regulating biodistribution, NPs' capacity to cross biological barriers, degradation, and toxicity.[68-70]

NPs have trouble interacting with the target area since they are typically injected intravenously into the circulation, where they are regularly eliminated from the body. The technical barriers for NPs are scale-up production, equal optimized performance, and efficacy projections. These are vital in assuring the beneficial accomplishment of nanoparticles. Many of the NPs used in in vivo and in vitro experiments are usually made in small batches, and scaling up for large volumes is occasionally impossible due to equipment and other factors. There is no systematic design or optimization process used to identify the lead therapeutic candidates that perform best in animal models. To circumvent this, we can practice certain procedures that can assess a range of nano-formulations after careful repetition and further choose one optimized formulation.[71-74] These hits, however, should be used later in human trials. It is challenging to predict the performance and effectiveness of nanoparticles, and it is impossible to reproduce in vivo outcomes in human investigations.

The fact that NPs are never used as first-line therapy is another serious issue. Nano-formulations have been successfully approved; however, they are normally saved for use in later treatment lines if disease progression is found. Most patients in the fictitious clinical trial have either gotten worse despite receiving multiple treatments or have developed drug resistance. The likelihood that NP treatment will help patients who are probably still curable is decreased in these circumstances, which frequently skew clinical trial outcomes.

Smart outlook on cancer nanomedicine

Smart thinking, logical reasoning, and realistic reasoning are all necessary to increase the therapeutic impact of cancer nanomedicines. In this vantage point, we outline four strategic objectives to enhance the performance, ease of application, and utilization of cancer nanomedicine.

- Probes and techniques for patient stratification are urgently required to improve cancer nanomedicine clinical trials, just as they are in other areas of oncology medication development.
- The likelihood that formulations created and tested in preclinical settings would eventually function well in patients will be increased through intelligent modular (pro)drug and nanocarrier design techniques, as well as library screening.
- The pharmacokinetic and/or pharmacodynamic advantages provided by entrapping pharmaceuticals in nanomedicine formulations will be amplified by rationally developed pharmacological and physical combination regimens.
- Last but not least, identifying the pathophysiological aspects that limit the effectiveness of current cancer immunotherapies and creating immuno-modulatory nanomedicines in response may assist in enhancing the results of immuno-oncological interventions and increase the number of long-term survivors.

The subject of cancer nanomedicine has rapidly grown in recent years. Only a handful of nano medicinal anticancer

medications, including antibody-drug conjugates, have made it to the market, in stark contrast to the numerous novel materials and publications being created. To remedy the situation, we must stop developing ever-more sophisticated nanomedicine materials and reevaluate our approach to translational cancer nanomedicine research. To ensure that nanomedicines are effective in as many people as possible, we must develop clever ways. This transition calls for consortia made up of academics, clinicians, pharmaceutical corporations, and regulatory agencies to think logically and realistically and to work together in concert. In addition to promoting the clinical impact and patient performance of nanomedicine-based anticancer medications, the strategic directions presented in this publication seek to streamline translational cancer nanomedicine research.

Conclusion

Numerous clinical settings for various disease types use cancer treatments based on the unique properties of NPs. NP-based DDS have been shown to have improved stability, tumor targeting, biocompatibility, and pharmacokinetics when compared to conventional medications. A hopeful new era in cancer treatment has begun with nanotechnology to deliver small molecules for cancer detection, diagnosis, and therapy. NPs also offer a superb platform for combination therapy that aids in conquering MDR. As a result of expanding research, many NP types, including metallic, hybrid, and polymeric NPs, have been shown to improve drug delivery effectiveness. The properties of the recommended nanoplatforms and those of medicinal drugs need to be closely studied by researchers.

In conclusion, we are quickly developing a far greater grasp of the difficulties and chances posed by cancer nanomedicine. For the more successful research and clinical translation of nanotherapeutics, this review has examined the significance of the convergence of nanotechnology and cancer biology. Additional effort must be made in “understand toxicity, cellular and physiological factors that regulate NP-based drug delivery, EPR, and PC mechanism” in the human body if one is to develop nanotechnology with any degree of rationality. We assume that the development of cancer therapies and, based on the results discussed above, nanotechnology will lead to a revolution in the clinical translation of NP-based cancer therapeutics. We anticipate that nanomedicines will change how cancer is treated and that in the not-too-distant future, the fundamental objective of cancer nanomedicine’s dramatic improvement in patient survival will become a reality.

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K.R. Padma and S.Mamatha Ramani solely drafted the paper under the guidance of Prof.P.Josthna. All the authors approved and are thankful to the Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam (Women’s University), Tirupati-Andhra Pradesh, India.

Conflict of interest

The authors do not have any conflict of interest (financial or other) other than those declared.

Consent for publication

NA.

References

[1] Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev. 2001; 22(2): 255-88. doi: 10.1210/edrv.22.2.0422.

[2] Zahedi P, Yoganathan R, Piquette-Miller M, Allen C. Recent advances in drug delivery strategies for treatment of ovarian cancer. Expert Opin Drug Deliv. 2012; 9(5): 567-83. doi: 10.1517/17425247.2012.665366.

[3] Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet. 2019; 393(10177): 1240-53. doi: 10.1016/S0140-6736(18)32552-2.

[4] Helen HW, Macus C, Kuo T. Improving radiotherapy in cancer treatment: Promises and challenges. Oncotarget. 2017; 8(37): 62742-58. doi: 10.18632/oncotarget.18409.

[5] Khosravi-Shahi P, Cabezón-Gutiérrez L , Custodio-Cabello S. Metastatic triple negative breast cancer: Optimizing treatment options, new and emerging targeted therapies. Asia Pac J Clin Oncol. 2018; 14(1): 32-9. doi: 10.1111/ajco.12748.

[6] Aggarwal RR, Feng FY, Small EJ. Emerging Categories of Disease in Advanced Prostate Cancer and Their Therapeutic Implications. Oncology (Williston Park). 2017; 31(6): 467-74.

[7] Gao X, Zhang M, Tang Y, Liang X. Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis. Onco Targets Ther. 2017; 10: 5219-28.

[8] Baselga NVJ, Hyman DM. A view on drug resistance in cancer. Nature. 2019; 575(7782): 299-309. doi: 10.1038/s41586-019-1730-1.

[9] Rueff J, Rodrigues AS. Cancer Drug Resistance: A Brief Overview from a Genetic Viewpoint. Methods Mol Biol. 2016; 1395: 1-18. doi: 10.1007/978-1-4939-3347-1_1.

[10] Hinohara K, Polyak K. Intratumoral Heterogeneity: More Than Just Mutations, Trends Cell Biol. 2019; 29(7): 569-79. doi: 10.1016/j.tcb.2019.03.003.

[11] Tian Y, Guo R, Wang Y, Yang W. Coordination-induced assembly of intelligent polysaccharide-based phototherapeutic nanoparticles for cancer treatment. Adv Healthc Mater. 2016; 5 (24): 3099-104. doi: 10.1002/adhm.201600877.

[12] Yang C, Xia BR, Zhang ZC, *et al*. Immunotherapy for ovarian cancer: adjuvant, combination, and neoadjuvant. *Front Immunol*. 2020; 11: 577869 doi: 10.3389/fimmu.2020.577869.

[13] Bozzer S, Dal Bo M, Grimaldi MC, Toffoli G, Macor P. Nanocarriers as a delivery platform for anticancer treatment: biological limits and perspectives in B-cell

malignancies. *Pharmaceutics*. 2022; 14 (9): 1965. doi: 10.3390/pharmaceutics14091965.

[14] Liu Y, Fns M, Lou B, et al. π-π-stacked poly(ε-caprolactone)-b-poly(ethylene glycol) micelles loaded with a photosensitizer for photodynamic therapy. *Pharmaceutics*. 2020; 12(4): 338 doi: 10.3390/pharmaceutics12040338.

[15] Tsouris V, Joo MK, Kim SH, Kwon IC, Won YY. Nano carriers that enable co-delivery of chemotherapy and RNAi agents for treatment of drug-resistant cancers. *Biotechnol Adv*. 2014; 32(5): 1037-50. doi: 10.1016/j.biotechadv.2014.05.00620.

[16] Shi Y, van der Meel R, Chen X, Lammers T. The EPR effect and beyond: strategies to improve tumor targeting and cancer nanomedicine treatment efficacy. *Theranostics*. 2020; 10(17): 7921-4. doi: 10.7150/thno.49577.

[17] Maeda H. Polymer therapeutics and the EPR effect. *J Drug Target*. 2017; 25(9-10): 781-5. doi: 10.1080/1061186X.2017.1365878.

[18] Rai S, Singh N, Bhattacharya S. Concepts on smart nano-based drug delivery system. *Recent Pat Nanotechnol*. 2022; 16(1): 67-89. doi: 10.2174/1872210515666210120113738.

[19] Li Y, Gao Y, Zhang X, Guo H, Gao H. Nanoparticles in precision medicine for ovarian cancer: from chemotherapy to immunotherapy. *Int J Pharm*. 2020; 591: 119986. doi: 10.1016/j.ijpharm.2020.119986.

[20] Wang X, Xiong T, Cui M, *et al*. A novel targeted co-delivery nanosystem for enhanced ovarian cancer treatment via multidrug resistance reversion and mTOR-mediated signaling pathway. *J Nanobiotechnol*. 2021; 19(1): 444. doi: 10.1186/s12951-021-01139-1.

[21] Barani M, Bilal M, Sabir F, Rahdar A, Kyzas GZ. Nanotechnology in ovarian cancer: diagnosis and treatment. *Life Sci*. 2021; 266: 118914. doi: 10.1016/j.lfs.2020.118914.

[22] Nishio S, Ushijima K. Clinical significance of primary debulking surgery and neoadjuvant chemotherapy-interval debulking surgery in advanced ovarian cancer. Jpn J Clin Oncol. 2020; 50(4): 379-86. doi: 10.1093/jjco/hyaa015.

[23] Prat J. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet. 2014; 124 (1): 1-5. doi: 10.1016/j.ijgo.2013.10.001.

[24] Kim S, Han Y, Kim SI, Kim HS, Kim SJ, Song YS. Tumor evolution and chemoresistance in ovarian cancer. NPJ Precis Oncol. 2018; 2: 20. doi: 10.1038/s41698-018-0063-0. eCollection 2018.

[25] Heintz AP, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT, et al. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. 2006; 95 (Suppl. S1), S161-S192. doi: 10.1016/S0020-7292(06)60033-7.

[26] Salani R, Axtell A, Gerardi M, Holschneider C, Bristow RE. Limited utility of conventional criteria for predicting unresectable disease in patients with advanced stage epithelial ovarian cancer. Gynecol Oncol. 2008; 108(2): 271-5. doi: 10.1016/j.ygyno.2007.11.004.

[27] Schorge JO, McCann C, Del Carmen MG. Surgical debulking of ovarian cancer: What difference does it make? Rev Obstet Gynecol. 2010; 3(3): 111-7.

[28] Du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: A combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: By the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer. 2009; 115(6): 1234-44. doi: 10.1002/cncr.24149.

[29] Basta ABM, Bie ´nkiewicz A, Blecharz P, Bodnar L, Jach R, Knapp P, et al. Recommendation of the Polish society of oncological gynaecology for the diagnosis and treatment of ovarian cancer. Oncol Clin Pract. 2017; 15 (1): 5-23. doi: 10.15557/CGO.2017.0001.

[30] Parmar MK, Ledermann JA, Colombo N, du Bois A, Delaloye JF, Kristensen, et al. Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: The ICON4/AGO-OVAR-2.2 trial. Lancet. 2003; 361(9375): 2099-106. doi: 10.1016/s0140-6736(03)13718-x.

[31] Ozaki S, Takigawa N, Ichihara E, Hotta K, Oze I, Kurimoto E, et al. Favorable response of heavily treated Wilms' tumor to paclitaxel and carboplatin. Onkologie. 2012; 35(5): 283-6. doi: 10.1159/000338532.

[32] Cristea M, Han E, Salmon L, Morgan RJ. Practical considerations in ovarian cancer chemotherapy. Ther Adv Med Oncol. 2010; 2(3): 175-87. doi: 10.1177/1758834010361333.

[33] Högberg T, Glimelius B, Nygren P. A systematic overview of chemotherapy effects in ovarian cancer. Acta Oncol. 2001; 40(2-3): 340-60. doi: 10.1080/02841860151116420.

[34] Aabo K, Adams M, Adnitt P, Alberts DS, Athanazziou A, Barley V, et al. Chemotherapy in advanced ovarian cancer: Four systematic meta-analyses of individual patient data from 37 randomized trials. Advanced Ovarian Cancer Trialists' Group. Br J Cancer. 1998; 78(11): 1479-87. doi: 10.1038/bjc.1998.710.

[35] Neijt JP, Engelholm SA, Tuxen MK, Sorensen PG, Hansen M, Sessa C, et al. Exploratory phase III study of paclitaxel and cisplatin versus paclitaxel and carboplatin in advanced ovarian cancer. J Clin Oncol. 2000; 18(17): 3084-92. doi: 10.1200/JCO.2000.18.17.3084.

[36] Pokhriyal R, Hariprasad R, Kumar, L, Hariprasad G. Chemotherapy resistance in advanced ovarian cancer patients. Biomark Cancer. 2019; 11: 1179299X19860815. doi: 10.1177/1179299X19860815.

[37] Damia G, Broggini M. Platinum Resistance in Ovarian Cancer: Role of DNA Repair. Cancers. 2019; 11(1): 119. doi: 10.3390/cancers11010119.

[38] Davis A, Tinker AV, Friedlander M. "Platinum resistant" ovarian cancer: What is it, who to treat and how to measure benefit? Gynecol Oncol. 2014; 133(3): 624-31. doi: 10.1016/j.ygyno.2014.02.038.

[39] Li H, Liu ZY, Wu N, Chen YC, Cheng Q, Wang J. PARP inhibitor resistance: The underlying mechanisms and clinical implications. Mol Cancer. 2020; 19(1): 107. doi: 10.1186/s12943-020-01227-0.

[40] Haley B, Frenkel E: Nanoparticles for drug delivery in cancer treatment. Urol Oncol. 2008; 26(1): 57-64. doi: 10.1016/j.urolonc.2007.03.015.

[41] Macchione MA, Biglione C, Strumia M. Design, synthesis and architectures of hybrid nanomaterials for therapy and diagnosis applications. Polymers. 2018; 10(5): 527. doi.org/10.3390/polym10050527.

[42] Senapati S, Mahanta AK, Kumar S, Maiti P. Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther. 2018; 3: 7. doi.org/10.1038/s41392-017-0004-3.

[43] Desai KGH. Polymeric drug delivery systems for intraoral site-specific chemoprevention of oral cancer. J Biomed Mater Res. 2018; Part B, 106(3): 1383-413. doi: 10.1002/jbm.b.33943. Epub 2017 Jun 26.

[44] Hekman MCH, Boerman OC, Bos DL, Massuger LFAG, Weil S, Grasso L, et al. Improved intraoperative detection of ovarian cancer by folate receptor alpha targeted dual-modality imaging. Mol Pharm. 2017; 14: 3457-63.

[45] Judy RP, Keating JJ, DeJesus EM, Jiang JX, Okusanya OT, Nie S, et al Quantification of tumor fluorescence during intraoperative optical cancer imaging. Sci Rep. 2015; 5: 16208. doi: 10.1038/srep16208.

[46] Falagan-Lotsch P, Grzincic EM, Murphy CJ. New Advances in Nanotechnology-Based Diagnosis and Therapeutics for Breast Cancer: An Assessment of Active-Targeting Inorganic Nanoplatforms. Bioconjug Chem. 2017; 18; 28(1): 135-52. doi: 10.1021/acs.bioconjchem.6b00591.

[47] Giulio Caraccioloa, Hojatollah Vali b, Anna Moorec, Morteza Mahmoudi. Challenges in molecular diagnostic research in cancer nanotechnology, Nano Today. 2019; 27: 6-10. doi.org/10.1016/j.nantod. 2019.06.001.

[48] www.who.int/gho/publications/worldhealthstatistics/2017.

[49] https://www.efpia.eu/.

[50] M, Lippi G. Cancer diagnostics: current concepts and future perspectives. Ann Transl Med. 2017; 5(13): 268. doi: 10.21037/atm.2017.06.20.

[51] Caracciolo G, Safavi-Sohi R, Malekzadeh R, Poustchi H, Vasighi M, Chiozzi RZ, et al. Nanoscale Horizons Correction: Disease-specific protein corona sensor arrays may have disease detection capacity nanoscale. Horiz. 2018; 5: 372. doi.org/10.1039/c9nh00097f.

[52] Smith B.R., Gambhir S.S., Engineered Manganese-Based Nanoparticles as MRI Contrast Agents for Early Tumor Detection. Chem Rev. 2017; 117(3): 901-86.

[53] Duvshani-Eshet M, Benny O, Morgenstern A, Machluf M. Therapeutic ultrasound facilitates antiangiogenic gene delivery and inhibits prostate tumor growth. Mol Cancer Ther. 2007; 6(8):2371-82. doi: 10.1158/1535-7163.MCT-07-0019.

[54]. Deng Z, Yan F, Jin Q, Li F, Wu J, Liu X, et al. Reversal of multidrug resistance phenotype in human breast cancer cells using doxorubicin-liposome-microbubble complexes assisted by ultrasound. J Control Release. 2014; 174, 109-16. doi: 10.1016/j.jconrel.2013.11.018.

[55] Bisi A, Cappadone C, Rampa A, Farruggia G, Sargenti A, Belluti F, et al. Coumarin derivatives as potential antitumor agents: Growth inhibition, apoptosis induction and multidrug resistance reverting activity. Eur J Med Chem. 2017; 127: 577-85. doi: 10.1016/j.ejmech.2017.01.020.

[56] Mignani S, Rodrigues J, Thomas H, Zablocka M, Shi X, Caminade AM, et al. Dendrimers in combination with natural products and analogues as anti-cancer agents. Chem Soc Rev. 2018; 47: 514-32.

[57] Liu X, Dong J, Cai W, Pan Y, Li,R, Li B. The effect of thymoquinone on apoptosis of SK-OV-3 ovarian cancer cell by regulation of Bcl-2 and bax. Int. J. Gynecol Cancer. 2017; 27(8): 1596-601. doi: 10.1097/IGC.0000000000001064.

[58] Qiu M, Xue C, Zhang L. Piperine alkaloid induces anticancer and apoptotic effects in cisplatin resistant ovarian carcinoma by inducing G2/M phase cell cycle arrest, caspase activation and inhibition of cell migration and PI3K/Akt/GSK3 signaling pathway. J Balkan Union Oncol. 2019; 24: 2316-21.

[59] Ren MX, Deng XH, Ai F, Yuan GY, Song HY. Effect of quercetin on the proliferation of the human ovarian cancer cell line SKOV-3 in vitro. Exp Ther Med. 2015; 10(2): 579-83. doi: 10.3892/etm.2015.2536.

[60] Xu X, Shi J, Gao H, Li Q. Zeylenone inhibits proliferation and promotes apoptosis in ovarian carcinoma cells via Janus kinase 2/signal transducers and activators of transcription 3 pathways. J Obstet Gynaecol Res. 2018; 44(8): 1451-7. doi: 10.1111/jog.13690.

[61] Pourhanifeh MH, Darvish M, Tabatabaeian J, Fard MR, Mottaghi R, Azadchehr MJ, Jahanshahi M, et al. Therapeutic role of curcumin and its novel formulations in gynecological cancers. J Ovarian Res. 2020; 13: 130. doi: 10.1186/s13048-020-00731-7.

[62] Teekaraman D, Elayapillai SP, Viswanathan MP, Jagadeesan A. Quercetin inhibits human metastatic ovarian cancer cell growth and modulates components of the intrinsic apoptotic pathway in PA-1cell line. Chem Biol Interact. 2019; 300: 91-100. doi: 10.1016/j.cbi.2019.01.008.

[63] Park S, Lim W, Jeong W, Bazer FW, Lee D, Song G. Sideroxylin (Callistemon lanceolatus) suppressed cell proliferation and increased apoptosis in ovarian cancer cells accompanied by mitochondrial dysfunction, the generation of reactive oxygen species, and an increase of lipid peroxidation. J

Cell Physiol. 2018; 233(11): 8597-604. doi: 10.1002/jcp.26540.

[64] Gao X, Wang B, Wei X, Men K, Zheng F, Zhou Y, *et al*. Anticancer effect and mechanism of polymer micelle-encapsulated quercetin on ovarian cancer. Nanoscale. 2012; 4(22): 7021-30. doi: 10.1039/c2nr32181e.

[65] Ryman-Rasmussen, Jessica P, *et al*. "Penetration of Intact Skin by Quantum Dots with Diverse Physicochemical Properties." Toxicol Sci. 2006; 91(1): 159-65. doi: 10.1093/toxsci/kfj122.

[66] Jia G, Han Y, An Y, Ding Y, He C, Wang X, *et al* . NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma in vitro and in vivo. Biomaterials. 2018; 178, 302-16. doi.org/10.1016/j.biomaterials.2018.06.029.

[67] Jiang J, Oberdörster, G, Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. J Nanopart Res. 2009; 11: 77-89. doi.org/10.1007/s11051-008-9446-4.

[68] Awasthi R, Pant I, T Kulkarni G, Satiko Kikuchi I, de Jesus Andreoli Pint T, *et al.* Opportunities and challenges in nanostructure mediated drug delivery: Where do we stand?. Curr Nanomed. 2016; 6(2): 78-104. doi: 10.2174/2468187306666160808160330

[69] Dobrovolskaia MA, Aggarwa P, Hall JB, McNeil SE. Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. Mol Pharmaceutics. 2008; 5(4): 487-95. doi: 10.1021/mp800032f.

[70] Akinc A, Zumbuehl A, Goldberg M, Leshchiner ES, Busini V, Hossain N, et al. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. Nat Biotechnol. 2008: 26(5), 561-9. doi.org/10.1038/nbt1402.

[71] Love KT, Mahon KP, Levins CG, Whitehead KA, Querbes W, Dorkin JR, et al. Lipid-like materials for low-dose, in vivo gene silencing. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(5): 1864-9. doi.org/10.1073/pnas.0910603106.

[72] Tansathien K, Dechsri K, Opanasopit P, Nuntharatanapong N, Sukma M, Rangsimawong W. Investigational lipid nanocarriers and microspicule gel for dermal delivery of porcine placenta extract. J Curr Sci Technol. 2022; 12(3): 505-16. doi: 10.14456/jcst.2022.39.

[73] Thungsatianpun N, Mavichak R, T-Thienprasert N, Unajak S, Sinthuvanich C. Cell-penetrating peptide nanocomplexes enhanced cellular uptake of dsRNA in Sf9 cell line. J Curr Sci Technol. 2021; 11(2): 299-310. doi: 10.14456/jcst.2021.30.

[74] Chamsai B, Opanasopit P, Samprasit W. Dual-drugs-loaded polymeric nanoparticles formulation design based on response surface methodology of particle size and zeta potential. J Curr Sci Technol. 2020; 10 (2): 143-53.

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Effects of promoting eating foods containing bitter vegetables on nutritional status in the elderly

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Bitter meal, elderly, non-communicable diseases, adipose tissue, fasting blood sugar

ABSTRACT

Background: Non-communicable diseases pose a considerable risk for the elderly population. This study aimed to investigate the impact of incorporating bitter vegetables into the diets of elderly individuals on their nutritional status. The study involved regular consumption of northern foods, such as Malidmai (Peka), salae, neem (neem), bitter gourd, and cassia, at least once a day for three months.

Materials and methods: A randomized clinical trial recruited eighty individuals aged 60 years and above. One group was allowed to eat ad libitum, while the other group followed a recommended menu that emphasized bitter flavors. The study assessed dietary intake, body composition, and blood biochemical parameters.

Results: The findings revealed a significant reduction in energy, saturated fat, and cholesterol intake, with the bitter group experiencing a statistically significant decrease in sugar consumption. The bitter group also demonstrated a considerable reduction in anthropometric and metabolic parameters when compared to the control group and baseline measures. These results indicate the potential benefits of bitter substances in managing or preventing obesity and type 2 diabetes in the elderly.

Conclusion: Encouraging the elderly to consume at least one bitter meal per day for 12 weeks resulted in a reduction in weight gain, adipose tissue, sugar levels, and LDL-C. The study highlights the importance of incorporating bitter vegetables into the diets of elderly individuals for better nutritional status and health outcomes.

Introduction

According to recent statistics, as of 2021, Thailand's total population is 66.7 million. There has been a significant increase in the number of elderly citizens in Thailand in recent years, with the older population growing at an unprecedented rate. Half a century ago, Thailand only had two million elderly citizens. However, as of 2021, this number has surged to 12.5 million, equivalent to 19% of the country's population. It is projected that by 2022, at least 20% of Thailand's population will be 60 years or older, making it a "completely aged society.[1]

As we age, our ability to perceive taste declines. Research conducted on healthy elderly individuals has demonstrated that the taste threshold begins to increase and can lead to dysgeusia after the age of 70. Additionally, chewing difficulties caused by tooth loss or denture use can interfere with taste sensation and reduce saliva

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production.[2] The reduction in taste recognition ability is attributed to various causes, including the reported decrease in the number and density of taste buds on the tongue.[3,4] The relationship between taste and diabetes is complex as several factors, including genetics, environment, behavior, and sensory perception, can influence eating habits, some of which may contribute to the development of diabetes. Patients with diabetes have been shown to have a general decrease in taste function, especially about sweet taste. Hyperglycemia has been linked to an increase in taste threshold.[3]

Recently, there has been an escalating interest in the ability of bitter substances to regulate energy intake and enhance glycaemic control. This interest is based on reports from preclinical models, such as studies conducted in cell cultures or animals, which have demonstrated that bitter substances have potent effects on the secretion of gastrointestinal hormones and the slowing of gastric emptying.[5-7] Studies have established that these gut functions play crucial roles in regulating acute energy intake and postprandial glycemia.[8-10] Given these findings, bitter substances may serve as a novel approach to managing or preventing obesity and its comorbidities, especially type 2 diabetes.

Over the last 4-5 years, while rendering academic services to the communities residing in Phayao Province, it has been observed that the elderly population has a predilection towards consuming bitter vegetables. As a result, it can be inferred that the sensation of bitterness in food may act as a deterrent to malnutrition and prevent obesity and type 2 diabetes.

This study is significant for the elderly population who are experiencing malnutrition or are at risk of malnutrition. It recommends regularly consuming Northern Thai foods such as Malidmai (Peka), Salae, Neem (Neem), Bitter Gourd, and Cassia at least once daily for three months. Anthropometric, biochemical, clinical, and nutritional assessments were utilized as indicators for the prevention of malnutrition and type 2 diabetes in elderly individuals.

Methods

Subjects

Eighty elderly people aged 60 years or over in Phayao Province, calculated by the G-power program. Family as t-tests and statistical tests selected as Means Difference between two independent means (two groups), specify α err prob equal to 0.05 and set Power equal to 0.87 and use effect size as recommended by Cohen (1988), effect size equal to 0.7. To calculate the total sample size, 80 subjects were required to test, divided into two groups of 40 subjects each.

Study design

This research was designed as a randomized clinical trial for 12 weeks, divided into two groups of 40 older adults with no differences, with the first group being a control group and the second group being a bitter group. Before recruitment, each participant was screened for eligibility. A flow chart of the study enrolment is demonstrated in Figure 1.

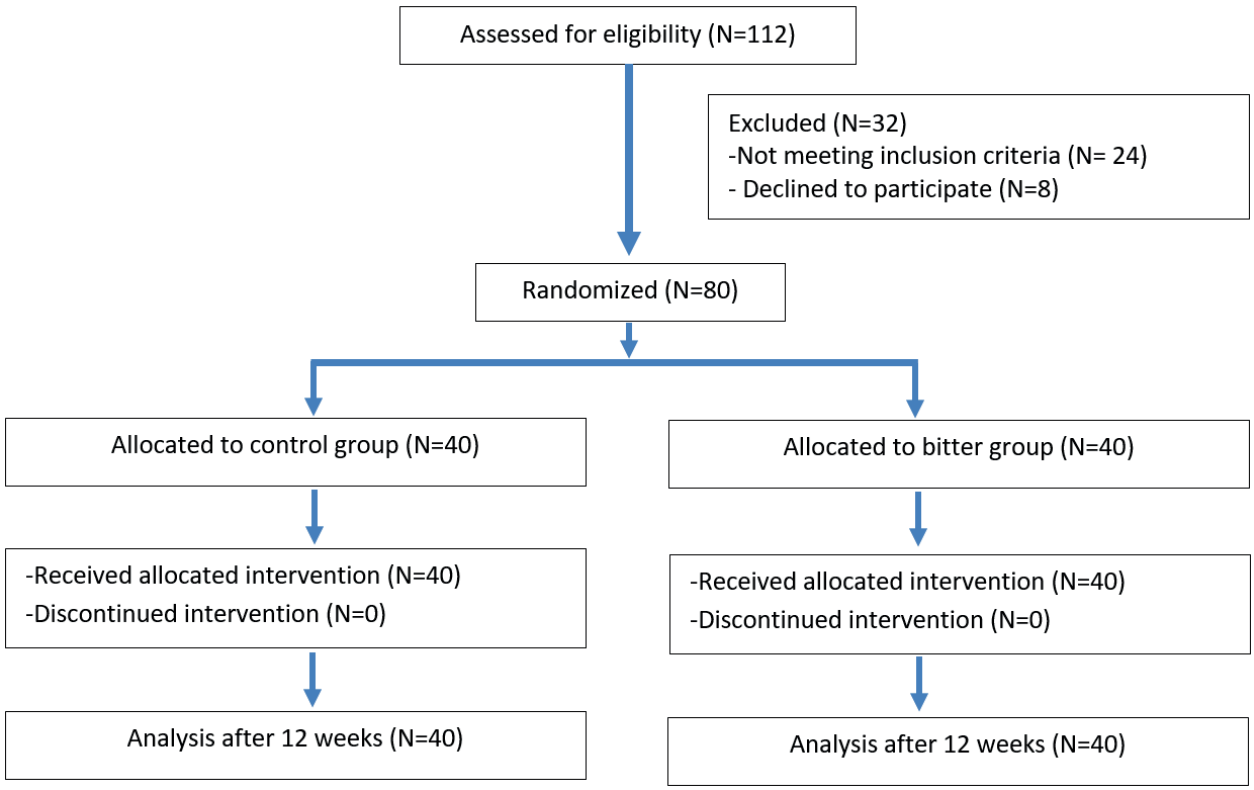

Figure 1. *Flow of participants in the study.*

Dietary

The control group was allowed to eat *ad libitum*. The experimental group, or the bitter group, was eating food according to the recommended menu. It is a local food menu that focuses on bitter tastes from Malidmai (Peka), salae, neem (neem), bitter gourd, cassia, sweet juice, gurmar, and Marsdenia glabra Cost, one meal a day. Both groups were trained before the start of the study so that they could make the right dietary choices for the elderly.

Inclusion criteria

An elderly person aged 60 years and over in Phayao Province. Malnutrition or at risk of malnutrition as elderly with greater than 23.0 kg/m^2, able to read and write well and willing to participate in the project.

Exclusion criteria

Elderly people with severe communicable diseases or severe NCDs, disabilities, chewing swallowing problems, or the inability to eat bitter foods were excluded from the study.

Body composition assessment

Height was measured by using a calibrated stadiometer. Body weight (kg), body fat (% of body weight), Visceral fat (level) were assessed using Tanita BC-418 ma segmental body composition analyzer (Tanita Co.Ltd., Japan).

Biochemical assessment

Biochemical measurements were conducted in both the control and experimental groups, including fasting blood sugar, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride. These parameters were analyzed using an automated blood BS-400 Chemistry Analyzer (Mindray bio-Medical Electronics Co. Ltd).

Dietary assessment

Energy and nutrient intakes were recorded 24 hrs. Dietary intake was calculated using INMUCAL-Nutrients version 3 software provided by the Institute of Nutrition, Mahidol University (Institute of Nutrition, 2016). All subjects were recorded for three days with a food record form at week 0 and week 12.

Ethics approval and permissions

This study, including protocol and consent forms for students and their parents, was approved by the Ethics committee at the University of Phayao (UP-HEC 1.3/010/65). Written informed consent was obtained from the subjects.

Statistical Analysis

Statistical analysis was performed using SPSS statistics version 18.0 (Levesque, 2007). Nutritional status (anthropometric, biochemical, and dietary data) was presented as mean (±SD) or percentage. The differences in body composition, biochemical data, and dietary data in the group were determined by the paired t-test. In addition, an independent t-test was employed to test the differences between the intervention and control groups. The $p<0.05$ was considered statistically significant.

Results

This study aimed to study the effect of encouraging the elderly to eat bitter vegetables on nutritional status. The elderly are at risk of developing non-communicable diseases. Therefore, 80 elderly people were recruited into the project to study the effects of promoting the consumption of bitter foods to reduce blood sugar and lipid levels.

At baseline, the characteristics of subjects in the two treatment groups do not differ in both body composition and biochemical assessment, as shown in Table 1.

Table 1. Baseline characteristics of subjects in the two treatment groups.

Parameters	Control group	Bitter group	*p* value*
Female gender, N (%)	72.5	70.8	
Age, years	68.4±5.41	67.24±4.81	0.312
Weight, kg	60.04±6.63	59.99±7.75	0.193
BMI, kg/m^2	24.88±1.75	24.37±2.44	0.076
FBS, mg/dL	92.12±11.12	92.90±17.38	0.214
Total cholesterol, mg/dL	198.83±40.06	197.54±49.57	0.907
HDL-C, mg/dL	51.20±11.08	51.88±14.49	0.924
LDL-C, mg/dL	124.20±35.16	125.83±37.63	0.298
LDL-C/HDL-C ratio	2.460±0.64	2.495±0.69	0.149
Triglyceride, mg/dL	117.83±55.05	118.15±39.77	0.658

*Note: Values are presented in mean (SD), *comparison between groups at baseline using Independent t-test, *statistically significant at p<0.05, FBS: fasting blood sugar, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.*

Table 2 shows that at week 0, subjects in both groups received enough energy from food according to the Dietary reference intake for Thais. At the same time, sugar and cholesterol were consumed more than the recommended daily amount.

After 12 weeks, there was a statistically significant decrease in energy intake, SFA, and cholesterol intake from baseline in both the control and bitter groups. When comparing groups, the bitter group had a statistically significant reduction in sugar consumption more than the control group at p=0.02, as shown in Table 3.

Anthropometric and metabolic parameters, including Weight, BMI, Body weight, Visceral fat, FBS, Total cholesterol, HDL-C, LDL-C, and Triglyceride after the 12-week nutritional intervention programmed both control and bitter groups were decreased when compared at baseline period as shown in Table 4.

After the 12-week nutritional intervention program, weight, BMI, body fat, FBS, and LDL-C in the bitter group were significantly reduced compared to baseline. When compared between groups, BMI and body fat in the bitter group were statistically significantly lower than the control group, as shown in Table 5.

Table 2. Comparison of dietary intakes between control and bitter groups at week 0 and week 12.

	Control group (N=40)		Bitter group (N=40)	
	Week 0	Week 12	Week 0	Week 12
Parameters	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
Energy intake, kcal	1599.91±161.35	1416.89±136.65	1601.86±162.51	1405.15±121.26
Energy distribution, % of total energy				
Carbohydrate	49.28±3.65	49.56±2.23	48.18±2.92	50.68±2.50
Protein	22.86±1.49	23.01±1.31	22.77±1.32	23.15±1.43
Fat	27.84±2.99	27.41±1.73	29.04±2.24	26.16±1.64
Sugar, gm	31.59±14.89	24.00±4.91	33.20±13.33	21.35±5.13
SFA, gm	18.26±3.13	15.34±2.44	18.70±2.85	14.73±1.85
Cholesterol, mg	361.17±95.86	295.47±26.28	369.18±87.67	284.50±23.15

Note: Values are presented in mean (SD), [a]comparison between week 0 and week 12 using the paired t-test, SFA: saturated fatty acid.

Table 3. Changes in dietary intakes in response to the 12-week nutritional intervention program.

	Control group			Bitter group			Control vs bitter group differences	
Parameters	**Δ 0-12 week**	**% Change from baseline**	***p* value**	**Δ 0-12 week**	**% Change from baseline**	***p* value**	**Mean**	***p* value**
Energy intake, kcal	-183.02	11.43	<0.001	-196.70	-12.27	<0.001	11.73	0.498
Energy distribution , % of total energy								
Carbohydrate	0.280	0.56	0.621	2.499	5.18	<0.001	1.114	0.038
Protein	0.151	0.66	0.513	0.382	1.67	1.133	0.140	0.647
Fat	-0.431	1.54	0.296	-2.881	9.92	<0.001	1.255	0.001
Sugar, gm	-7.592	24.03	0.002	-11.848	35.68	<0.001	2.647	0.020
SFA, gm	-2.920	15.99	< 0.001	-3.965	21.20	<0.001	0.605	0.212
Cholesterol, mg	-65.701	18.19	< 0.001	-84.680	22.93	<0.001	10.963	0.050

Note: Values are presented in mean (SD), Significant differences in the control vs bitter group between 0-12 weeks without adjustment for the baseline value (p≤0·05), SFA: saturated fatty acid.

Table 4. Anthropometric and metabolic parameters at baseline (time=0) and after the 12-week nutritional intervention program. (Mean±SD and CV).

	Control group (N=40)				Bitter group (N=40)			
	Week 0		Week 12		Week 0		Week 12	
Parameters	**Mean±SD**	**CV**	**Mean±SD**	**CV**	**Mean±SD**	**CV**	**Mean±SD**	**CV**
Weight, kg	60.04±6.63	0.11	58.29±8.76	0.15	59.99±7.75	0.12	56.50±6.25	0.11
BMI, kg/m^2	24.88±1.75	0.07	23.90±3.09	0.12	24.37±2.44	0.10	22.36±1.51	0.06
Body fat, %	32.38±6.77	0.20	30.48±8.29	0.27	30.94±7.75	0.25	26.79±6.66	0.24
Visceral fat, level	9.70±3.28	0.33	8.45±3.46	0.40	9.58±3.21	0.33	8.82±3.50	0.39
FBS, mg/dL	92.12±11.12	0.12	89.15±13.08	0.14	92.90±17.38	0.18	86.73±10.80	0.12
Total cholesterol, mg/dL	198.83±40.06	0.20	192.90±39.35	0.20	197.54±49.57	0.25	185.36±38.79	0.20
HDL-C, mg/dL	51.20±11.08	0.21	51.45±11.31	0.21	51.88±14.49	0.27	50.22±11.99	0.23
LDL-C, mg/dL	124.20±35.16	0.28	119.08±33.86	0.28	125.83±37.63	0.29	112.20±33.33	0.29
LDL-C/HDL-C ratio	2.46±0.64	0.26	2.33±0.52	0.22	2.49±0.69	0.27	2.27±0.61	0.26
Triglyceride, mg/dL	117.83±55.05	0.46	112.50±36.61	0.32	118.15±39.77	0.33	115.20±36.62	0.31

Note: Values are presented in mean (SD), [a]comparison between week 0 and week 12 using the Paired t-test, FBS: fasting blood sugar, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, coefficient of v(CV).

Table 5. Changes in anthropometric and metabolic parameters in response to the 12-week nutritional intervention program.

Parameters	*Control group*			*Bitter group*			Control vs bitter group differences	
	Δ 0-12 week	**% Change from baseline**	***p* value**	**Δ 0-12 week**	**% Change from baseline**	***p* value**	**Mean**	***p* value**
Weight, kg	-1.74	-2.89	0.209	-3.47	-5.78	0.024	1.790	0.292
BMI, kg/m^2	-0.98	-3.93	0.029	-2.00	-8.20	<0.001	1.534	0.006
Body fat, %	-1.90	-1.90	0.207	-4.15	-4.15	0.004	3.694	0.030
Visceral fat, level	-1.25	-12.88	0.102	-0.75	-7.82	0.299	0.379	0.626
FBS, *mg/dL*	-2.98	-3.23	0.077	-6.17	-6.64	0.016	2.418	0.366
Total cholesterol, *mg/dL*	-5.93	-2.98	0.169	-12.17	-6.16	0.077	7.534	0.388
HDL-C, *mg/dL*	0.25	0.49	0.800	-1.65	-3.18	0.306	1.230	0.636
LDL-C, *mg/dL*	-5.13	-4.13	0.239	-13.63	-10.83	0.008	6.879	0.360
LDL-C/HDL-C ratio	-0.12	4.87	0.149	-0.21	-8.43	0.014	0.055	0.666
Triglyceride, *mg/dL*	-5.33	-4.52	0.522	-2.95	-2.50	0.685	2.695	0.741

Note: Values are presented in mean (SD), Significant differences in the control vs bitter group between 0-12 weeks without adjustment for the baseline value (p≤0·05), FBS: fasting blood sugar, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, Δ 0-12: changes in anthropometric and metabolic parameters week 12 - week 0.

Discussion

Taste function in humans decreases with age. A reduction in taste recognition ability was described with increasing age. Older persons have a reduced taste of food, making them more likely to eat foods that are too sweet, salty, or fat, increasing the risk of NCDs. Encouraging older people to eat bitter foods may result in lower intakes of sugar, sodium, or fat from their diet. This reduces the risk of NCDs.

During the baseline assessment, it was observed that the elderly participants in the study exhibited an elevated body mass index while their blood sugar and lipid levels were within normal ranges.[11] However, the average values for these parameters were quite high and almost exceeded the standard thresholds. These findings are consistent with previous studies which have shown that a majority of the elderly population tends to have a higher body weight that exceeds the recommended BMI.[12] This is often associated with elevated levels of blood sugar and lipids.[13,14]

According to the 24-hr food questionnaire, it was found that the energy intake from food among the elderly was normal. However, their consumption of sugar, saturated fat, and cholesterol was higher than the recommended daily intake.[15] Overconsumption of sugar, saturated fat, and cholesterol can lead to an increase in

blood sugar, cholesterol, and LDL-C levels in the elderly, similar to the study in 2020, which can exceed the normal value.[16] This finding is consistent with the study conducted by Witek K *et al*., which suggests that consuming sweet-tasting foods with high sugar content is associated with diabetes and high blood sugar levels.[17]

Encouraging the elderly in the bitter group to consume at least one bitter meal per day for 12 weeks resulted in a statistically significant decrease in their daily sugar intake and LDL-C blood sugar levels compared to the control group. The quantity of bitter vegetables consumed in both groups was documented. The daily consumption of bitter vegetables was recorded for three days per week. The results revealed that the experimental group consumed an average of 40-50 grams per day, whereas the control group consumed approximately 5-10 grams per day. The possible mechanism underlying the reduction of blood sugar levels after consuming bitter-tasting food may conducted on preclinical models. It has been observed that bitter substances have a potent impact on upper gastrointestinal functions. This impact is primarily seen in the secretion of gut hormones such as CCK, GLP-1, and ghrelin. These hormones are associated with a decrease in food intake and body weight, as well as a reduction in postprandial blood glucose excursions. This beneficial effect has been observed in models of obesity and type 2 diabetes. However, when it comes to clinical studies, the outcomes have needed to be more consistent and varied. These clinical studies have only been conducted on healthy individuals. Nevertheless, the studies suggest that bitter compounds stimulate GLP-1, reduce postprandial glucose, and modestly reduce energy intake.[18]

In addition to the monitoring of blood glucose levels, the group who consumed bitter foods exhibited a statistically significant reduction in LDL-C levels. This reduction is believed to be due to the suppression of saturated fatty acid intake and subsequent decrease in cholesterol levels during the 12-week study period. The underlying mechanisms responsible for this effect are likely similar to those observed in previous studies.[18]

The study found that a decrease in energy intake from food, as well as a reduction in energy intake from fat and carbohydrates, led to a significant statistical reduction in body weight, BMI, and body fat among participants in the bitter group. Additionally, there was a noticeable decrease in abdominal fat, consistent with previous research, which concluded that weight loss of 6-7% with diet or with exercise plus diet reduced both subcutaneous and intra-abdominal fat.[19]

Based on the study, it can be observed that promoting the consumption of at least one bitter meal per day among the elderly can lead to a reduction in the intake of sugar, fat, and cholesterol from their diet. This reduction in consumption consequently leads to lower blood sugar and LDL-C levels, as well as a decrease in body weight and adipose tissue over a 12-week duration.

Conclusion

Encouraging the elderly to eat at least one bitter meal daily for 12 weeks resulted in weight loss. Adipose tissue, sugar levels, and LDL-C were statistically significantly reduced.

Conflict of interest

No

Funding

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References

[1] Pramote Prasartkul, Jongjit Rittirong, Sutthida Chuanwan, Napaphat Satchanawakul, Suporn Jaratsit, Kanchana Thianlai (2022). Situation of the Thai Older Persons 2021. Institute for Population and Social Research, Mahidol University; Nakhon Pathom. ISBN: 978-616-443-686-2.

[2] Shilpa Amarya, Kalyani Singh and Manisha Sabharwal. Aging Process and Physiological Changes. Reviewed: March 6th, 2018 Published: July 4th, 2018 doi: 10.5772/intechopen.76249

[3] Pugnaloni S, Alia S, Mancini M, Santoro V, Di Paolo A, Rabini RA, Fiorini R, Sabbatinelli J, Fabri M, Mazzanti L, Vignini A. A Study on the Relationship between Type 2 Diabetes and Taste Function in Patients with Good Glycemic Control. Nutrients. 2020; 12(4): 1112. doi: 10.3390/nu12041112.

[4] Sergi G., Bano G., Pizzato S., Veronese N., Manzato E. Taste loss in the elderly: Possible implications for dietary habits. *Crit Rev Food Sc Nutr.* 2017; 57(17): 3684-9, doi: 10.1080/10408398.2016.1160208.

[5] Avau B, Bauters D, Steensels S, Vancleef L, Laermans J, Lesuisse J, Buyse J, Lijnen HR, Tack J, Depoortere I. The Gustatory Signaling Pathway and Bitter Taste Receptors Affect the Development of Obesity and Adipocyte Metabolism in Mice. PLoS One. 2015; 10(12): e0145538. doi: 10.1371/journal.pone.0145538.

[6] Chen MC, Wu SV, Reeve JR Jr, Rozengurt E. Bitter stimuli induce Ca2+ signaling and CCK release in enteroendocrine STC-1 cells: role of L-type voltage-sensitive Ca2+ channels. Am J Physiol Cell Physiol. 2006; 291(4): C726-39. doi: 10.1152/ajpcell.00003.2006.

[7] Kim KS, Egan JM, Jang HJ. Denatonium induces secretion of glucagon-like peptide-1 through activation of bitter taste receptor pathways. Diabetologia. 2014; 57(10): 2117-25. doi: 10.1007/s00125-014-3326-5.

[8] Marathe CS, Rayner CK, Jones KL, Horowitz M. Relationships between gastric emptying, postprandial glycemia, and incretin hormones. Diabetes Care. 2013; 36(5): 1396-405. doi: 10.2337/dc12-1609.

[9] Steinert RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, Geary N. Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After

RYGB. Physiol Rev. 2017; 97(1): 411-63. doi: 10.1152/physrev.00031.2014.

[10] Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature. 2006; 444(7121): 854-9. doi: 10.1038/nature05484.

[11] Pan WH, Yeh WT. How to define obesity? Evidence-based multiple action points for public awareness, screening, and treatment: an extension of Asian-Pacific recommendations. Asia Pac J Clin Nutr. 2008; (3): 370-4.

[12] Kıskaç M, Soysal P, Smith L, Capar E, Zorlu M. What is the Optimal Body Mass Index Range for Older Adults? Ann Geriatr Med Res. 2022; 26(1): 49-57. doi: 10.4235/agmr.22.0012.

[13] Bae YJ, Shin SJ, Kang HT. Body mass index at baseline directly predicts new-onset diabetes and to a lesser extent incident cardio-cerebrovascular events, but has a J-shaped relationship to all-cause mortality. BMC Endocr Disord. 2022; 22(1): 123. doi: 10.1186/s12902-022-01041-3.

[14] Bai K, Chen X, Song R, Shi W, Shi S. Association of body mass index and waist circumference with type 2 diabetes mellitus in older adults: a cross-sectional study. BMC Geriatr. 2022; 22(1): 489. doi:10.1186/s12877-022-03145-w.

[15] Dietary reference intake for Thais 2020. The Committee on Allowances, Recommended Daily Dietary Nutrition Division, Department of Health, Ministry of Public Health. A.V. Progressive LTD., Bangkok. 2020. (in Thai).

[16] Carson JAS, Lichtenstein AH, Anderson CAM, Appel LJ, Kris-Etherton PM, Meyer KA, Petersen K, Polonsky T, Van Horn L; American Heart Association Nutrition Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Peripheral Vascular Disease; and Stroke Council. Dietary Cholesterol and Cardiovascular Risk: A Science Advisory From the American Heart Association. Circulation. 2020; 141(3): e39-e53. doi: 10.1161/CIR.0000000000000743.

[17] Witek K, Wydra K, Filip M. A High-Sugar Diet Consumption, Metabolism and Health Impacts with a Focus on the Development of Substance Use Disorder: A Narrative Review. Nutrients. 2022; 14(14): 2940. doi: 10.3390/nu14142940.

[18] Rezaie P, Bitarafan V, Horowitz M, Feinle-Bisset C. Effects of Bitter Substances on GI Function, Energy Intake and Glycaemia-Do Preclinical Findings Translate to Outcomes in Humans? Nutrients. 2021; 13(4): 1317. doi: 10.3390/nu13041317.

[19] van Gemert WA, Peeters PH, May AM, Doornbos AJH, Elias SG, van der Palen J, Veldhuis W, Stapper M, Schuit JA, Monninkhof EM. Effect of diet with or without exercise on abdominal fat in postmenopausal women - a randomised trial. BMC Public Health. 2019; 19(1): 174. doi: 10.1186/s12889-019-6510-1.

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Atorvastatin increases autophagic flux and p62/SQSTM1 of kidney cells in hyperglycemic conditions and treatment in combination with insulin improves renal function of streptozotocin (STZ)-induced diabetic rats

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ABSTRACT

Background: Although atorvastatin is commonly used as a hypolipidemic agent, it confers many health benefits in which the underlying mechanisms are not fully understood. We have previously shown that combined treatment of atorvastatin and insulin effectively restored renal function of streptozotocin (STZ)-induced diabetic rats; nevertheless, the underlying mechanism was not known.

Objective: To determine whether the reno-protective effect of atorvastatin and insulin is mediated through its impact on autophagy.

Materials and methods: Markers of autophagy, LC3, and p62/SQSTM1, in rat kidney tissues and cell lines treated with atorvastatin and/or insulin were determined by Western blot analysis.

Results: Levels of both LC3-I and LC3-II proteins in kidney tissues of STZ-diabetic rats treated with atorvastatin and insulin were significantly increased. The autophagic flux was examined in *vitro* and showed that high glucose culture conditions suppressed the autophagic flux in kidney cells. Treatment with insulin moderately increased the conversion of LC3-I to LC3-II. Interestingly, atorvastatin increased autophagic flux only in the hyperglycemic but not in the normoglycemic condition. p62/SQSTM1 protein level was decreased in response to high glucose treatment but increased with the addition of insulin and/or atorvastatin.

Conclusion: This study has demonstrated that atorvastatin may represent a novel regimen in providing prevention and protection for diabetic nephropathy through the underlying mechanisms of inducing autophagy and p62/SQSTM1.

Introduction

Diabetes is a major threat to human health worldwide. The spiraling epidemic of metabolic syndrome and obesity has primarily driven this. Diabetic nephropathy is a distressing complication of diabetes. It affects about 15-25% of type 1[1] and 30-40% of type 2 diabetic patients[2] and is responsible for a significant increase in morbidity and mortality in adults with diabetes. Therapeutic interventions to prevent the development and progression of diabetic kidney disease are urgently needed.

Chronic hyperglycemia or high blood glucose is a serious problem in diabetic patients. The pathogenesis of diabetic

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nephropathy is believed to be hyperglycemia-driven alterations of various metabolism, including the activation of protein kinase C, accumulation of advanced glycation end-products, and oxidative stress.[2-4] Current medications for diabetic nephropathy aim to control blood glucose levels and blood pressure to repeal the development of albuminuria and the progression of diabetic nephropathy.[5] However, the incidence of diabetic kidney disease continues to rise, and many patients with diabetic nephropathy experience a progressive decline of kidney function, highlighting a need for better therapeutic interventions and management.

Autophagy, a self-degradative process important for balancing energy sources at critical times in response to stress, serves as a critical mechanism to maintain homeostasis of glomeruli and tubules.[6] Impairment of autophagy is implicated in the pathogenesis of diabetic nephropathy.[7,8] An Emerging body of evidence suggests that targeting the autophagic pathway to activate and restore autophagy activity may be renoprotective.[9] We have previously shown that combined treatment between insulin and atorvastatin effectively restored renal function in streptozotocin (STZ)-induced diabetic rats.[10] Moreover, the combined treatment also decreased pancreatic inflammation and apoptosis in diabetic rats. In this study, we focused on the effect of atorvastatin and/or insulin on autophagy.

Material and methods

Animal treatment

The animal treatment protocol was approved by the Laboratory Animal Care and Use Committees at the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (Permit No: 12/2557) and carried out in male Wistar rats as described previously.[10] In brief, thirty-six male Wistar rats (200-250 gm) were randomly divided into control (12 rats) and diabetic (24 rats) groups. The control group was divided into two groups, control (C), and control that received atorvastatin (CS) (six rats per group). Rats in the diabetic group were intraperitoneally (i.p.) injected with 50 mg/kg body weight of streptozotocin in 10mM citrate buffer pH 4.5 while the control rats received the equivalent dose of citrate buffer as a vehicle. After seven days, rats with fasting blood glucose ≥250mg/dl were included in the diabetic group and assigned into four sub-groups (six rats per group): diabetic (DM), diabetic plus insulin (DMI), diabetic plus atorvastatin (DMS), and diabetic plus insulin and atorvastatin (DMIS). Insulin (4 units/day) was injected subcutaneously while 10 mg/kg/day of atorvastatin dissolved in saline was administered orally for four weeks. Rats were killed after being anesthetized using isofurane inhalation. The kidneys were removed immediately and kept at -80 °C for further western blot analysis.

Cell culture and treatment

The effect of atorvastatin and/or insulin on the autophagy of kidney cells was investigated using an African green monkey kidney cell line (Vero cells) kindly gifted by Dr. Khajornsak Tragoolpua.[11] Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 15 mM HEPES, 2 mM L-glutamine, and antibiotics. Approximately $1x10^6$ Vero cells were sub-cultured in a 60 mm culture dish overnight to allow cell attachment with about 65-80% cell confluent. Cells were subjected to culture in the control condition (5 mM glucose) or various additional concentrations of glucose (control, 10, 20, and 40 mM) or atorvastatin (0, 10, 20, 40 µM) or insulin (0, 100, 200, 400 nM) with or without lysosomal inhibitors E-64d and pepstatin A, added to the culture media 6 hours before cell harvesting. The cell lysate was stored at -20 °C until autophagy analysis by Western blotting.

Western blot analysis

The kidney tissues from experimental rats or Vero cells treated with various conditions were homogenized in SDS lysis buffer and heated at 95 °C for 10 minutes. Cell lysates (25 µg) were resolved on SDS polyacrylamide gels under reducing conditions and electrotransferred onto a PVDF membrane. After blocking, the membrane was incubated with primary antibodies for 1 hour at room temperature (LC3 antibody (Santa Cruz, sc-398822) and anti-p62 antibody (Santa Cruz, sc-28359)). Bound antibodies were detected with horseradish peroxidase (HRP)-conjugated goat anti-mouse (Dako, cat.no. P0447) for 1 hour. After extensive washing, immunoreactive protein was visualized using the Clarity ECL detection kit (Biorad Laboratories). After band visualization, the PVDF membrane was stripped and re-probed with anti-beta actin antibody to verify the amount of protein loaded into each lane. Band intensities of LC3-I, LC3-II, and beta-actin form each sample were quantified using Image J.[12] Means and standard deviations (SDs) of the relative LC3-II/LC3-I ratio from three independent experiments of each treatment condition were graphically represented as a bar graph, in which the ratio of LC3-II/LC3-I from cells without the lysosomal inhibitor treatment was adjusted to 1.0.

Statistical analysis

Statistical analyses were performed using SPSS software version 18 (SPSS, Inc., Chicago, IL, USA). The Mann-Whitney U test was used to analyze the comparison of means between groups, with $p<0.05$ considered statistically significant.

Results

Effects of atorvastatin and insulin on autophagy of kidney cells in STZ-induced diabetic rats.

We have previously shown that combined atorvastatin (10 mg/kg/day) and low-dose insulin treatment (4 units/day) exhibit protective effects on kidney cells and lead to the reversal of pancreatic β-cell function in streptozotocin-induced diabetic rats.[10] To understand the effects of atorvastatin and insulin on autophagy, cell lysate prepared from kidney tissues of the experimental animals was subjected to Western blot analysis to determine LC3 protein level. The Western blot results showing the level of LC3-I and LC3-II are shown in Figure 1. LC3 is a microtubule-associated protein light chain three widely used to monitor autophagy. Determination of autophagy level is frequently carried out

by detecting LC3 conversion from LC3-I to a smaller form LC3-II because the amount of LC3-II is reported to correlate with the number of autophagosomes. However, when interpreting the results of LC3 immunoblotting, one needs to consider that LC3-II itself is also degraded by autophagy. From Figure 1, there were significant changes in LC3 protein in rats receiving insulin and autophagy. However, a simple ratio determination of LC3-II/ LC3-I would indicate a similar ratio as non-treatment and atorvastatin control. In contrast, a simple comparison of LC3-II between samples would indicate a significant induction of autophagy, an opposite effect. Therefore, we investigated the effect of insulin and atorvastatin on autophagy of kidney cells *in vitro* by the determination of LC3-II delivered to lysosomes by comparing LC3-II levels in the presence and absence of lysosomal protease inhibitors, which is a more reliable method for monitoring autophagy.[13]

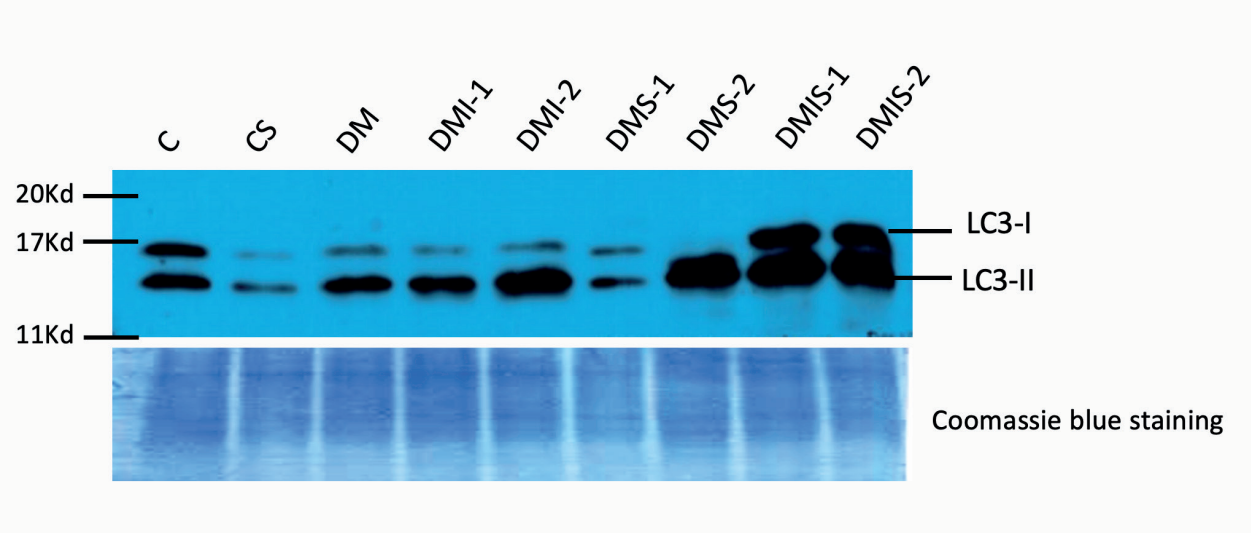

Figure 1. *Western blot analysis showing the levels of autophagy marker (LC3-I and LC3-II) in renal tissues of STZ-induced diabetic rats receiving atorvastatin and/or insulin. C: control group, DM: diabetic group, DMI: diabetic plus insulin group, DMS: diabetic plus atorvastatin group. DMIS: diabetic plus insulin and atorvastatin group, CS: control atorvastatin group.*

Effect of insulin and atorvastatin on autophagy flux under normoglycemic condition.

To test the contribution of insulin and atorvastatin on autophagy of kidney cells, an *in vitro* experiment was performed using African green monkey kidney (Vero) cells. Overnight 60 mm cultured dishes of Vero cells in DMEM were treated with various concentrations of insulin (0, 100, 200 nm) and/ or atorvastatin (0, 10, 20 µM) for 12-16 hours in duplicated. Lysosomal protease inhibitor (E64d and pepstatin A, 10 µg/ml each) was added to one of the two culture dishes 4 hours before cell harvesting. Then, the levels of LC3 in the presence and absence of lysosomal inhibitor were determined by Western blot analysis. Detected bands were quantified using ImageJ software[12] and the ratio of LC3-II/LC3-I band intensities was calculated. The values obtained in the absence of lysosomal inhibitors were adjusted as 1.0, and relative change in the presence of lysosomal inhibitors was then calculated and considered to represent autophagic flux. The results showed that although modestly, insulin can increase autophagic flux (Figure 2B), whereas atorvastatin did not show any impact (Figure 2A) under the normoglycemic culture condition.

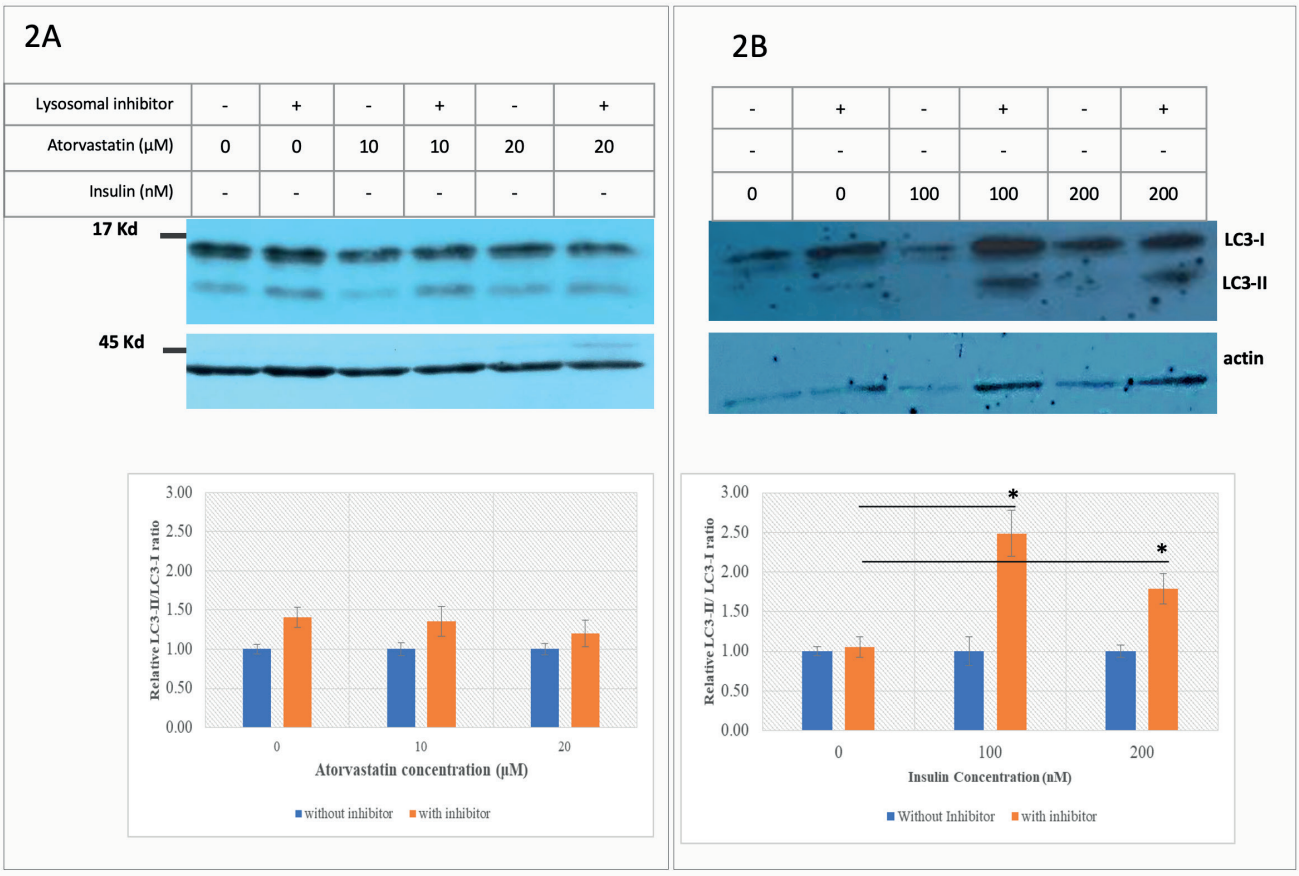

Figure 2. *Representative western blots and bar graph of relative LC3-II/ LC3-I ratio showing the effect of insulin (2A) and atorvastatin (2B) on autophagy flux of kidney cells cultured in normoglycemic (basal level of 5 mM glucose) condition. *Significant difference from non-treated control ($p<0.05$ by Mann Whitney U test)*

High glucose culture condition suppresses autophagy flux in vitro.

To test the contribution of glucose concentration on autophagy level, Vero cells were either cultured in standard Dulbecco's Modified Eagles Medium (DMEM) (5 mM glucose) or with various additional concentrations of glucose (10, 20, 40 mM) overnight. Level of LC3 protein was detected in the presence and absence of lysosomal protease inhibitors by Western blot analysis. The results showed that autophagy was drastically suppressed with the supplement of 40 mM glucose. Therefore, this concentration was used for further experiments (Figure 3).

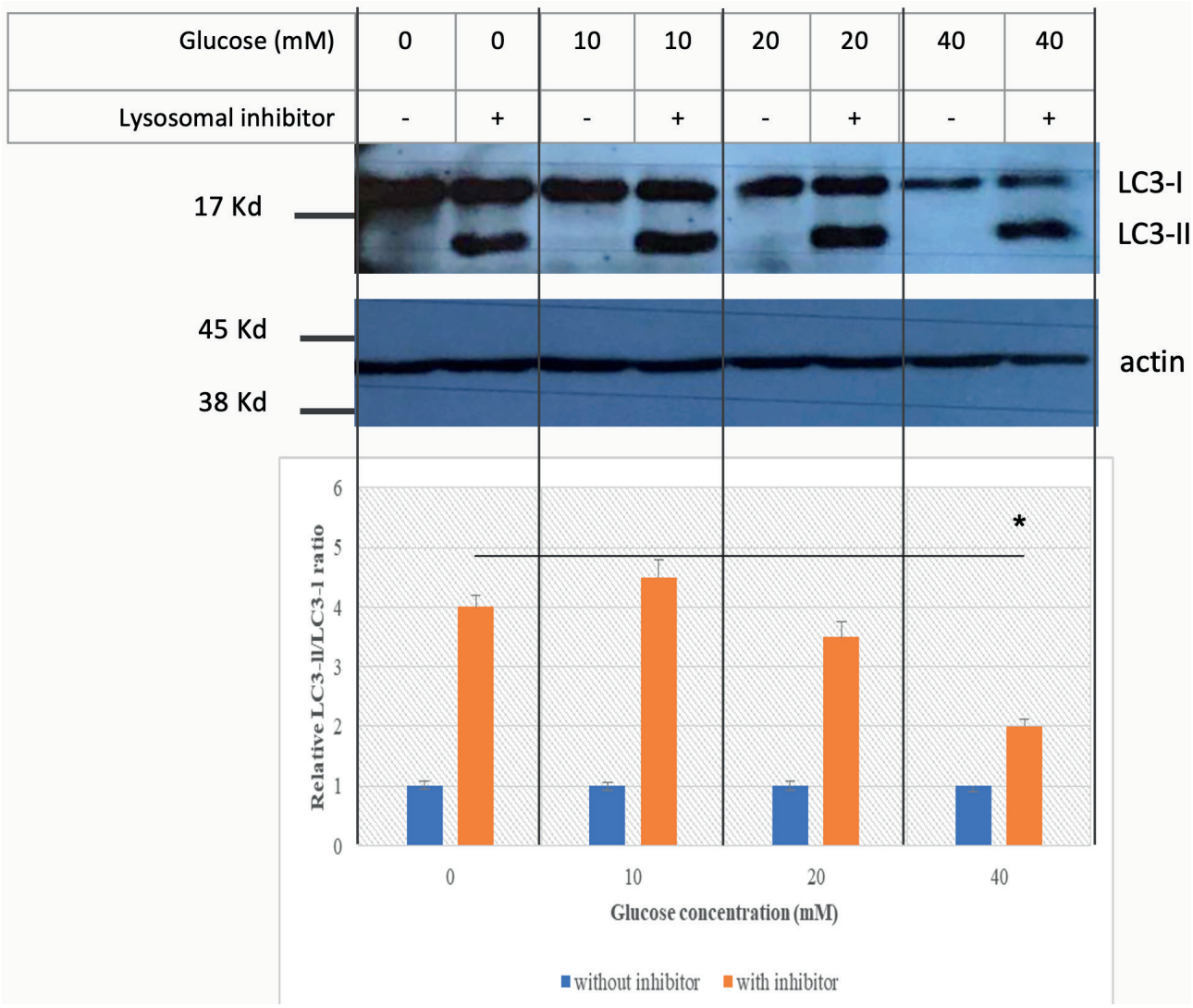

Figure 3. *Representative western blots and bar graph of relative LC3-II/ LC3-I ratio showing the effect of glucose concentration on autophagy flux of kidney cells. *Significant difference from non-treated control ($p<0.05$ by Mann Whitney U test)*

Atorvastatin increased autophagic flux under hyperglycemic condition.

Overnight 60mm cultured dish of Vero cells in DMEM supplemented with 40 mM glucose were treated with various concentrations of insulin (0, 100, 200 nm) or atorvastatin (0, 10, 20 µM) for 12-16 hours. Lysosomal protease inhibitor was added to one of the culture media 4 hours before cell harvesting, and LC3 levels were examined by Western blot analysis. Similar to its effect under normoglycemic condition, insulin increased autophagic flux under hyperglycemic condition. Although atorvastatin did not impact autophagy under normoglycemic conditions, interestingly, it increased autophagic flux under hyperglycemic conditions. Nevertheless, although the initial dose of insulin or atorvastatin was able to induce autophagy, further increased doses did not have any significant impact (Figure 4A-B).

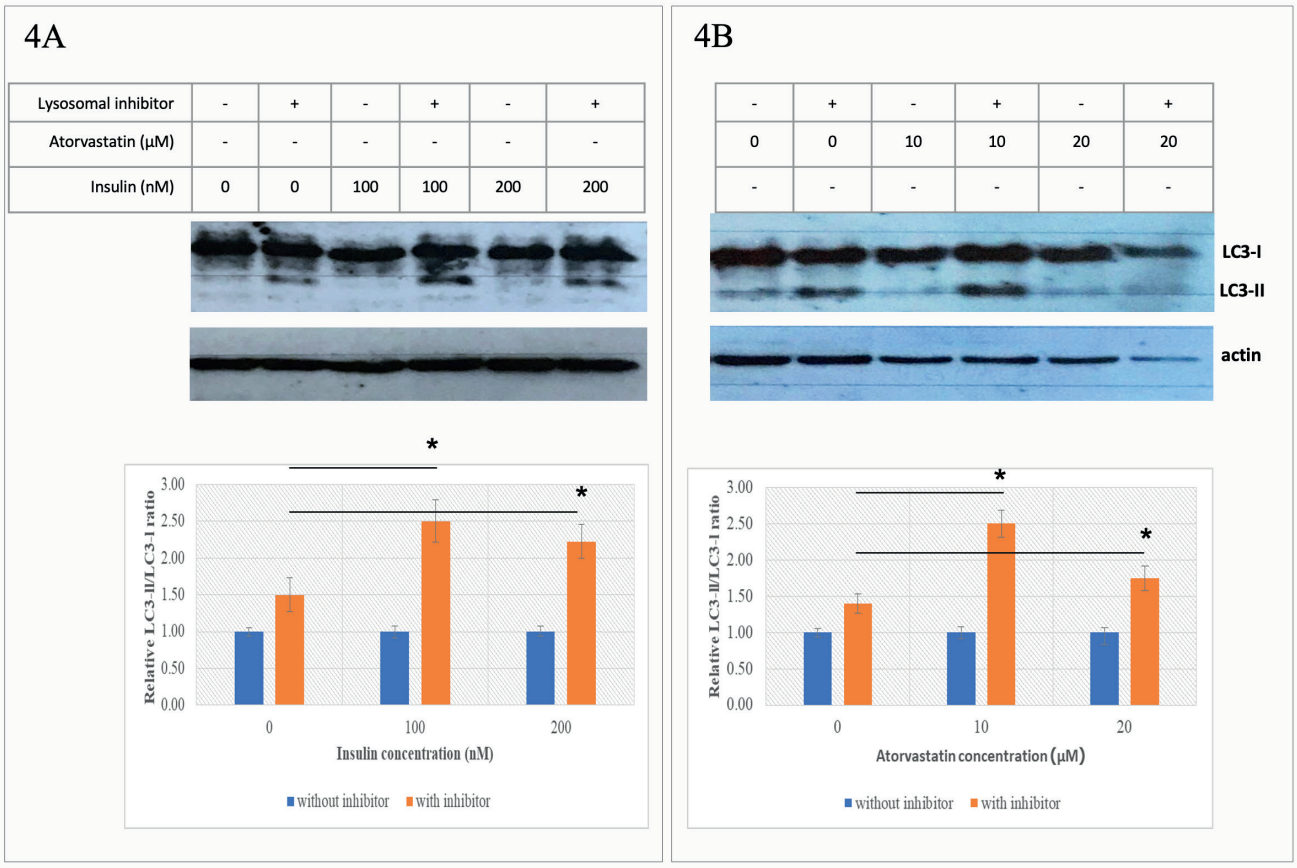

Figure 4. *Representative western blots and bar graph of relative LC3-II/ LC3-I ratio showing the effect of insulin (4A) and atorvastatin (4B) on autophagy flux of kidney cells cultured in hyperglycemic condition (supplemented with 40 mM glucose). *Significant difference from non-treated control ($p<0.05$ by Mann Whitney U test)*

The combination treatment of insulin and atorvastatin provides an additional effect on autophagy induction.

Overnight 60mm cultured dish of Vero cells in DMEM supplemented with 40 mM glucose were treated with various concentrations of insulin (0, 100, 200 nm) in combination with atorvastatin (0, 10, 20 µM) for 12-16 hours. Lysosomal protease inhibitor was added to one of the culture media 4 hours before cell harvesting, and LC3 levels were examined by Western blot analysis. The results show that combined treatment of insulin and atorvastatin to dose-dependently increased autophagic flux, thus indicating that these two compounds could help each other by providing additional effect on autophagic induction under hyperglycemic condition (Figure 5).

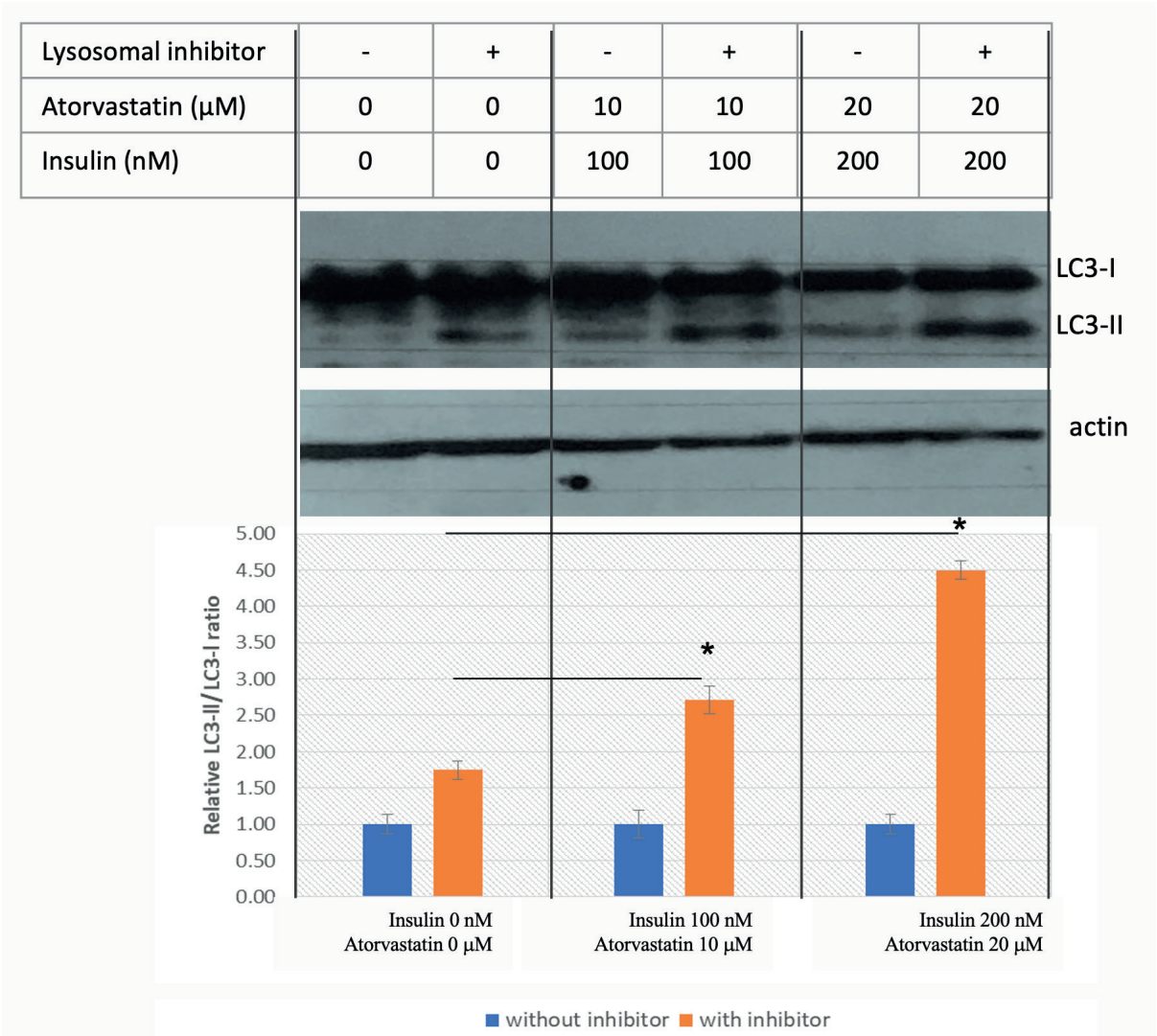

Figure 5. *Representative western blots and bar graph represented quantified LC3-II/ LC3-I ratio showing the effect of atorvastatin in combination with insulin on autophagy flux of kidney cells cultured in hyperglycemic condition (supplemented with 40 mM glucose). *Significant difference from non-treated control (p<0.05 by Mann Whitney U test).*

Effect of insulin and atorvastatin on level of p62/SQSTM1 (sequestosome 1).

The effect of insulin and atorvastatin on autophagy was also determined using a different autophagy indicator, p62. This protein encoded by *SQSTM1* gene controls autophagic pathway by mediating ubiquitinated cargoes to autophagosomes for degradation. Figure 6 showed that kidney cells cultured in DMEM supplemented with 40 mM glucose possessed a reduced level of p62/SQSTM1. The addition of insulin was able to induce its expression back to above the basal level, but not in a dose dependent manner. Interestingly, atorvastatin dose dependently increased p62/SQSTM1 protein level. Combine treatment of insulin and atorvastatin help further increase the level of p62/SQSTM1.

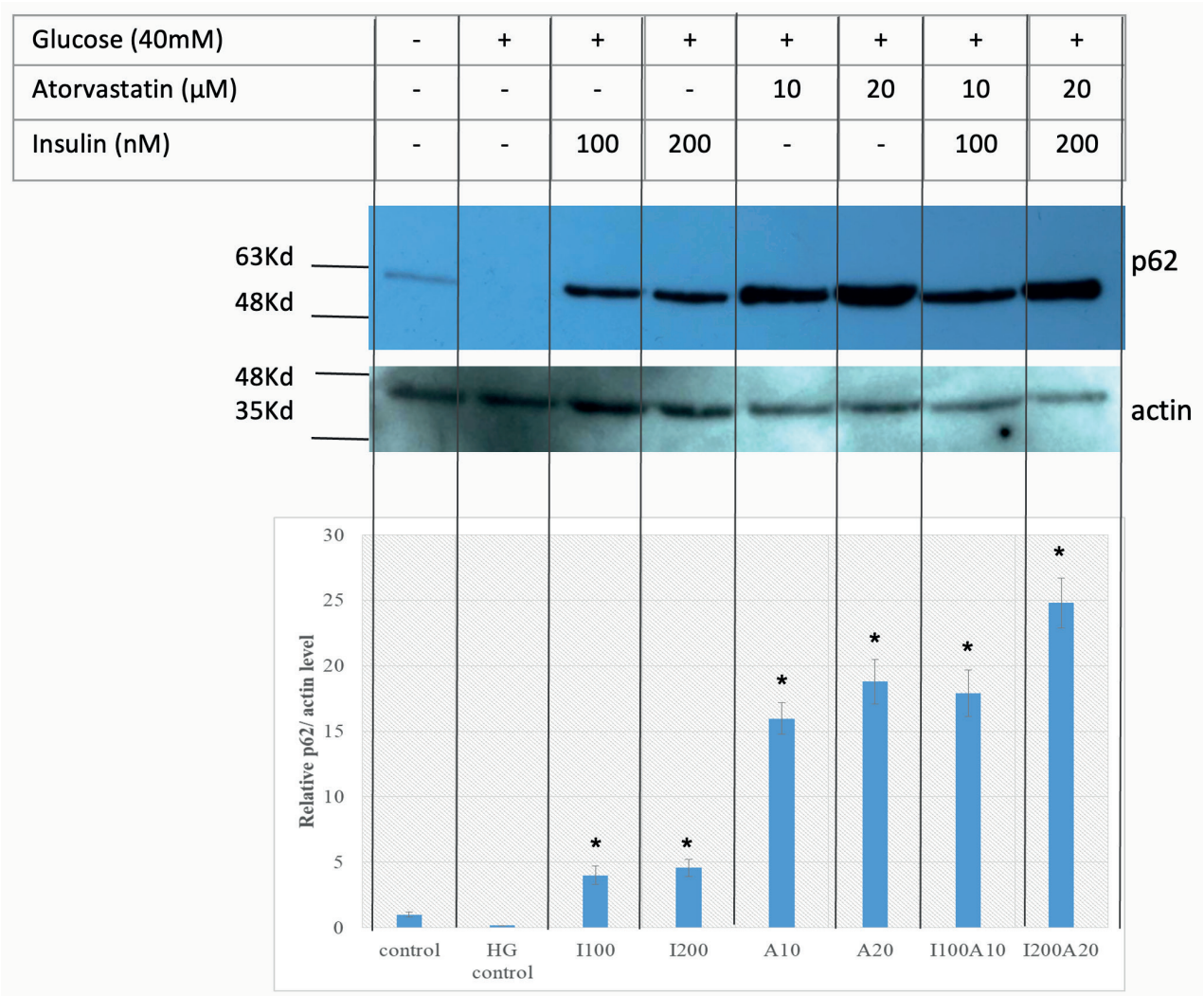

Figure 6. *Representative western blots and bar graphs showing the effect of atorvastatin and insulin on p62/SQSTM1 of kidney cells cultured in normoglycemic (5 mM glucose) or hyperglycemic condition (supplemented with 40 mM glucose) *Significant different from high glucose treatment control ($p<0.05$ by Mann Whitney U test).*

Discussion

Diabetic nephropathy is a common cause of end-stage kidney disease responsible for an increased rate of morbidity and mortality in both type 1 and type 2 diabetic patients worldwide. With a drastic increasing prevalence of diabetes, identifying new therapeutic targets for the prevention and treatment of this serious complication is urgently needed. Impairment of autophagy has been implicated in the pathogenic process of diabetic nephropathy.[14,15] There is increasing evidence that autophagy regulates many aspects of the kidney in normal and pathologic conditions.[16] We have shown in this study that high glucose (40 mM) condition negatively regulated autophagy of kidney cells, and the addition of insulin increased the process moderately. Consistent with our report, high glucose has also been previously shown to reduce autophagic flux of cardiomyocytes[17] provides a link between glycemic condition and autophagic activity of cells from various tissues. Multiple reports have suggested that autophagic activity was impaired in diabetic kidneys.[15,18] One study showed that autophagy was inhibited in the kidney cortex tubules of STZ-induced diabetic rats, and this was reversed through insulin replacement.[19] Studies have shown that the pathogenesis of diabetic nephropathy is associated with the decrease of autophagic activity via the activation of the mTOR pathway. This pathway regulates autophagic activity by associating with mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Nutrient deprivation induces autophagy through the inhibition of mTORC1,[20] while an excess of nutrient components (i.e., glucose) increases mTOR activity, either by direct interactions or indirectly via metabolic signaling pathways, including insulin and other growth factors.[20,21]

A number of generally used medications have been reported to up-regulate autophagy, including resveratrol and statins.[22,23] Atorvastatin is of particular interest due to the vast clinical use and their beneficial effect against dyslipidemia,[24,25] hypertension[26,27] and myocardial infarction.[28] Atorvastatin also shows significant cardio-protective[29] and we have previously demonstrated its reno-protective effect.[10] Several studies have reported that the various statin derivatives impacted autophagy by inhibiting mTOR.[30,31] Atorvastatin has also been reported to activate autophagy and consequently protect mesenchymal stem cells from oxygen and serum deprivation via AMP-activated protein kinase (AMPK)/ mTOR pathway.[23] Our results showed that atorvastatin only induced autophagic flux under hyperglycemic condition where this process was drastically suppressed, but not in the normoglycemic condition. Interestingly, atorvastatin has been demonstrated to have differential effects on autophagy in ischemic and non-ischemic myocardium. Atorvastatin stimulated autophagy in non-ischemic myocardium but inhibited autophagy in ischemic myocardium.[32] This difference suggests that the effect of atorvastatin on autophagy is dependent on both the stimulus and the cellular environment.

Apart from LC3-II/ LC3-I measurement, p62/SQSTM1 has also been widely used to assess autophagic activity. The best-known function of p62/SQSTM1 is its ability to target poly-ubiquitylated proteins for degradation through interaction with ubiquitin and LC3 during autophagy process. Activation of autophagy has been shown to co-exist with the reduction of p62/SQSTM1 level.[33] In contrast, pharmacological and genetic inhibition of autophagy resulted in an increase of p62/SQSTM1 level in various cell lines.[34,35] Unexpectedly, our results showed that p62/SQSTM1 in kidney cell line was reduced in response to high glucose culture condition, where measurement of LC3-II/ LC3-I indicated a reduction of autophagic flux. On the other hand, a supplement of insulin and/or atorvastatin increased p62/SQSTM1 level, where measurement of LC3-II /LC3-I indicated an increase of autophagic flux. Although p62/SQSTM1 is used as a marker of autophagic flux, it is important to consider other factors before using p62/SQSTM1 to assess autophagic activity, particularly as this protein interacts with multiple signaling molecules that affect its transcriptional synthesis. For example, transcription of p62/SQSTM1 is modulated by oxidative stress, Ras/MAPK pathway, JNK/c-Jun pathway, and many chemical compounds.[36,37] Overexpression of p62/SQSTM1 increased protein aggregation, and this has a protective effect on cell survival.[38-40] The deletion of p62/SQSTM1 resulted in the impaired formation of LC3-II, aggresome, and autophagosome, thereby exacerbating cell injury and lowering cell viability.[41] Therefore, we hypothesized that the induction of p62/SQSTM1 may be one of the atorvastatin and insulin underlying mechanisms in providing a protective effect and restoring the renal function of type 1 diabetic rats in our previous report.[10]

Conclusion

In conclusion, our results have demonstrated that atorvastatin increased autophagic flux and p62/SQSTM1 protein level of kidney cells cultured in hyperglycemic condition and co-treatment with insulin provided an additional effect on these inductions, which may explain their additional protective effect on renal function of STZ-induced diabetic rats we previously described. Nevertheless, further investigations are needed to clarify the involvement of p62/SQSTM1 induction toward this reno-protective effect.

References

[1] Hovind P, Tarnow L, Rossing K, Rossing P, Eising S, Larsen N, *et al*. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. Diabetes Care. 2003; 26(4): 1258-64 doi: 10.2337/diacare.26.4.1258.

[2] Ritz E, Keller C, Bergis K, Strojek K. Pathogenesis and course of renal disease in IDDM/NIDDM: differences and similarities. Am J Hypertens. 1997; 10(9 Pt 2): 202S-7S doi: 10.1016/s0895-7061(97)00154-4.

[3] Burdo JR, Chen Q, Calcutt NA, Schubert D. The pathological interaction between diabetes and pre-symptomatic Alzheimer's disease. Neurobiol Aging. 2009; 30(12): 1910-7 doi: 10.1016/j.neurobiolaging. 2008.02.010.

[4] Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107(9): 1058-70 doi: 10.1161/CIRCRESAHA.110.223545.

[5] Ruggenenti P, Remuzzi G. What blood-pressure level provides greatest renoprotection in patients with diabetic nephropathy and hypertension? Nat Clin Pract Nephrol. 2006; 2(5): 250-1 doi: 10.1038/ncpneph0157.

[6] Yamamoto T, Takabatake Y, Kimura T, Takahashi A, Namba T, Matsuda J, *et al*. Time-dependent dysregulation of autophagy: Implications in aging and mitochondrial homeostasis in the kidney proximal tubule. Autophagy. 2016; 12(5): 801-13 doi: 10.1080/15548627.2016.1159376.

[7] Jiang Y, Zhao Y, Zhu X, Liu Y, Wu B, Guo Y, *et al*. Effects of autophagy on macrophage adhesion and migration in diabetic nephropathy. Ren Fail. 2019; 41(1): 682-90 doi: 10.1080/0886022X.2019.1632209.

[8] Liu WJ, Huang WF, Ye L, Chen RH, Yang C, Wu HL, *et al*. The activity and role of autophagy in the pathogenesis of diabetic nephropathy. Eur Rev Med Pharmacol Sci. 2018; 22(10): 3182-9 doi: 10.26355/eurrev_201805_15079.

[9] Xu Y, Liu L, Xin W, Zhao X, Chen L, Zhen J, *et al*. The renoprotective role of autophagy activation in proximal tubular epithelial cells in diabetic nephropathy. J. Diabetes Complicat. 2015; 29(8): 976-83 doi: 10.1016/j.jdiacomp.2015.07.021.

[10] Thongnak L, Pongchaidecha A, Jaikumkao K, Chatsudthipong V, Chattipakorn N, Lungkaphin A. The additive effects of atorvastatin and insulin on renal function and renal organic anion transporter 3 function in diabetic rats. Sci Rep. 2017; 7(1): 13532 doi: 10.1038/s41598-017-13206-5.

[11] Iadnut A, Mamoon K, Thammasit P, Pawichai S, Tima S, Preechasuth K, *et al*. In Vitro Antifungal and Antivirulence Activities of Biologically Synthesized Ethanolic Extract of Propolis-Loaded PLGA Nanoparticles against Candida albicans. Evid Based Complement Alternat Med. 2019; 2019: 3715481 doi: 10.1155/2019/3715481.

[12] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012; 9(7): 671-5 doi: 10.1038/nmeth.2089.

[13] Tanida I, Ueno T, Kominami E. LC3 and Autophagy. Methods Mol Biol. 2008; 445: 77-88 doi: 10.1007/978-1- 59745-157-4_4.

[14] Cui J, Bai X, Chen X. Autophagy and Diabetic Nephropathy. Adv Exp Med Biol. 2020; 1207: 487-94 doi: 10.1007/978-981-15-4272-5_36.

[15] Koch EAT, Nakhoul R, Nakhoul F, Nakhoul N. Autophagy in diabetic nephropathy: a review. Int Urol Nephrol. 2020; 52(9): 1705-12 doi: 10.1007/s11255-020-02545-4.

[16] Zhang MZ, Wang Y, Paueksakon P, Harris RC. Epidermal growth factor receptor inhibition slows progression of diabetic nephropathy in association with a decrease in endoplasmic reticulum stress and an increase in autophagy. Diabetes. 2014; 63(6): 2063-72 doi: 10.2337/db13-1279.

[17] Kobayashi S, Xu X, Chen K, Liang Q. Suppression of autophagy is protective in high glucose-induced cardiomyocyte injury. Autophagy. 2012; 8(4): 577-92 doi: 10.4161/auto.18980.

[18] Bhatia D, Choi ME. Autophagy in kidney disease: Advances and therapeutic potential. Prog Mol Biol Transl Sci. 2020; 172: 107-33 doi: 10.1016/bs.pmbts.2020.01.008.

[19] Han K, Zhou H, Pfeifer U. Inhibition and restimulation by insulin of cellular autophagy in distal tubular cells of the kidney in early diabetic rats. Kidney Blood Press Res. 1997; 20(4): 258-63 doi: 10.1159/000174155.

[20] Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol. 2011; 12(1): 21-35 doi: 10.1038/nrm3025.

[21] Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol Cell. 2010; 40(2): 310-22 doi: 10.1016/j.molcel.2010.09.026.

[22] Sabe AA, Elmadhun NY, Dalal RS, Robich MP, Sellke FW. Resveratrol regulates autophagy signaling in chronically ischemic myocardium. J Thorac Cardiovasc Surg. 2014; 147(2): 792-8; Discussion 8-9 doi: 10.1016/j.jtcvs.2013.06.062.

[23] Zhang Q, Yang YJ, Wang H, Dong QT, Wang TJ, Qian HY, *et al*. Autophagy activation: a novel mechanism of atorvastatin to protect mesenchymal stem cells from hypoxia and serum deprivation via AMP-activated protein kinase/mammalian target of rapamycin pathway. Stem Cells Dev. 2012; 21(8): 1321-32 doi: 10.1089/scd.2011.0684.

[24] Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, *et al*. Additive beneficial effects of fenofibrate combined with atorvastatin in the treatment of combined hyperlipidemia. J Am Coll Cardiol. 2005; 45(10): 1649-53 doi: 10.1016/j.jacc.2005.02.052.

[25] Yang F, Wang J, Li F, Cui L. Atorvastatin Combined Nitroglycerin Therapy Confer Additive Effects on Rabbits with Dyslipidemia. Exp Clin Endocrinol Diabetes. 2016; 124(6): 367-71 doi: 10.1055/s-0042-104496.

[26] Koh KK, Quon MJ, Han SH, Lee Y, Park JB, Kim SJ, *et al*. Additive beneficial effects of atorvastatin combined with amlodipine in patients with mild-to-moderate hypertension. Int J Cardiol. 2011; 146(3): 319-25 doi: 10.1016/j.ijcard.2009.07.002.

[27] Lu JC, Cui W, Zhang HL, Liu F, Han M, Liu DM, *et al*. Additive beneficial effects of amlodipine and atorvastatin in reversing advanced cardiac hypertrophy in elderly spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 2009; 36(11): 1110-9 doi: 10.1111/j.1440-1681.2009.05198.x.

[28] Ke X, Ke B, Wang X, Wu S, Yang R, Hu C. Additive effects of atorvastatin combined with sitagliptin on rats with myocardial infarction: a pilot study. Arch Med Sci. 2017;1 3(4): 956-61 doi: 10.5114/aoms.2017.68143.

[29] Elmadhun NY, Lassaletta AD, Chu LM, Liu Y, Feng J, Sellke FW. Atorvastatin increases oxidative stress and modulates angiogenesis in Ossabaw swine with the metabolic syndrome. J Thorac Cardiovasc Surg. 2012; 144(6): 1486-93 doi: 10.1016/j.jtcvs.2012.08.065.

[30] Wei YM, Li X, Xu M, Abais JM, Chen Y, Riebling CR, *et al*. Enhancement of autophagy by simvastatin through inhibition of Rac1-mTOR signaling pathway in coronary arterial myocytes. Cell Physiol Biochem. 2013;31(6):925-37 doi: 10.1159/000350111.

[31] Gao K, Wang G, Wang Y, Han D, Bi J, Yuan Y, *et al*. Neuroprotective Effect of Simvastatin via Inducing the Autophagy on Spinal Cord Injury in the Rat Model. Biomed Res Int. 2015;2015:260161 doi: 10.1155/2015/260161.

[32] Sabe AA, Elmadhun NY, Sadek AA, Chu LM, Bianchi C, Sellke FW. Differential effects of atorvastatin on autophagy in ischemic and nonischemic myocardium in Ossabaw swine with metabolic syndrome. J Thorac Cardiovasc Surg. 2014;148(6):3172-8 doi: 10.1016/j.jtcvs.2014.07.104.

[33] Song YM, Song SO, Jung YK, Kang ES, Cha BS, Lee HC, *et al*. Dimethyl sulfoxide reduces hepatocellular lipid accumulation through autophagy induction. Autophagy. 2012;8(7):1085-97 doi: 10.4161/auto.20260.

[34] Riley BE, Kaiser SE, Kopito RR. Autophagy inhibition engages Nrf2-p62 Ub-associated signaling. Autophagy. 2011;7(3):338-40 doi: 10.4161/auto.7.3.14780.

[35] Sha Z, Schnell HM, Ruoff K, Goldberg A. Rapid induction of p62 and GABARAPL1 upon proteasome inhibition promotes survival before autophagy activation. J Cell Biol. 2018;217(5):1757-76 doi: 10.1083/jcb.201708168.

[36] Puissant A, Fenouille N, Auberger P. When autophagy meets cancer through p62/SQSTM1. Am J Cancer Res. 2012;2:397-412

[37] Seglen PO, Gordon PB. 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. Proc Natl Acad Sci U S A. 1982;79(6):1889-92 doi: 10.1073/pnas.79.6.1889.

[38] Paine MG, Babu JR, Seibenhener ML, Wooten MW. Evidence for p62 aggregate formation: role in cell survival. FEBS Lett. 2005;579(22):5029-34 doi: 10.1016/j.febslet.2005.08.010.

[39] Gal J, Strom AL, Kilty R, Zhang F, Zhu H. p62 accumulates and enhances aggregate formation in model systems of familial amyotrophic lateral sclerosis. J Biol Chem. 2007;282(15):11068-77 doi: 10.1074/jbc.M608787200.

[40] Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, *et al*. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol. 2005;171(4):603-14 doi: 10.1083/jcb.200507002.

[41] Su H, Wang X. Autophagy and p62 in cardiac protein quality control. Autophagy. 2011;7(11):1382-3 doi: 10.4161/auto.7.11.17339.

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Efficacy of the voice therapy protocol (VTP) for adult patients with unilateral vocal fold mobility impairment; a feasibility study

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ABSTRACT

Background: Unilateral vocal fold mobility impairment (UVFMI) causes dysphonia and/or dysphagia, which can significantly affect a patient's ability to communicate and perform regular daily life activities as well as the quality of life. Voice therapy offers a less invasive and more preferential method for patients. However, there are limitations concerning the integration of multiple therapy approaches. Voice therapy exercises with clear methodologies are required to plan and conduct therapy systematically, and frequency would be required for each exercise. Therefore, this study was conducted by applying the protocols of voice therapy in adult patients with unilateral vocal fold mobility impairment.

Objective: This feasibility study is a prospective cohort, pre-post single arm, designed to determine whether the voice therapy protocol (VTP) can enhance voice quality in adult patients with unilateral vocal fold mobility impairment (UVFMI) in a pilot study.

Materials and methods: All subjects received 12 sessions of voice therapy protocol, with each session conducted weekly for 45 minutes. The voice therapy protocol applied in this study consisted of vocal hygiene education, abdominal breathing exercises, vocal function exercises, pushing exercises, muscle relaxation exercises, and applied resonance voice therapy. The outcomes of protocols for voice therapy were measured before and after treatment using subjective voice assessments (GIRBAS scale) and objective voice assessments (Dr. Speech program and electro-glottography-EGG).

Results: Cases 2, 7, 10, 11, and 13 improved after receiving VTP. As for other participants, there are still some voice parameters that need to be monitored. Overall, it was found that the participants' voice parameters were changing within the acceptable range, with MPT, jitter, shimmer, and HNR values significantly different (p<0.05).

Conclusion: The findings of this study indicated that the voice therapy protocol was a worthwhile alternative and could be used to develop further treatment guidelines for adult patients with UVFMI at a speech clinic.

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Introduction

Unilateral vocal fold mobility impairment (UVFMI) is a condition in which one of the vocal folds suffers from paralysis or paresis, thus causing incomplete glottal closure while speaking.[1,2] This condition usually occurs because of damage to the vagus nerve (tenth cranial nerve; CN.X), which may be a consequence of trauma, iatrogenic

trauma,[3,4] tumor,[5] a viral or bacterial infection, neurotoxic drugs,[4,6] and/or neuritis or idiopathic diagnosis.[4,5,7] This impairment causes vocal abnormalities and/or dysphagia,[3,4,8,9] that significantly affects the patient's ability to communicate and perform regular daily life activities, including social interactions and professional employment, as well as their emotional and physical health.[10-12] Nouraei *et al*. reported that the incidence of adult unilateral vocal fold mobility impairment (UVFMI) in England is approximately 1.2%. The most common causes of UVFMI were idiopathic.[13] In Thailand, there hasn't been any research on the prevalence or incidence of this issue. A previous study found that patients with vocal impairment caused by nervous system abnormalities scored a very low Voice Handicap Index (VHI) compared with patients with vocal impairment from other causes.[10-12] It was also shown that they were more likely to develop a reduction in communication needs or experience difficulties in maintaining interpersonal relationships.[12]

Common symptoms among patients with UVFMI include hoarseness, breathy voice, variations in pitch and loudness, and intermittent diplophonia.[1,2,6,14-16] A breathy voice and reduced loudness of speech were shown to vary according to the emission of air between the glottis while talking. Hoarseness and diplophonia were also caused by the inability of the vocal folds to maintain normal tension within the affected fold, which resulted in asymmetric vocal fold vibrations.[14,17] Furthermore, patients usually exhibited exhaustion after extensive talking and needed more energy to maintain talking in a long conversation. Moreover, they could develop throat pain,[15,16] globus sensation, and neck discomfort.[9] These symptoms were caused by the patient's compensatory behavior to the incomplete folding of their vocal folds.[16,17] Particularly, hoarse voices, strained voices, and low-pitched sounds represented the leading symptoms that caused patients to seek recovery.[9]

Voice therapy presents a less invasive and more preferential method for patients; they would not be subjected to the aforementioned surgical risks.[18] This also offers advantageous results to the patients, such as glottal gap reduction,[9] improved vocal quality,[9,19-21] and quality of life,[19,20] and beneficial results that could last for years.[22] Therefore, voice therapy could be a treatment scheme to help patients recover without unnecessary surgery. Consequently, several techniques have been recommended for treating UVFP, including hard glottal attack exercises (HGA), pushing exercises, and lateral digital pressure. Many other approaches are available, including the head tilt method, half-swallow boom technique, vocal function exercise (VFE), abdominal breathing, head and neck relaxation, lip and tongue trill, resonant voice, accent method, head position, chin tuck, focus, tongue protrusion /i/, yawn-sigh, pitch shift up, and inhalation phonation.[14,23] Each exercise helps to adjust the structures and functions of a patient's vocalization mechanism, which would affect vocal quality.

Voice therapy has typically been conducted as a combination of exercises rather than just relying on a single method.[6,19,24] These practices have also been adopted by speech clinics in Thailand. Although several studies have combined multiple voice therapies that verified the positive treatment results of UVFP, distinguishing which therapies contributed effective results has yet to be determined. In addition, there are limitations to integrating multiple therapies, such as heterogeneity in voice therapy techniques and different methodologies that result in the heterogeneity and inconsistency of the study results. Hence, voice therapy exercises with clear methodologies are required to systematically plan and conduct which frequency would be required for each exercise.[20,22] However, there are no current systematic voice therapy protocols (VTP) for patients with UVFMI in Thailand. Therefore, the purpose of this feasibility study is to inquire if the VTP could improve voice quality for these patients before proceeding with the protocol in a future definitive randomized controlled trial (RCT) study.

Methods

Participants

This pilot study was conducted with 18 new patients diagnosed with unilateral vocal fold paralysis or paresis at the Outpatient Department (OPD) in the Department of Otolaryngology, Faculty of Medicine Ramathibodi Hospital, Mahidol University between October 2018 and November 2019. Purposive subject sampling was applied based on the diagnosis of the otolaryngologist. However, three participants left the study due to health problems, transportation inconvenience, and surgical procedures. Therefore, the study comprised 15 participants (13 females and two males aged between 37-72 years; an average of 58.8 years) who had normal speech, language, and cognitive perception ability and had not been diagnosed with any psychological disorders or neurological diseases, had no communicable-level hearing impairment, no vision problems, and had never previously received voice therapy. Patients being administered medication that affected changes in the laryngeal muscles, laryngeal tissue, and laryngeal functions or had been diagnosed with an allergy, asthma, lung diseases, or other respiratory tract diseases were excluded from the study.

Assessments

In this study, two types of vocal assessments were used, namely subjective vocal assessments and objective vocal assessments.

Subjective Vocal Assessment: Voice samples of each participant were recorded one time with a voice recorder, before and after voice therapy, by reading the Fonfa passage (similar to Rainbow passage), which includes all Thai consonant sounds. The audio files were randomized and recorded onto a compact disc. Subsequently, the files were assessed in terms of the GIRBAS scale by an experienced SLP who had a minimum of 10 years of experience in voice therapy treating patients with voice disorders. In order to perform auditory-perceptual assessments based on the GIRBAS scale (G = grade/degree of the voice disorder, I = instability,

R = roughness, B = breathiness, A = asthenia, and S = strain), the scoring for each parameter can be divided into 4 levels, with 0 = normal, 1 = slightly abnormal, 2 = moderately abnormal, and 3 = severely abnormal.[24] The severity of hoarseness could be inferred from parameter G (Grade), which indicated overall voice quality, thus integrating all the deviant components.[25]

Objective Vocal Assessments

Each participant was assessed using the vocal assessment program of Dr. Speech Software version 5 by Tiger DRS, Inc. The participants were seated 15 cm away from a microphone and performed a prolonged /a:/ sound after inhalation to find the f0, jitter, shimmer, and HNR. The assessment was repeated three times to identify the longest /a:/, selected to represent the patient's MPT. After that, the researcher attached EGG electrodes to the participant's neck around the thyroid cartilage and then prompted the participant to perform another prolonged /a:/ for three seconds to find the CQ.

The voice parameters with descriptions that were derived from objective assessment are presented in Table 1.

Table 1. Voice parameters and description.

Parameters			Norm	Abnormal status
Mean fundamental frequency (mean f0)[26]	An average of the rate of the vocal fold vibration.[25]	Age Male Female	51-60 years 115±6 Hz 191±9 Hz	increasing in value
Jitter[28]	The deviation of pitches or f0 from one cycle to another. In other words, the jitter reflects the abnormality of the vocal fold vibration.[27]		≤0.5%	increasing in value
Shimmer[28]	The deviation of the amplitude waveform from one cycle to another, which reflects the glottal resistance and mass lesion of the vocal folds. [27]		≤3.0%	increasing in value
Harmonic-to-noise ratio (HNR) [26]	The proportion between harmonic energy and noise energy that indicates the regularity of the overall voice signals. [27]	Age Male Female	51-60 years 19.94±0.86 dB 22.37±0.61 dB	decrease in value
Maximum phonation time (MPT) [29]	The aerodynamics analysis assesses the ability to control the respiratory function, laryngeal control, and glottal efficiency of the patients by producing the longest vowel sounds after inhalation.[1]		11.27±3.30 seconds	decrease in value
Closed quotient (CQ) [28]	The level of the vocal fold contacts, but not the glottal width. The CQ could determine the contact of the vocal fold from the hypoadducted "breathy" to hyperadducted "pressed" only.[28]		50-70%	decrease in value

Voice therapy protocol

This study was conducted by applying voice therapy protocols adopted from Kao *et al*.[20] It used voice therapies by the three systems for the physiologies of speech mechanisms, i.e., respiration, phonation, and resonation.[23] Although the study of Kao *et al*. conducted the voice therapy protocol and yielded positive outcomes, there would be limitations in adapting such protocols in the context of a study in Thailand, primarily due to differences in stature.[14]

The voice therapy protocol (VTP) consists of three stages. Each stage took four weeks, meaning the total duration was 12 weeks. Each session was performed once a week for a total of 45 minutes in each of 12 sessions. The stages of the voice therapy protocol (VTP) were as follows.

Stage 1: Appropriate respiratory system adjustment for vocalization (Weeks 1-4): abdominal breathing exercise and muscle relaxation exercise.

Stage 2: Strengthening of the phonatory system (Weeks 5-8): vocal function exercises, pushing exercises, and muscle relaxation exercises.

Stage 3: Appropriate resonation adjustment for vocalization (Weeks 9-12): applied resonant voice therapy (RVT).

Statistical analysis

The vocal assessment data from before and after therapy were computed by utilizing the statistical package IBM SPSS Statistics 22. The results of comparing the mean MPT, f0, jitter, shimmer, HNR, and CQ were analyzed. The data were analyzed using a Wilcoxon signed-rank test (significance level of $p<0.05$). Meanwhile, the GIRBAS were shown as descriptive data.

Results

The results of the vocal assessment that was performed on the 15 adult patients with unilateral vocal fold mobility impairment before therapy, case No. 11, had no abnormal voice and could keep her voice at a normal level until the end of therapy. An evaluation using the GIRBAS Scale after therapy, it was found that when the participants received the voice therapy protocol (VTP), cases No. 6, 7, 8, 12, 13, and 15 had improved G values. When analyzed by objective vocal assessment, it was found that the voice parameters of cases No. 7 and 13 had a better change, except for cases No. 6 and 15, which had higher f0, case No. 8, which had lower HNR values, and case No. 12, who had slightly lower MPT values. Cases No. 1, 2, 5, and 10 had G values unchanged at the moderate level of severity. When analyzed by objective vocal assessment, it was found that the voice parameters of cases No. 2 and 10 tended to improve, except for cases No. 1 and 5, which have a lower HNR value. Cases No. 3 and 4 had G values unchanged in mild severity. When analyzed by objective vocal assessment, it was found that the voice parameters of case No. 3 tended to improve, except for the CQ value, which decreased very low, and case No. 4, in which the f0 value is slightly higher than the normal range. Case No. 14 had an unchanged G value at the severe level. When analyzed by objective vocal assessment, it was found that the CQ value dropped below the normal range. Furthermore, it was found that after the VTP, case No. 9 had more abnormalities, with I, R, and B values and f0 and MPT values not very much changing (Table 2).

In summary, cases No. 2, 7, 10, 11, and 13 improved after receiving VTP. As for other participants, there were still some voice parameters that needed to be monitored. Overall, it was found that the participants' voice parameters were changing within the acceptable range, with MPT, jitter, shimmer, and HNR values significantly different (p<0.05) (Table 3).

Table 2. Demographic characteristics of the participants and speech parameters before and after therapy.

Case No.	F/M	E	O	C	G		I		R		B		A		S		MPT (s)		F0 (Hz)		Jitter (%)		Shimmer (%)		HNR (dB)		CQ (%)	
					Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	F	Ia	L	Bo	2	2	1	1	1	1	2	2	0	0	2	2	3.00	6.40	274.03	275.60	1.25	0.46	5.29	3.34	14.30	19.60	64.43	56.72
2	F	Ia	M	Lo	2	2	1	2	2	3	1	1	0	0	2	3	8.29	9.57	238.41	201.22	0.60	0.49	7.53	2.33	14.51	24.83	64.41	56.75
3	F	Id	M	Bo	1	1	0	1	0	1	1	1	0	0	1	1	6.69	8.28	174.81	158.79	0.16	0.15	1.13	0.74	28.91	31.77	64.31	35.01
4	M	Id	M	Lo	1	1	0	1	1	1	1	2	0	0	1	1	11.39	14.31	127.09	125.59	0.28	0.20	2.09	1.62	25.60	30.45	77.37	49.44
5	M	Id	M	Lo	2	2	1	0	3	3	2	2	0	0	1	1	7.62	10.07	144.86	126.45	0.41	0.35	4.12	2.96	17.72	20.48	44.73	42.09
6	F	Id	M	Lo	2	1	1	1	1	1	3	1	0	0	1	1	6.07	10.79	214.34	230.37	0.14	0.14	0.94	0.87	34.31	34.69	54.20	61.55
7	F	Id	M	Bo	1	0	0	0	1	0	0	0	0	0	1	0	5.77	7.91	162.76	186.00	0.20	0.15	1.95	1.04	24.94	30.16	62.76	53.33
8	F	Ia	L	Bo	3	2	3	1	3	3	3	3	0	0	3	3	3.21	7.40	284.57	278.34	2.15	0.44	7.74	5.89	10.45	15.85	61.71	55.26
9	F	Id	M	Lo	1	2	0	1	1	2	1	2	0	0	1	1	6.49	5.43	202.21	203.80	0.27	0.28	1.65	1.24	29.07	29.92	54.61	56.04
10	F	Id	M	Bo	2	2	1	1	2	3	2	2	0	0	2	1	2.80	7.58	164.35	168.63	0.25	0.36	2.50	2.14	27.31	25.43	42.25	53.66
11	F	Ne	L	Lo	0	0	0	0	0	0	0	0	0	0	0	0	5.17	11.72	199.83	185.89	0.32	0.19	1.16	1.75	30.47	27.10	49.78	59.51
12	F	Vi	L	Lo	2	1	1	1	3	2	2	1	0	0	1	1	5.09	4.78	213.23	230.68	0.40	0.18	4.17	2.75	20.74	22.40	48.98	53.63
13	F	Ia	L	Lo	2	1	1	0	3	1	3	0	0	0	2	1	5.45	12.02	180.58	183.52	0.37	0.15	1.76	0.90	24.24	27.21	46.03	66.33
14	F	Ia	L	Bo	3	3	2	3	3	3	3	2	0	0	3	3	2.53	5.50	190.02	163.15	0.47	0.24	3.56	2.50	23.79	25.90	73.94	43.42
15	F	En	M	Lo	3	1	2	1	3	1	3	1	0	0	3	2	4.31	14.07	216.34	260.10	0.32	0.11	2.08	1.96	25.81	24.86	44.00	65.43

Note: F: Female, M: Male, E: etiology, Ia: iatrogenic, Id: idiopathic, Ne: neuritis, Vi: viral infection, En: encephalopathy

O: Onset:< L: less than or equal to 6 months, M: more than six months, C: Configuration, Lo: longitudinal gap, Bo: bowing

Table 3. Comparison of the results for objective vocal assessment before and after VTP therapy.

Objective vocal assessment	Pre-Tx			Post-Tx			Z	*p* values	95%CI
	Min.	Max.	Median (IQR)	Min.	Max.	Median (IQR)			
MPT (s)	2.53	11.39	5.45 (3.48)	4.78	14.31	8.28 (5.32)	-3.23	0.00*	(1.86-4.77)
Mean f0 (Hz)	127.09	284.57	199.83 (51.99)	125.59	278.34	186.00 (67.53)	0.00	1.00	(-12.32-10.19)
Jitter (%)	0.14	2.15	0.32 (0.22)	0.11	0.49	0.20 (0.21)	-2.79	0.00*	(-0.40-(-0.05))
Shimmer (%)	0.94	7.74	2.09 (2.52)	0.74	5.89	1.96 (1.71)	-3.01	0.00*	(-1.42-(-0.39))
HNR (dB)	10.45	34.31	24.94 (11.19)	15.85	34.69	25.90 (7.76)	-2.38	0.01*	(0.61-4.13)
CQ (%)	42.25	77.37	54.61 (18.38)	35.01	66.33	55.26 (10.07)	-0.51	0.60	(-12.32-6.88)

*Note: Statistical analysis using the Wilcoxon signed-rank test, p<0.05**

Discussion

Case No. 11, who was the otolaryngologist diagnosed with vocal fold paralysis due to neuritis and had voice disorders. She might have recovered from the disease during the referral period from the otolaryngologist to the speech therapist. Therefore, it is possible that the participant had normal voice quality before therapy. However, she kept her voice normal until the end of therapy. This was probably because the participant received instruction on vocal hygiene before beginning the voice therapy protocol. Vocal hygiene prevents undesirable vocal behavior, facilitating the improvement of behaviors that might result in vocal fold trauma.[15,30]

Five participants (cases No. 2, 7, 10, 11 and 13) had improved voice outcomes after receiving VTP, indicating that the voice therapy protocol was systematically selected and organized voice approaches may help balance all three stages of speech mechanisms. Stage I adjusts the respiratory system for vocalization using abdominal breathing and muscle relaxation exercises. Treatment would first begin with the abdominal breathing exercise, as this would provide the general basis for vocalization. Moreover, insufficient air to produce speech would be a common problem for patients with vocal fold mobility impairment.[31] Therefore, the abdominal breathing exercise would help the participants maintain the appropriate subglottic pressure,[10] regulating the length of the utterance,[6] avoiding chest breathing, and vocalizing with residual air.[19] However, as participants already had limited breath support, they might exhibit tightening of the laryngeal muscles during the abdominal breathing exercises, affecting phonation and voice quality.[23] Consequently, participants should also be subsequently treated with muscle relaxation exercises. Stage 2 enhances the strengthening of the phonatory system consisting of VFEs, pushing exercises, and muscle relaxation exercises. Participants would perform a relaxed vocalization to relieve the tension of the supraglottic laryngeal muscles through a low-impact adductory power exercise. A pushing exercise would be conducted to increase the glottal closure. However, these steps require high exertion, and participants might experience supraglottic hyperfunction. Therefore, they would be encouraged to practice muscle relaxation exercises at the end of the treatment. After that, participants should be able to increase the strength of the phonatory systems. Stage 3: An appropriate resonation adjustment for vocalization could be done by employing applied resonant voice therapy (RVT), a form of holistic voice therapy that could assist patients in having forward resonance and easy phonation.[23] This would result in the reduction of the laryngeal hyperfunction.[32] Regular speech mechanisms would require all three stage systems to function together integrally. The abnormality of one or more systems could cause voice disorders.[23] However, other participants persisted in some voice parameters that still need to be monitored; there may be changes. So, intensive voice therapy and continuous therapy can help them have better voice quality.[33]

After receiving the voice therapy protocol, the group of participants had improved G values; there are also cases No. 6 and 15 that still have high f0 values. This may be because both cases had voice disorders for two years, causing the participants to engage in compensatory behavior to reduce the air leakage, resulting in a falsetto voice, which can be a high-pitched sound. As reported by the study of Patel and Parsram.[34] However, it's in contrast with the findings of Bielamowicz *et al.,* which reported that the f0 value decreased as dysphonia increased.[35] Case No. 8 had a low HNR value. This may be because the participant has had the severity of an abnormal voice of R, B, or S at a severe level before entering the program. This can be seen from the roughness (R) caused by irregular vibration cycles of the vocal folds due to the paralyzed vocal fold not moving or being limited. Breathiness (B) is the occurrence of air leaks during vocalization caused by incomplete glottal closure, causing a breathy voice, and strain (S) is an attempt to increase vocal effort, causing the participant to have compensatory behaviors, so the HNR value decreases. HNR is the proportion between harmonic and noise energy that indicates the regularity of the overall voice signals.[27] For UVFMI patients, the HNR value is low compared to normal people.[36] In case No. 8, the HNR value could increase if she received the VTP with more training sessions. In case No. 12, the MPT value decreased slightly because she had a common cold on the day of the therapy assessment. The larynx's mucous membrane is inflamed in this case due to upper respiratory tract infections. Incomplete glottal closure during phonation can be caused by the membranous covering swelling and going red, the vocal folds thickening, and the vocal fold mass becoming stiffer. Air flowed through the glottis, but the vocal folds were not fully adducted. The MPT decreases when glottal airflow increases.[14,37]

A group of the participants had G values unchanged in the severity of moderate; cases No. 1 and 5, which have a lower HNR value. In case No. 1, it may be due to the participant's abnormal voice quality (B, S) being moderately severe before therapy. As can be seen from air leaks during voicing caused by incomplete glottal closure, a breathy voice (B) and attempts to increase vocal effort cause compensatory behavior in the participant's voice (S). These are causing the participant to have a low HNR value. In case No. 1, gaining VTP with more training sessions may cause the HNR value to increase and the S value to decrease. For case No. 5, the HNR value was low, possibly because the participant had a problem with the level of glottal closure. Hypoadduction causes a breathy voice, which corresponds to the abnormal R at a severe level and B at a moderate level of severity before therapy.

A group of the participants had G values unchanged in the severity of mild; case No. 3 tends to improve in all values except for the CQ value, which decreased very low. It is highly likely that she still has glottic insufficiency. As for case No. 4, the f0 value was slightly higher than the normal range. This may be due to the patient being an elderly male whose mass of vocal folds decreases due to changes in the sex hormone estrogen, laryngeal cartilage, and muscle function that also change with age. Therefore,

in males, the f0 value rises with age.[38,39] In addition, the duration of voice disorders has been 1.6 years, causing the patient to have compensatory behavior to reduce air leaks, resulting in the tenseness of the vocal fold.

Case No. 14 had G values unchanged in the severity of severe. Her CQ dropped below the normal range. This may be due to aging, age 72.7 years, possibly having problems with presbylaryngis or aging voice, causing changes in the structure and function of the vocal folds such as vocal fold edge stiffness, atrophy, and bowing, which make the vocal folds unable to close together, resulting in still having glottic insufficiency.[40]

In addition, case No. 9 found more abnormalities after receiving VTP, with f0 and MPT values that did not change much and I, R, and B values that worsened. This may be due to the participant's aging, age 63, and presbylaryngis, causing changes in the structure and function of the vocal folds, such as vocal fold edge stiffness, atrophy, and bowing, which make the vocal folds unable to close together, resulting in still having glottic insufficiency which affects vocal parameters.[40] This is consistent with Vaca *et al.,* who reported that elderly patients had short phonation times, worse jitters, and worse GRBAS scores.[41]

This study used a small number of participants; therefore, its positive results can still be observed. Moreover, the selection and organization of voice therapy techniques, the duration of the therapy, and the number of sessions of voice therapy protocol are important.

Limitations and recommendations

To reach the protocol's efficiency, future research on this aspect should be investigated with a large sample size, using a control group for comparison, utilizing an extended duration of VTP, and following training at home.

Conclusions

Adult patients with unilateral vocal fold mobility impairment (UVFMI) benefit from the voice therapy protocol (VTP), which enhances voice quality. The results of this investigation may be used as preliminary evidence to help develop guidelines for a prospective intervention program.

Ethics approval

Data were collected after receiving approval from the Ethics Committee of Ramathibodi Hospital, COA. No. MURA2018/596.

References

[1] Colton RH, Casper JK, Leonard R. Understanding voice problems: a physiological perspective for diagnosis and treatment. 4th Edition. Philadelphia: Lippincott Williams&Wilkins; 2011.

[2] Kitzing P. Stroboscopy-a pertinent laryngological examination. J Otolaryngol. 1985; 14(3): 151-7.

[3] Bergamini G, Alicandri-Ciufelli M, Molteni G, Villari D, Luppi MP, Genovese E, *et al*. Therapy of unilateral vocal fold paralysis with polydimethylsiloxane injection laryngoplasty: our experience. J Voice. 2010; 24(1): 119-25. doi.org/10.1016/j.jvoice.2008. 05.003

[4] Sulica L, Blitzer A. Vocal fold paresis: evidence and controversies. Curr Opin Otolaryngol Head Neck Surg. 2007; 15(3): 159-62. doi.org/10.1097/MOO.0b013e32814b0875

[5] Sulica L. Vocal fold paresis: an evolving clinical concept. Curr Otorhinolaryngol Rep. 2013; 1(3): 158-62. doi.org/10.1007/s40136-013-0019-4

[6] D'Alatri L, Galla S, Rigante M, Antonelli O, Buldrini S, Marchese MR. Role of early voice therapy in patients affected by unilateral vocal fold paralysis. J Laryngol Otol. 2008; 122(9): 936-41. doi.org/10.1017/S0022215107000679

[7] Kelchner LN, Stemple JC, Gerdeman B, Borgne WL, Adam S. Etiology, pathophysiology, treatment choices, and voice results for unilateral adductor vocal fold paralysis: a 3-year retrospective. J Voice. 1999; 13(4): 592-601. doi.org/10.1016/s0892-1997(99)80013-7

[8] Walton C, Conway E, Blackshaw H, Carding P. Unilateral vocal fold paralysis: a systematic review of speech-language pathology management. J Voice. 2017; 31(4): 509 e7-e22. doi.org/10.1016/j.jvoice.2016.11.002

[9] El-Banna M, Youssef G. Early voice therapy in patients with unilateral vocal fold paralysis. Folia Phoniatr Logop. 2014; 66(6): 237-43. doi.org/10.1159/000369167

[10] Cantarella G, Viglione S, Forti S, Pignataro L. Voice therapy for laryngeal hemiplegia: the role of timing of initiation of therapy. J Rehabil Med. 2010; 42(5): 442-6. doi.org/10.2340/16501977-0540

[11] Harris G, O'Meara C, Pemberton C, Rough J, Darveniza P, Tisch S, *et al*. Vocal fold paresis - a debilitating and underdiagnosed condition. J Laryngol Otol. 2017; 131(S2): S48-S52. doi.org/10.1017/S0022215117000810

[12] Cohen SM, Dupont WD, Courey MS. Quality-of-life impact of non-neoplastic voice disorders: a meta-analysis. Ann Otol Rhinol Laryngol. 2006; 115: 128-34. doi.org/10.1177/000348940611500209

[13] Nouraei SA, Middleton SE, Butler CR, Sandhu GS. An estimation of the population incidence of adult unilateral vocal fold mobility impairment in England. Logoped Phoniatr Vocol. 2015; 40: 93-4. doi.org/10.3109/14015439.2014.902497

[14] Boone DR, McFarlane SC, Von Berg SL. The voice and voice therapy. 7th edition. Boston, MA: Allyn & Bacon; 2005.

[15] Rubin AD, Sataloff RT. Vocal fold paresis and paralysis. Otolaryngol Clin North Am. 2007; 40(5): 1109-31. doi.org/10.1016/j.otc.2007.05.012

[16] Koufman JA, Postma GN, Cummins MM, Blalock PD. Vocal fold paresis. Otolaryngol Head Neck Surg. 2000; 122(4): 537-41. doi.org/10.1067/mhn.2000.102574

[17] Stewart CF, Allen E. Voice therapy for unilateral vocal fold paralysis. In: Sulica L, Blitzer A, editors. Vocal fold paralysis. Germany: Springer; 2010. p. 87-93.

[18] Desjardins M, Halstead L, Cooke M, Bonilha HS. A systematic review of voice therapy: what "effectiveness" really implies. J Voice. 2017; 31(3): 392 e13-e32. doi.

org/10.1016/j.jvoice.2016.10.002
[19] Schindler A, Bottero A, Capaccio P, Ginocchio D, Adorni F, Ottaviani F. Vocal improvement after voice therapy in unilateral vocal fold paralysis. J Voice. 2008; 22(1): 113-8. doi.org/10.1016/j.jvoice.2006. 08.004
[20] Kao YC, Chen SH, Wang YT, Chu PY, Tan CT, Chang WD. Efficacy of voice therapy for patients with early unilateral adductor vocal fold paralysis. J Voice. 2017; 31(5): 567-75. doi.org/10.1016/j.jvoice.2017. 01.007
[21] Mattioli F, Menichetti M, Bergamini G, Molteni G, Alberici MP, Luppi MP, *et al*. Results of early versus intermediate or delayed voice therapy in patients with unilateral vocal fold paralysis: our experience in 171 patients. J Voice. 2015; 29(4): 455-8. doi.org/10.1016/j.jvoice.2014.09.027
[22] Busto-Crespo O, Uzcanga-Lacabe M, Abad-Marco A, Berasategui I, Garcia L, Maravi E, *et al*. Longitudinal voice outcomes after voice therapy in unilateral vocal fold paralysis. J Voice. 2016; 30(6): 767 e9-e15. doi.org/10.1016/j.jvoice.2015.10.018
[23] Stemple JC, Glaze LE, Klaben BG. Clinical voice pathology: theory and management. 3rd Edition. San Diego, CA: Singular Publishing Group; 2000.
[24] Mattioli F, Bergamini G, Alicandri-Ciufelli M, Molteni G, Luppi MP, Nizzoli F, *et al*. The role of early voice therapy in the incidence of motility recovery in unilateral vocal fold paralysis. Logoped Phoniatr Vocol. 2011; 36(1): 40-7. doi.org/10.3109/14015439. 2011.554433
[25] Felippe AC, Grillo MH, Grechi TH. Standardization of acoustic measures for normal voice patterns. Rev Bras Otorrinolaringol. 2006; 72(5): 659-64. doi.org/10.1016/s1808-8694(15)31023-5
[26] Smits I, Ceuppens P, De Bodt MS. A comparative study of acoustic voice measurements by means of Dr. Speech and Computerized Speech Lab. J Voice. 2005; 19(2): 187-96. doi.org/10.1016/j.jvoice. 2004.03.004
[27] Teixeira JP, Fernandes PO. Acoustic analysis of vocal dysphonia. Procedia Comput Sci. 2015; 64: 466-73. doi.org/10.1016/j.procs.2015.08.544
[28] Huang DZ, Lin S. Vocal assessment: user's manual. In: Tiger DRS I, Editor. 1995.
[29] Prathanee B, Watthanathon JI, Ruangjirachuporn PA. Phonation time, phonation volume and air flow rate in normal adults. J Med Assoc Thai. 1994; 77(12): 639-45.
[30] Stemple JC. A holistic approach to voice therapy. In Seminars in Speech and Language 2005. p. 131-7. doi.org/10.1055/s-2005-871209
[31] Schneider SL. Behavioral management of unilateral vocal fold paralysis and paresis. Perspect ASHA Spec Interest Groups. 2012; 22: 112-20. doi.org/10.1044/vvd22.3.112
[32] Miller S. Voice therapy for vocal fold paralysis. Otolaryngol Clin North Am. 2004; 37(1): 105-19. doi.org/10.1016/S0030-6665(03)00163-4
[33] Godoy J, Silverio K, Brasolotto A. Effectiveness of vocal therapy for the elderly when applying conventional and intensive approaches: a randomized clinical trial. J Voice. 2019;33(5):809-e19. doi.org/10.1016/j.jvoice.2018.03.017
[34] Patel R, Parsram KS. Acoustic analysis of subjects with vocal cord paralysis. Indian J Otolaryngol Head Neck Surg. 2005; 57: 48-51. doi.org/10.1007/BF02907629
[35] Bielamowicz S, Kreiman J, Gerratt BR, Dauer MS, Berke GS. Comparison of voice analysis systems for perturbation measurement. J Speech Hearing Res. 1996 ;39: 126-34. doi.org/10.1044/jshr.3901.126
[36] Jesus LM, Martinez J, Hall A, Ferreira A. Acoustic correlates of compensatory adjustments to the glottic and supraglottic structures in patients with unilateral vocal fold paralysis. Biomed Res Int. 2015. doi.org/10.1155/2015/704121
[37] Ng ML, Gilbert HR, Lerman JW. Some aerodynamic and acoustic characteristics of acute laryngitis. J Voice. 1997; 11(3): 356-63. doi.org/10.1016/s0892-1997(97)80015-x
[38] Stathopoulos ET, Huber JE, Sussman JE. Changes in acoustic characteristics of the voice across the life span: Measures from individuals 4-93 years of age. J Speech Hearing Res. 2011; 54: 1011-21. doi.org/10.1044/1092-4388(2010/10-0036)
[39] Gugatschka M, Kiesler K, Obermayer-Pietsch B, Schoekler B, Schmid C, Groselj-Strele A, Friedrich G. Sex hormones and the elderly male voice. J Voice. 2010; 24(3): 369-73. doi.org/10.1016/j.jvoice.2008.07.004
[40] Park J, Alnouri G, Eichorn D, Sataloff RT. Correlation between presbylarynx and laryngeal EMG. J Voice. 2022; 36(3):413-6. doi.org/10.1016/j.jvoice.2020.06.029
[41] Vaca M, Mora E, Cobeta I. The aging voice: influence of respiratory and laryngeal changes. Otolaryngol Head Neck Surg. 2015; 153(3): 409-13. doi.org/10.1177/0194599815592373

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Prevalence and associated factors of dyslipidemia among university students in Central Thailand: a cross-sectional study

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ABSTRACT

Background: Dyslipidemia is regarded as a significant risk factor for atherosclerotic cardiovascular diseases (ASCVDs). Currently, there is limited data regarding dyslipidemia among Thai university students.

Objective: This study aimed to examine the prevalence of dyslipidemia and its related factors among university students in the central region of Thailand.

Materials and methods: In this cross-sectional study, a total of 434 students aged 18-25 years at Thammasat University and Kasetsart University were recruited using simple random sampling. Fasting venous blood samples were obtained, and plasma lipid profiles were assessed by an automated analyzer. Dyslipidemia was formally characterized according to the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP III) guidelines. Demographic information, dietary behavior, and physical activity were collected using questionnaires. Anthropometric measures were also performed according to a standard protocol.

Results: The prevalence of elevated total cholesterol, high-risk HDL-C, elevated LDL-C, and elevated triglycerides was 30.4, 18.2, 20.0, and 11.8%, respectively. The most common adverse lipid parameter was total cholesterol in both genders. Obesity and high fat intake were significantly associated with dyslipidemia (p<0.05).

Conclusion: This research revealed a significant occurrence of dyslipidemia, primarily driven by elevated total cholesterol, among university students in Thailand. Obesity and high fat intake are significant risk contributors to dyslipidemia. These findings emphasize the need for awareness, prevention, and management strategies targeting this population.

Introduction

Dyslipidemia is a medical condition characterized by abnormal levels of lipids in the blood, including high levels of total cholesterol (TC), triglycerides (TGs), and low-density lipoprotein (LDL-C), or low level of high-density lipoprotein cholesterol (HDL-C). Dyslipidemia represents a significant risk factor for atherosclerotic cardiovascular diseases (ASCVDs), which stand as a prominent cause of death globally.[1] Individuals afflicted by dyslipidemia face a twofold higher risk of developing cardiovascular diseases (CVDs) compared to those with normal lipid levels.[2] In 2019, the World Health Organization (WHO) reported that CVDs accounted for 1.7 million fatalities worldwide, with heart attacks and strokes responsible for 85% of these cases.[3]

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Previous studies have investigated the prevalence of dyslipidemia among university students in different countries. It has been reported to be relatively high, from 76.5% to 86.7%.[4-6] Common factors associated with dyslipidemia in university students include physical inactivity, unhealthy diet, smoking, and obesity. It has been proposed that half of the adolescents with elevated total cholesterol levels face a fivefold greater risk of developing coronary heart disease at the ages of 30 and 40 in comparison to those with normal total cholesterol levels.[7,8]

Considering the widespread occurrence of dyslipidemia among university students and its possible long-term health consequences, early detection and prevention of dyslipidemia have become crucial strategies for reducing both current and future health risks. Therefore, this study aims to assess the prevalence of dyslipidemia and its associated factors among university students located in the Central Thailand.

Materials and methods

Study design and sample group

This was an observational cross-sectional study involving 434 university students of both genders aged between 18 and 23 years. Participants were recruited from Thammasat University in Pathum Thani Province, Kasetsart University, Bangkhen campus in Bangkok, and Kamphaeng Saen campus in Nakhon Pathom Province. Each participant was informed and provided written consent before participating in the study. This study was conducted between December 27, 2021 and December 27, 2022.

Data collection

There were two phases of data collection.

First, all study participants have been requested to complete a questionnaire. Students were informed that they could skip questions and could discontinue the survey at any time. The questionnaire was divided into the following two parts.

Part I Demographics: The questions covered the following aspects: age, gender, academic year, living or housing (alone or with friends or family), dietary behavior, alcohol habits, and smoking status.

Part II Physical activity: The Global Physical Activity Questionnaire (GPAQ) was used to assess the level of physical activities related to occupation, transportation, and leisure time.

Second, anthropometric measures were performed according to a standard protocol. A student's height was measured while standing erect and barefoot using a stadiometer. A bioelectrical impedance analyzer (Tanita Corporation, USA) measured weight and percent body fat. The extreme dehydration or overhydration can impact bioelectrical impedance analyzer readings; the measurements of the subjects were taken in the morning after an overnight fast, with an empty bladder, no vigorous exercise, and no alcohol or stimulant consumption.

Body mass index (BMI) was calculated using the equation BMI = weight (kg)/height $(m)^2$ and classified according to the WHO's criteria as normal (BMI<23.0 kg/m^2), overweight (BMI 23.0-24.9 kg/m^2), or obesity (BMI≥25.0 kg/m^2).[9]

After an overnight fast, venous blood samples were drawn into vacutainer containers containing sodium heparin. All blood samples were transported on ice at certain times to the Medical Technology Service and Research Center laboratory, Faculty of Allied Health Sciences, Thammasat University. Plasma concentrations of TC, HDL-C, LDL-C, and TG were evaluated using a biochemical automated analyzer (Furuno, Japan). The value of TC ≥200 mg/dL, TG ≥150 mg/dL, and LDL-C ≥130 mg/dL were considered high. HDL-C levels of <40 and <50 mg/dL were considered high risk for males and females, respectively.[10] Dyslipidemia was defined as the presence of at least one abnormal lipid parameter.

Statistical analysis

Data were analyzed and shown as frequencies, percentages, and means. The means of two distinct samples were compared using an independent samples *t*-test. The chi-square test was employed to assess the categorical data. Multivariable logistic regression was employed to determine the variables that were associated independently with anomalies in lipid markers. The dependent factors in the regression models consisted of dyslipidemia and abnormal lipid profiles, while the independent variables encompassed anthropo-demographics and behavioral traits. A *p*-value less than or equal to 0.05 was considered statistically significant.

Results

Characteristics of the student participants

This study included 434 university students, of which 340 were female (78.3%), 47.5% were Thammasat University students, and 52.5% were from Kasetsart University. The average (SD) age was 20.6±1.3 years, and half of the participants were over 20 (80.2%). The mean BMI of the student sample was 22.2±4.8, indicating a relatively healthy weight range. However, it is noteworthy that approximately 32.7% of the students were classified as overweight/obese, as indicated in Table 1.

Table 1. Characteristics and prevalence of dyslipidemia in study participants (N=434).

	Total (%)	Male (%)	Female (%)	*p* value
N	434 (100)	94 (21.7)	340 (78.3)	
University				
Thammasat University	206 (47.5)	48 (23.3)	46 (20.2)	0.430
Kasetsart University	228 (52.5)	158 (76.7)	182 (79.8)	
Age (years)	20.6±1.3	20.6±1.2	20.6±1.3	0.733
≤19 years	86 (19.8)	20 (21.3)	66 (19.4)	0.607
20-21 years	241 (55.5)	48 (51.1)	193 (56.8)	
21 years	107 (24.7)	26 (27.7)	81 (23.8)	
BMI (kg/m^2)	22.2 ± 4.8	23.5 ± 6.3	21.9 ± 4.2	0.002
Normal (<23)	292 (67.3)	58 (61.7)	234 (68.8)	0.153
Overweight (23.00-24.99)	57 (13.1)	11 (11.7)	46 (13.5)	
Obese (≥25.00-29.99)	85 (19.6)	25 (26.6)	60 (13.8)	
Percent body fat	23.8±6.9	16.7±6.7	25.8±5.6	<0.001
Normal (M<21.0, F<34.0)	380 (87.6)	72 (76.6)	308 (90.6)	<0.001
Overfat/Obese (M≥22.0, F≥34.0)	54 (12.4)	22 (23.4)	32 (9.4)	
Physical activity (MET)[a]	4819.8±6367.7	7029.4±6381.5	4196.3±6234.7	<0.001
Low	105 (29.2)	7 (8.9)	98 (35.0)	<0.001
Medium	177 (32.6)	23 (29.1)	94 (33.6)	
High	137 (38.2)	49 (62.0)	88 (31.4)	
Living/housing				
Alone	118 (27.2)	42 (44.7)	76 (22.4)	<0.001
With friend	198 (45.6)	34 (36.2)	164 (48.2)	
With family	118 (27.2)	18 (19.1)	100 (29.4)	
Smoking[b]				
No	23 (5.4)	79 (87.8)	327 (96.5)	0.001
Yes	406 (94.6)	11 (12.2)	12 (3.5)	
Alcohol consumption[c]				
No	245 (57.5)	48 (53.9)	197 (58.5)	0.442
Yes	181 (42.5)	41 (46.1)	140 (41.5)	
Sweet intake				
Low	339 (78.1)	70 (74.5)	269 (79.1)	0.335
High	95 (21.9)	24 (25.5)	71 (20.9)	
Lipid intake				
Low	216 (49.8)	47 (50.0)	169 (49.7)	0.960
High	218 (50.2)	47 (50.0)	171 (50.3)	
Expenses (THB)				
<5,000	116 (26.9)	20 (21.5)	96 (28.3)	0.008
5001-10,000	235 (54.4)	46 (49.5)	189 (55.8)	
10,000-15,000	70 (16.2)	21 (22.6)	49 (14.5)	
>15,000	11 (2.5)	6 (6.5)	5 (1.5)	

Note: [a,b,c] The number of participants was not equal to 434, resulting from missing data.

Lipid profiles and prevalence of dyslipidemia

The average plasma concentration of TC, HDL-C, LDL-C, and TGs among 434 student participants is shown in Figure 1. The average HDL-C levels were significantly greater in females (p<0.001). The average TG was slightly higher in males, whereas the average TC and LDL-C levels were higher in females.

The prevalence of high TC, LDL-C, and TG levels was 30.4, 20.0, and 11.8% (N=132, 87, and 51), respectively. Furthermore, 79 participants (18.2%) exhibited low HDL-C levels, indicating a high-risk condition. In the sample group, 214 individuals (49.3) had normal lipid levels, while the remaining 220 participants had at least one type of abnormal lipid level, resulting in a dyslipidemia prevalence of 50.7%. Notably, the prevalence of dyslipidemia was 52.4% in females (178/340) and 44.7% in males (42/94), with a p-value of 0.118, indicating no significant difference between the genders (Figure 2). The prevalence of high TC, high-risk HDL, and high LDL was higher in females (p=0.015, 0.065, and 0.159, respectively). Meanwhile, the prevalence of high TG was more frequently observed in males (p=0.012). The numbers of participants with single and multiple lipid abnormalities are shown in Figure 3.

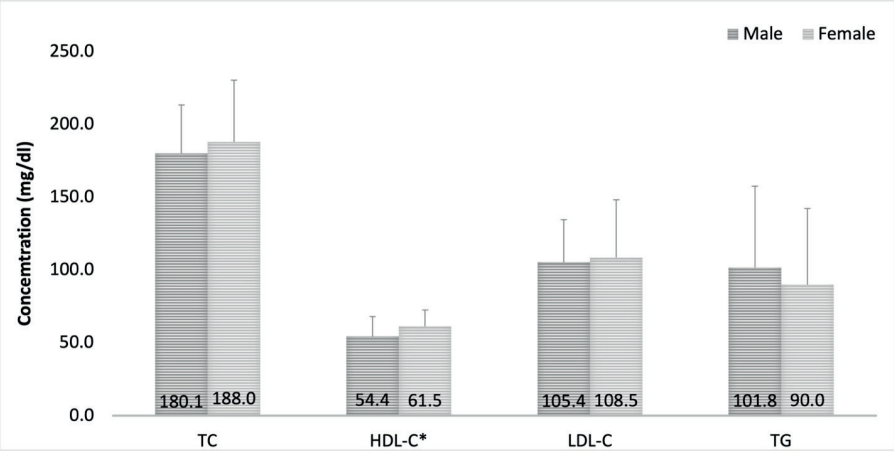

Figure 1. *Plasma concentrations of TC, HDL-C, LDL-C, and TG among Thai university students. *p<0.001 obtained from independent sample t-test.*

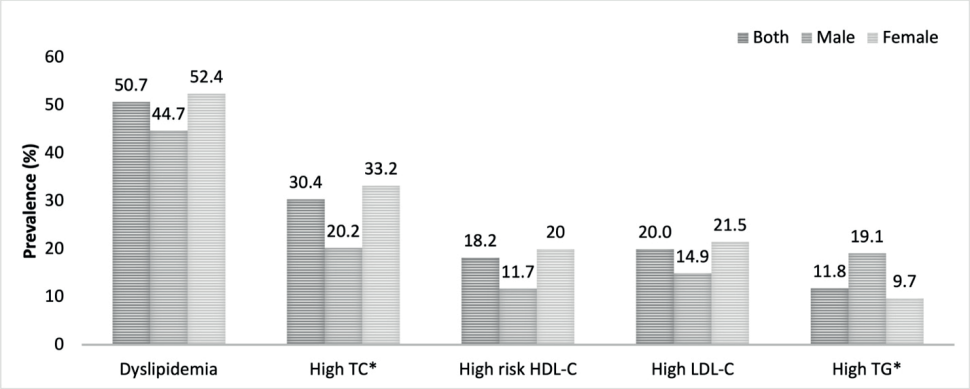

Figure 2. *Prevalence of dyslipidemia and abnormal lipid markers among Thai university students. *p<0.05 was obtained from the chi-square test.*

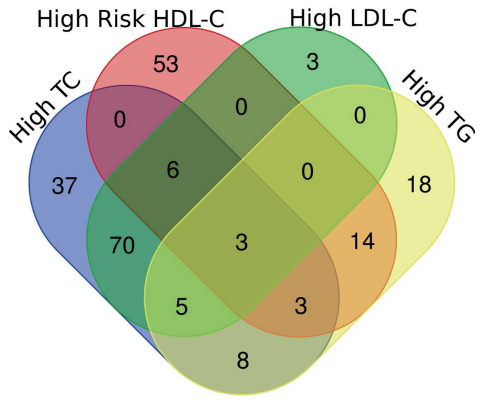

Figure 3. *Co-occurrence of abnormal lipid abnormalities among university students in Thailand. The values demonstrate the abnormal levels of TC, HDL-C, LDL-C, and TG.*

Participants who were categorized as obese according to their BMI and body fat percentage had a notably greater prevalence of dyslipidemia, high-risk HDL, and high TG (p<0.05). Furthermore, a positive correlation of high-risk HDL-C with high fat intake was observed, while a negative correlation was found between the prevalence of high-risk HDL-C and physical activity. (Table 2)

Table 2. The prevalence of dyslipidemia among Thai university students according to different factors (N=434).

Variable	N (%)				
	Dyslipidemia	High TC	High-risk HDL-C	High LDL-C	High TGs
Age (years)					
≤19 years	47 (54.7)	24 (27.9)	15 (17.4)	15 (17.4)	12 (14.0)
20-21 years	112 (46.5)	70 (29.0)	39 (16.2)	49 (20.3)	23 (9.5)
>21 years	61(57.0)	38 (35.5)	25 (23.4)	23 (21.5)	16 (15.0)
p value	0.138	0.410	0.271	0.772	0.274
BMI (kg/m^2)					
Normal (<23)	**129 (44.2)**	85 (29.1)	**34 (11.6)**	54 (18.5)	**24 (8.2)**
Overweight (23.00-24.99)	**32 (56.1)**	17 (29.8)	**14 (24.6)**	13 (22.8)	**5 (8.8)**
Obese (≥25.00-29.99)	**59 (69.4)**	30 (35.3)	**31 (36.5)**	20 (23.5)	**22 (25.9)**
p value	**< 0.001**	0.549	**< 0.001**	0.508	**< 0.001**
Percent body fat					
Normal (M<21.0, F<34.0)	**181 (47.6)**	112 (29.5)	**55 (14.5)**	**70 (18.4)**	**36 (9.5)**
Overfat/Obese (M≥22.0, F≥34.0)	**39 (72.2)**	20 (37.0)	**24 (44.4)**	**17 (31.5)**	**15 (27.8)**
p value	**<0.001**	0.258	**<0.001**	**0.025**	**<0.001**
Physical activity[a]					
Low	57(54.3)	32 (30.5)	**27 (25.7)**	27 (25.7)	7 (6.7)
Medium	60 (51.3)	37 (31.6)	**19 (16.2)**	23 (19.7)	18 (15.3)
High	61 (44.5)	37 (27.0)	**16 (11.7)**	21 (15.3)	17 (12.4)
p value	0.291	0.701	**0.016**	0.132	0.124
Living/housing					
Alone	57 (48.3)	40 (33.9)	15 (12.7)	29 (24.6)	11 9.3)
With friend	100 (45.5)	54 (40.9)	41 (20.7)	32 (16.2)	21 (10.6)
With family	63 (28.6)	38 (32.2)	23 (19.5)	26 (22.0)	19 (16.1)
p value	0.735	0.411	0.187	0.160	0.215
Smoking[b]					
No	210 (51.7)	127 (31.3)	74 (18.2)	83 (20.4)	48 (11.8)
Yes	8 (1.9)	3 (13.0)	5 (21.7)	2 (8.7)	2 (4.0)
p value	0.114	0.064	0.672	0.169	0.649
Alcohol consumption[c]					
No	132 (53.9)	82 (33.5)	45 (18.4)	52 (21.2)	33 (13.5)
Yes	83 (45.9)	47 (26.0)	33(18.2)	33 (18.2)	16 (8.8)
p value	0.102	0.096	0.972	0.445	0.139
Sweet intake					
Low	172 (50.7	105 (31.0)	59 (17.4)	68 (20.1)	35 (10.3)
High	48 (50.5)	27 (28.4)	20 (21.1)	19 (20.0)	16 (16.8)
p value	0.971	0.633	0.415	0.990	0.081
Fat intake					
Low	102 (47.2)	67 (31.0)	**31 (14.4)**	43 (19.9)	22 (10.2)
High	118 (54.1)	65 (29.8)	**48 (22.0)**	44 (20.2)	29 (13.3)
p value	0.150	0.786	**0.038**	0.943	0.313
Expenses (Thai Bht)					
<5,000	117 (49.8	33 (28.4)	**27 (23.3)**	20 (17.2)	12 (2.8)
5001-10,000	61 (52.7)	76 (32.3)	**38 (16.2)**	49 (20.9)	28 (6.5)
10,000-15,000	32 (45.7)	19 (27.1)	**9 (12.9)**	14 (20.0)	7 (1.6)
>15,000	9 (81.8)	3 (27.3)	**5 (45.5)**	3 (27.3)	3 (0.7)
p value	0.158	0.794	**0.024**	0.796	0.389

[a,b,c] *The number of participants was not equal to 434 as a result of missing data*

Associated factors of dyslipidemia among Thai university students

Table 3 shows the results of the multivariable logistic regression analysis. Compared to participants with normal weight, the odds of dyslipidemia, high-risk HDL-C, and high TG were 2.08, 2.69, and 3.50 times among participants with obese (p<0.05), respectively. Regarding percent body fat, participants reporting overfat/obese (M≥22.0%, F≥34.0%) were 3.07 and 2.73 times more likely to develop high-risk HDL-C and high LDL than those reporting normal percent body fat (p=0.09). High-fat consumption is significantly associated with dyslipidemia and high TG (p<0.05). The risk of high TG of participants drinking alcohol was decreased by 55% compared to participants who did not drink alcohol.

Table 3. Association of dyslipidemia to different factors among university student in Thailand by multivariable logistic regression analysis.

Variable	Dyslipidemia	*p* value	High TC	*p* value	High risk HDL-C	*p* value	High LDL-C	*p* value	High TG	*P* value
	OR (95% CI)		OR (95% CI)		OR (95% CI)		OR (95% CI)		OR (95% CI)	
Gender										
Male	Reference		Reference		Reference		Reference		Reference	
Female	1.70 (0.94-3.07)	0.080	**2.04 (1.04-4.02)**	**0.038**	2.42 (0.97-6.06)	0.059	1.62 (0.75-3.53)	0.222	0.77 (0.33-1.79)	0.545
Age (year)										
≤19 years	Reference		Reference		Reference		Reference		Reference	
20-21 years	0.58 (0.33-1.02)	0.059	1.01 (0.55-1.85)	0.978	0.74 (0.34-1.63)	0.459	1.02 (0.50-2.04)	0.966	0.42 (0.18-1.02)	0.055
>21 years	0.96 0.49-1.86)	0.899	1.26 (0.62-2.54)	0.524	1.14 (0.48-2.72)	0.764	1.07 (0.47-2.40)	0.879	0.82 (0.31-2.13)	0.678
BMI (kg/m²)										
Normal (<23)	Reference		Reference		Reference		Reference		Reference	
Overweight (23.00-24.99)	1.39 (0.72-2.70)	0.324	0.92 (0.45-1.90)	0.820	2.09 (0.91-4.79)	0.083	1.08 (0.48-2.43)	0.845	0.77 (0.23-2.55)	0.665
Obese (≥25.00-29.99)	**2.08 (1.06-4.11)**	**0.034**	1.06 (0.52-2.17)	0.873	**2.69 (1.17-6.20)**	**0.020**	1.02 (0.45-2.30)	0.965	**3.50 (1.37-8.89)**	**0.009**
Percent body fat										
Normal (M<21.0,F<34.0)	Reference		Reference		Reference		Reference		Reference	
Overfat (M≥22.0, F≥34.0)	2.24 (0.94-5.31)	0.068	1.93 (0.83-4.49)	0.125	**3.07 (1.21-7.79)**	**0.018**	**2.73 (1.11-6.71)**	**0.029**	1.55 (0.52-4.58)	0.428
Physical activity										
High	Reference		Reference		Reference		Reference		Reference	
Medium	1.14 (0.66-1.95)	0.637	1.10 (0.62-1.96)	0.733	1.31 (0.60-2.89)	0.501	1.20 (0.61-2.37)	0.594	1.39 (0.62-3.12)	0.426
Low	1.04 (0.58-1.87)	0.895	0.91 (0.49-1.68)	0.757	1.85 (0.84-4.08)	0.125	1.52 (0.76-3.06)	0.239	0.41 (0.14-1.18)	0.097
Smoking										
No	Reference		Reference		Reference		Reference		Reference	
Yes	0.39 (0.13-1.18)	0.094	0.32 (0.07-1.51)	0.151	1.44 (0.37-5.56)	0.594	0.49 (0.10-2.39)	0.376	0.44 (0.05-3.99)	0.464
Alcohol consumption										
No	Reference		Reference		Reference		Reference		Reference	
Yes	0.77 (0.48-1.23)	0.272	0.79 (0.48-1.30)	0.349	1.06 (0.56-2.02)	0.849	0.94 (0.54-1.65)	0.835	**0.45 (0.20-0.98)**	**0.045**
Sweet intake										
Low	Reference		Reference		Reference		Reference		Reference	
High	0.74 (0.42-1.23)	0.282	0.74 (0.40-1.35)	0.324	1.15 (0.55-2.02)	0.706	0.80 (0.40-1.60)	0.523	1.33 (0.58-3.04)	0.494
Fat intake										
Low	Reference		Reference		Reference		Reference		Reference	
High	**1.65 (1.04-2.64)**	**0.035**	1.06 (0.65-1.73)	0.818	1.59 (0.84-3.03)	0.156	1.11 (0.63-1.94)	0.716	**2.33 (1.07-5.04)**	**0.032**
Expenses (Thai Bht)										
<5,000	Reference		Reference		Reference		Reference		Reference	
5001-10,000	0.90 (0.54-1.52)	0.696	1.24 (0.71-2.17)	0.444	0.55 (0.28-1.08)	0.083	1.32 (0.69-2.52)	0.399	0.94 (0.41-2.19)	0.893
10,000-15,000	0.92 (0.45-1.86)	0.809	1.10 (0.50-2.40)	0.809	0.44 (0.16-1.24)	0.120	1.25 (0.51-3.04)	0.621	1.21 (0.39-3.70)	0.741
>15,000	8.67 (0.89-84.67)	0.063	1.48 (0.25-8.86)	0.670	2.96 (0.51-17.14)	0.226	2.29 (0.38-13.99)	0.368	6.02 (0.80-45.63	0.082

Discussion

This study represents the initial investigation into the prevalence of dyslipidemia and its related factors among Thai university students between 18 and 25. The prevalence of dyslipidemia among Thai university students (50.7%.) was relatively lower than that among Yemeni students, with a prevalence rate of 86.7%, and that among Bangladeshi students, with a prevalence rate of 76.5%.[4,5] Previous studies have demonstrated that young adult females exhibit higher levels of TC, HDL-C, and LDL-C than males.[11] However, TG levels are higher in males. Our data demonstrate similar findings to previous reports. Mean levels of TC (188.0 vs. 180.1, p=0.056), LDL-C (108.5 vs. 105.4, p=0.390), and HDL-C (61.5 vs. 55.4; p<0.001) were higher in females, whereas TG levels in males were slightly higher than those in females (101.8 vs. 90.0, p=0.067). A national survey conducted on the Thai population found that females showed higher mean levels of TC, HDL-C, and LDL-C, while average TG levels were greater in males.[12] Their results showed that the means of all lipid markers were slightly higher than those in our results. This may be due to the older age group of participants used in their survey (20-34 years).

In this study, the most common prevalence of abnormal lipid markers was high TC at 30.4%, followed by high LDL-C at 26.5%, high-risk HDL at 18.2%, and high TG at 11.8%. Our result aligns with the findings of an earlier investigation conducted in Thailand, which found that the prevalence of dyslipidemia in the Thai population was primarily based on the presence of high TC and high LDL-C.[13,14] Abnormal values have been observed in comparable studies conducted in various countries. In Yemen, the prevalence of abnormal values for TC, HDL-C, LDL-C, and TG were 21.7%, 81.7%, 31.7%, and 23.8%, respectively.[4] Similarly, in China, the prevalence of high TC, low HDL-C, high LDL-C, and high TG in medical university students was 1.3%, 18.7%, 6.7%, and 5.1%, respectively.[5] In Bangladesh, the prevalence of abnormal values in university students for TC, HDL-C, LDL-C, and TG was 25.6%, 69.3%, 26.5%, and 39.0%, respectively.[6] The variations observed in the overall dyslipidemia prevalence and the rate of abnormal lipid parameters across different countries and studies can be caused by several factors, including variations in methodologies, such as differences in age group selection, cutoff reference values, consideration of fasting state, and lifestyle factors, such as demographic features, dietary habits, and genetic backgrounds.

As expected, our study results have shown a correlation between a high-fat diet and dyslipidemia, particularly high triglycerides. It's widely recognized that a significant portion of triglycerides in the body originates from dietary sources, including fatty meat and cooking oils.[15] Various studies have demonstrated that being overweight or obese is an important factor for developing dyslipidemia in university students and adolescent[4-6,16]. Kwon *et al*. revealed that the prevalence of dyslipidemia increased in line with BMI among adolescents in South Korea.[16] The findings of our investigation indicate a significant association between general obesity (BMI of ≥25 kg/m^2) and dyslipidemia, especially high-risk HDL and high TG levels. Moreover, high percent body fat contributed to high-risk HDL and high LDL-C. The mechanisms underlying this phenomenon suggest that an elevation in plasma triglyceride (TG) levels can be attributed to an augmented hepatic synthesis of very-low-density lipoprotein (VLDL) particles, along with a reduction in the removal of triglyceride-rich lipoproteins. In contrast, diminished levels of HDL-C are linked to reduced concentrations of adiponectin, a hormone that is often reduced in individuals with obesity.[17] Another proposed mechanism is that when total body fat mass increases, the rate of fat accumulation tends to be higher in the central body but lower in the peripheral.[18] Insulin resistance is mediated by excess central fat and caused an increase in hepatic lipase and the reduction of lipoprotein lipase. This alteration led to impair maturation and increase catabolism of HDL-C.[19] Previous study reported the strong association of percent body fat with LDL-C.[20,21] Nevertheless, the causal mechanism remains inconclusive. There is indication that obesity during this life stage posed a risk factor for developing coronary disease in adulthood.[22] Hence, an increased BMI and higher levels of body fat are regarded as key screening markers for the identification of persons with dyslipidemia. For obese people with dyslipidemia, treatment should focus on life-style changes such as weight loss, weight control, a healthy diet and physical exercise. The introduction of lifestyle adjustments has a synergistic and positive effect on insulin resistance and dyslipidemia.[23]

We also found that the participant currently consuming alcohol had a 55% decrease in their TG level. This finding was consistent with earlier research.[24,25] There is evidence from certain research that moderate alcohol consumption may benefit elevated HDL and decreased LDL-C.[26] However, the relationship between alcohol intake and lipid levels might be complex and unique to each person. Importantly, we stressed the need for individuals to consult with healthcare professionals and consider their specific health circumstances before deciding on alcohol consumption. Additionally, we emphasized that any potential benefits should not serve as a justification to initiate alcohol consumption if it is not already a part of one's lifestyle.

The study on dyslipidemia among Thai university students in the central region possesses notable strengths. Firstly, it addresses a critical gap in the existing literature by providing novel insights into the prevalence of dyslipidemia and its associated factors, specifically among university students in Thailand. The comprehensive approach, which includes the assessment of demographics, lifestyle factors, and anthropometric measures, enriches the depth of understanding regarding the studied population. This study has some limitations. First, because of the nature of the cross-sectional study design, it is impossible to establish a causality in our study. Second, the sample size was relatively small, and the data was exclusively collected from Central Thailand. Consequently, the findings might need to reflect the overall dyslipidemia scenario throughout the country accurately. Further large-scale

studies are needed to gather a broader understanding of dyslipidemia prevalence among Thai university students and to explore potential interventions to reduce the burden of this condition.

Conclusion

The data provided indicate a high prevalence of dyslipidemia among university students in Central Thailand. The dyslipidemia is primarily based on high TC and LDL-C levels among the participants. The significant risk factors were obesity and high fat consumption, emphasizing the need for awareness, prevention, and management strategies targeting this population.

Conflict of interest

The authors declare that there is no conflict.

Ethics approval

This study's protocol was approved by the Ethical Committee of Thammasat University (project no. 088/2564; COA no.106/2564)

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References

[1] Sandesara P.B., Virani S.S., Fazio S., Shapiro M.D. The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk. Endocr Rev. 2019; 40: 537-57. doi: 10.1210/er.2018-00184.

[2] Mozaffarian D, Benjamin EJ, et al. Heart disease and stroke statistics-2016 Update: A Report From the American Heart Association. Circulation. 2016; 133(4): e38-e360. doi:10.1161/CIR.0000000000000350.

[3] World Health Organization Western Pacific Region. Cardiovascular diseases (CVDs). Geneva: World Health Organization; 2021. [cited 2023 Sep 7]. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

[4] Al-Duais MA, Al-Awthan YS. Prevalence of dyslipidemia among students of a Yemeni University. J Taibah Univ Med Sci. 2019; 14(2): 163-71. doi: 10.1016/j.jtumed.2018.12.003.

[5] Abuzhalihan J, Wang YT, Adi D, et al. Prevalence of dyslipidemia in students from Han, Uygur, and Kazakh ethnic groups in a Medical University in Xinjiang, China. Sci Rep. 2019; 9(1): 19475. doi:10.1038/s41598-019-55480-5.

[6] Ali N, Kathak RR, Fariha KA, Taher A, Islam F. Prevalence of dyslipidemia and its associated factors among university academic staff and students in Bangladesh. BMC Cardiovasc Disord. 2023; 23(1): 366. doi:10.1186/s12872-023-03399-1.

[7] Cleeman JI, Grundy SM. National cholesterol Education Program recommendations for cholesterol testing in young adults. A science-based approach. Circulation. 1997; 95: 1646-50.

[8] Washington R.L. Interventions to reduce cardiovascular risk factors in children and adolescents. Am Fam Phys. 1999; 59(8): 2211-8.

[9] World Health Organization Western Pacific Region. The Asia-Pacific perspective: redefining obesity and its treatment. Geneva: World Health Organization; 2000. [cited 2023 Sep 7]. Available from: https://apps.who.int/iris/bitstream/handle/10665/206936/09577082 11_eng.pdf.

[10] National cholesterol education program. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation. 2002; 106(25): 3143-421.

[11] Eissa MA, Mihalopoulos NL, Holubkov R, Dai S, Labarthe DR. Changes in fasting lipids during puberty. J Pediatr. 2016; 170: 199-205. doi:10.1016/j.jpeds.2015.11.018.

[12] Aekplakorn W, Taneepanichskul S, Kessomboon P, et al. Prevalence of dyslipidemia and management in the Thai population, National Health Examination Survey IV, 2009. J Lipids. 2014; 2014: 249584. doi:10.1155/2014/249584.

[13] Jeenduang N, Whanmasae S, Seepawin P, Kullabootr S. The prevalence of dyslipidemia among a rural Thai population in the Nakhon Si Thammarat province. J Med Assoc Thai. 2013; 96(8): 992-1000.

[14] Theeratrakoolchai O., Chaimanee A. Prevalence of dyslipidemia and associated factors among medical personnel in Nopparat Rajchathani hospital. Thammasat Med J. 2017; 17: 9-17. (in Thai).

[15] Wang L, Wang H, Zhang B, Popkin BM, Du S. Elevated fat intake increases body weight and the risk of overweight and obesity among Chinese adults: 1991-2015 trends. Nutrients. 2020; 12(11): 3272. doi:10.3390/nu12113272.

[16] Kwon M, Kim J, Cha E. Obesity-status-linked affecting factors of dyslipidemia in Korean young-adult men: based on the Korea national health and nutrition examination survey (2019-2021). Healthcare (Basel). 2023; 11(14): 2015. doi:10.3390/healthcare11142015.

[17] Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013; 5(4): 1218-40. doi:10.3390/nu5041218.

[18] Wells JC, Fewtrell MS, Williams JE, Haroun D, Lawson MS, Cole TJ. Body composition in normal weight, overweight and obese children: matched case-control analyses of total and regional tissue masses, and body composition trends in relation to relative weight. Int J Obes (Lond). 2006; 30(10): 1506-13.

doi:10.1038/sj.ijo.0803402.

[19] Dullaart R, Tol AV. Role of phospholipid transfer protein and prebeta-high density lipoproteins in maintaining cholesterol efflux from Fu5AH cells to plasma from insulin-resistant subjects. Scand J Clin Lab Invest. 2001; 61: 69-74. 10.1080/00365510151068027.

[20] Oda E. LDL cholesterol was more strongly associated with percent body fat than body mass index and waist circumference in a health screening population. Obes Res Clin Pract. 2018; 12(2): 195-203. doi:10.1016/j.orcp.2017.05.005

[21] Martakis K, Stark C, Rehberg M, Jackels M, Schoenau E, Duran I. Association of muscle mass and fat mass on low-density-lipoprotein cholesterol and triglyceride plasma concentration in children and adolescents. J Pediatr Endocrinol Metab. 2021; 34(10): 1273-82. Published 2021 Jul 19. doi:10.1515/jpem-2021-0254

[22] Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents, National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 2011; 128(Supl. 5): S213-S256. doi: 10.1542/peds.2009-2107C.

[23] Klop B., Castro Cabezas M. Chylomicrons: A key biomarker and risk factor for cardiovascular disease and for the understanding of obesity. Curr Cardiovasc Risk Rep. 2012; 6: 27-34. doi: 10.1007/s12170-011-0215-z.

[24] Song P, Zha M, Yang X, et al. Socioeconomic and geographic variations in the prevalence, awareness, treatment and control of dyslipidemia in middle-aged and older Chinese. Atherosclerosis. 2019; 282: 57-66. doi:10.1016/j.atherosclerosis.2019.01.005.

[25] Xi Y, Niu L, Cao N, et al. Prevalence of dyslipidemia and associated risk factors among adults aged ≥35 years in northern China: a cross-sectional study. BMC Public Health. 2020; 20(1): 1068. doi:10.1186/s12889-020-09172-9.

[26] Brinton EA. Effects of ethanol intake on lipoproteins and atherosclerosis. Curr Opin Lipidol. 2010; 21(4): 346-51. doi:10.1097/MOL.0b013e32833c1f41.

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Reliability of running parameters using fitness watches synced with accelerometers during outdoor runs

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ABSTRACT

Background: To prevent running related injuries and return to sport activities, monitoring the running dynamic parameters (cadence, stride length, ground contact time and vertical oscillation) especially outdoor running is crucial. Previous studies investigated the reliability of these parameters in laboratory settings. However, the nature of outdoor runs is different (curve, uphill, other runners, etc.) and challenging in terms of equipment (simple) and environments (grass, asphalt, rubber, etc.). Therefore, the reliability of these parameters using a fitness watch synced with accelerometer needed to be investigated.

Objective: To investigate the reliability of running parameters measured using fitness watches and accelerometers during outdoor runs.

Materials and methods: 30 healthy volunteers (age 25.8±9.6 years, height 167.2±9.3 cm, weight 62.4±14.2 kg, and body mass index 22.2±3.8 kg/m^2) participated in the study. They wore a fitness watch and attached a synced accelerometer at their pants. They completed 2 running laps (800 meters each) at their comfortable speeds. Resting periods were provided between laps. To control the speed for the second lap, the watch was set the maximum and minimum speed and set vibration and sound alarm mode. Running parameters include cadence, stride length, vertical oscillation, and ground contact time.

Results: The reliability of the four running parameters (cadence, stride length, ground contact time, and vertical oscillation), indicated by the intraclass correlation coefficients (ICC (3,k)) was 0.94, 0.97, 0.98 and 0.99, respectively. Very high reliability values were confirmed.

Conclusion: Using a fitness watch synced with an accelerometer during outdoor runs, running dynamic parameters (cadence, stride length, ground contact time, and vertical oscillation) illustrated very high levels of reliability.

Introduction

The most common running-related injuries (RRIs) include iliotibial band syndrome, Achilles tendinopathy, plantar fasciitis, medial tibial stress syndrome, patellofemoral pain syndrome, and tibial stress fracture.[1] The etiology of RRI is directly related to high impact loads, repetitive use over long periods of time and/or poor structure and biomechanics such as leg length discrepancy, flat foot, tightness and weakness of leg muscles and connective tissues.[2,3] A previous systematic review and meta-analysis of 18 studies involving 1172 volunteers

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reported that high-impact runners were more likely to have injuries than low-impact runners.[4] Therefore, reducing the impact load and loading rate are methods that have been widely researched. Among runners with poor structure and biomechanics, monitoring running biomechanics, especially spatiotemporal or running dynamic parameters, could be a useful strategy to prevent RRIs. The running dynamic parameters examined herein include cadence, stride length, vertical oscillation, and ground contact time.

Several previous studies examined the modification of running dynamic parameters to prevent or reduce RRIs and reported that increasing the number of steps per minute or increasing the cadence while controlling the speed could immediately reduce the impact on the hip and knee joint.[5-9] In addition, reducing the stride length, especially in the overstrike pattern, could reduce the likelihood of the knee being in a very stretched position and decrease the ground reaction force to the knee joint. A ten-percent reduction in stride length can reduce the average ground reaction force on the knee by up to 14.9%.[8,10] The rearfoot strike pattern leads to more injuries, especially patella injuries, than midfoot or forefoot strikes. A decrease in vertical oscillation could also reduce the impact force from the ground by 46-75%[7] and reduce the risk of tibial stress fracture.[11,12] Finally, reducing the ground contact time could reduce the impact from the ground.[13] Therefore, these parameters could be monitored as strategies for the prevention of RRIs and the improvement of running performance.

Fitness watches are especially popular among health-conscious people and runners. They have an optical sensor that penetrates the skin of the wrist to measure heart rate,[14] and they have motion sensors on the wrist to count the number of steps during walking and running.[15] They also use a highly accurate global positioning system (GPS) via satellites to measure running distance. When they are connected or synced to an accelerometer, they can monitor more important running dynamic parameters, including stride length, vertical oscillation, and ground contact time. They can also be used as real-time feedback while running and have vibratory feedback and auditory feedback features to alert runners while training.[16] Interestingly, a feature called lap, which sets all running parameters in the range of distances needed, such as 200, 300, or 400 meters, could be used to analyze the selected data.

Accelerometers with smaller sizes and attached locations have been developed. Initially, these accelerometers were designed to attach at the xiphoid process using a chest strap to monitor heart rate and running dynamic parameters. The Garmin HRM-pro plus and the Polar H10 are two such examples. Because the accelerometer and strap are in direct contact with the skin, when runners sweat, particularly during long-distance running, the devices can move and cause friction with the skin, thereby leading to discomfort and skin lesions. Additionally, accelerometers that can be attached to shoes have been developed; these devices are known as foot pods, and examples include the Stryd foot pods and Garmin foot pods. However, while running, there is a considerable amount of impact on the foot, thus causing the sensor to detach or bounce. Recently, an accelerometer named the run pod was developed with a smaller size. It is designed to be clipped on the edge of a runner's pants, thus avoiding the abovementioned problems. However, further research is needed to determine the reliability of running-related dynamic parameters measured by fitness watches and run pod accelerometers.

Previous studies related to the reliability of running parameters were conducted using treadmill runs in the laboratory setting. Running parameters (cadence, vertical oscillation, and foot contact time) measured using a fitness watch combined with an accelerometer mounted on the chest using a chest strap demonstrated very high levels of reliability (ICC>0.95 in all three variables), consistent with our previous research.[18] It was found that the reliability of running parameters (leg cadence, vertical oscillation, stride length and ground contact time) measured using a fitness watch combined with an accelerometer (run pod) was also very high (ICC>0.95 in all four variables).[17,18] Both studies focused on reliability while running on a treadmill at a constant speed. However, this situation is clearly different from running outdoors, where environmental conditions are constantly changing, e.g., the nature of the running surface (stone, ground, sand, road, tire, and swampy terrain), the slope of the terrain (flat, uphill, or downhill), the process of cornering, and the need to avoid people or obstacles. Recently, a systematic review and meta-analysis of 33 studies involving 494 volunteers reported statistically significant differences in the running parameters between treadmill and outdoor runs.[19] The results indicated a decrease in vertical oscillation but an increase in ground contact time while running on a treadmill compared to outdoor runs. These differences could be due to the propulsive nature of treadmill running, during which the belt pushes your legs and body forward. In contrast, when running outdoors, the torso and legs push forward during the propulsive phase. Thus, running on a treadmill involves less forward momentum than running on a real track. There is also the issue of the stiffness of the belt being different from the stiffness of outdoor running surfaces.[19-21] Therefore, this study aimed to investigate the test-retest reliability of running parameters measured using fitness watches and accelerometers during outdoor runs. We hypothesized that even the outdoor runs, the running dynamic parameters would have high levels of reliability.

Materials and methods

Participants

A priori power analysis was conducted using G power version 3.1.9.7 for sample size estimation, based on the intraclass correlation coefficient (ICC) for a one-way random effects model. The ICC was obtained from our previous study by Prasartwuth *et al.* (N=20), which measured the agreement among one rater on twenty subjects.[18] The ICC in Prasartwuth *et al.* study was 0.94-0.98. With significance, criterion of alpha was 0.05 and

power was 0.80, the minimum sample size needed with this ICC was approximately thirty.

Thirty healthy volunteers aged 18 years and over participated in this study. All participants engaged in at least 150 minutes of physical activity per week to avoid muscle soreness as unaccustomed to running. During the experiment, they wore comfortable clothes and running shoes and refrained from eating large meals or drinking alcoholic beverages at least 2 hours before the test. They also abstained from vigorous exercise for at least 30 minutes before the test. They completed the history questionnaire, and the researcher collected data such as sex, height, and the arm on which the watch was worn. All volunteers signed informed consent forms before participating in the study. This research was approved by the Research Ethics Committee (AMSEC-64EX-110). Before the actual run, the volunteers performed a warm-up by stretching their lower leg muscles (e.g., calf, hamstrings, quadriceps, etc.) 10 repetitions 3 sets for each muscle, and jogging for at least 10 minutes, and then resting for 5-10 minutes.

Procedures

The volunteers wore a fitness watch (Garmin Forerunner 245, Switzerland) as well as a synced accelerometer (Running Dynamics Pod, United States), which was attached to the top of the backside of their sport pants. The volunteers were then asked to run at the standard 400-meter oval track with lanes in the University running field with other runners for two laps (800 meters each) at a pace that could be run continuously without breaks and without being too tired (comfortable speed), i.e., at moderate intensity, as assessed by the talk test when running. Between laps, there was a rest period equivalent to at least 2-3 times the running time or until the volunteer was no longer tired and was ready to run the second lap. The researchers read the average speed and maximum speed of the volunteers based on the data collected from the watch or from mobile and computer applications (Garmin Connect and Garmin Express). Then, the difference between the two speeds was calculated, and the resulting value from the average speed was subtracted as the lowest speed. Then, the researcher set an alarm on the watch using vibration and auditory signals to control the lowest and maximum speed; this process aimed to ensure that the speed of the second lap was similar to that of the first lap. When the volunteers completed the second lap, they were asked to cool down by stretching and walking slowly for at least 10 minutes.

Statistical analysis

In this study, the watch was set to have a run lap every 200 meters to omit the first 200 meters and the last 200 meters. The average data was chosen at the mid-400 m range, and the SPSS statistics version 26 program was used to analyze the data. The test-retest reliability was determined using intraclass correlation coefficients (ICC (3,k)). The ICC values below 0.50 indicate poor reliability, between 0.5 and 0.75 moderate reliability, between 0.75 and 0.9 good reliability, and any values above 0.9 indicate excellent reliability. The absolute reliability was determined using standard error of measurement (SEM), calculated as SD/√n, where SD=standard deviation. Additionally, a Bland-Altman plot was constructed to show the difference in parameters between the first and second runs and to calculate the limit of agreement (LoA). The mean, standard deviation, minimum and maximum values for each running parameter were obtained from the fitness watch and accelerometer.

Results

The test-retest reliability of the running parameters measured using a fitness watch synced with an accelerometer was examined. Among thirty healthy volunteers (19 males and 11 females), the mean age was 25.8 ± 9.6 years, the mean weekly duration of physical activity was 184.67±69.37 minutes. Demographic data of the volunteers are shown in Table 1.

Table 1. Demographic data of volunteers (mean±SD and range).

	Volunteers (N=30)	
	Mean±SD	**Range**
Age (years)	25.8±9.6	18.0-58.0
Height (cm)	167.2±9.3	150.0-186.0
Weight (kg)	62.4±14.2	41.0-107.0
Body mass index (kg/m^2)	22.2±3.8	15.9-35.0

In the first run, the average speed was 10.1±0.7 kilometers per hour. The minimum and maximum speeds were 8.0 and 15.6 kilometers per hour, respectively. For the first and second run, the running parameters (cadence, stride length, ground contact time, and vertical oscillation) were shown in Table 2. The speed of the second run was controlled using a vibrating and auditory alarm setting to control the minimum and maximum speed; therefore, the average speed for the second run was 10.0±0.72 kilometers per hour. The minimum and maximum speeds were 7.1 and 15.6 kilometers per hour, respectively. The reliability of the four running parameters (cadence, stride length, ground contact time, and vertical oscillation), indicated by the intraclass correlation coefficients (ICC (3,k)), was 0.94, 0.97, 0.98 and 0.99, respectively. The standard error of measurement (SEM) values was 1.38, 0.04, 5.68 and 0.28, respectively. All four parameters had very high reliability values and low standard error of measurement (SEM) values, as shown in Table 2. In addition, the Bland-Altman plot showed the reliability of these parameters. Two laps at the 95% confidence level of limits of agreement (LoA) are shown in Figure 1. The ground contact time, stride length, and vertical oscillation were overestimated in the second run, whereas the cadence was underestimated in the second run.

Table 2. Mean and SD of running parameters in the first and second run, ICC(3,k), limits of agreement (LoA), and standard error of measurement (SEM).

Measured variables	First run	Second run	Bland-Altman (LoA)	ICC (3, k)	SEM
Cadence (steps/min)	172.4±7.5	174.6±8.3	-2.23 (-8.90)	0.94	1.38
Stride length (m)	1.0±0.2	1.0±0.2	0.02 (-0.07)	0.97	0.04
Ground contact time (msec)	239.6±31.1	237.7±30.0	1.93 (-17.87)	0.98	5.68
Vertical oscillation (cm)	9.3±1.5	9.2±1.5	0.10 (-0.58)	0.99	0.28

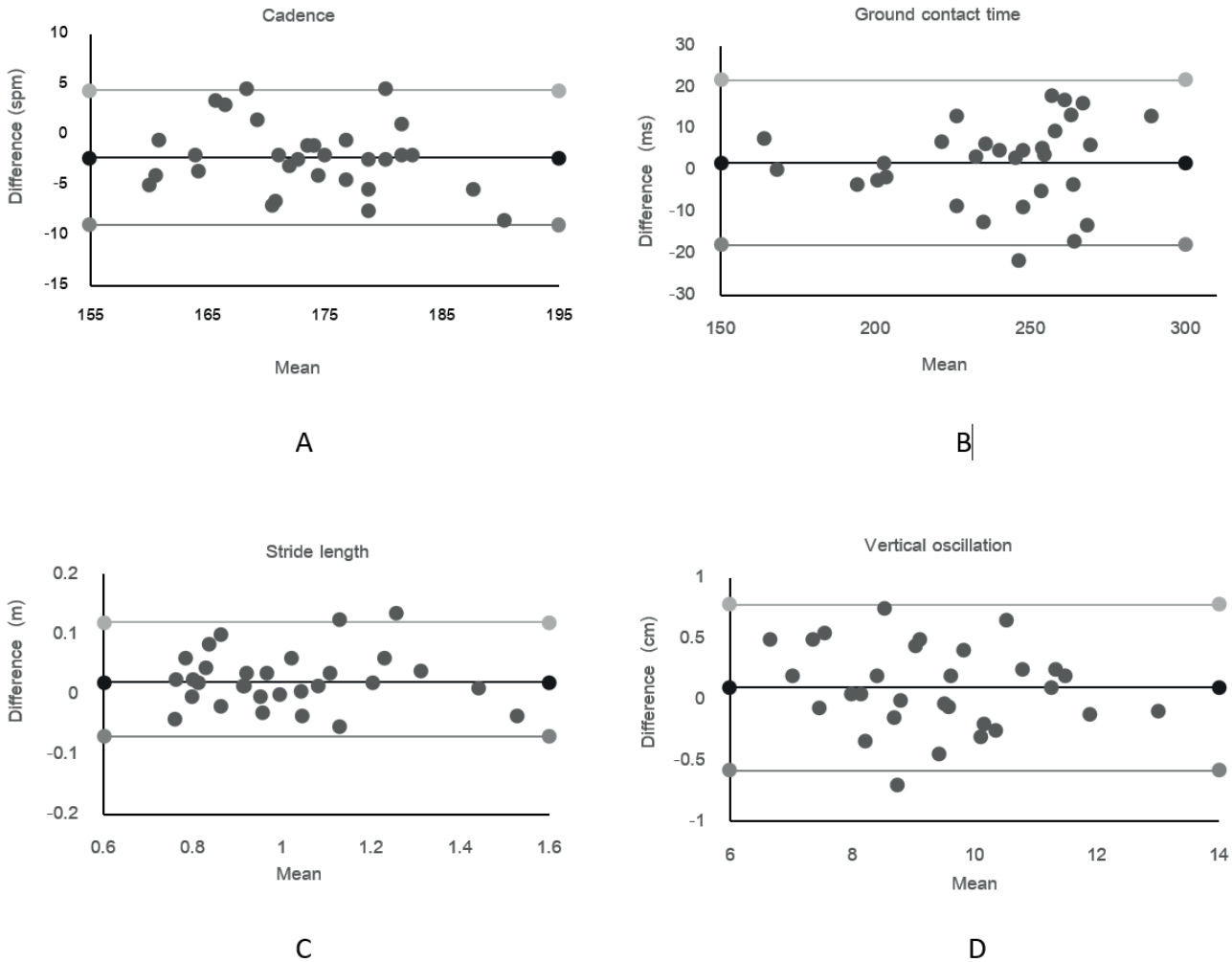

Figure 1. *Bland-Altman plots of the reliability of running parameters. The horizontal gray line shows 95% limits of agreement (LoA) of the mean difference. The horizontal black line represents the bias of the mean difference. A: cadence, B: ground contact time, C: stride length, D: vertical oscillation.*

Discussion

This study used a within-subjects design to examine runners without musculoskeletal injuries and to determine the reliability of running dynamic parameters recorded from fitness watches in combination with accelerometers during outdoor runs. The running parameters examined herein included cadence, stride length, ground contact time, and vertical oscillation. To assess test-retest reliability, the volunteers ran 2 rounds on the actual track with uncontrollable environments, e.g., the curvature of the field, and/or ran to avoid crowds. This study attempted to control the speed of the second round to be close to the speed of the first round by setting vibration- and sound-based alerts on the watch to indicate the minimum and maximum speed. The results showed that the speeds of the first and second laps were 10.1 and 10.0 km/h, respectively. This difference of only 0.1 km/h indicated that the speeds were very similar. The results of this study revealed that the ICC (3,k) of all four running dynamic parameters from two rounds ranged from 0.94-0.99, indicating excellent reliability, and there was a low standard error of measurement (SEM). Bland-Altman analysis revealed that most of the data were within the limits of the agreement, indicating that the measured parameters were consistent across the two rounds. Our findings were consistent with a recent study from Sama et al 2022, which found that an Apple smart watch had excellent reliability (ICC=0.94-0.97); however, their study did not explicitly state the running parameters examined.[22] In addition, in a previous study in the field, test-retest reliability was measured by an accelerometer (Myotest), and the level of reliability was good (ICC>0.75) for cadence and moderate (ICC> 0.50) for ground contact time.[23] Another study conducted at an indoor facility (60 meter run) used an accelerometer (Myotest) among individuals running at different speeds (12, 15, 18, and 21 km/h), and the levels of reliability for ground contact time and cadence were good (ICC>0.80).[24] Even studies in a laboratory setting (i.e., treadmill runs, during which the belt speed can be adjusted as needed and kept constant) have shown excellent reliability when the running parameters were measured by a fitness watch combined with a chest-mounted accelerometer as well as a fitness watch combined with a pants-mounted accelerometer.[17,18] Taken together, even if there are the differences in brands of the fitness watch or accelerometer, in positions to attach with the body, and in environments (indoors or outdoors), the running parameters showed excellent level of reliability and could be useful for future research.

The reliability was very high when measuring running dynamic parameters with fitness watches and accelerometers in this study. The standard error of measurement was low and there was consistency across all parameters measured over the two rounds during outdoor runs. Possible explanations could be that for cadence, the fitness watch uses a motion sensor attached to the watch (wrist), and cadence is calculated based on how many times the arm swings up and down per minute. This arm swing is related to the number of steps while running. The swing of the arm wearing the watch up equals 1 step, and when the arm is down, the stride of the other leg equals 1 more step. Therefore, in 1 minute of running, for example, 170 arm swings up and down results in a cadence of 170 steps per minute. Even when runners turned a curve, ran on different surfaces, or changed speeds to avoid other runners, the cadence was not affected in both rounds. The stride length in running is defined as the distance between the left and the right leg, i.e., the distance between the heels. The pants-mounted accelerometer synced with the fitness watch was used and played an important role in measuring the stride length, vertical oscillation, and ground contact time. Therefore, the combination of the accelerometer and the fitness watch can be used to measure stride length due to the accuracy of the global positioning system (GPS), which is used to measure the running distance. The stride length can be easily calculated by dividing the running distance by the cadence. For example, if someone runs 200 meters using 200 steps, the stride length would be 1 meter. Both an accurate distance (obtained via GPS on the accelerometer) and a reliable cadence (measured using the motion sensor of the fitness watch) could explain a very high reliability in the stride length. The vertical oscillation was calculated as the difference in the position of the accelerometer that moves the maximum and minimum while running. Similarly, the ground contact time was calculated by the amount of time between when the feet started to touch the ground until the time the feet started moving off the ground. This parameter is indicated by the average amount of time (in milliseconds) the right and left feet touch the ground. We observed that the measurement of three running dynamic parameters using the accelerometer had very high reliability (ICC>0.97), while the measurement of the cadence using only the motion sensor in the fitness watch had lower reliability (ICC=0.94). Finally, in this study, we only analyzed data from the middle 400 meters of the 800-meter run; we did not analyze data from the first 200 meters or the last 200 meters. This technique of selecting data analysis could also contribute to a very high level of running parameter reliability.

Limitations

The limitations of this study are as follows: the running track used herein is a smooth surface with an oval path and a constant distance of 400 meters per lap, and it is used for exercise purposes only. If possible, future studies should consider using natural routes with slopes and rough surfaces, such as trails. Additionally, it could be challenging to examine longer distances, such as 5K runs, or to examine runs at different speeds (slow to high speed). Therefore, there are still issues that require further research. In addition, future studies should test the validity of these running parameters with the gold standard instruments e.g., 3-dimensional motion capture and inertial measurement unit (IMU). Lastly, from now on, as excellent reliability, the application of the fitness

watch and accelerometer could be used to monitor the progression of training either in rehabilitation program for returning to sport or in performance training of athletes.

Conclusion

Running dynamic parameters (cadence, stride length, ground contact time, and vertical oscillation) measured using a fitness watch synced with an accelerometer were found to have very high test-retest reliability during outdoor runs.

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Conflict of interest

No

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References

[1] Lopes AD, Hespanhol Junior LC, Yeung SS, Costa LO. What are the main running-related musculoskeletal injuries? A Systematic Review. Sports Med. 2012; 42(10): 891-905. doi: 10.1007/BF03262301.

[2] Davis IS, Bowser BJ, Mullineaux DR. Greater vertical impact loading in female runners with medically diagnosed injuries: a prospective investigation. Br J Sports Med. 2016; 50(14): 887-92. doi: 10.1136/bjsports-2015-094579.

[3] Ferna´ndez-Lo´ pez I, Rojano-Ortega D. Lower limb biomechanical factors related to running injuries: A review and practical recommendations. Strength and Cond J. 2020; 42(1): 24-38. doi: 10.1519/SSC.0000000000000497.

[4] van der Worp H, Vrielink JW, Bredeweg SW. Do runners who suffer injuries have higher vertical ground reaction forces than those who remain injury-free? A systematic review and meta-analysis. Br J Sports Med. 2016; 50(8): 450-7.doi: 10.1136/bjsports-2015-094924.

[5] Schubert A, Kempf, J, Heiderscheit, BC. Influence of stride frequency and length on running mechanics: A systematic review. Sports Health. 2014; 6(3): 210-7. doi: 10.1177/ 1941738113508544.

[6] Lenhart RL, Thelen DG, Wille CM, Chumanov ES, Heiderscheit BC. Increasing running step rate reduces patellofemoral joint forces. Med Sci Sports Exerc. 2014; 46(3): 557-64. doi: 10.1249/MSS.0b013e3182a78c3a.

[7] Adams D, Pozzi F, Willy RW, Carrol A, Zeni J. Altering cadence or vertical oscillation during running: effects on running related injury factors. Int J Sports Phys Ther. 2018; 13(4): 633-42. doi: 10.26603/ijspt20180633.

[8] Musgjerd T, Anason J, Rutherford D, Kernozek TW. Effect of Increasing Running Cadence on Peak Impact Force in an Outdoor Environment. Int J Sports Phys Ther.2021;16(4):1076-83.doi:10.26603/001c.25166.

[9] Wang J, Luo Z, Dai B, Fu W. Effects of 12-week cadence retraining on impact peak, load rates and lower extremity biomechanics in running. Peer J. 2020; 8: e9813. doi: 10.7717/peerj.9813.

[10] Boyer ER, Derrick TR. Lower extremity joint loads in habitual rearfoot and mid/forefoot strike runners with normal and shortened stride lengths. J Sports Sci. 2018; 36(5): 499-505. doi: 10.1080/02640414.2017.1321775.

[11] Popp KL, McDermott W, Hughes JM, Baxter SA, Stovitz SD, Petit MA. Bone strength estimates relative to vertical ground reaction force discriminates women runners with stress fracture history. Bone. 2017; 94: 22-8. doi: 10.1016/j.bone.2016.10.006.

[12] Willy RW, Buchenic L, Rogacki K, Ackerman J, Schmidt A, Willson JD. In-field gait retraining and mobile monitoring to address running biomechanics associated with tibial stress fracture. Scand J Med Sci Sports. 2016; 26(2): 197-205. doi: 10.1111/sms.12413.

[13] Phan X, Grisbrook TL, Wernli K, Stearne SM, Davey P, Ng L. Running quietly reduces ground reaction force and vertical loading rate and alters foot strike technique. J Sports Sci. 2017; 35(16): 1636-42. doi: 10.1080/02640414.2016.1227466.

[14] Karmen CL, Reisfeld MA, McIntyre MK, Timmermans R, Frishman W. The clinical value of heart rate monitoring using an apple watch. Cardiol Rev. 2019; 27(2): 60-2. doi: 10.1097/ CRD.0000000000000243.

[15] Held NJ, Perrotta AS, Mueller T, Pfoh-MacDonald SJ. Agreement of the Apple Watch(R) and Fitbit Charge(R) for recording step count and heart rate when exercising in water. Med Biol Eng Comput. 2022; 60(5): 1323-31. doi: 10.1007/s11517-022-02536.

[16] Van Hooren B, Goudsmit J, Restrepo J, Vos S. Real-time feedback by wearables in running: Current approaches, challenges and suggestions for improvements. J Sports Sci. 2020; 38(2): 214-30. doi: 10.1080/02640414.2019.1690960.

[17] Adams D, Pozzi F, Carroll A, Rombach A, Zeni J, Jr. Validity and Reliability of a Commercial Fitness Watch for Measuring Running Dynamics. J Orthop Sports Phys Ther. 2016; 46(6): 471-6. doi: 10.2519/jospt.2016.6391.

[18] Prasartwuth O, Nanthoraphak N, Kaseantadanon A, Chawawisuttikool J, Phirawatthakul P Reliability and minimal detectable change of running parameters monitored by fitness watch and accelerometer. Thai J Phys Ther 2022; 44(2): 97-105.

[19] Van Hooren B, Fuller JT, Buckley JD, Miller JR, Sewell K, Rao G, et al. Is motorized treadmill running biomechanically comparable to overground running? A systematic review and meta-analysis of cross-over studies. Sports Med. 2020; 50(4): 785-813. doi: 10.1007/s40279-019-01237-z.

[20] Brahms CM, Zhao Y, Gerhard D, Barden JM. Stride

length determination during overground running using a single foot-mounted inertial measurement unit. J Biomech. 2018; 71: 302-5. doi: 10.1016/j.jbiomech.2018.02.003.

[21] Benson LC, Clermont CA, Ferber R. New considerations for collecting biomechanical data using wearable sensors: the effect of different running environments. Front Bioeng Biotechnol. 2020; 8: 86. doi: 10.3389/fbioe.2020.00086.

[22] Sama AJ, Hillstrom H, Daluiski A, Wolff A. Reliability and agreement between two wearable inertial sensor devices for measurement of arm activity during walking and running gait. J Hand Ther. 2022; 35: 151-4. doi: 10.1016/j.jht.2020.08.001.

[23] Gouttebarge V, Wolfard R, Griek N, de Ruiter CJ, Boschman JS, van Dieën JH. Reproducibility and validity of the Myotest for measuring step frequency and ground contact time in recreational runners. J Hum Kinet. 2015; 45: 19-26.doi: 10.1515/hukin-2015-0003.

[24] Gindre C, Lussiana T, Hebert-Losier K, Morin J. Reliability and validity of the Myotest® for measuring running stride kinematics. J Sports Sci. 2016; 34(7): 664-70. doi: 10.1080/02640414.2015.1068436.

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The development and content validity of the emotional recognition memory training program (ERMTP) for children with autism spectrum disorder: A trial phase

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ABSTRACT

Background: Facial expression, tone of voice, body language, and context are unrecognizable to children with autism. Emotional arousal and emotion recognition (required emotion empathy and cognitive processing empathy) induce downstream illnesses in children with ASD. Thus, the proposed study aimed to develop a computer-based Emotional Recognition Memory Training Program (ERMTP) for ASD.

Objective: Firstly, to develop and validate the ERMTP for social cognitive abilities in children with ASD and secondly, to conduct pilot-tested it in typically developing children and children with ASD.

Materials and methods: This study consisted of 3 phases. The first phase was developing the ERMTP from the literature review. The second phase was analyzed for content validity with five experts about Task 1 (two activities) and Task 2 (nine activities) comprising ERMTP. Computer-based learning of six fundamental facial emotions (happy, sad, angry, fear, disgusted, and surprised) improves social cognition. Finally, the pilot test was analyzed to discover the ERMTP's challenges for five children with typical development and ASD.

Results: The ERMTP's activity items have good content validity, especially regarding clarity and relevance. All five raters gave the intervention a 1.0 IOC for its distinct components. In the training program, we followed the expert instructions regarding background music or voice and the generalization task. Descriptive analysis indicated that all five normal-developing children followed emotional expressions and instructions (100%). All five parents reported there were changes in focus and memory skills. Emotion regulation, memory abilities, and the social cognition index demonstrated statistically significant (p<0.05) effects before and after ERMTP treatment in ASD.

Conclusion: ERMTP seeks to improve the social cognition of children with ASD by the use of feedback from both specialists and the children themselves. However, further research will be necessary to investigate ASD using a randomized control trial.

Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that start in utero and last a lifetime.[1] In the first three years of life, ASD causes communication, social interaction, and stereotyped behavior issues.[2] Individuals with autism spectrum disorders with excessive emotional arousal and cognitive issues.[3] Heart rate, pupil dilation, and skin color vary with emotional arousal. These include six strong fundamental emotions (happy, sad, anger, fear, disgusted, and surprised) and many complicated emotions (arising from mental

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state, situational context, and culture) via emotional arousal.[4] Emotional arousal is a fight-or-flight reaction. The emotional arousal drive is the fundamental process that initiates and executes all actions.[5] Emotional arousal reflects both positive and negative expressions of experience. The significant role of emotional arousal is to make a person pay attention and develop memory skills.[6,7] Hyper levels of emotional arousal trigger stress and cause poor emotion recognition (ER) memory in children with ASD.[8]

ER is the ability to understand and identify human emotions.[9,10] ER is the aptitude to attend to social interactions by understanding the individual's facial expressions, tone of voice, and body language. These abilities arise from emotional empathy (affective empathy/cognitive empathy) and mental functions (social orientation/attention, the theory of mind, working memory, episodic memory, and social awareness). Regarding emotional empathy, the 'systemizing' (cognitive) empathy component is the capacity to identify or recognize other people's problems and respond appropriately to their mental state and emotions. For example, it is the ability to share an emotional experience, feel distressed in response to someone's pain, and be willing to help someone.[10] The affective (emotion) empathy component is the emotional reaction (neutral, happy, sad, anger, fear, disgusted, and surprised) to another person's mental state.[11] In social cognition, ER is essential for people to be successful in their everyday functions of communication and social interactions.[12]

The occurrence of emotional regulation (ER) difficulties has the potential to contribute to the development of mental health challenges, particularly in those diagnosed with autism spectrum disorder (ASD). Children with ASD have trouble recognizing emotions from facial expressions,[11-13] tone of voice, body language, and situation contexts.[14-17] The meta-analysis study reveals that impairments in facial affect recognition are associated with autism spectrum disorders.[18] Another study showed that there was a relationship between executive functions and sensory processing with emotional recognition in autism spectrum disorder.[19] ER may increase in older adults than in younger ASD.[20] ER modulation causes repeated behavior, social isolation, and peer rejection. ER may impact episodic memory, social interaction, vocational performance, and mental comorbidity.[21,22] Due to emotional dysregulation, most ASD individuals have shown obsessive behavior, self-injury behavior, irritability, aggression, screaming, mood swings, temper tantrums, poor anger control, maladaptive behavior, avoidance, escaping, and crying.[23,24] The study showed maladaptive behavior may lead to impaired school functioning, which could affect the long-term outcome.[25] Individuals must learn to control their emotional arousal via cognition capabilities and appropriately respond in a social context.[26]

Emotional arousal control and ER (affective/cognitive empathy) work together as brain-mind functions for human wellness.[27] Studies have stated that emotional arousal (low) control and ER (affective empathy, such as attention, working memory, episodic memory) are linked to self-awareness and awareness of others.[28,29] Previous literature has also reported that people with ASD have impaired attention, working memory, and episodic memory due to preserved rote memory and reduced use of organizational strategies to support recall. Additionally, the study showed a correlation between facial recognition, sensory perception, attention, and memory.[30]

Hyper-emotional arousal in ASD children causes emotional dysregulation and cognitive impairment.[31] Emotional arousal governs both positive and negative reactions and is more associated with ER memory.[32,33] Emotion recognition memory (ERM) is the feeling of personal experience in everyday life. For example, a person (i.e., joyful, stressful), a place (i.e., safe, threatening), and everyday activities (i.e., fun, exciting, challenging, or dull).[34] ERM has a significant role in everyday functioning for children, but it is affected by ASD. ERM allows an individual to favorably or adversely understand people's emotions, places, and activities. When ERM is impaired, a child may not try to know a person (self and others), places, and activities.[35] Occupational therapists and parents may help children with ASD participate in daily activities by knowing ERM. ERM is also linked to visual working memory, spatial memory, future thinking, mentalizing/ theory of mind (ToM), cognitive flexibility, planning, prospective memory (event-based and time-based), and social cognition.[20,28,35-38]

Numerous interventions for ASD have been proposed; however, most current research data are lacking to support their effectiveness in emotion recognition in generalization. A few ER interventions have improved emotion expression, but not in real clinical settings. For example, using animated vehicles with real emotional faces, 'reading the mind in films, and reading the mind in the voice test.[14-16] A systematic review of ER treatment and generalizability among people with ASD. They identified 13 randomized control trial (RCT) studies with cohorts aged 4 to 18 with an IQ >70. The outcomes and treatments reported in the reviewed studies were profoundly different, and many of the studies showed that the training effectively improved the participants' ER. However, all the ER treatments were conducted using computed-based programs, not in real-world clinical settings. Consequently, it was concluded that the findings were unclear and, thus, they cannot be generalized.[39]

Self-relaxation (mindfulness) is regulated by a person's conscious mind and the flow of emotions and thoughts in the mind and body.[40] Few studies have reported that mindful body training modulates functional neural connectivity and improves memory function in children with ASD.[41,42] Another study found that music regulates emotion recognition memory for social cognition in children with ASD.[43] Treatment using mellow, rhythm-based music with a slow tempo (60 beats per minute) regulates the appropriate emotions and reduces aggressive and repetitive behavior in ASD.[44] Therefore, applying mellow music, mindfulness relaxation (deep breathing), and mind-body exercises (slow body movement) enhances low emotional arousal, thereby supporting empathy.[45-47]

Previous studies have found that children with ASD are affected by the underlying components of emotion and cognition. Consequently, this may affect the essential problems these children face in relation to social interaction, communication, and repetitive/ interest behavior. However, no studies have developed an intervention strategy for synchronizing emotion control (low arousal) and ER (affective empathy and cognitive empathy of attention, working memory, and episodic memory) for social cognition among people with ASD. Emotional regulation and cognitive function are closely related to social cognition for social interaction. Therefore, the current study was to develop underlying components for ASD children and parents' well-being using the occupational therapy frame of reference of cognitive behavior therapy,[48] the dynamic interaction model (DIM),[49] and Empathic Systemic (E-S) theory.[50] E-S theory addresses both systemizing and empathizing with ER in ASD. Systemizing is the drive to analyze or form a system. There are many systems to analyze in humans, such as natural, mechanical, numerical, collectible, motor, sensory, spatial, social, music, action sequence, environmental, and vocal/ auditory/verbal. While every person has specialized skills in any one system, children with ASD are more obsessed with some specific systems; for example, spinning wheels or objects and stereotypical movements are desirable to children with ASD. The males were better at systemizing (logically) than empathizing. For example, males are better at working with motor tasks or concepts (tools, computers, handling phones, and car mechanics). However, females are better at empathizing (emotionally) than systemizing. For example, females are particularly good at caring for others. They are well-socialized, understand other people's feelings, and are good at helping others. Thus, while ASD affects both systemizing and empathizing, the degree to which it does so varies with gender.[50]

The proposed study incorporates E-S theory by integrating an emotion and cognition intervention using computer-based or multimedia formats. The combined method of CBT breathing techniques and mind-body exercises (simple body movements) with mellow instrumental music is used for processing emotional arousal skills. DIM is used to help integrate the underlying cognitive process for emotion recognition memory to enhance social cognitive performance among children with ASD.[51.52] As a result, the present study required the development of a training program to identify emotional states in the memory of children aged 5 to 10 years. The development of emotional recognition occurs within this particular age range (5 to 10 years).

In phases 1 and 2, the study aimed to construct the theoretical conceptualization framework of the emotional recognition memory training program (ERMTP) and examine the content validity. In phase 3, the pilot test evaluated the ERMTP feasibility of typically developing children. Moreover, the study explores the typically developing children and their parent's feedback about the ERMTP. Finally, the study also evaluated the preliminary efficacy of ERMTP on emotion regulation, memory, and social cognition among ASD.

Materials and methods

The current research is divided into three phases. Phase one described the theoretical conceptualizations of the development of the emotion recognition memory training program (ERMTP). The expert review of the content validity of the generated ERMTP is presented in phase two. The pilot study was done in the third phase to explore the viability of the ERMTP on five typical developing children and the preliminary efficacy of ERMTP in ASD. The following are the specifics of the process in three steps.

Phase 1. *Theoretical conceptualization of ERMTP development*

The Development of the ERMTP for social cognitive skills among children with ASD was based on the theoretical literature review. The framework was developed with the age range of 5 to 10 years. Hyper emotional arousal and emotional recognition memory (ERM) deficits have significantly affected the development of social cognition in children with ASD.[53] Dysregulation of emotional arousal may also be influenced by hyperresponsivity to environmental (e.g., loud background noise) and sensory stimuli (e.g., visual and auditory), with the sensory stress acting as emotional triggers.[54,55]

The cognitive-behavioral frame of reference (FOR) is primarily used in occupational therapy to understand better a person's needs with emotional and mental disturbance.[56] It has five components: emotion, thought, behavior, response, and environment. These individual components are integrated and function meaningfully in a social context. One of the critical elements in cognitive behavioral therapy (CBT) is the hierarchical levels of cognition. Occupational therapists use this conceptualization to understand the problems faced by ASD based on the children's emotions, thinking process, physiological response, behaviors, and environment. Children with ASD have an emotional empathy imbalance that may lead to cognitive disturbance.[57] Autistic children have emotion dysregulation but intact emotions. They can show their feelings (emotions) by themself, such as enjoyment, pleasure, and joy. Adults with ASD have a stress level ranging from 25% to 55%; 42-84% experience anxiety, 30–56% experience physical violence, 30-50% experience depression, and 40% suffer from chronic unhappiness.[58,59] The cognitive-behavioral frame of reference (FOR) is a more unified approach in occupational therapy rather than only using CBT (i.e., deep breathing) for stress and anger management for people with ASD. Especially relaxation technique of deep breathing is a preparation method used for the emotion regulation impairment of ASD.[48] Deep breathing is a useful method for calming down emotion. Children with ASD often get angry and frustrated. Therefore, deep breaths with a relaxing period and positive reinforcement (classic conditioning) may be helpful to the children.[60] A study that reviewed CBT (deep breathing/ relaxation) and mind-body movement with listening to rhythmic mellow instrumental music showed that there

is an association between changes in the cortical area for emotion regulation in children with ASD.[51,52,60]

The proposed ERMTP (figure 2) was developed based on a review of previous articles. It consists of a combination of several techniques available from CBT approaches,[56] E-S theory,[50,52] information processing model of cognition,[61] working memory model,[62] and the DIM (computer-based memory recognition training),[63] and as well as newly formed material. Therefore, two activities of deep breathing and slow body movement with rhythmic mellow instrumental music are recommended to reduce emotional hyperarousal. According to the information processing model of cognition[61] and working memory model,[62] which requires nine activities related to underlying components of sensory reception-perceptual memory, selective/sustained attention, working memory storage (visual/auditory), and episodic memory to improve emotion recognition. The ERM of six basic facial emotional expressions (happy, sad, angry, fearful, disgusted, and surprised) improved social cognition via computer-based training.[50,63] The ERMTP components consist of Task 1 (two activities) and Task 2 (nine activities).

Phase 2. *Expert evaluation method*

An expert panel evaluated the procedures utilized to assess the produced ERMTP's content validity. Five professionals received the initial draft of the intervention tool that had been created. An occupational therapist, a teacher, a special educator, a pediatrician, and a clinical psychologist were among the professionals who took part in this investigation for the construct of emotional recognition memory performance. All five professionals had master's degrees and five years of clinical experience in the field of children with ASD. The teacher worked in a school with children ranging in age from 5 to 10 years old.

Each assessment tool item was given a score on a scale of clear (+1), uncertain (0), or unclear (-1) by the five experts. The rating for each item's Item Objective Congruence (IOC) index was determined. The IOC values between 0.5 and 1 were considered acceptable.[64,65] Following that, all intervention program tasks were updated following the specialists' suggestions. Following any advice from the experts, the researchers revised the intervention program. Finally, pilot research with a sample group of 5 typically developing children evaluated the feasibility of the intervention for children with ASD. The training program was approved as a result of the pilot study's findings. To determine whether the intervention is suitable for ASD based on its viability in typical developing children. Furthermore, the study controls the ceiling effect by observing the typical developing children, who experience all six emotions independently without any prompts.

Phase 3. *Pilot testing*

Participants

The pilot study was conducted to investigate the feasibility of the ERMTP on five typically developing children. Institutional review board (IRB) approval (IRB/2176/22, ICT21R/010/03) granted by the Research Ethics Committees of the King Abdullah International Medical Research Center (KAIMRC), Saudi Arabia. The parent's written consent form was obtained, and the research procedure and methodologies were thoroughly explained to the participants.

Typical developing children

The study included the typical developing children in the age range of 5 to 10. The five children were collected from the Riyadh, Saudi Arabia International School. Children who are able to speak and comprehend English were involved in the research. Children with normal eyesight and hearing were included in the study. The study did not include any children who received memory training or supplements. The children who had medical diagnoses in the past are not included in the study.

Children with ASD

The children with ASD (age range 5 to 10) were collected from the clinical center of ASD. Based on a licensed psychiatrist report, all five children with mild-to-moderate ASD were included. The study included participants who could follow simple verbal directions and instructions. Their IQ ranged from 69 to 83 (borderline) according to the clinical psychological report of the Wechsler Intelligence Scale for Children–Fourth Edition.[66] Furthermore, the children must sufficiently understand spoken English to comprehend the testing procedures.

Instruments

The study examined the effect ERMTP effects on emotion regulation, memory, and social cognition among ASD by using the following measures. All outcome measures were completed at pre-intervention and post-intervention time points only. Emotional regulation was evaluated using the Childhood Autism Rating Scale (CARS) with two domains: imitation and emotional response.[67] All scale subtests were reliable and valid. The Children Memory Questionnaire-Revised and the Observation Memory Questionnaire-Parent Form (OMQ-PF) were used to measure the emotion recognition memory.[68,69] The OMQ-PF was internally consistent and positively linked with learning. The Social Responsiveness Scale, Second Edition was used to assess social cognition. All four outcome measures have good reliability and validity.[70] In addition, measures to track pilot study protocol adherence.

Data collection procedure

Five children with typical development and five children with ASD completed two activities in Task I and nine activities in Task II (Table 1). The children were instructed to remain upright with their bodies and legs crossed on the floor to complete Task 1. The children were seated in a serene area with adequate ventilation and illumination. Every child participated in two activities. The children were then instructed to position themselves progressively in front of the computer for Task II. When setting the 15.7-inch laptop monitor screen, the child's

60-centimeter visual field was considered. Using an adjustable chair and a footstool for proper seating, the chair is positioned at the appropriate height in relation to the workstation. The illumination was adequate and free of reflections on the computer screen. With the children, eleven varied activities were conducted. Each child practiced the ERMTP for 10 minutes of self-relaxation (deep breathing), 10 minutes of gradual body movements accompanied by soothing instrumental music, and then six basic emotion expressions (Figure 1-6) for 40 minutes. The intervention consisted of 14 appointments, each of which lasted 60 minutes and occurred seven times per week for two weeks.[62-64] The study measured the training average timing for two weeks for each child. After the session, the child and parents were asked for feedback and suggestions about the training. The study aimed to determine whether the typical developing children encountered any difficulties during the session. For ASD, outcome measures were conducted at pretest, treatment (14 sessions), and posttest.

Table 1 Emotional Recognition Memory Training Program (ERMTP).

Tasks	Domains	Activities
Task one (2 activities)	**Emphasizing/emotional component** (Sharing of emotional expressions with others & regulation of low emotion arousal)	1. **Cognitive behavior therapy (10 mins):** - Fourteen slow, deep breathing counts with rhythmic mellow instrumental listening music. 2. **Mind-body exercise (10 mins):** - Ten slow, simple bodily movements (1 to 3 count) with rhythmic mellow instrumental listening music. 1. Neck: bend (flex) and straight (extend) 2. Face: eyebrow-raising, opening/closing mouth, lip smiling/blowing. 3. Eye gaze: closing/opening eye, up, down, inward, outward, rotation movement. 4. Shoulder: up and down 5. Elbow: bend (flex) and straight (extend) 6. Wrist: bend (flex) and straight (extend) 7. Finger: close and open 8. Knee: bend (flex) and straight (extend) 9. Ankle: bend (flex) and straight (extend) 10. Body: bend (flex) and straight (extend)
Task two (9 activities) 1	**Systemizing/cognitive component** (Understanding others' emotions: Theory of mind) Visual-auditory reception and perception of emotional face recognition	**Computer-based training:** - The child is instructed to silently watch and listen carefully to six different (happy face, sad face, angry face, fearful face, disgusted face, and surprised face) emotional visual-auditory pictures of human facial expressions (30 secs for each slide).
2	Visual selective and sustained attention of emotional face recognition	**Computer-based training:** - The child is instructed to silently observe and focus on six pictures of human facial expressions depicting emotions (30 secs for each slide) and to discriminate the visual stimuli of the appropriate emotional response.
3	Visual Working memory (Short term memory) storage of emotional face recognition	**Computer-based training:** - The child is instructed to recall and express the six facial expressions in the slide show. - The child may express either verbally (telling) or non-verbally (motor action, pointing in flashcards).
4	Auditory selective and sustained attention of emotional face recognition	**Computer-based training:** - The child is instructed to listen carefully and silently to six different human vocal emotion tones (i.e., happy, sad, anger, fear, disgusted, and surprise) in each slide (white screen) shown for a 30-second without being distracted. The slide shows how to apply different auditory stimuli of human sound to discriminate the appropriate emotional response.
5	Auditory Working memory storage of emotion face recognition	**Computer-based training:** - The child is instructed to recall six different vocal emotional tones. - The child may express verbally (telling) or non-verbally (motor action, pointing in flashcards).
6	Episodic memory of emotion face recognition	**Computer-based training** - The child is instructed to express the person's emotion, place, and event after watching the different context pictures in the slide show.
7	Self-emotion facial recognition memory (child)	**Real-world clinical setting:** - The child is instructed to express (verbally or non-verbally) their facial expression by looking in the mirror.
8	Other emotions facial recognition memory (therapist/ caretaker)	**Real-world clinical setting:** - The child is instructed to express (verbally or non-verbally) the therapist/caretaker's facial expressions by seeing them in the presented images.
9	Generalization	**Real-world clinical setting:** - The child is instructed to express other children's emotions by seeing and listening to their tone of voice and body language in the occupational therapy department.

Figure 1. *Smile expression.*

Figure 2. *Sad expression.*

Figure 3. *Anger expression.*

Figure 4. *Fear expression.*

Figure 5. *Disgust expression.*

Figure 6. *Suprise expression.*

Data analysis

1. The IOC Index was used to analyze the Content's validity. The IOC values below 0.5 are deemed unsatisfactory, but IOC values over 0.5 are considered acceptable.[71]

2. The initial pilot testing was conducted using descriptive analysis and Wilcoxon signed-rank test. The Wilcoxon signed-rank test was used to identify the statistically significant difference in pre and post-ERMTP intervention on emotion regulation, memory skill, and social cognition among ASD. The statistical significance was set at $p<0.05$.

Results

The item-objective congruence (IOC) test was used to assess the content validity of the ERMTP (Table 2). We used IOC indices for multidimensional items to determine if the eleven task items that comprised the intervention had content validity. This was done so that we could see if the intervention was beneficial or not. According to the findings, the ERMTP exhibited excellent levels of content validity in the different activity items, particularly regarding the clarity and relevance of the activities themselves. When looking at each intervention component separately, all five raters concluded that the IOC for the intervention as a whole was 1.0. Despite this, the experts provided some helpful recommendations concerning the generalization of information, the use of flash card pictures, the addition of calming music, the addition of background voice in task 1 (activity 1 and 2), and the timing of each assignment. The ERMTP was finally modified due to the recommendations given by the experts, which led to a higher degree of content validity as a direct result of these modifications.

Table 2 Index of Item-Objective Congruence (IOC) from the five experts of the survey for ERMTP.

Activity Items in terms of clarity	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Item Objective Congruence (IOC)
1. The objective of the training is stated clearly.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
2. The purpose of every task activity item is clear.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
3. The task activity item is appropriate.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
4. The instruction is clear.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
Activity Items in terms of relevancy	**Expert 1**	**Expert 2**	**Expert 3**	**Expert 4**	**Expert 5**	**Item Objective Congruence (IOC)**
Task 1: 1. Cognitive behavior therapy - Deep breathing with mellow music (10 mins).	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
2. Mind-body exercise - Slow body movements with mellow music (10 mins).	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
Task 2: 1. Visual-auditory reception and perception of emotional face recognition	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
2. Visual selective and sustained attention of emotion face recognition.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
3. Visual Working memory storage of emotion face recognition.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
4. Auditory selective and sustained attention of emotion face recognition.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
5. Auditory Working memory. Storage of emotion face recognition.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
6. Episodic memory of emotion face recognition.	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
7. Self-emotion facial recognition memory (child)	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
8. Others' emotional facial recognition memory (therapist/ caretaker).	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)
9. Generalization	+1	+1	+1	+1	+1	+5/+ 5= +1 (Acceptable)

Table 3 shows the characteristics of participants of typical developing children and ASD in the study. There were four girls and one boy in the age range of six to eight years old in typical developing children. Two students were enrolled in Grade 1 and three in Grade 2 in regular school. There were five children with ASD children aged 6 to 8 years old (Mean; SD =6.8; 0.836). Two children with ASD had mild symptoms, whereas three had moderate symptoms.

Table 3 Characteristics of typical developing children's (N=5) and children with ASD (N=5).

Participant characteristics of typical developing children's	Girls (%)	Boys (%)	Total (%)
Age 6	1 (50.0%)	1(50.0%)	2 (100.0%)
Age 7	1 (100.0%)	0 (0.0%)	1(100.0%)
Age 8	2 (100.0%)	0 (0.0%)	2 (100.0%)
Regular school - Grade 1	1(50.0%)	1(50.0%)	2 (100.0%)
Regular school - Grade 2	3 (100.0%)	0 (0.0%)	3 (100.0%)
Participant characteristics of ASD			
Age 6	2 (100.0%)	0 (0.0%)	2 (100.0%)
Age 7	0 (0.0%)	2 (100.0%)	2(100.0%)
Age 8	0 (0.0%)	1 (100.0%)	1 (100.0%)
Diagnosis Mild ASD	1 (50.0%)	1 (50.0%)	2 (100.0%)
Diagnosis Moderate ASD	1 (25.0 %)	2 (75.0%)	3 (100.0%)

An analysis of the use concerning difficulty with the ERMTP is shown in Table 4. All five children could follow all of the emotional expressions and tasks. One child attended the 10 days of instruction, while the other four participated for the full 14 days. The teaching took an average of 54 minutes for Child 1, 50 minutes for Child 2, 45 minutes for Child 3, 56 minutes for Child 4, and 42 minutes for Child 5. The therapy session was described as engaging, joyful, enjoyable, growing in concentration ability, and calming, and Child 5 proposed adding extra timing for memory recall tasks. Additionally, following ERMTP, the parents of all 5 children reported that their children's concentration, relaxation, and memory skills had improved. For example, one parent said, "My child brings the pencil box without fail in the classroom and pays more attention to the daily task skill of dressing and brushing." One parent reports that her child obeys their instructions more easily than before.

Table 4 Results of the use concerning difficulty with the ERMTP.

N	Gender (Years, month)	Difficulty in using the ERMTP		Number of training session days	Two-week training average	Child feedback & suggestion
		Able to follow emotional expressions & instructions	Not able to follow emotional expressions & instructions			
1	Female (6 years 9 months)	Yes	-	14/14	54 mins	Interesting and happy
2	Female (7 years 6 months)	Yes	-	14/14	50 mins	Fun and enjoying
3	Female (8 years 2 months)	Yes	-	14/14	45mins	Increasing focusing skill
4	Male (6 years 4 months)	Yes	-	10/14	56 mins	Fun game
5	Female (8 years 4 months)	Yes	-	14/14	42 mins	Relaxing and the child suggested to increase the timing for recall memory task

Table 5 compares pre- and post-ERMTP intervention for ASD (N=5). Emotional response and imitation were the two subdomains used by CARS to evaluate emotional regulation. A statistically significant difference was found in both imitation (Z=-2.070, p=0.038) and emotional response (Z=-2.121, p=0.034). CMQ-R and OMQ-PF were used to measure the emotion recognition memory. A statistically significant difference showed in CMQ-R total score (Z=-2.023, p=0.043) and subdomains of episodic memory (Z=-2.032, p=0.042), visual memory (Z=-2.033, p=0.043), and working memory (Z=-2.032, p=0.042) was found. Furthermore, the total score of the Observation Memory Questionnaire- parent form (Z=-2.033, p=0.043) revealed a statistically significant difference. The social responsiveness scale was used to assess social cognition. Social awareness (Z=-2.060, p=0.039), social motivation (Z=-2.032, p=0.042), restricted interests and repetitive behavior (Z=-2.033, p=0.043), social cognitive index (Z=-2.033, p=0.043), and total score (Z=-2.032, p=0.042) were all statistically significant. Social cognition (Z=-1.769, p=0.077) and social communication (Z=-1.604, p=0.109) showed no statistically significant differences.

Table 5 Comparison of pre-and post-ERMIP intervention for ASD.

Measures	Pre-intervention	Post-intervention	95% confidence interval		Z	*p*
	Median (min, max)	Median (min, max)	Lower	Upper		
CARS: Imitation	3 (2.5, 3.0)	2 (2.0, 2.5)	0.068	0.032	-2.070[b]	0.038*
CARS: Emotional response	3 (2.3, 3.0)	2.5 (2.0, 2.5)	0.064	0.032	-2.121[b]	0.034*
CMQ-R: Episodic memory	33 (46,162)	42 (48, 204)	0.068	0.033	-2.032[d]	0.042*
CMQ-R: Visual memory	15 (12, 26)	25 (20, 35)	0.067	0.031	-2.023[d]	0.043*
CMQ-R: Working memory	29 (12, 36)	36 (25, 45)	0.068	0.033	-2.032[d]	0.042*
CMQ-R: Total score	81 (42, 108)	95 (81, 126)	0.062	0.030	-2.023[d]	0.043*
OMQ-PF Total score	73 (70, 103)	92 (87, 110)	0.068	0.033	-2.023[b]	0.043*
SRS: Social awareness	72 (68, 74)	63 (61, 65)	0.061	0.071	-2.060[b]	0.039*
SRS: Social cognition	68 (60, 76)	63 (54, 70)	0.115	0.128	-1.769[b]	0.077
SRS: Social communication	76 (72, 77)	74 (70, 76)	0.240	0.257	-1.604[b]	0.109
SRS: Social motivation	65 (62, 67)	64 (56, 64)	0.057	0.067	-2.032[b]	0.042*
SRS: Restricted interests and repetitive behavior	68 (62, 73)	65 (60, 67)	0.060	0.069	-2.041[b]	0.041*
SRS: Social cognitive index	73 (68, 75)	65 (59, 72)	0.058	0.068	-2.023[b]	0.043*
SRS: Total score	75 (67, 80)	70 (63, 76)	0.062	0.028	-2.032[b]	0.042*

*Note: CARS: childhood autism rating scale, CMQ-R: children memory questionnaire-revised, OMQ-PF: observation memory questionnaire-parent from, SRS: social responsiveness scale, Wilcoxon singed ranks test, *significant at p<0.05*

Discussion

We developed a theoretical framework for the ERMT program to enhance social cognition in children with autism spectrum disorder (ASD). The previous studies of emotion recognition intervention programs do not include real-world clinical settings.[39] Nevertheless, no research has developed an intervention strategy that synchronizes ER (affective empathy and cognitive empathy of attention, working memory, and episodic memory) and emotion recognition (low arousal) for social cognition in individuals with ASD. The study present study developed the ERMTP components consisting of Task 1 (two activities) and Task 2 (nine activities).

In the second phase, we analyzed the content validity of the ERMTP. Five subject matter experts assessed the validity of ERMTP's material. The highest score possible was awarded to each of the five experts and recommendations.[65] All five experts were given +1, and due to emotion pictures were developed in accordance with age-specific standards. During the training program, we were responsible for following the instructor's directions regarding the generalization work and the background music or voice. In the end, the modification was approved by all five of the specialists, and it was incorporated into the most recent version of the ERMTP for children.

In the third phase, we conducted pilot testing with children whose development was normal. The breakdown of the issue discovered during the pilot test of the ERMTP can be found in Table 4, which explains the problem. The five children had no problem comprehending any of the actions that were taking place or the facial expressions that were being employed. There was a total of five children who took part in the training session that lasted for fourteen days, while there was only one child who took part in the session that lasted for ten days. The ERMTP timing ranged from a minimum of 42 minutes to a maximum of 56 minutes, and all five children finished it. On average, the total amount of time (duration) spent on each activity by each of the five children was 49.4 minutes. Early studies supported measuring the duration of outcomes across treatment domains for children with autism spectrum disorder.[71] Child number 5 proposed including a more extended period within the recall memory task. Only two weeks' worth of training (14 total sessions) was applied to the children. According to studies conducted in the past, a successful intervention should occur six times per week for four weeks, with sessions lasting either 24 or 60 minutes.[62-64] We only provided training for two weeks training (14 sessions). In further research, we plan to lengthen the period to sixty minutes.

All five children commented that the treatment session was interesting and fun and included an enjoyable game. In addition, they reported feeling more relaxed and having an increased capacity for focused attention. Moreover, the ERMTP resulted in favorable comments and suggestions from the children's parents regarding changes in their abilities to relax and enhance their memory. These results may be attributed to the ERMTP. The training program was described as straightforward in its instructions and useful by all five children and their parents. On the other hand, the children maintained their attention and motivation for the entirety of the session.

Table 5 showed the analysis of the ASD treatment received during and after ERMTP. The emotional regulation was measured using the two subdomains of the CARS. ERMTP demonstrated improvements in the imitation and emotional response subdomains. Early studies also supported the idea that emotional regulation was improved by providing emotion recognition training.[63,64] The emotion recognition memory was evaluated using two outcome measures, the CMQ-R and the OMQ-PF. The CMQ-R total score and subdomains of episodic memory, visual memory, and working memory revealed a statistically significant difference between pre- and post-ERMTP treatment. Furthermore, the total score of the Observation Memory Questionnaire-Parent form showed a statistically significant difference in ERMTP. The current study identified the underlying components of emotion recognition memory based on previous research, and training was given accordingly.[41,53] As a result, children with ASD improved their memory domains. Finally, social cognition showed a statistically significant difference in social awareness, social motivation, restricted interests, repetitive behavior, social cognitive index, and total score of SRS. However, social cognition and social communication did not improve after ERMTP was implemented. This could be because the intervention was applied for a shorter period. The current pilot study only employed an intervention period of two weeks. Previous research has demonstrated that the minimum number of weekly sessions should be three to sixteen weeks.[63,64,71] Overall, the pilot study found that ERMTP affected emotion regulation, memory, and social cognition in children with ASD.

Limitations and future recommendations

The pilot study only lasted two weeks for the intervention training. The duration should be increased to four weeks in subsequent research, and randomized controlled trials with both children with ASD and other atypical developing children should be conducted. Furthermore, the sample size that was used was not very large. The researcher did not have control over the biases in the parent report since the parent was already aware that the children had achieved the program's goal.

Conclusion

The ERMTP improves the emotion recognition and social involvement of children with ASD by teaching the six facial expressions using technology and real-world clinical settings. According to parent reports of typically developing children from the pilot study, taking ERMTP for two weeks can increase a child's ability to pay attention and short-term memory. When we conduct future studies, we must use RCT methods and lengthen the period children with ASD are treated.

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Conflict of interest

The authors reported no potential conflict of interest.

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References

[1] Achenbach TM. DSM-III in light of empirical research on the classification of child psychopathology. J Am Acad Child Psychiatry. 1980; 19(3): 395-412. doi: 10.1016/s0002-7138(09)61061-x

[2] American Psychiatric Association DS, American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5. Washington, DC: American Psychiatric Association; 2013 May 22. doi: 10.1176/appi.books.9780890425596

[3] Markram K, Markram H. The intense world theory–a unifying theory of the neurobiology of autism. Front Hum Neurosci. 2010; 4: 224. doi: 10.3389/fnhum.2010.00224

[4] Ekman P. Basic emotions. Handbook of Cognition and Emotion. 1999; 98(45-60): 16.

[5] Mather M. Emotional arousal and memory binding: An object-based framework. Perspect Psychol Sci. 2007; 2(1): 33-52. doi: 10.1111/j.1745-6916.2007.00028.x

[6] Bishop SJ. Trait anxiety and impoverished prefrontal control of attention. Nat Neurosci. 2009; 12(1): 92-8. doi: 10.1027/1618-3169/a000083

[7] Brosch T, Scherer KR, Grandjean D, Sander D. The impact of emotion on perception, attention, memory, and decision-making. Swiss Med Wkly. 2013;143: w13786. Published 2013 May 14. doi: 10.4414/smw.2013.13786

[8] Maras KL, Gaigg SB, Bowler DM. Memory for emotionally arousing events over time in Autism Spectrum Disorder. Emotion. 2012; 12(5): 1118-28. doi: 10.1037/a0026679

[9] Banziger T, Grandjean D, Scherer KR. Emotion recognition from expressions in face, voice, and body: the Multimodal Emotion Recognition Test (MERT). Emotion. 2009; 9(5): 691. doi: 10.1037/a0017088

[10] Baron-Cohen, Golan O, Wheelwright S, Hill J. Mind reading: The interactive guide to emotions. London,

UK: Jessica Kingsley Press; 2004.

[11] Rymarczyk K, Żurawski Ł, Jankowiak-Siuda K, Szatkowska I. Emotional Empathy and Facial Mimicry for Static and Dynamic Facial Expressions of Fear and Disgust. Front Psychol. 2016; 7: 1853. doi: 10.3389/fpsyg.2016.01853

[12] Baron-Cohen, Golan O, Chakrabarti B, Belmonte MK. Social cognition and autism spectrum conditions. In: Fonagy P, Goodyer I, editors. Social Cognition and Developmental Psychopathology. Oxford, UK: Oxford University Press; 2013.

[13] Itier RJ, Batty M. Neural bases of eye and gaze processing: the core of social cognition. Neurosci Biobehav Rev. 2009; 33(6): 843-63. doi: 10.1016/j.neubiorev.2009.02.004

[14] Golan O, Ashwin E, Granader Y, McClintock S, Day K, Leggett V, *et al*. Enhancing emotion recognition in children with autism spectrum conditions: An intervention using animated vehicles with real emotional faces. J Autism Dev Disord. 2010; 40(3): 269-79. doi: 10.1007/s10803-009-0862-9

[15] Golan O, Baron-Cohen S, Golan Y. The 'reading the mind in films' task [child version]: Complex emotion and mental state recognition in children with and without autism spectrum conditions. J Autism Dev Disord. 2008; 38(8): 1534-41. doi: 10.1007/s10803-007-0533-7

[16] Golan O, Baron-Cohen S, Hill JJ, Rutherford M. The 'Reading the Mind in the Voice'test-revised: a study of complex emotion recognition in adults with and without autism spectrum conditions. J Autism Dev Disord. 2007; 37(6): 1096-106. doi: 10.1007/s10803-006-0252-5

[17] Philip R, Whalley H, Stanfield A, Sprengelmeyer R, Santos I, Young A, et al. Deficits in facial, body movement and vocal emotional processing in autism spectrum disorders. Psychol Med. 2010; 40(11): 1919-29. doi: 10.1017/S0033291709992364

[18] Lozier LM, Vanmeter JW, Marsh AA. Impairments in facial affect recognition associated with autism spectrum disorders: a meta-analysis. Dev Psychopathol. 2014; 26(4): 933-45. doi: 10.1017/S0954579414000479

[19] Erfanian F, Hashemi Razini H, Ramshini M. The relationship between executive functions and sensory processing with emotional recognition in autism spectrum disorder. Int J Sport Stud Health. 2018; 1: e74071. doi: 10.5812/intjssh.74071

[20] Kuusikko S, Haapsamo H, Jansson-Verkasalo E, Hurtig T, Mattila M-L, Ebeling H, *et al*. Emotion recognition in children and adolescents with autism spectrum disorders. J Autism Dev Disord. 2009; 39(6): 938-45. doi: 10.1007/s10803-009-0700-0

[21] Hill EL, Frith U. Understanding autism: insights from mind and brain. Philos Trans R Soc Lond B Biol Sci. 2003; 358(1430): 281-9. doi: 10.1098/rstb.2002.1209

[22] Howlin P. Interventions and outcome in autism. J Intellect Disabil Res. 2004; 48(4-5). doi: 10.1016/S1874-5911(02)80004-5

[23] Lecavalier L. Behavioral and emotional problems in young people with pervasive developmental disorders: Relative prevalence, effects of subject characteristics, and empirical classification. J Autism Dev Disord 2006; 36: 1101-14. doi: 10.1007/s10803-006-0147-5

[24] Mayes SD, Kokotovich C, Mathiowetz C, Baweja R, Calhoun SL, Waxmonsky J. Disruptive mood dysregulation disorder symptoms by age in autism, ADHD, and general population samples. J. Ment. Health Res. Intellect. Disabil. 2017; 10(4): 345-59. doi: 10.1080/19315864.2017.1338804

[25] Fulton E, Eapen V, Crnčec R, Walter A, Rogers S. Reducing maladaptive behaviors in preschool-aged children with autism spectrum disorder using the early start denver model. Front Pediatr. 2014; 2: 40. doi: 10.3389/fped.2014.00040.

[26] Inzlicht M, Bartholow BD, Hirsh JB. Emotional foundations of cognitive control. Trends Cogn Sci. 2015; 19(3): 126-32. doi: 10.1016/j.tics.2015.01.004.

[27] Gaigg SB. The interplay between emotion and cognition in autism spectrum disorder: implications for developmental theory. Front Integr Neurosci. 2012; 6: 113. doi: 10.3389/fnint.2012.00113

[28] Gasbarri A, Pompili A, d'Onofrio A, Cifariello A, Tavares MC, Tomaz C. Working memory for emotional facial expressions: role of the estrogen in young women. Psychoneuroendocrinology. 2008; 33(7): 964-72. doi: 10.1016/j.psyneuen.2008.04.007

[29] Mallory C, Keehn B. Implications of Sensory Processing and Attentional Differences Associated with Autism in Academic Settings: An Integrative Review. Front Psychiatry. 2021; 12: 695825. doi: 10.3389/fpsyt.2021.695825.

[30] Fabio RA, Esposito S, Carrozza C, Pino G, Caprì T. Correlations between facial emotion recognition and cognitive flexibility in autism spectrum disorder. Adv. Autism. 2020; 6(3): 195-204.

[31] Mazefsky CA, Herrington J, Siegel M, Scarpa A, Maddox BB, Scahill L, White SW. The role of emotion regulation in autism spectrum disorder. J Am Acad Child Adolesc Psychiatry. 2013; 52(7): 679-88. doi: 10.1016/j.jaac.2013.05.006.

[32] Nielson KA, Bryant T. The effects of non-contingent extrinsic and intrinsic rewards on memory consolidation. Neurobiol Learn Mem. 2005; 84(1): 42-8. doi: 10.1016/j.nlm.2005.03.004

[33] Nielson KA, Powless M. Positive and negative sources of emotional arousal enhance long-term word-list retention when induced as long as 30 min after learning. Neurobiol Learn Mem. 2007; 88(1): 40-7. doi: 10.1016/j.nlm.2007.03.005

[34] Crane L, Goddard L. Episodic and semantic autobiographical memory in adults with autism spectrum disorders. J Autism Dev Disord. 2008; 38(3): 498-506. doi: 10.1007/s10803-007-0420-2

[35] Lind SE, Bowler DM. Recognition memory, self-other source memory, and theory-of-mind in children with autism spectrum disorder. J Autism Dev Disord. 2009; 39(9): 1231. doi: 10.1007/s10803-009-0735-2

[36] Lind SE. Memory and the self in autism: A review and theoretical framework. Autism. 2010; 14(5): 430-56. doi: 10.1177/1362361309358700

[37] Lind SE, Williams DM, Bowler DM, Peel A. Episodic memory and episodic future thinking impairments in high-functioning autism spectrum disorder: An underlying difficulty with scene construction or self-projection? Neuropsychology. 2014; 28(1): 55. doi: 10.1037/neu0000005

[38] Pellicano E. Links between theory of mind and executive function in young children with autism: Clues to developmental primacy. Dev Psychol. 2007; 43(4): 974. doi: 10.1037/0012-1649.43.4.974

[39] Berggren S, Fletcher-Watson S, Milenkovic N, Marschik PB, Bölte S, Jonsson U. Emotion recognition training in autism spectrum disorder: A systematic review of challenges related to generalizability. Dev Neurorehabil. 2018; 21(3): 141-54. doi: 10.1080/17518423.2017.1305004

[40] Chan, Cheung M-c, Sze SL. Effect of mind/body training on children with behavioral and learning problems: a randomized controlled study. Mind-Body and Relaxation Research Focus. 2008; 8: 165-93.

[41] Chan, Han YM, Sze SL, Lau EM. Neuroenhancement of memory for children with autism by a mind–body exercise. Front Psychol. 2015; 6: 1893. doi: 10.3389/fpsyg.2015.01893

[42] Chan, Han YMY, Cheung M-C. Chinese Chan-based prospective neuropsychological intervention for autistic children. In: Patel V, Preedy V, Martin C, editors. Comprehensive guide to autism. New York, NY: Springer; 2014. p. 2333-55. doi: 10.1007/978-1-4614-4788-7_142

[43] Molnar-Szakacs I, Wang MJ, Laugeson EA, Overy K, Wu W-L, Piggot J. Autism, emotion recognition and the mirror neuron system: the case of music. McGill Journal of Medicine: MJM. 2009; 12(2). MID: 21264050; PMCID: PMC2997252.

[44] Berger DS. Pilot study investigating the efficacy of tempo-specific rhythm interventions in music-based treatment addressing hyper-arousal, anxiety, system pacing, and redirection of fight-or-flight fear behaviors in children with autism spectrum disorder (ASD). J. Biomed. Eng. 2012; 2: 1-15. doi: 10.4303/jbe/M110902

[45] Orr TJ, Myles BS, Carlson JK. The impact of rhythmic entrainment on a person with autism. Focus Autism Other Dev Disabl. 1998; 13(3): 163-6. doi: 10.1177/108835769801300304

[46] Woodman AC, Breviglia E, Mori Y, Golden R, Maina J, Wisniewski H. The Effect of Music on Exercise Intensity among Children with Autism Spectrum Disorder: A Pilot Study. J. Clin. Med. 2018; 7(3): 38. doi: 10.3390/jcm7030038

[47] Kim, Wigram T, Gold C. The effects of improvisational music therapy on joint attention behaviors in autistic children: a randomized controlled study. J Autism Dev Disord. 2008; 38(9): 1758. doi: 10.1007/s10803-008-0566-6

[48] Scarpa A, Reyes N, Attwood T. Cognitive-behavioral therapy for stress and anger management in young children with ASD. Cuban Landscapes: Heritage, Memory, and Place. 2009: 147.

[49] Toglia JP. The dynamic interactional model of cognition in cognitive rehabilitation (2011).

[50] Baron-Cohen. The empathising-systemising theory of autism: implications for education. Tizard Learn. Disabil. Rev. 2009; 14(3): 4-13. doi: 10.1108/13595474200900022

[51] Sequeira S, Ahmed M. Meditation as a potential therapy for autism: A review. Autism res. treat. 2012; 2012: 1-11. doi: 10.1155/2012/835847

[52] Greenberg DM, Rentfrow PJ, Baron-Cohen S. Can music increase empathy? Interpreting musical experience through the empathizing–systemizing (ES) theory: Implications for autism. Musicol. rev. Empirical Musicology Review. 2015 Apr 8; 10(1-2): 80-95.

[53] White, Mazefsky CA, Dichter GS, Chiu PH, Richey JA, Ollendick TH. Social-cognitive, physiological, and neural mechanisms underlying emotion regulation impairments: Understanding anxiety in autism spectrum disorder. Int J Dev Neurosci. 2014; 39: 22-36. doi: 10.1016/j.ijdevneu.2014.05.012

[54] Bal E, Harden E, Lamb D, Van Hecke AV, Denver JW, Porges SW. Emotion recognition in children with autism spectrum disorders: Relations to eye gaze and autonomic state. J Autism Dev Disord. 2010; 40(3): 358-70. doi: 10.1007/s10803-009-0884-3

[55] Hilton CL, Harper JD, Kueker RH, Lang AR, Abbacchi AM, Todorov A, *et al*. Sensory responsiveness as a predictor of social severity in children with high functioning autism spectrum disorders. J Autism Dev Disord. 2010; 40(8): 937-45. doi: 10.1007/s10803-010-0944-8

[56] Duncan EA, Editor. Foundations for Practice in Occupational Therapy-E-BOOK. Elsevier Health Sciences; 2011 Apr 15

[57] Smith A. The empathy imbalance hypothesis of autism: A theoretical approach to cognitive and emotional empathy in autistic development. Psychol. Rec. 2009; 59(3): 489-510. doi: 10.1007/BF03395675

[58] Bradley EA, Summers JA, Wood HL, Bryson SE. Comparing rates of psychiatric and behavior disorders in adolescents and young adults with severe intellectual disability with and without autism. J Autism Dev Disord. 2004; 34: 151-61. doi: 10.1023/B:JADD.0000022606.97580.19

[59] Joshi G, Wozniak J, Petty C, Martelon MK, Fried R, Bolfek A, Kotte A, Stevens J, Furtak SL, Bourgeois M, Caruso J. Psychiatric comorbidity and functioning in a clinically referred population of adults with autism spectrum disorders: a comparative study. J Autism Dev Disord. 2013; 43: 1314-25. doi: 10.1007/s10803-012-1679-5

[60] Baker J. Preparing for life: The complete guide for transitioning to adulthood for those with autism and Asperger's syndrome. Future Horizons; 2005.

[61] Deffenbacher K, Brown E. Memory and cognition: An

information processing model of man. Theory Decis. 1973; 4(2): 141-78. doi: 10.1007/BF00145150

[62] Baddeley A. The episodic buffer: a new component of working memory? Trends Cogn Sci. 2000; 4(11): 417-23. doi: 10.1016/s1364-6613(00)01538-2

[63] Golan O, Sinai-Gavrilov Y, Baron-Cohen S. The Cambridge Mindreading Face-Voice Battery for Children (CAM-C): complex emotion recognition in children with and without autism spectrum conditions. Mol Autism. 2015; 6(1): 1-9. doi: 10.1186/s13229-015-0018-z

[64] Portney LG, Watkins MP. Foundations of clinical research: applications to practice: Pearson/Prentice Hall Upper Saddle River, NJ; 2009.

[65] Graham JE, Karmarkar AM, Ottenbacher KJ. Small sample research designs for evidence-based rehabilitation: issues and methods. Arch Phys Med Rehabil. 2012; 93(8): S111-S6. doi: 10.1016/j.apmr.2011.12.017

[66] Wechsler D. Wechsler intelligence scale for children–Fourth Edition (WISC-IV). (No Title). 2003.

[67] Schopler E, Reichler RJ, Renner BR. The childhood autism rating scale (CARS). Los Angeles, CA: Western Psychological Services; 2010.

[68] Hedges R, Drysdale K, Levick WR. The Children's Memory Questionnaire-Revised. Appl. Neuropsychol. Child. 2015; 4(4): 285-96. doi: 10.1080/21622965.2014.925806

[69] Gonzalez LM, Anderson VA, Wood SJ, Mitchell LA, Heinrich L, Harvey AS. The Observer Memory Questionnaire-Parent Form: Introducing a new measure of everyday memory for children. J Int Neuropsychol Soc. 2008; 14(2): 337-42.

[70] Bruni TP. Test review: Social responsiveness scale–Second edition (SRS-2). J. Psychoeduc. Assess. 2014 Jul; 32(4): 365-9. doi: 10.1177/0734282913517

[71] Linstead E, Dixon DR, Hong E, Burns CO, French R, Novack MN, Granpeesheh D. An evaluation of the effects of intensity and duration on outcomes across treatment domains for children with autism spectrum disorder. Transl Psychiatry. 2017; 7(9): e1234. doi: 10.1038/tp.2017.207.

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[1] Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes Dis. 2019; 6(2): 109-19. doi: 10.1016/j.gendis.2019.04.001.

[2] Hung Kn G, Fong KN. Effects of telerehabilitation in occupational therapy practice: A systematic review. Hong Kong J Occup Ther. 2019; 32(1): 3-21. doi: 10.1177/1569186119849119.

[3] Wijesooriya K, Liyanage NK, Kaluarachchi M, Sawkey D. Part II: Verification of the TrueBeam head shielding model in Varian VirtuaLinac via out-of-field doses. Med Phys. 2019; 46(2): 877-884. doi: 10.1002/mp.13263.

[4] Velayati F, Ayatollahi H, Hemmat M. A systematic review of the effectiveness of telerehabilitation interventions for therapeutic purposes in the elderly. Methods Inf Med. 2020; 59(2-03): 104-9. doi: 10.1055/s-0040-1713398.

[5] Junmee C, Siriwachirachai P, Chompoonimit A, Chanavirut R, Thaweewannakij T, Nualnetr N. Health status of patients with stroke in Ubolratana District, Khon Kaen Province: International Classification of Functioning, Disability and Health-based assessments. Thai J Phys Ther. 2021; 43(1): 45-63 (in Thai).

Journal Articles on the Internet

[1] Siegel PM, Bojti I, Bassler N, Holien J, Flierl U, Wang X, et al. A DARPin targeting activated Mac-1 is a novel diagnostic tool and potential anti-inflammatory agent in myocarditis, sepsis and myocardial infarction. Basic Res Cardiol [Internet]. 2021; 116(1): 1-25. Available from: https://doi.org/10.1007/s00395-021-00849-9.

Journal Articles from an Online Database

[1] Jackson D, Firtko A, Edenborough M. Personal resilience as a strategy for surviving and thriving in the face of workplace adversity: A literature review. J Adv Nurs [serial online]. 2007;60(1):1-9. DOI: 10.1111/j.1365-2648.2007.04412.x.

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[1] Grove SK, Cipher DJ. Statistics for nursing research: A workbook for evidence-based practice. 3rd ed. St. Louis, Missouri: Elsevier; 2019.

[2] Haznadar M, editor. Cancer metabolism: Methods and protocols. New York: Humana Press; 2019. doi: 10.1007/978-1-4939-9027-6.

[3] Perrin DH. The evaluation process in rehabilitation. In: Prentice WE, editor. Rehabilitation techniques in sports medicine. 2nd ed. St Louis, Mo: Mosby Year Book; 1994: 253–276.

E-book

[1] Dehkharghani S, editor. Stroke [e-book]. Brisbane (AU): Exon Publications; 2021 [cited 2021 Jul 31]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK572004/ doi: 10.36255/exonpublications.stroke.2021

[2] Tran K, Mierzwinski-Urban M. Serial X-Ray radiography for the diagnosis of osteomyelitis: A review of diagnostic accuracy, clinical utility, cost-effectiveness, and guidelines [e-book]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2020 [cited 2021 Jul 31]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562943/

Dissertation/Thesis

[1] On-Takrai J. Production of monoclonal antibody specific to recombinant gp41 of HIV-1 subtype E [Term paper]. Faculty of Associated Medical Sciences: Chiang Mai University; 2001 [in Thai].

[2] Seale AC. The clinical and molecular epidemiology of streptococcus agalactiae in Kenya: maternal colonization and perinatal outcomes [Dissertation on the Internet]. [Oxford (England)]: University Oxford; 2015 [cited 2015 Jul 28]. Available from: http://ora.ox.ac.uk/objects/uuid:6e7d952a-dc5b-4af0-b0bb-f2ae2184eed0.

.

Conference Proceedings

[1] Lake M, Isherwood J, Clansey. Determining initial knee joint loading during a single limb drop landing: reducing soft tissue errors. Proceedings of 34th International Conference of Biomechanics in Sport; 2016 Jul 18-22; Tsukuba, Japan, 2016. Available from: https://ojs.ub.uni-konstanz.de/cpa/article/view/7126

[2] Ellis MD, Carmona C, Drogos J, Traxel S, Dewald JP. Progressive abduction loading therapy targeting flexion synergy to regain reaching function in chronic stroke: preliminary results from an RCT. Proceedings of the 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society; 2016: 5837-40. doi: 10.1109/EMBC.2016.7592055.

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[1] Australian Government, Department of Health. Physical activity and exercise guidelines for all Australian. 2021 [updated 2021 May 7; cited 2021 Jul 15]. Available from: https://www.health.gov.au/health-topics/physical-activity-and-exercise/physical-activity-and-exercise-guidelines-for-all-australians.

[2] Department of Health. Situation survey on policy and implementation of physical activity promotion in schools for first year 2005. Nonthaburi: Ministry of Public Health; 2005. (in Thai).

[3] Department of Local Administration, Ministry of Interior Affairs. Standard of Sports Promotion. Bangkok. 2015: 7–9. (in Thai).

[4] World Health Organization. WHO guidelines on physical activity and sedentary behaviour. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

[5] World Health Organization. The epidemiology and impact of dementia: current state and future trends. 2015 [cited 2021 Mar 8]. Available from: http://www.who.int/mental_health/neurology/dementia/dementia_thematicbrief_epidemiology.pdf.

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