JAMS

JOURNAL OF ASSOCIATED MEDICAL SCIENCES

Volume 54 Number 2 May-August 2021 E-ISSN: 2539-6056

Journal of Associated Medical Sciences

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, physical therapists, occupational therapists, radiologic technologists, 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. Physical Therapy
3. Occupational Therapy
4. Radiologic Technology
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.

Objectives

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.

Types of manuscript

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

- **Review articles** must not exceed 20 journal pages (not more than 5,000 words), including 6 tables/figures, and references (maximum 75, recent and relevant).
- **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).
- **Short communications** including technical reports, notes, and letters to the editor must not exceed 5 journal pages (not more than 1,500 words), including 2 tables/figures, and references (maximum 10, recent and relevant).

Peer review process

By submitting a manuscripts to Journal of Associated Medical Sciences, the authors agree to subject it to the confidential double-blinded peer-review process. Editors and reviewers are informed that the manuscripts must be considered confidential. After a manuscripts is received, it is assigned by a specific Associate Editor. The Associate Editor prepares a list of expert reviewers, which may include some suggested by the Editor-in-Chief. Authors can indicate specific individuals whom they would like to have excluded as reviewers. Generally, requests to exclude certain potential reviewers will be honored except in fields with a limited number of experts. All potential reviewers are contacted individually to determine availability. Manuscripts files are sent to at least two expert reviewers. Reviewers are asked to complete the review of the manuscripts within 2 weeks and to return a short review form. Based on the reviewers' comments, the Associate Editor recommends a course of action and communicates the reviews and recommendations to the Editor-in-Chief for a final decision.

The Associate Editor considers the comments made by the reviewers and the recommendation of the Editor-in-Chief, selects those comments to be shared with the authors, makes a final decision concerning the manuscripts, and prepares the decision letter for signature by the Editor-in-Chief. If revisions of the manuscripts are suggested, the Associate Editor also recommends who should review the revised paper when resubmitted. Authors are informed of the decision by e-mail; appropriate comments from reviewers and editors are appended.

Publication frequency

Journal of Associated Medical Sciences publishes 3 issues a year

Issue 1: January-April
Issue 2: May-August
Issue 3: September-December

Journal Impact Factor
The journal's 2017 Impact Factor is 0.237

Journal website
Homepage https://www.tci-thaijo.org/index.php/bulletinAMS/index

Journal E-ISSN:
2539-6056

Editorial Office
Faculty of Associated Medical Sciences, Chiang Mai University
110 Inthawaroros Road, Suthep, Muang, Chiang Mai, 50200
Phone 053 935072 Facsimile 053 936042

Journal of Associated Medical Sciences

Vol. 54 No. 2: May 2021

Content

1 Comprehensive esophageal speech training for laryngectomees: Substantial benefits
Benjamas Prathanee[1] Tawitree Pumnum[1] Nantiya Ooppanasak[1] Patravoot Vatanasapt[1] Nichanun Punyaek[2]*

8 Monitoring and evaluation for quality of Thailand SARS-CoV-2 laboratory network: Lessons learnt for policy drive and new guidelines
Surasak Muenphon[1] Patravee Soisangwan[1] Nattakarn Laieddee[1] Nutthanun Nammontri[1] Kanokwan Kittiniyom[2] Sumonmal Uttayamakul[3]

17 A survey of radiation released from patients treated with radioiodine-131 therapy
Chaisunthorn Wisetnan[1] Patamaporn Molee[2] Panatsada Awikunprasert[2] Khajornkiat Srichachet[3] Vithit Pungkun[4]*

22 A preliminary performance evaluation of 3D facial image reconstruction from computed tomography scan
Nontanun Moonsan[1] Suchart Kiatwattanacharoen[2] Komsanti Chokethawai[3]*

29 A preliminary study of myofascial release technique effect on the range of hip flexion, knee flexion, and ankle dorsiflexion motion at affected lower extremity in individuals with chronic stroke
Sataporn Intanon Peanchai Khamwong[*] Sothida Nantakool

35 Associations between urinary excretion of cadmium with alpha-1 microglobulin and microalbuminuria: a cross-sectional study in northwestern Thai population
Sujitra Sikaphan[1] Ratchaneekorn Boonthum[2] Siriwan Leudang[1] Sittiporn Parnmen[1]*

42 Cytotoxic and antiproliferative effects of crude ethanolic extract from *Piper betle* leaves on leukemic cell lines
Methee Rungrojsakul[1] Siriporn Okonogi[2] Pawaret Panyajai[3] Songyot Anuchapreeda[3*]*

Journal of Associated Medical Sciences 2021; 54 (2): 1-7

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Comprehensive esophageal speech training for laryngectomees: Substantial benefits

Benjamas Prathanee[1] Tawitree Pumnum[1*] Nantiya Ooppanasak[1] Patravoot Vatanasapt[1] Nichanun Punyaek[2]

[1]Department of Otorhinolaryngology, Faculty of Medicine, Khon Kaen University, Khon Kaen Province, Thailand
[2]Department of Rehabilitation, Faculty of Medicine, Khon Kaen University, Khon Kaen Province, Thailand

ARTICLE INFO

Article history:
Received 8 April 2020
Accepted as revised 21 January 2021
Available online 21 January 2021

Keywords:
Esophageal speech, laryngectomy, laryngeal cancer, speech rehabilitation

ABSTRACT

Background: Head and neck cancer is one of the major cancer burdens in Thailand and Southeast Asia. Most patients with laryngeal cancer present at an advanced stage and require laryngectomy. Esophageal speech (ES) is an option for effective communication.

Objectives: To determine the effectiveness of comprehensive esophageal speech under the "Training for The Trainer" project.

Materials and methods: Ninety-four laryngectomees (patients with laryngeal cancer undergoing total laryngectomy) received esophageal speech training by speech and language pathologists (SLPs) and the trainers or laryngectomee volunteers. ES was instructed on a monthly for 4 years. Project "Camp of Hope and Spirit for Laryngectomees", which provides support to improve quality of life by integration of music, art, nutrition, and dharma with healthy activities, was also conducted.

Results: Thirty-six patients who attended ES classes for 6 sessions (1 session compose of 5 periods) had an average improvement in their level of ES of 3.27 levels and 19 laryngectomees who attended ES classes for 12 sessions had average improvement of their level of ES of 4.74 levels. The success rate for ES at level 1 (belch 1-5 times in 10 attempts) was 72% and level 5 (2-syllable words/phases for 1-5 times in 10 attempts) was 34 of 94 (36%) within 12 sessions. Srinagarind ES score was found improved significantly between the 1st and 6th visit (MD=4, 95%CI=2-4); the 1st and 12th visit (MD=6 , 95%CI=2.5-7). Twenty-eight of a total 34 patients (82.35%), who could use ES at level 5, did not require an electrolarynx. Therefore, a saving of the cost of electrolarynx purchase of 896,000 Baht (32,000 Baht/case). Satisfactions with ES training and "Camp of Hope and Spirit for Laryngectomees" were scored as good to excellent.

Conclusion: The success rate for ES at level 1 was 72% and level 5 was 36% within 12 sessions. A saving of the cost of electrolarynx purchase of 896,000 Baht (32,000 Baht/case) within 4 years. Satisfactions with ES training and "Camp of Hope and Spirit for Laryngectomees" were scored as good to excellent.

* **Corresponding author.**
Author's Address: *Department of Otorhinolaryngology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.*
** **E-mail address:** matodabovvy@gmail.com
doi: 10.14456/jams.2021.10
E-ISSN: 2539-6056

Introduction

Head and neck cancer is one of the major cancer burdens in Thailand and Southeast Asia. The estimated incidence of head and neck cancer in Thailand is 15.7 per 100,000 in males and 10.7 per 100,000 in females.[1] Among these, laryngeal cancer is known to have the greatest negative impact on survivors, both from cancer per se, and its treatment.[2] The majority of patients with laryngeal or hypopharyngeal cancer in the northeast of Thailand are diagnosed at an advanced disease stage[3] and most cases end up having their larynx totally removed (total laryngectomy). Those who undergo this surgery, so called "laryngectomees", they need to breathe through their tracheostoma and swallow through their neopharynx. Normal speech is lost, and there is a permanent stoma in the middle of the neck. Even though the survival rate after surgery is acceptable after surgical treatment,[4] several functional deficits including speech, swallowing, breathing, and physical disabilities around the head and neck region, not to mention the disfiguring effects of surgery and emotional and behavioral disturbances, result in negative effects on daily living.[5]

One of the most critical disabilities is aphonia.[5] This is the most important social problem that the patients face.[6] As a consequence, many patients develop emotional and psychological problems and some of them need psychological management. Voice rehabilitation (esophageal speech, electrolarynx and tracheoesophageal prosthesis) is a challenge that these patients must overcome.

The electrolarynx is the easiest vocal rehabilitation method for total laryngectomy patients to use, as it hardly requires training, but patients' satisfaction rates were lower because of the mechanical, low frequency, monotonous and unnatural voice that it produces.[7] A tracheoesophageal prosthesis is most commonly used for voice rehabilitation in developed countries. It is a surgical method that could be performed as either a primary or secondary procedure. Reported patient quality of life and satisfaction data following tracheoesophageal puncture are the best[8, 9] because the tracheoesophageal prosthesis significantly contributes to the acquisition of speech and intelligibility in alaryngeal speakers.[8] However, complications may occur in as many as 42.6% of cases[10] and the frequent need for replacement of the prostheses is a major burden for patients. Esophageal speech (ES) is one of voice rehabilitation that might cost and safe benefit, however, it might take long duration for training success.

In our institution, the primary modality for speech rehabilitation is esophageal speech (ES), which is more natural, cost effective and does not require a device for electrolaryngeal speech, or surgery to facilitate esophageal speech.[8] The disadvantage of ES is mainly the long time taken to achieve a communicable level of speech, and it is not feasible in some cases due to a poor anatomical structure or rigidity of the neopharynx. The success rate of ES is reported to be 6-32%.[11, 12]

Most laryngectomees in the northeast of Thailand are in low socioeconomic status with limited access to speech services because of a shortage of professional speech and language pathologists (SLPs) in the country [13] and because they are unable to afford living expenses and travel. Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, serves as a super tertiary center for cancer treatment in this region covering approximately one-third of the population of Thailand. Therefore, a multidisciplinary team for a comprehensive rehabilitation for laryngectomees was developed. Besides speech rehabilitation, all other aspects of their quality of life were taken into account.

The purpose of this study was: 1) to determine the effectiveness of a comprehensive ES training program before and after at 6 and 12 sessions of training, respectively; and 2) to investigate the costs and benefits of comprehensive ES training.

Materials and methods

This research was a retrospective descriptive study. Data were retrieved from 1) medical records, Department of Medical Records and Statistics, Srinagarind Hospital; 2) 4 annual reports of comprehensive esophageal speech training in 2010- 2013; and 3) 2 reports of the Art 4' Mee camp in 2011 and 2013.

Subjects recruited for this study were the patients who had undergone laryngectomy for laryngeal or hypopharyngeal cancer treatment and were enrolled in ES training at Srinagarind Hospital between January 2010 and December 2013. Three cases who did not participate ES program were excluded. Ninety-four of 97 laryngectomees were included in this study. Following to objective of this study, laryngectomees who attended ES training for less than 6 sessions were also excluded. At the end of the study 56 laryngectomees continued with ES while 38 of them chose electrolarynxes.

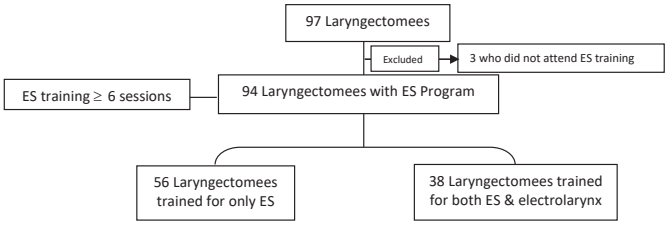

Figure 1. *Flow chart of subjects.*

Comprehensive ES training

The comprehensive ES training was a program for training in ES integrated with establishing hope, spirit and support to promote quality of life. The program composed of activities as follows:

ES training

The ES training was conducted by speech and language pathologists (SLPs), as well as speech assistants (SAs), volunteers who were laryngectomees and had finished a course entitled "Training for the Trainer". They spoke by ES fluently. In the training program, the laryngectomees were divided into 5 groups according to their level of ES ability follow to Srinagarind ES Levels. Srinagarind ES Levels have done and used for clinical outcomes and accepted among

multidisciplinary approaches for more than 20 years. It's composed of 17 levels of ES as follows:

Groups	**Srinagarind ES Levels**
I	0 = cannot belch or make any sound after swallowing air;
	1 = belch 1-5 times in 10 attempts;
	2 = belch 6-10 times in 10 attempts;
II	3= speak 1-word 1-5 times in 10 attempts;
	4 = speak 1-word for 6-10 times in 10 attempts;
	5 = speak 2-words for 1-5 times in 10 attempts;
	6 = speak 2-words for 6-10 times in 10 attempts;
III	7 = speak 3-words for 1-5 times in 10 attempts;
	8 = speak 3-words for 6-10 times in 10 attempts;
	9 = speak 4-words for 1-5 times in 10 attempts;
	10 = speak 4-words for 6-10 times in 10 attempts;
IV	11 = speak 5-words for 1-5 times in 10 attempts;
	12 = speak 5-words for 6-10 times in 10 attempts;
	13 = speak 6-words for 1-5 times in 10 attempts;
	14 = speak 6-words for 6-10 times in 10 attempts;
V	15 = fluently read or speak approximately 50%;
	16 = fluently read or speak approximately 51-100%;
	17 = fluently speak or sing songs.

Three groups were trained by 3 SAs and 2 groups were trained by 2 SLPs. Each group was trained ES 5 30-minute periods in 1 session/month. SAs and SLPs rotated to other groups every session. Therefore, each SA and SLP trained ES for every laryngectomee group. ES training compose of 5 steps:

Step 1: belch and 1-syllable word
- Open mouth and take a breath into the mouth and nose
- Close mouth, then raises the tongue to the palate and pushing the air to upper esophagus or swallow air (try to hold the air at the upper esophageal sphincter)
- Open mouth and then belch
- Repeat several attempts until belch with /a/ sound
- Repeat several attempts until produce monothongs
- Repeat several attempts until produce simple 1-word sentences

Step 2: 2-syllable words/phrases/sentences
- Belch 2 syllables of monothongs such as /a-a/, /u-u/, /u-a/, /u-i/
- Repeat several attempts until produce 2-syllable words/phrases/sentences

Step 3: 3-syllable words/phrases/sentences
- Belch 3 syllables of monothongs such as /a-a-a/, /u-u-u/, /u-a-i/, /u-i-e/
- Repeat several attempts until produce 3-syllable words/phrases/sentences

Step 4: 4-syllable words/phrases/sentences
- Belch 4 syllables of monothongs such as /a-a-a-a/, /u-u-u-u/, /u-a-i-e/
- Repeat several attempts until produce 4-syllable words/phrases/sentences

Step 5: 5-syllable words/phrases/sentences
- Belch 5 syllables of monothongs such as /a-a-a-a-a/, /u-u-u-u-u/, /u-a-i-e-o/
- Repeat several attempts until produce 5-syllable words/phrases/sentences

Step 6: reading and conversation
- Reading practice
- Conversation practice

Step 7: Singing
- Singing practice

The Art 4'Mee Camp

The Art 4'Mee Camps (the arts for the laryngectomees) were held in 2011 and 2013. They were two-day programs designed specifically for the laryngectomees and their caregivers and aimed to support and empower them in order that they might regain their normal livelihoods. Music, dance, and art in this camp were encouraged to promote internal connection between their body and mind, and external connection between the participants. Besides music, dance, and art; the program included meditation, yoga based exercise, and a workshop for self-care.[14]

A music therapy program was integrated with the ES training. This hospital-based program was conducted by musicians, physiotherapists, SLPs, and laryngectomee volunteers. The music therapy was aimed to support the ES training, including creative music making, music and movement and music and breathing. Music was also used to promote esophageal voice projection and concluded with singing and dancing to traditional songs by esophageal voice (depending on their ability).

The main outcome was graded by the 1st author, a senior SLP. This level was identified based on the Srinagarind ES Levels, which was consensus of grading criteria and was established by a group of SLPs who have worked more than 20-year experiences with laryngectomee and esophageal speech for quantitative and subjective assessment of ES in the Speech Clinic at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University.

Satisfaction and evaluation of program

Questionnaires were filled by laryngectomees who enrolled program and caregivers on topics based on 5- category scores from 1-5 (1= should be revised or not good; 2= fair; 3= moderate; 4= good; 5= very good).

Regarding to The Art 4'Mee Camp, similarity score was rated based on the same category as good – excellent for each item including activities in the camp, duration,

accommodation, coordination, food. The Art 4'Mee Camp was established every 2 years (2011 and 2013). The number of laryngectomees and caregivers who responded questionnaire for 2011, and 2013 were the same group to number of participants who responded to questionnaires for satisfaction and evaluation of program.

Descriptive analysis was used to assess the laryngectomee's characteristics and general information about their esophageal speech level. The comparison of ES level before and after training for 6 and 12 sessions was analyzed using The Wilcoxon Signed-Rank Test.

Results

This was a retrospective study from 2010-2013. Patients were recruited in the study in each year displayed as Table 1. New cases were enrolled in the study any time that they were consulted from physician for speech rehabilitation. The ES program was begun within the 1st visit and number of ES training counted from the number of visits (not counted from the number of months after enrollment). The participants were 91 males and 3 females, average age 61.6 (39-87) years old. The general characteristics of the laryngectomees are displayed in Table 2.

Table 1 Number of patients in this study.

Date	Old Case	New Case	Total
2010	20	10	30
2011	19	11	30
2012	27	20	42
2013	29	22	51

Table 2 General characteristics of laryngectomees.

Characteristics		Number (N=94)	Percentage
Diagnosis	Larynx	73	77.66
	Hypopharynx	20	21.28
	Undetermined	1	1.06
Stage	Stage I	3	3.19
	Stage II	5	5.32
	Stage III	36	38.30
	Stage IV	46	48.94
	Unknown	4	4.26
Neck Dissection	Unilateral	35	37.23
	Bilateral	30	31.91
	No dissection	26	27.66
	Unknown	3	3.19

Table 2 General characteristics of laryngectomees. (continued)

Characteristics		Number (N=94)	Percentage
Reconstruction	Regional flap	8	8.51
	Gastric pull-up	3	3.19
	No reconstruction	80	85.11
	Unknown	3	3.19
Post-operative Radiotherapy	Yes	91	96.81
	No	2	2.13
	Unknown	1	1.06
Laryngectomy as a salvage	Yes	11	11.7
	No	81	86.17
	Unknown	2	2.13

ES level was assessed every time that patients attended ES program and displayed data on the 6th and 12th sessions. New laryngectomees could access ES program any time that they were consulted. Therefore, assessment level depends on the number of times that they attend.

Some laryngectomees could not visit ES program monthly, therefore, the ES training was counted from the number of sessions. Thirty-six of them, who had attended six sessions of ES training had average progression of Srinagarind ES levels = 3.27 levels, while 19, who had 12 sessions, had progression of Srinagarind ES levels = 4.74 levels. Sixty-eight of 94 laryngectomees (72%) achieved ES scores of at least level 1 within 12 sessions (Figure II). Thirty-four of 94 laryngectomees (36%) achieved ES scores of at least level 5 within 12 sessions (Figure III).

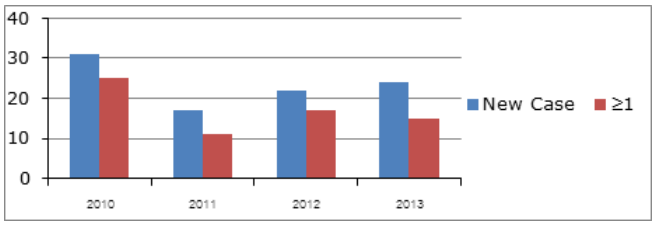

Figure 2. *Number of laryngectomees who succeeded in developing ES at least to level 1 within 12 sessions.*

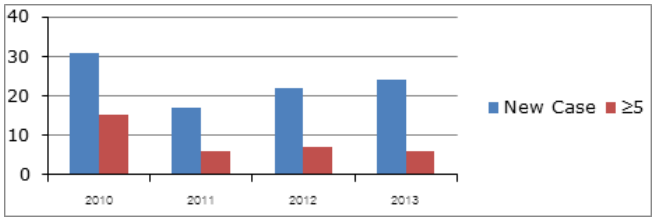

Figure 3. *Number of laryngectomees who succeeded in developing ES at least level 5 within 12 sessions.*

Twenty-eight of 34 laryngectomees (82%) who achieved Srinagarind ES level 5 within 12 sessions did not request an electrolarynx.

Some laryngectomees could not visit the esophageal speech program every month for personal reasons, such as financial problems, sickness or unavailable caregivers, etc. Therefore, the success rates for 6 and 12 visits were analyzed. The Srinagarind ES score was found to improved significantly between the 1st and 6th visit; the 1st and 12th visit; and the 6th and 12th visit for training (Table 3 –5).

Table 3 Comparison of Srinagarind ES Levels between the 1st and 6th sessions.

Parameter	Before	After	n	Median difference	Z	*p* value*	95% Confident interval
Median	0	4	37	4	3	<0.001	2-4
Maximum	15	16					
Minimum	0	0					

** The Wilcoxon Signed Rank Test*

Table 4 Comparison of Srinagarind ES Levels between the 1st and 12th sessions.

Parameter	Before	After	n	Median difference	Z	*p* value*	95% Confident interval
Median	0	4	19	6	4.5	0.009	2.5-7
Maximum	15	16					
Minimum	0	0					

** The Wilcoxon Signed Rank Test*

Table 5 Comparison of Srinagarind ES Levels between the 6th and 12th sessions.

Parameter	Before	After	n	Median difference	Z	*p* value*	95% Confident interval
Median	4	6	19	2	15	0.0498	0-3
Maximum	16	16					
Minimum	0	0					

** The Wilcoxon Signed Rank Test*

The existing data of laryngectomees who attended ES program presented the improvement of ES level varied to the number of visits showed as Table 6.

Table 6 ES Level improvement.

Number of ES program	Number*	ES Level improvement		
		min	max	mean
1	20	-	-	-
2-5	32	-1	8	1.81
6-11	18	0	14	4.28
≥12	19	0	16	6

** Not included SAs*

It was surprising that 2/3 of laryngectomees who were underwent gastric pull up reconstruction had progression of the ES level from 4 and 6 levels with 5 ES visits.

Patients' satisfaction with the ES training program was assessed. Most laryngectomees gave average scores of good – excellent for each item as 94-100%, 88.24-100%, 82.76-100% and 90-100% in 2010, 2011, 2012, and 2013, respectively (Table 7).

For satisfaction with the 2-day Art 4'Mee Camps, score was ranged 91.43-100% and 88-100% in 2011 and 2013, respectively. The existing data on each topic are available only in 2013 which are presented in Table 8.

Table 7 Satisfaction of ES program.

Topics	Satisfaction of ES program			
	2010 N=23 (L=19: C=4)	2011 N=28 (L=19: C=9)	2012 N=48 (L=27: C=21)	2013 N=34 (L=25: C=9)
Interesting activities	4.76	4.53	4.41	4.60
Interesting lectures or descriptions	4.82	4.65	4.31	4.45
Duration of program	4.35	4.05	3.76	3.90
Activity demonstration	4.88	4.76	4.34	4.40
Place or meeting room	4.64	4.71	4.28	4.55
Meterials	4.59	4.76	4.31	4.45

N=Number, L: Laryngectomy, C: Caregivers.

Table 8 Satisfaction of Art 4'Mee Camps.

Topics	Satisfaction of Art 4'Mee Camps in 2013 N=34 (L=25: C=9)
Interesting activities	4.70
Accommodation	4.38
Food	4.44
Place or meeting room	4.85
Coordination	4.52
Duration of program	4.54

N=Number, L: Laryngectomy, C: Caregivers.

Discussion

Examination of data from 2010-2013 shows that most laryngectomees were able to achieve Srinagarind ES Level 1 (72%) and level 5 (36%) within 12 sessions (Figure 1 and 2), respectively. It was interesting to find that those undergoing regional flap or gastric pull up for reconstruction could produce ES, although the pharyngeal tissue was distorted by the surgical reconstruction. It is possible that sphincter pressures and/or esophageal motility patterns do not have any predictive for ES.[6] Comparison of data after 6 and 12 sessions for ES showed significant improves in Srinagarind ES Levels. The longer the laryngectomee attended, the greater the outcome of ES. These results can be used for counseling new laryngectomees who enroll into the ES training program.

Twenty-eight of 34 laryngectomees (82%), who achieved ES Score 5 (2-syllable meaningful word), did not request an electrolarynx. It appeared that they were motivated to persist with ES. This saved the cost of an electrolarynx, which needs to be claimed from the Thai National Security Office. An electrolarynx cost 32,000 Baht (US $ 1,000). The cost of 28 electrolarynxes is 896,000 Baht or US $ 28,000 (approximately 32,000 Baht/electrolarynx, 32 Baht=1 US$) in 4 years. Besides improving the speech capacity from the ES training program, the patients' satisfaction was found to be achieved.

For satisfaction for enrolling the program, the scores were range good to very good or excellent activities (82.76-100%) on items of 1) interesting activities; 2) interesting lectures or descriptions; 3) duration of program; 4) activity demonstration; 5) place or meeting room; 6) materials. Most of the participants were happy to join activities in each year. Participants' impressions were e.g., professionals were very kind and good instructors; good friends and society; very good and interesting activities etc. Regarding to participants' suggestion, included more often sessions from once a month to be every week, providing more transportation compensation for participants who had low economic status, more soft diet for aging laryngectomee etc. These suggestions were information to providing better care in the future.

Even though ES is the most challenging method for vocal rehabilitation, with a long period of time to gain the skills,[9] and has limited voice quality, such as a restriction in F_0, increase in Jitter and Shimmer, decreasing of HNR values, and reduced intensity compared to the voice of normal laryngeal speakers,[15] the results of this study, unlike those of a previous one,[10] indicate a reasonable success rate, good patient satisfaction and cost benefits. The Voice-Related Quality of Life measure is needed for further assessment of this technique comparing to other speaking device or normal laryngeal voice; and to determine the perceived level of influence on vocal communication[10] and the self-assessed vocal handicap.[10] No single method is considered to be the best for all patients. Selection of a method should be based on the patient, the surgeon and the SLPs.[16]

Healthcare workers should understand the advantages and disadvantages of each voice rehabilitation method to assist people with total laryngectomy in making the most appropriate decision, taking into consideration their age, sex, physical condition, job, economic status and other relevant factors.[10]

Although this study was primary report in Thailand and summarized that the comprehensive ES training program might be an effective program in terms of communication in daily, there were some limitations including individual background and characteristics of participants such as stage of cancer, areas of cancer invasion, surgery size, as well as reconstruction, type of treatments (surgery, surgery with radiation or chemotherapy or surgery with both radiation and chemotherapy etc). These factors might affect clinical outcome of ES. It needs to carefully interpret and the further study might be more concern about these factors.

Conclusion

The success rate of ES was 72% for laryngectomees who achieved ES at level 1 within 12 sessions; 36% for laryngectomees who achieved ES at level 5 within 12 sessions. The comprehensive ES training program is an effective program in terms of both speech and economic benefits. This program can be applied in practice for developing to gain their capacity to live in their society. Participants' suggestions should be considered for the further program.

Conflicts of interests

This study has no conflicts of interest.

Acknowledgements

We gratefully acknowledge the Northeastern Laryngectomee Society and the interdisciplinary team of Srinagarind Hospital as well as laryngectomee volunteers involved in this comprehensive program for their great support. We give special thanks to Srinagarind Hospital, Khon Kaen University for financial support. We thank Professor Robert Mills for editing English language of the manuscript via Publication Clinic KKU, Thailand.

References

1] Tangjaturonrasme N, Vatanasapt P, Bychkov A. Epidemiology of head and neck cancer in Thailand. Asia Pac J Clin Oncol 2018 Feb; 14(1): 16-22. doi: 10.1111/ajco.12757.

[2] Furtado de Araújo Neto VJ, Cernea CR, Aparecido Dedivitis R, Furtado de Araújo Filho VJ, Fabiano Palazzo J, Garcia Brandão L. Cervical metastasis on level IV in laryngeal cancer. Acta Otorhinolaryngol Ital 2014 Feb; 34(1): 15-8.

[3] Vatanasapt P, Lertsinudom S, Sookprasert A, Phunmanee A, Pratheepawanit N, Wattanaudomrot S, et al. Prevalence and management of cancer pain in Srinagarind Hospital, Khon Kaen, Thailand. J Med Assoc Thai 2008; 91(12): 1873-7.

[4] Ma Y, Liu L, Huang D, Wang J, Wu W, Liu M, et al. [Retrospective analyses of postoperative survival of laryngeal carcinoma patients at late stage]. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2013 Aug; 27(15): 844-6.

[5] Braz DS, Ribas MM, Dedivitis RA, Nishimoto IN, Barros AP. Quality of life and depression in patients undergoing total and partial laryngectomy. Clinics (Sao Paulo) 2005 Apr; 60(2): 135-42.

[6] Sahin M, Vardar R, Kirazli T, Ogut F, Akyildiz S, Bor S. Predictive value of esophageal motility test in the proficiency of esophageal speech. Dis Esophagus 2015 feb-Mar; 28(2): 151-5.

[7] Meltzner GS, Hillman RE. Impact of aberrant acoustic properties on the perception of sound quality in electrolarynx speech. J Speech Lang Hear Res 2005 Aug; 48(4): 766-79.

[8] Stajner-Katusic S, Horga D, Musura M, Globlek D. Voice and speech after laryngectomy. Clin Linguist Phon 2006 May; 20(2-3): 195-203.

[9] Xi S. Effectiveness of voice rehabilitation on vocalisation in postlaryngectomy patients: a systematic review. Int J Evid Based Healthc 2010 Dec; 8(4): 256-8.

[10] Imre A, Pinar E, Calli C, Sakarya EU, Oztürkcan S, Oncel S, et al. Complications of tracheoesophageal puncture and speech valves: retrospective analysis of 47 patients. Kulak Burun Bogaz Ihtis Derg 2013 Jan-Feb; 23(1): 15-20.

[11] Hillman RE, Walsh MJ, Wolf GT, Fisher SG, Hong WK. Functional outcomes following treatment for advanced laryngeal cancer. Part I--Voice preservation in advanced laryngeal cancer. Part II--Laryngectomy rehabilitation: the state of the art in the VA System. Research Speech-Language Pathologists. Department of Veterans Affairs Laryngeal Cancer Study Group. Ann Otol Rhinol Laryngol Suppl 1998 May; 172: 1-27.

[12] Sloane PM, Griffin JF, O'Dwyer TP. Esophageal insufflation and videofluoroscopy for evaluation of esophageal speech in laryngectomy patients: clinical implications. Radiology 1991 Nov; 181(2): 433-7.

[13] Thai Speech and Hearing Association. A directory of speech pathologist, audiologist, medical scientist in audiology and audiotechnician. Bangkok: Thai Speech and Hearing Association; 2014 (in Thai).

[14] Vatanasapt P. Music for Bridging the Gaps in Cancer Care. J Urban Culture Res 2012(5): 84-90.

[15] Ferrat K, Guerti M. A study of sounds produced by Algerian esophageal speakers. Afr Health Sci 2012; 12(4): 452-8.

[16] Rosso M, Sirić L, Tićac R, Starcević R, Segec I, Kraljik N. Perceptual evaluation of alaryngeal speech. Coll Antropol 2012 Nov;36 Suppl 2: 115-8.

Journal of Associated Medical Sciences 2021; 54 (2): 8-16

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Monitoring and evaluation for quality of Thailand SARS-CoV-2 laboratory network: Lessons learnt for policy drive and new guidelines

Surasak Muenphon[1] Patravee Soisangwan[1] Nattakarn Laieddee[1*] Nutthanun Nammontri[1] Kanokwan Kittiniyom[2] Sumonmal Uttayamakul[3]

[1]Bureau of Laboratory Quality Standards (BLQS), Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.
[2]Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.
[3]Bamrasnaradura Infectious Diseases Institute, Department of Disease Control, Ministry of Public Health, Nonthaburi Province, Thailand.

ARTICLE INFO

Article history:
Received 16 November 2020
Accepted as revised 14 February 2021
Available online 15 February 2021

Keywords:
COVID-19, SARS-CoV-2 laboratory network, assessment checklist

ABSTRACT

Background: Due to the COVID-19 outbreak, Department of Medical Sciences, Ministry of Public Health has taken responsibility for SARS-CoV-2 laboratories using Real-Time RT-PCR in network accreditation. Monitoring and evaluation are important to ensure the quality of SARS-CoV-2 laboratory networks after receiving accreditation.

Objectives: This study aims to summarize and provide policy proposals and new laboratory guidelines from on-site-assessment key points to elevate lab quality and support laboratory development to meet the international standard, ISO 15189 accreditation.

Materials and methods: The coaching-type-assessment checklist for SARS-CoV-2 laboratory network was prepared by an expert team and contained 44 requirements in Analytical technique and Biological safety. It was then sent to the laboratory network nationwide for self-assessment. The assessor team used this checklist for on-site assessment from May to September 2020.

Results: On-site assessment of 38 SARS-CoV-2 laboratories showed a total of 156 nonconformities (NCs). The top three NCs in the Analytical technique requirements were 1) accommodation and environment condition (27.6%), (involved performing Pre-PCR activities in the same area as Post-PCR and unidirectional workflow), 2) laboratory equipment, reagents, and consumables (13.5%) and 3) post-examination process (13.5%). In Biological safety requirements, personal protective equipment (PPE) was the most frequently found NC with 8.3%, and involved area constraints with no suitable places to put on or remove PPE.

Conclusion: The monitoring and evaluation for the SARS-CoV-2 laboratory network in Thailand has been developed according to the PCR standards both in terms of management and technical including biological safely for improving and developing the quality of SARS-CoV-2 laboratory network response to an emerging situation. To strengthen the quality of laboratory network in Thailand and preparedness to emerging situation, integrated tools are proposed including laboratory's self-assessment (coaching-type checklist), the proficiency testing (PT) programs, re-accreditation, special assessment from particular request and organizing training to disseminate knowledge to all network laboratories. In addition, this system may apply for other emerging diseases in the future and should encourage laboratories to continue for the ISO 15189 accreditation.

* **Corresponding author.**
Author's Address: *Bureau of Laboratory Quality Standards (BLQS), Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.*
** **E-mail address:** nattakarn.l@dmsc.mail.go.th
doi: 10.14456/jams.2021.11
E-ISSN: 2539-6056

Introduction

Due to the COVID-19 outbreak, Department of Medical Sciences, Ministry of Public Health has taken responsibility for laboratories implementing SARS-CoV-2 testing. The project named "one laboratory one province" has been launched to develop Laboratories with SARS-CoV-2 testing in every provinces of Thailand serving for diagnosis, treatment, monitoring, disease investigation and surveillance preparedness for peoples of the whole country. The Department of Medical Sciences was the host for work integration between internal and external organizations in establishing the network system for laboratories implementing SARS-CoV-2 testing using Real-Time RT-PCR.[1] The laboratories requesting laboratory network accreditation, need to pass assessment by the Department of Medical Sciences in the capacities of personnel, instrument, equipment, reagents, in a BSL 2 enhanced facility and proficiency testing by giving correct reports for all PT samples provided. Only laboratories in the accredited list of SARS-CoV-2 laboratory networks could join the program of the National Health Security Office (NHSO) for supporting lab analysis and PPE costs. Currently, over 200 laboratories have been accredited leading to rapid diagnosis, infection control and prevention by timely management. However, to ensure the quality of laboratories in the network after receiving accreditation, a monitoring team has been set up to play roles in lab evaluation for re-accreditation and for special assessment on particular request after discordant results from reference labs, and to support laboratories to meet the international standard, ISO 15189 accreditation. During May 2020 to September 2020, there were 4 lab assessments based on particular request, 12 labs with random assessment and 22 labs co- assessed with the National Health Security Office (NHSO). Lab assessment activities brought up the key points that could strengthen the network through development of policy and new guidelines such as reducing test sensitivity by using autolysis in the extraction step, risk of contamination from lab workspace and workflow designs, knowledge and understanding while interpreting test results, management guidelines for the many samples processed during an outbreak, arrangement of examination round, incorrectly opening the sample box outside the BSL2 enhanced room, and putting on and taking off PPE in limited spaces

Objective

1. To summarized assessments and outline the policy proposal and new laboratory guidelines, from key points of on-site assessment, that will strengthen the SARS-CoV-2 laboratory network using real-time RT PCR in Thailand.

2. To support laboratory development to meet the international standard, ISO 15189 accreditation.

Materials and methods

1. The monitoring and evaluation team for the SARS-CoV-2 laboratory network was appointed by dividing into 2 subgroups; 1) expert team (10 members) and 2) assessor team (67 members). The expert team was composed of representatives from the Department of Medical Sciences, specialists from the Faculty of Medicine Siriraj Hospital and representatives from the National Health Security Office (NHSO). Assessor team were assessors from the Department of Medical Sciences, Reginal Medical Sciences Center nationwide, Bamrasnaradura infectious diseases institute, Rajavithi Hospital, and Faculty of Medicine Ramathibodi Hospital.

2. The coaching-type-assessment checklist for the SARS-CoV-2 laboratory network was prepared by the expert team and contained 44 requirements in Analytical technique and Biological safety. Among these, 24 requirements were "The must" representing the critical points with high impact to the correction of analytical results and/or biological safety such as well-trained and knowledgeable personnel, separate designated rooms for Pre-PCR and Post-PCR, and appropriate cleaning for preventing contamination. The laboratories in the network must follow all "The must" requirements. The rest of the requirements can be added in the plan/ directives for improvement with a specific due date in the case of not being able to perform or only partially perform. The outcome of this checklist was of the semi-coaching type with detailed guidelines that laboratories could directly use in practice.

3. The coaching-type- assessment checklists were sent to the laboratory network nationwide for self-assessment, improving and fulfilling the requirements.

4. The assessor team used the coaching-type- assessment checklist for on-site assessment from May 2020 to September 2020. A total of 38 laboratories were assessed by divided into 3 categories; 1) special assessment from particular request according to the order of the Director-General of the Department of Medical Sciences for 4 laboratories, 2) random assessment for 12 laboratories, and 3) co-assessment with the National Health Security Office (NHSO) for 22 laboratories.

5. The expert team analyzed the assessment results and summarized into a policy proposal and new guidelines for the SARS-CoV-2 laboratory network in Thailand.

Results

The assessment checklist

The coaching-type-assessment checklist for the SARS-CoV-2 laboratory network in Thailand waspproposed by the expert team with a total of 44 requirements covered Analytical technique and Biological safety. 24 of the 44 were "the must" requirements. The Analytical technique part was composed of 8 topics arranged in order for the analytical process; 1) personnel, 2) accommodation and environment conditions, 3) laboratory equipment, reagents, and consumables, 4) pre-examination process, 5) examination process, 6) quality control, 7) post-examination process, and 8) interpretation and reporting of results. The Biological safety part consisted of 3 topics; 1) procedure document, 2) personal protective equipment (PPE) and 3) tools and other equipment. An example of a coaching-type-assessment checklist for SARS-CoV-2 laboratory network in Thailand is shown in Table 1 and the Thai version can be downloaded by QR code.

Table 1 Example of the coaching-type-assessment checklist for a SARS-CoV-2 laboratory network in Thailand.

No.	Requirement	Evidence	Recommendations for the assessment
	Analytical technique requirements		
	1. Personnel		
1 The must	A list of personnel who have been assigned to inspect SARS-CoV-2 as a medical technician /medical scientist who have experience in biomolecular examination and biosafety	>Contact list >Education background >Biomolecular experience (… Years) >Biosafety experience	Random inspection of personnel files/ Focused on new personnel/Observe performance/Interview/ In the case of a scientist, it can be performed under supervision of a medical technician or a medical scientist
	2. Accommodation and environment conditions		
2 The must	Semi-Automated PCR Separated areas to prevent cross-contamination by separating into 2 parts: Pre-PCR and Post-PCR reaction as follows: 1. Pre-PCR must provide a working area divided into 2 parts: • Master mix preparation room • Sample preparation and extraction of genetic material room In the case that there is 1 Pre-PCR room, separate activities and must not be performed at the same time as follows: 1) Prepare the master mix solution in a PCR cabinet with UV lamp. 2) Prepare and extract samples of genetic material in a Biosafety cabinet (BSC) Class II by cleaning the area and equipment before and after each operation with the appropriate reagents and methods. For example, 1% sodium hypochlorite for 30 minutes and then wipe off with clean water, 70% ethanol for 10 minutes followed by at least 30 minutes of UV exposure, RNA-destroying liquid, etc. 2. Provide space/area for Post-PCR set up real-time PCR machines for increasing genetic content and analyzing test results. - Organize the workflow chart in one direction from Pre-PCR to Post-PCR - Separate PPE sets, material equipment.	PCR Laboratory layout, instrument layout and direction of wind source/ workflow path	Investigation >Laboratory layout requires separated Pre-PCR and Post-PCR rooms. >Equipment layout / workflow path / practice at every step must ensure that it does not lead to contamination In the case that there is 1 Pre-PCR room, there must be clear procedures using separate times to perform each activity. Avoid preparing Master Mix reagents and preparing samples and extracting genetic material at the same time.
	Biological safety requirements (Additional)		
	2. Personal Protective Equipment (PPE)		
38	>Use a waterproof, disposable lab coat (front fastening type), or a lab coat to be covered with a plastic apron >Appropriate handling methods for lab coats, such as storage and proper disposal. >Provide an appropriate lab coat changing room.	Attach picture	

Opportunity for improvements from the on-site assessment

On-site assessment of 38 SARS-CoV-2-testing laboratories by the assessor team using the 44-requirement checklist found a total of 156 nonconformities (NCs) which enabled the opportunity for improvement. The top 3 NCs in the Analytical technique requirements were 1) accommodation and environment conditions (27.6%) (Table 2, Figure 1), 2) laboratory equipment, reagents, and consumables (13.5%) and 3) post-examination process (13.5%). In Biological safety requirements, personal protective equipment (PPE) was the most frequently found NC with 8.3% (Table 2, Figure 1).

Table 2 Nonconformity from on-site assessment of 38 laboratories using the coaching-type-assessment checklist for SARS-CoV-2 laboratory network in Thailand.

Order	Requirement	Number of NC*	Percentage of NC*
I. Analytical technique requirements			
1.	personnel	17	10.9%
2.	accommodation and environment condition	43	27.6%
3.	laboratory equipment, reagents, and consumables	21	13.5%
4.	pre-examination process	8	5.1%
5.	examination process	15	9.6%
6.	quality control	5	3.2%
7.	post-examination process	21	13.5%
8.	interpretation and reporting results	3	1.9%
II. Biological safety requirements (additional)			
1.	procedure document	5	3.2%
2.	personal protective equipment (PPE)	13	8.3%
3.	tools and other equipment	5	3.2%

**NC = nonconformity*

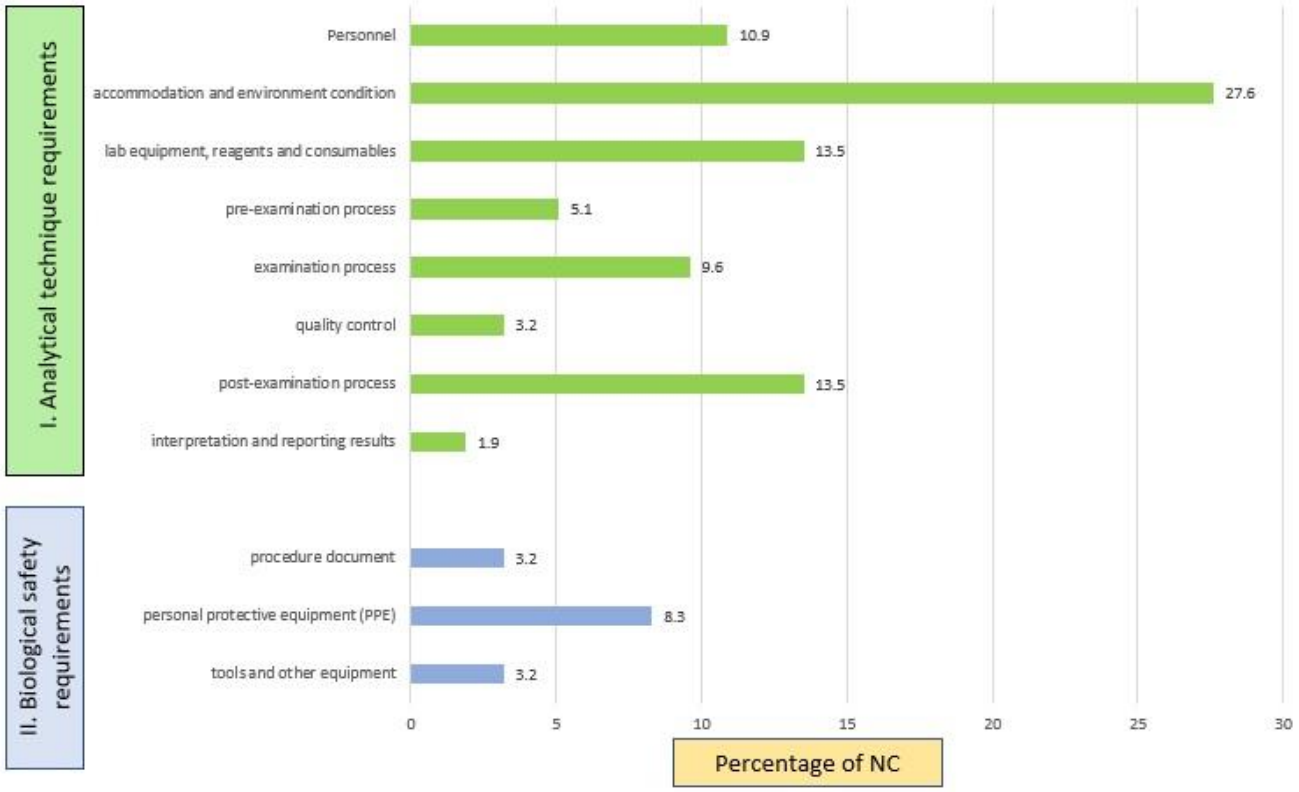

Figure 1. *Percentage of nonconformity from on-site assessment of 38 laboratories using the coaching-type-assessment checklist for SARS-CoV-2 laboratory network in Thailand where it was classified requirements into 2 parts: 1) Analytical technique (green bar) and 2) Biological safety (blue bar).*

Policy proposals and new guidelines

Key points of nonconformity (NC) from on-site assessment of 38 laboratories are listed in Table 3 along with the practical guidelines and suggestions for problem solving and lab improvement. 17 observed key points from both sections, Analytical technique and Biological safety, were analyzed for the causes of problems (Table 3). Three main causes were classified as A) insufficient knowledge (7 key points), B) inappropriate management (4 key points) and C) inappropriate quality of test and control (6 key points). These issues could be guides for developing policy proposals and strengthening the SARS-CoV-2 laboratory network in the future.

Table 3 Key points of nonconformity (NC) from on-site assessment, assessed causes and suggestions for lab improvement.

Observed key point and (cause of problem)	Guideline/Suggestion
Section I: Analytical technique	
Personnel	
1. Practitioners lack knowledge and understanding of the main issues such as result interpretation, and prevention from contamination causing false positive. (A: insufficient knowledge)	1. Provide online training course for the staff of SARS-CoV-2 testing laboratories including the important topics both analytical technique and laboratory safety such as analysis of SARS-CoV-2 by Real Time RT-PCR, sample preparation, extraction of genetic material, reagent preparation, examination process, interpretation and reporting following the guideline of Ministry of Public Health, performing of internal quality control (IQC)/proficiency testing (PT) and solving the problem in the case that the result is out of the acceptable criteria, management and prevention of contamination for PCR, managing/ wearing and removal of PPE, etc. 2. Provide the channel for questions and answers between the laboratory and the expert team by online coaching.
2. During the epidemic period, a large number of samples causing the workload overload of staff. (B: Inappropriate management)	Fatigue from continuous working 24 hours a day during heavy outbreaks may cause an error in the result. The Department of Medical Sciences therefore issues recommendations to organize no more than 3 examination rounds per day in order to allow personnel to have time to rest. It also has time to clean the laboratory and all systems to prevent contamination.
Accommodation and environment conditions	
3. Designing of laboratory area was not suitable; Pre-PCR and Post-PCR were in the same area causing contamination risks such as preparing Master mix reagents and adding a template which was Pre-PCR part in the room where the PCR machine was located as part of Post-PCR includes non-directional workflow from Pre-PCR to Post-PCR. (A: insufficient knowledge)	The PCR room layout depends on the type of equipment used and is divided into 2 types as follows[2]. I. Semi-Automated PCR The laboratory shall use the separated room to prevent Cross-contamination, divide the area into two parts including the Pre-PCR and Post-PCR as following; 1. Pre-PCR, the laboratory shall provide the area divided into two parts; • room for preparing of Master mix reagent • room for sample preparation and extraction But in case of the laboratory have 1 room for Pre-PCR, the laboratory shall separate the following activities and shall not process at the same time. 1.1) Master mix reagent preparation in PCR cabinet which has UV 1.2) sample preparation and extraction in BSC Class II. Clean the room and equipment every time before and after work using suitable disinfectants such as 1% sodium hypochlorite for 30 min, then clean with water or 70% ethanol for 10 min, after that using the UV light at least 30 min or using RNA destructive solution, etc. 2. Post-PCR is the area for the installation of a Real-time PCR analyzer, amplification, and analysis of results.

Table 3 Key points of nonconformity (NC) from on-site assessment, assessed causes and suggestions for lab improvement. (continued)

Observed key point and (cause of problem)	Guideline/ Suggestion
	Separate PPE and equipment for each room. Provide directional workflow from Pre-PCR to Post-PCR. II. Fully Automated PCR The laboratory can prepare the sample in BSC Class II and use Fully Automated PCR to prepare reagent, extraction of RNA, RNA amplification, and analysis in the same analyzer, no need to separate the room. But in case of the analyzer doesn't have the system to prevent the amplicon, the laboratory shall separate the sample preparation room and the PCR room.
4. The doors and walls of the room were blacked out. Can't see the operator inside. (A: insufficient knowledge)	The laboratory shall have the part visible to the operator from the outside. The door can be closed to prevent unauthorized persons and designate the authorized persons who have the right to enter and exit[3,4].
5. The cleaning staff had no knowledge of PCR laboratory cleaning. (A: insufficient knowledge)	Since PCR testing has a chance of contamination from the sample or PCR product that may be left in the room, so cleaning the floor must be careful to not cause diffusion, such as using a dust mop instead of a broom, separate cleaning equipment for each area and cleaning staff must be trained to clean the molecular biology laboratory including prevention of infection.
6. There were no records of those who accessed the laboratory. (B: Inappropriate management)	A laboratory is a place where is a risk of exposure to infection. Therefore, the name, contact address, and the time of being in the laboratory of those who contacted must be recorded. Whether it is an assessor, equipment maintenance technicians, etc. So that the laboratory can follow up in the case of suspected infection in the laboratory.
7. The BSC cabinet was installed in an inappropriate location, causing the air in front of the cabinet to be disturbed by an air conditioner. (A: insufficient knowledge)	To reduce diffusion of samples or PCR products in a BSC cabinet, BSC cabinet placement should be positioned and directed away from doors, windows, corridors, fans, air conditioners, fume hoods and other types of air sources to ensure that the outside wind does not disturb the BSC cabinet air curtain.
Laboratory equipment, reagents, and consumables	
8. Use a wide variety of brands of reagent and extraction kit without verification before use. (C: Inappropriate quality of test and control)	Select reagents and test kits which are registered with the Food and Drug Administration (FDA) and the reagent shall be verified before use with the parameters of analytical sensitivity and specificity. But if there is no such verification result, IQC results and PT results may be used instead, and PT results must be verified as current reagents.
Pre-examination process	
9. Open the sample container outside the BSL-2 Enhanced laboratory. (A: insufficient knowledge & B: Inappropriate management)	Opening of the sample container must be done in a BSC Class II cabinet with the corrected technique in a provided room. Staff must wear the correct and suitable PPE. Use a waterproof, disposable lab coat or use a lab coat cover with a plastic apron, a hair cap, N-95 face-shield, double gloved, closed toe shoes (Practices like BSL3 practice). In case of a large number of samples, other room spaces may be used. There must be a hanging sign to warn those who are not involved in the work area. Also, the sample container must be opened in a BSC Class II cabinet, as well as to wear the correct PPE as required.

Table 3 Key points of nonconformity (NC) from on-site assessment, assessed causes and suggestions for lab improvement. (continued)

Observed key point and (cause of problem)	Guideline/ Suggestion
Examination process	
10. Autolysis was used instead of extraction of genetic material. Making it unsure whether to extract RNA or not. (C: Inappropriate quality of test and control)	Since the evaluation of the test kits and reagents related for the diagnosis of SARS-CoV-2 infection by Real-time RT-PCR method for registration with the Food and Drug Administration (FDA) is covered only the test using extracted RNA sample, Extraction reagent and autolysis step is not included in that evaluation. Therefore, if the manufacturer/ importer refered to Autolysis they must notify the FDA and Department of Medical Sciences to evaluate whether if it covers such procedures. Therefore, if there is no evaluation result, the laboratory shall extract the RNA from the specimen for PCR reaction.
11. There were modifications of the method, which may affect the sensitivity of the test. (C: Inappropriate quality of test and control)	- In some cases, the laboratory may be advised by the staff of the company that is selling reagents to modify test methods to reduce the process and time, but it does not comply with the manufacturer's instruction. Without supported validation, it may decrease sensitivity of the assay, and this may cause a false negative. So the laboratory must strictly follow the protocol specified in the package instructions. - Communication and insisting on an understanding of the staff of the reagent companies are needed in order to provide correct laboratory advice
12. Examination by pooled samples without validation results. (C: Inappropriate quality of test and control)	The pooled sample will cause the sample to be diluted and the genetic material will be reduced. There is a high probability of false-negative effects. Therefore, testing by using a pooled sample must always have the results of the method validation.
Quality control	
13. Using a reagent that had not passed Proficiency Testing with the Department of Medical Sciences (C: Inappropriate quality of test and control)	PT is an important tool in evaluating the laboratory competency. The lab must clearly identify each batch of reagents. If there is a change in the reagent, the laboratory shall inform the Department of Medical Sciences to evaluate the proficiency test (PT) with a new set of reagents before using it.
14. The laboratory used external QC to control the quality of the PCR process. Therefore, the sample quality should also be examined. (C: Inappropriate quality of test and control)	In quality control for Semi-Automated PCR there must be a performed positive control and non-template control for each test. And, where possible, internal control (housekeeping genes) should be performed to examine sample quality. Because the virus multiplies in the cell, the cells also carry housekeeping genes, so when the housekeeping gene is detected, it can indicate that the sample is of quality because the infected cells can be collected[5].
Post-examination process	
15. The laboratory translated results only from the machine, did not neither read the graph nor look at the Ct range for the interpretation of positive control. (A: insufficient knowledge)	Interpretation of results according to "Guidelines for the management of the laboratory testing and reporting system for COVID-19 with a single laboratory system (DMSc_P05)"[6] of the Department of Medical Sciences. Up-to-date version, which set the guidelines as follows

Table 3 Key points of nonconformity (NC) from on-site assessment, assessed causes and suggestions for lab improvement. (continued)

Observed key point and (cause of problem)	Guideline/ Suggestion
	1) Confirm the results of the gene test at least 2 positions 2) Ct value not more than 36, if it exceeds, the lab shall repeat test by starting the process of extraction of new genetic material, if the result is positive, then the report can be released. 3) Interpretation of the result should also consider the curve, which should look like a S-shaped curve or sigmoid curve, as shown in the Figure 2. However, the user should study the instructions given in the test package and follow it strictly.
16. Incomplete reporting information, unspecified reagent name, target gene with Ct value, and summary method, etc. (B: Inappropriate management)	The report shall identify the name of the reagent, target gene with Ct value and the method of summarize of result as set in "Guidelines for the Management of the Laboratory Testing and Reporting System for Covid-19" With a single laboratory system (DMSc_P05)"[6] of the Department of Medical Sciences.
Section II: Biological safety	
17. No appropriate designated area for put on and removal of PPE due to area constraint (B: Inappropriate management)	Lab should provide area for dress up PPE before entry in the working area. In case of space limitation and no outside lab area available, should set up clean corner in the lab for putting on PPE and provide area for taking off PPE and discarding into the infectious waste bin before exiting the lab. However, should provide separate room for PPE removal with hand washing sink in the specimen preparation area followed BSL-2 enhance.

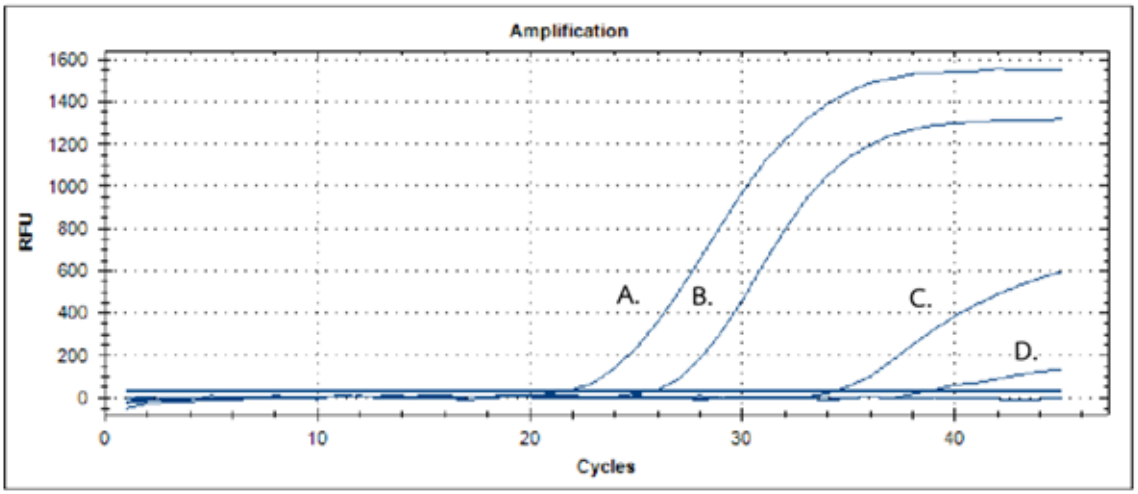

Figure 2. *Curves of SARS-CoV-2 Ct by Real-time RT-PCR method. A: S-curve, Ct 21.41, B: S-curve, Ct 25.50, C: S-curve, Ct 34.23, D: no S-curve format, Ct 38.56.*

Discussion and Conclusion

This coaching-type-assessment checklist for SARS-CoV-2 laboratory network used in Thailand was developed based on the main idea to use this tool for assessment and for practical coaching at the same time. Therefore, each requirement in the checklist also contained the detail of practice guidelines that could be directly applied for use in the laboratories. This advantage could help in rapid lab setup in response to an emergency situation. This checklist, specifically designed to assess capacities of laboratories implementing SARS-CoV-2 testing, was more specific than the WHO questionnaire which focused on overall management at the nation level and competency of lab analysis.[7,8]

The results from on-site assessment in 2020 of 38 laboratories from the total of 237 laboratories with SARS-CoV-2 testing in Thailand showed the main opportunity of improvement (OFIs) for 156 NCs. The most OFI in the Analytical technique part was accommodation and environment conditions 43 NCs (27.6%). The main cause was not the insufficient workspace but from insufficient knowledge and understanding of practitioners such as no separation of Pre- and Post-PCR working areas. The Pre-PCR processes such as master mix preparation and template adding were performed in the room with the PCR machine which counted as Post-PCR area. Also, inappropriate workflow by overlapping between Pre-PCR and Post-PCR activities may lead to cross contamination. In the Biological safety part, the most OFI found was requirements of personal protective equipment (PPE) 13 NCs (8.3%). Most OFIs were related to inappropriate designated area for putting on and taking off PPE. The suggestions for this issue were; 1) should provide area for dress up in PPE before entry to the lab, 2) in case of limitation of space with no outside lab area, should set up clean corner in the lab for putting on PPE and provide area for taking off PPE and then drop into infectious waste before exiting the lab.

Most key points from on-site assessment referred to the main causes of incidents focusing on insufficient knowledge, inappropriate management and inappropriate quality of test and control. Solutions are policy proposals and additional guideline development to improve the quality of the SARS-CoV-2 laboratory network such as a SARS-CoV-2 laboratory guidance document in a Question & Answer format, and online training for nation-wide SARS-CoV-2 laboratories and related stakeholders such as assessors, sale representative, etc. to strengthen the quality of SARS-CoV-2 laboratory network. Many tools were recommended and could be used together. The African Society for Laboratory Medicine recommended use of 3 tools including Quality Control (QC), External Quality Assessment (EQA) and Quality Improvement (QI).[9] WHO proposed the Laboratory Assessment Tool.[8] The nation-wide laboratory network in Taiwan was rapidly established and kept monitoring to achieve reports within 24-hours for infection control efficiency but quality assurance was not clearly mentioned.[10] For Thailand, the committee proposed a policy by using tools that covered all dimensions which were 1) self-assessment checklist, 2) proficiency testing program, 3) on-site assessment for re-accreditation and special assessment on particular request due to the quality of report and 4) training/ coaching program for the entire laboratory network. All integrated tools could be implemented to strengthen the SARS-CoV-2 laboratory network in Thailand and ensure the quality of analytical results to use for diagnosis, surveillance, control and prevention of diseases with efficiency and rapid response. In addition, this molecular detection system may be applied for use for other emerging diseases in the future and should further encourage well-prepared laboratories to apply for international standard ISO 15189.

References

[1] Department of Medical Sciences. Ministry of Public Health. Manual for Diagnosis of Coronavirus Disease 2019 (COVID-19) for SARS-CoV-2 Laboratory. [in Thai]. https://www3.dmsc.moph.go.th/post-view/700. accessed on 1 April 2020.

[2] Department of Medical Sciences. Ministry of Public Health. Guidelines for establishing a COVID-19 laboratory. DMSc_P06 Revision 1. 16 July 2020. [in Thai].

[3] Ministry of Public Health. Pathogens and Animal Toxins Act. Published in the Government Gazette, 2015; 132(Part 80a): 9.

[4] Ministry of Public Health. Notification of the place of produce or possess and the operation on Pathogens and Animal Toxins Act. [in Thai]. Published in the Government Gazette, Vol. 137, Part 117d, Page 44, date 19th May B.E. 2563. (2020).

[5] Department of Medical Sciences. Ministry of Public Health. Pitfall for detection of SARS-CoV-2 by real-time RT PCR. ISBN 978-616-11-4482-1; 2020. [in Thai].

[6] Department of Medical Sciences. Ministry of Public Health. Guidelines for the management of laboratory testing and reporting of COVID-19 with a single-lab system. [in Thai]. DMSc_P05 Revision 1. 23 March 2020.

[7] World Health Organization. Assessment tool for laboratories implementing SARS-CoV-2 testing: Interim guidance. Geneva: World Health Organization; 2020.

[8] World Health Organization. Laboratory Assessment Tool. Geneva: World Health Organization; 2012.

[9] Guidance on Quality Assurance for COVID-19 Molecular Laboratory Testing. African Society for Laboratory Medicine.

[10] Ji-Rong Yang, Ming-Tsan Liu, Hsin-I Huang, Hwa-Jen Teng, Jou-Han Chen, and Shu-Ying Li. Building the National SARS-CoV-2 Laboratory Diagnostic Capacity in Taiwan. Health Security 2020; 18(5): 383-91. doi: 10.1089/hs.2020.0056.

Journal of Associated Medical Sciences 2021; 54 (2): 17-21

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

A survey of radiation released from patients treated with radioiodine-131 therapy

Chaisunthorn Wisetnan[1] Patamaporn Molee[2*] Panatsada Awikunprasert[2] Khajornkiat Srichachet[3] Vithit Pungkun[4]

[1]Division of Nuclear Medicine, Department of Diagnostic Radiology and Nuclear Medicine, Udonthani cancer Hospital, Udonthani Province, Thailand.
[2]Department of Radiological Technology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand.
[3]Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen Province, Thailand.
[4]Ionizing Radiation Metrology Group, Office of Atoms for Peace, Bangkok, Thailand.

ARTICLE INFO

Article history:
Received 11 January 2021
Accepted as revised 2 May 2021
Available online 2 May 2021

Keywords:
I-131, sewer systems, OSL

ABSTRACT

Background: Radioactive iodine 131 (I-131) is used as an alternative to treat thyroid cancer. Patients receiving I-131 must be separated in provided hospital rooms until radiation level falls below the specified threshold. The knowledge of the amount of radiation in patient rooms along with outlying areas, together with the building's sewer systems will help monitoring and controlling the radiation hazard.

Objectives: This study was conducted to investigate the radiation exposure from ward of patients treated with I-131, and the effects upon general public.

Materials and methods: OSL devices were placed on the outer surface of sewer line and external walls of patient rooms. Accumulated radiation was measured for a period of one month.

Results: The results showed that radiation exposure from I-131 patient rooms located on the 5th floor of Srinagarind Hospital was 7.24 µSv/hr. However, the radiation detected from both sides of drainage pipe were unequal. Radiations on the 2nd, 3rd, 4th, and 5th floors were 1.70, 1.28, 2.97, and 7.24 µSv/hr, respectively.

Conclusion: It could be concluded that accumulated radiation in a single year exceeded the ICRP specified limit and poses a safety hazard for staff and general public. Recommendations to rectify the problem included increasing awareness of staff and public through warning signs, as well as adding of lead shields surrounding the patient rooms. Nonetheless, further measurements should be performed again after reconstruction.

Introduction

Radioactive iodine-131 (I-131) is orally or intravenously administered to patients for the treatment of hyperthyroidism and thyroid cancer. The radiation dosage for hyperthyroidism treatment is about 1 GBq, while treatment of thyroid cancer patients requires a higher I-131 radiation dosage of about 3-6 GBq.[1] During the period in which patients are admitted as hospital in-patients, the measured dose rate outside the room or public area should not be higher than 6 µSv/hr. To protect the public, it is required to isolate these patients until the retained radioactive drug in the patient's body is below 1 GBq, or the measured dose rate from the patient is lower than 5 µSv/hr at one meter. The maximum activity at which a patient is allowed to return home depends on the national practice and individual patient's condition, where it usually ranges between 0.2 and 1 GBq.[1]

The excretion of radioiodine from the patient subsequently enters the hospital's sewer system as waste, which is carried away from the patient ward and may be harmful to staff, relatives, patients, and the general public.[2, 3] There are various recommended methods for the management of these kinds of waste. The most convenient method is to dispose the

* **Corresponding author.**
Author's Address: *Department of Radiological Technology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand.*
** **E-mail address:** patamaporn.m@nmu.ac.th
doi: 10.14456/jams.2021.12
E-ISSN: 2539-6056

patient's excretion of radioiodine directly into the public sewer system. However, the International Commission on Radiological Protection (ICRP) (1990) has suggested to reduce the dose disposed to the public sewer system to an acceptable level.[4] Therefore, the I-131 treatment ward and the radioactive waste water management system must be specially designed and certified to meet the national radiation safety standard by Office of Atoms for Peace (OAP), Thailand. Moreover, hospital's sewer systems that have been used for a long period of time are subjected to deteriorate and require regular maintenance.

The physical properties of I-131 include its decay through beta and gamma emissions. The decayed beta particles produce a maximum energy of 606 keV (89% abundance, 248-807 keV), and the 364 gamma-ray emits 81% abundance and 723 keV.[2, 5-7] Because beta particles have a shorter range in tissue, the ionizing effects of beta-radiation are restricted to the cancer cells. However, high-penetrating gamma radiation can escape the patient's body, leading to unwanted exposure to those around them.[3, 7-9]

Our study aims to investigate the radiation exposure from the radioiodine therapeutic ward. I-131 is released from the treated patient into the environment, such as building, corridor, staff room, and untreated patient rooms, as well as the sewage waste drainage system.[2, 3, 9] OSL dosimeters were used to measure radiation emitted from the I-131 therapeutic patients' ward.

Materials and methods

InLight® dosimetry systems using optically stimulated luminescence (OSL) were installed at 17 points, two pieces each, totaling to 34 pieces. This provides users with reliable and accurate radiation monitoring (Figure 1). The system measures radiation via aluminum oxide doped with carbon (Al_2O_3: C), and the detectors read optically through stimulated luminescence.[10, 11] The OSL devices were mounted on 1) corridor, staff room, and patient rooms adjacent to the rooms treated with I-131 on the 5th floor; 2) sewer line connected to the exposed rooms; and 3) patient rooms and adjacent rooms on the 4th floors of the building in Srinagarind Hospital. The accumulated radiation was measured for a period of one month. InLight Automatic Reader® (Ionizing Radiation Metrology Group, Office of Atoms for Peace, Bangkok, Thailand) was used to measure the radiation received by the OSL dosimeters (Figure 2).

Figure 1. *OSL dosimeters installed on different places to measure radiation doses.*

Figure 2. *InLight Automatic Reader®.*

Results

Twenty-eight patients, between the ages of 17 and 78 years were treated with radioactive, I-131 at levels between 3.7 - 7.4 GBq. The total radiation activity was approximately 166.5 GBq. Table 1 shows that the exposed radiation level from the I-131 patient room located on the 5th floor was between 0.09-5.21 mSv/month, or 0.12-7.24 µSv/hr (Figure 3). The amount of radiation from both sides of the sewer drainage pipe were unequal. Radiation results on the 2nd, 3rd, and 4th floors were between 0.19-2.14 mSv/month, or 0.26-2.97 µSv/hr (Figure 4).

Table 1 Maximum radiation levels measured in different layers.

Area	Staff room mSv/mo.	Patient room mSv/mo.	Corridor mSv/mo.	Patients who do not receive I-131 mSv/mo.	Waste drainage area mSv/mo.	
					Left wing	Right wing
5th floor	1.48	5.21	0.47	0.09	n/a*	n/a*
4th floor	n/a*	n/a*	n/a*	0.46	0.73	2.14
3rd floor	n/a*	n/a*	n/a*	n/a*	0.28	0.92
2nd floor	n/a*	n/a*	n/a*	n/a*	0.19	1.23

**n/a means: no measurement according to no specified area on that floor.*

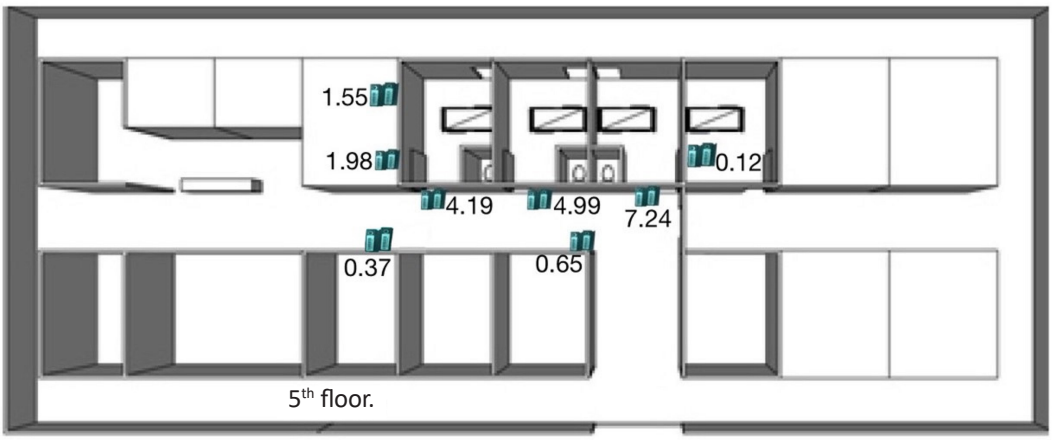

Figure 3. *Placement of OSL plates used to measure radiation on the 5th floor.*

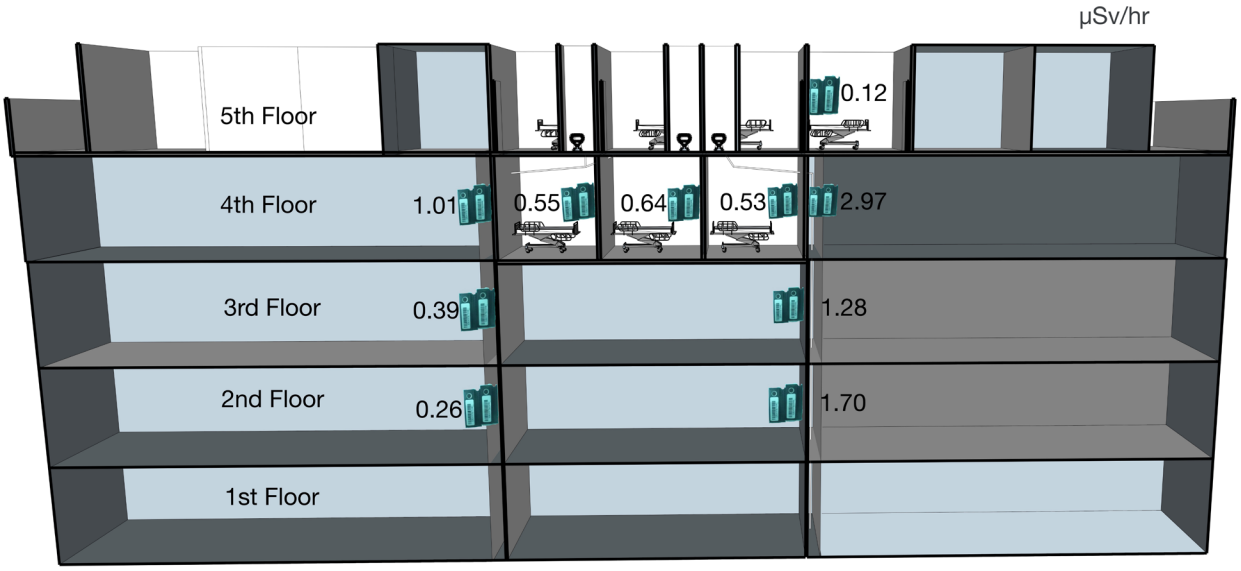

Figure 4. *Positions of OSL plates used to measure the amount of radiation along the sewer line and patient rooms adjacent to I-131 treated rooms on the 2nd through 5th floors.*

Discussion

The cancer treatment ward at Srinagarind hospital is located on the 5th floor of a five-story building. Thyroid cancer patients receiving I-131 treatments are distributed into six patient bends in three rooms. Patients treated with high levels of radioactive I-131 generally remain as in-patients until their radiation level is lower than 5 μSv/hr at 1 meter.[2, 6, 8] In 2017, a total of 275 patients received radiation doses total of 1,472 GBq/year. This high radiation activity may be harmful to people and the environment. Our study examined the radiation levels emitted from radiotherapy patients and their effects upon adjacent areas. We determined that patient rooms involved in the administration of I-131, along with the connecting sewage system were not designed to prevent the ramifications of the radiation therapy. Our study, which we believe to be the first to employ OSL measurements in radiation detection, strives to identify as well as offer solutions to these radiation levels; which can be harmful to staff, relatives, and the general population.

In 2014, Memon SA, *et al*. had conducted a study to record and examine the exposure rates of I-131 to the general public in the corridors and within the non-radioactive patients in adjacent rooms; calibrated by an LAMSE RM1001-RD survey meter. The average exposure rate in the corridors was about 5.17 μSv/hr (2.14 μSv/hr to 8.15 μSv/hr), and the cumulative exposure level of nonradioactive patients residing in adjacent rooms was 0.647 mSv (0.192 mSv to 1.664 mSv). The exposure rates to the general public, especially those non-radioactive patients admitted in adjacent rooms, were slightly beyond the acceptable limit (1 mSv) as specified by both national and international standards.[2]

The current study determined that the adjacent, untreated I-131 patient rooms and staff rooms on the 2nd to 5th floors of Srinagarind Hospital, as well as the associated waste pipeline, exhibited levels of radiation in excess according to the ICRP recommended limits. Levels affecting the public (>1 mSv/y) and the hospital staff (>20 mSv/y)[12] have prompted the necessity to advise them of the associated health concerns, specifically to children and women during pregnancy or breastfeeding; and to reduce radiation exposure levels,[2, 3, 6, 7, 9] as provided by the guidelines and restrictions provided by the IAEA (Safety Report Series 63).[13]

Conclusion

The accumulated results of the radiation exposure determined through our study, ranging from 1.08 - 62.52 mSv/y, exceed the safety limits recommended by the ICRP, which produce an unsafe environment for other patients, staff, and general public. We recommend that lead shields should be added to each thyroid cancer patient room, and warning signs should be posted to alert the general public. We further recommend that radiation exposure measurements should be carried out regularly along with associated water leakage (through sewage) inspections.

References

[1] Manual on Therapeutic Uses of Iodine-131. Vienna: INTERNATIONAL ATOMIC ENERGY AGENCY.

[2] Memon SA, Laghari N, Qureshi ST, Ahmad A, Khan A, Hussain M. Public exposure from I-131 hospitalized isolated patients in NIMRA Jamshoro Pakistan. International Journal of Cancer Therapy and Oncology. 2014;2.

[3] Tavakoli MB. Radioactive discharge from patients with thyroid cancer under 131l treatment and its safe disposal to the public sewer system. 2005; 9: 38-41.

[4] 1990 Recommendations of the International Commission on Radiological Protection. Ann ICRP. 1991; 21(1-3): 1-201.

[5] Cayir D. Radioiodine Therapy of Benign Thyroid Diseases. International Journal of Nuclear Medicine Research. 2017; 4.

[6] Kim CB, Jung JW, Jeong KH, Ahn B-C, Lee HH, Lee J. Measurements and prediction of the ambient dose rate from patient receiving radioiodine administration after thyroid ablation. Radiation Protection Dosimetry. 2012; 151(1): 158-61.

[7] Hamizah N, Juliana M, Waidi A, Isa SNI, Ahmad Z. Surface Contamination in Skin and Room during Hospitalization of Thyroid Cancer Patient Receiving Radioiodine Ablation. IOSR Journal of Dental and Medical Sciences (JDMS). 2012; 2: 27-33.

[8] Dehkordi F, Rasuli B, Mahmoud-Pashazadeh A. An evaluation of deviation from the international atomic energy agency-international commission on radiological protection proposed equation for calculation of radiation dose rate emanating from the patients with differentiated thyroid cancer undergoing radioiodine (I-131) therapy. World Journal of Nuclear Medicine. 2017; 16(2): 150-5.

[9] Zehtabian M, Dehghan N, Danaei Ghazanfarkhani M, Haghighatafshar M, Sina S. Measurement of the Dose to the Family Members Taking Care of Thyroid Cancer Patients Undergoing I-131 Therapy in Nuclear Medicine Using TLD-100. Radiation Protection Dosimetry. 2017; 174(4): 541-4.

[10] Sudchai W, Sa-Ngan-Sat A. Inlight Optically Stimulated Luminescence for Occupational Monitoring Service in Thailand. Progress in Nuclear Science and Technology. 2012; 3: 94-6.

[11] Nagase Landauer. InLight® systems & OSL TECHNOLOGY: Nagase Landauer, Ltd.; [2020 December 23]. Available from: https://www.nagase-landauer.co.jp/english/inlight/technology.html.

[12] The 2007 Recommendations of the International Commission on Radiological Protection. ICRP publication 103. Ann ICRP. 2007;3 7(2-4): 1-332.

[13] Release of Patients After Radionuclide Therapy. Vienna: INTERNATIONAL ATOMIC ENERGY AGENCY; 2009.

Journal of Associated Medical Sciences 2021; 54 (2): 22-28

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

A preliminary performance evaluation of 3D facial image reconstruction from computed tomography scan

Nontanun Moonsan[1] **Suchart Kiatwattanacharoen**[2] **Komsanti Chokethawai**[3*]

1Master of Science Program in Forensic Science, The Graduate School, Chiang Mai University, Chiang Mai Province, Thailand.
2Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai Province, Thailand.
3Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai Province, Thailand.

ARTICLE INFO

Article history:
Received 15 December 2020
Accepted as revised 9 March 2021
Available online 9 March 2021

Keywords:
Facial reconstruction, CT scan image, facial soft tissue thickness, forensic science

ABSTRACT

Background: As a result of all kinds of disasters, people might be injured or killed. Most people could not be able to identify their family members dying in disasters. 3D facial image reconstruction from skull could narrow down the recognition of the faces from their family or close friends.

Objectives: The purpose of this preliminary study is to receive facial soft tissue thickness for forming a preliminary database and to create a 3D facial image reconstruction from Thai people.

Materials and methods: 3D facial image reconstruction based on facial soft tissue thickness data and paranasal sinuses CT scan images from Thai people was studied. Firstly, 15 anatomical facial landmarks to form a facial soft tissue thickness data were collected from Maharaj Nakorn Chiang Mai hospital CT scan database, 45 cases were used (22 males and 23 females). A 3D skull model matrix from CT scan DICOM files was then generated. Finally, the 3D facial soft tissue model matrix was combined with the 3D skull model matrix to create a 3D facial image reconstruction.

Results: The result showed Thai male facial soft tissue was thicker than the female at mid-philtrum and rhinion, respectively. Median values of 15 landmarks were used to create a 3D facial skin on a 3D skull. The random survey of 100 people showed 11% matched a 3D facial image reconstruction with a real 3D face of that skull.

Conclusion: The results also revealed that some people could recognize the real faces from the 3D facial images through facial soft tissue on the skull without any facial organs such as eyes, nose, or lips. It is suggested that facial soft tissue sampling and other facial organ studies could be helpful to create a big database for rebuilding more perfectly facial reconstruction in Thai people.

Introduction

In general, we can identify a dead person using various ways such as DNA or fingerprints matching. However, it is difficult to apply both methods in some cases such as a disaster responsible for appalling massacre. It is a very hard work for running the identification of all bodies through a short period of time. To solve this problem, the facial reconstruction can be helpful by narrowing a finding scope of a pair matching DNA or fingerprints.[1] To recreate face of the unknown skull, facial soft tissue thickness is very important. Therefore, in several years, many researchers in the field have studied about facial soft tissue thickness form a database for facial reconstruction in each nationality, specifically.[2-6]

* **Corresponding author.**
Author's Address: *Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai Province, Thailand.*
** **E-mail address:** komsanti.chokethawai@cmu.ac.th
doi: 10.14456/jams.2021.13
E-ISSN: 2539-6056

It is noticeable that most of the researchers reported that facial soft tissue thickness in their studies was different from the other studies even in the same country. Thus, it is necessary to prescribe the facial soft tissue thickness differences in ethnic diversity related to age, sex and nutritional condition.[7-8]

The facial soft tissue thickness measurement could be done by several methods using facial soft tissue.[9-11] In the past, needle puncture on dead body was applied.[12,13] But once medical imaging technology has been developed, the imaging based method known as CT scan became an illustrious method for measurement because it provides more accurate and reliable results.[14-17] Moreover, this method can use in the living while the needle puncture cannot be performed.[18] According to this study, the facial soft tissue thickness at 15 anatomical facial landmarks collected from paranasal sinuses CT scan DICOM file was used to created 3D facial image reconstruction of Thai people. The main purposes are to get an initiative facial soft tissue thickness data for Thai population and to create a 3D facial image reconstruction.

Materials and methods

Data Collecting

Prior to the measurement, this study was reviewed and approved by the Ethics Committee of Faculty of Associated Medical Sciences, Chiang Mai University. Thus, the researchers did not receive any personal information, except for the sex and age of the patients. The data was obtained from facial soft tissue thickness in 45 cases of 22 males and 23 females from Maharaj Nakorn Chiang Mai hospital CT scan database. The cases in which deformations such as the fracture of the skull or facial bones, tumors, swollen or damages of facial tissue were not included. The ages of the case studies are between 13 – 72, making an average of 37.91 years. One examiner measured and recorded the facial soft tissue thickness data twice, leaving a measurement gap for two weeks to control an intra-observer bias. 15 anatomical facial landmarks of the skull were measured, 5 of which were on the midline and 5 on bilateral (Figure 1).[2] The measurement software used was eFilm Workstation version 3.4. The DICOM images of CT scan had 0.4 mm voxel size (Figure 02).

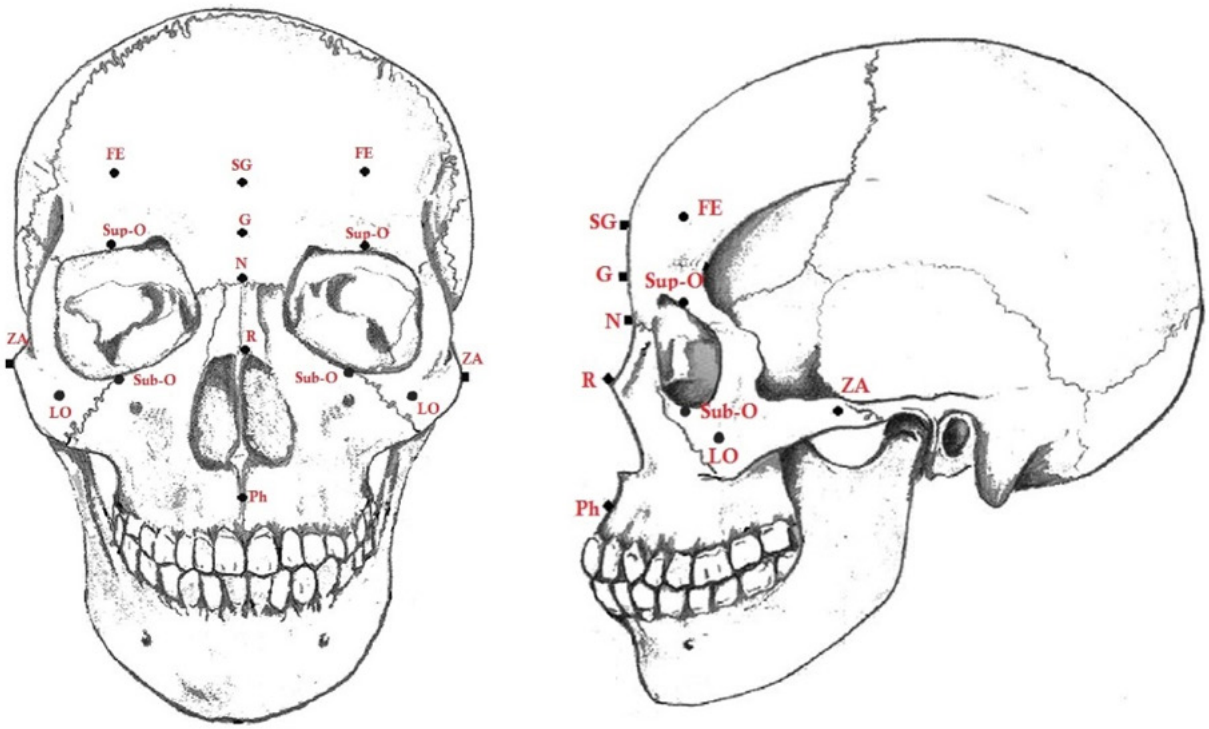

Figure 1. *Anatomical facial landmark of skull. FE: frontal eminence (left and right), SG: supra-glabella, Sup-O: supraorbital (left and right), G: glabella, Sub-O: suborbital (left and right), N: nasion, ZA: zygomatic arch (left and right), R: rhinion, LO: lateral orbit (left and right), and Ph: mid-philtrum.*[2]

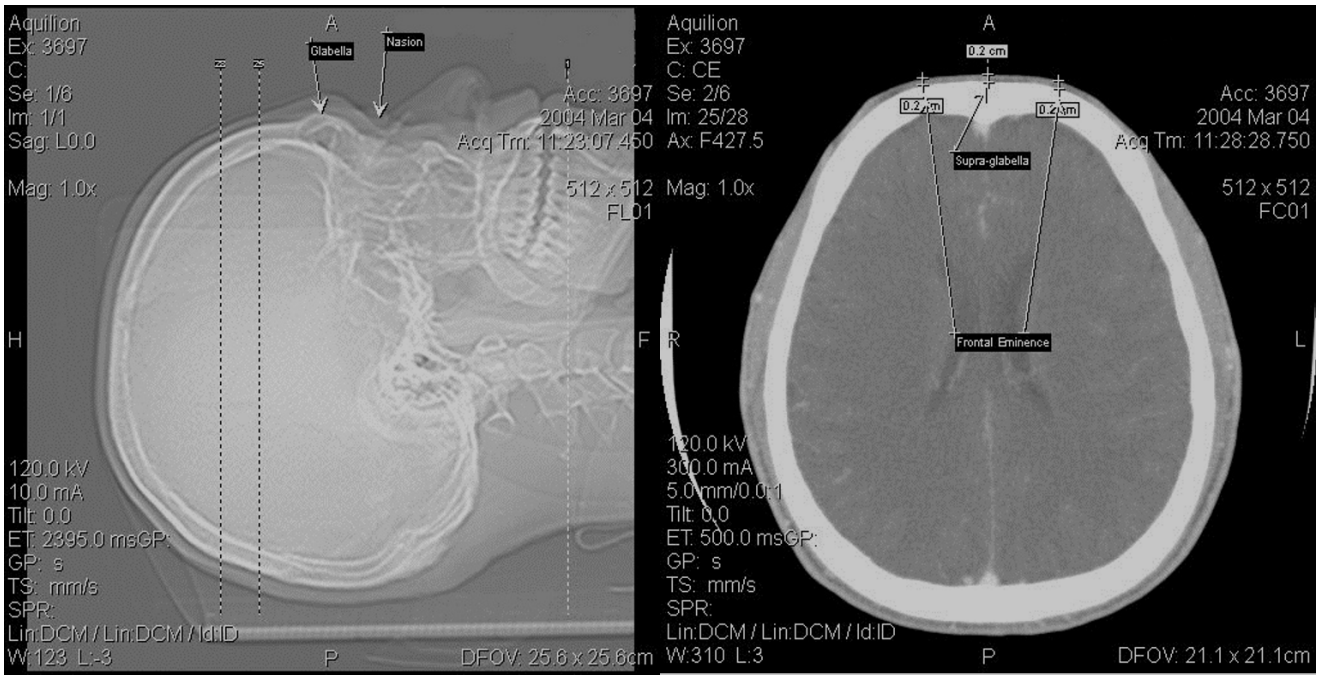

Figure 2. *Measurement window from eFilm Workstation version 3.4.*

Data analysis

The SPSS software version 22.0 (SPSS Inc., Chicago, USA) was applied to analyze the facial soft tissue thickness data. The Kolmogorov-Smirnov and Shapiro-Wilk were used to assess the distribution of the data showing its being distributed not normally. Median was represented as a central value. Wilcoxon Signed Ranks Test was used to assess Intra-observer reliability of the facial soft tissue thickness measurement. Mann-Whitney and Wilcoxon were used to compare the two groups of male and female patients involved in the study. Chi-Square was used to test their age differences. Kruskal Wallis Test was used to compare the differences more than two groups, and a statistically significant difference level, *p*-value <0.05, was used for all statistical analyses.

Generate 3D facial image reconstruction

Firstly, a median of Thai facial soft tissue thickness was collected, then a 3D facial soft tissue of the skull on the 3D model program was created from the central values. Secondly, the software InVesalius, version 3.1 (CTI, Campinas, Brazil) was used to generate a 3D skull image from CT Scan DICOM files and to attach the 3D facial soft tissue model matrix on 3D skull model matrix in software Autodesk Meshmixer platform (Autodesk Inc., California, USA). It should be noted that all 3D programs tools in this work were free software.

Test the recognition of face

In order to prove that the 3D facial image reconstruction of Thai people could be effective, able to be used for the recognition of real faces in any people, the survey paper was developed to evaluate 3D facial images reconstruction. A hundred people were randomly selected to answer 4 questions. They were asked to match the pictures between the 3D real faces model of the skull and our 3D facial image reconstruction.

Results

Facial Soft Tissue Thickness Results

Table 2 demonstrates that the thickest facial soft tissue thickness landmark is mid-philtrum (about 1.00 centimeter) and the thinnest landmark is rhinion (about 0.25 centimeter). Moreover, Figure 3 shows the tissue thickness of the male is thicker than that of the female of both landmarks.

Table 1 Explains the definitions of anatomical facial landmarks.

No.	Landmarks	Definition
1.	SG (Supra-glabella)	The most anterior point on midline of forehead.
2.	G (Glabella)	The most prominent point between the supraorbital ridges in the midsagital plane.
3.	N (Nasion)	Midpoint of the fronto-nasal suture.
4.	R (Rhinion)	The anterior tip of the nasal bone.
5.	Ph (Mid-philtrum)	Point at the intermaxillary suture, placed as high as possible before the curvature of the anterior nasal spine begins.
6, 7	Left and Right FE (Frontal Eminence)	Centered on eye pupil, most anterior point of the forehead.
8, 9	Left and Right Sup-O (Supraorbital)	Center upper part of margin of the orbit.
10, 11	Left and Right Sub-O (Suborbital)	Center lower part of margin of the orbit.
12, 13	Left and Right ZA (Zygomatic Arch)	Maximum, most lateral curvature of the zygomatic bone.
14, 15	Left and Right LO (Lateral Orbit)	Lateral side of the eye, point on the zygomatic bone.

Table 2 Median values of 15 anatomical facial landmarks.

No.	Landmarks	Median 1st	Median 2nd	Average of Median
1	Supra-glabella	0.4	0.4	0.4
2	Glabella	0.5	0.5	0.5
3	Nasion	0.4	0.4	0.4
4	Rhinion	0.3	0.2	0.25
5	Mid-philtrum	1.0	1.0	1.0
6	Left FE (Frontal eminence)	0.4	0.3	0.35
7	Right FE (Frontal eminence)	0.3	0.3	0.3
8	Left (Supraorbital)	0.6	0.6	0.6
9	Right (Supraorbital)	0.6	0.6	0.6
10	Left (Suborbital)	0.6	0.6	0.6
11	Right (Suborbital)	0.6	0.6	0.6
12	Left (Zygomatic arch)	0.8	0.8	0.8
13	Right (Zygomatic arch)	0.8	0.8	0.8
14	Left (Lateral orbit)	0.4	0.4	0.4
15	Right (Lateral orbit)	0.4	0.4	0.4

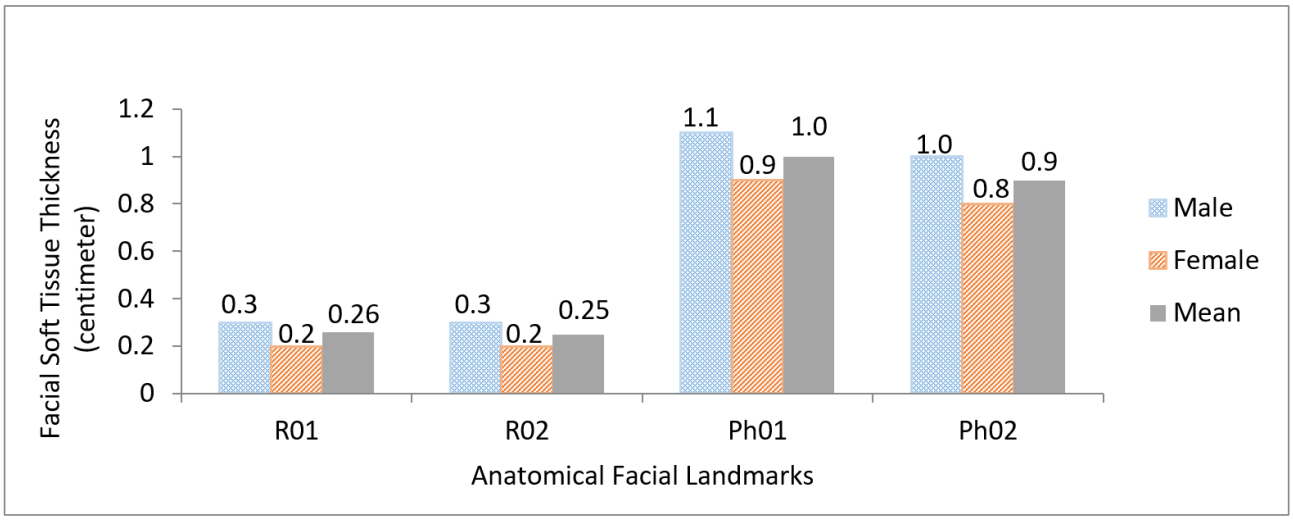

Figure 3. *Thicknesses of rhinion and mid-philtrum between male and female. R01: rhinion first measurement values, R02: rhinion second measurement values, Ph01: mid-philtrum first measurement values, Ph02: mid-philtrum second measurement values. The values are in centimeters.*

Facial image reconstruction result

Figure 4 demonstrates the 3D facial images before and after the facial reconstruction process. Four examples of original 3D skulls before facial reconstruction were shown in Figures 4.1(a)-4.4(a). These skull images were extracted from the original CT scan images of known people whereas Figures 4.1(b)-4.4(b) are 3D skull matrices after the facial soft tissue reconstruction on each original 3D skull image. Thin and soft tissue layer generated from 15 anatomical facial landmarks can be noted in the resulting images. However, these constructed facial images are generated as a preliminary work at this point. The more advanced the techniques or imaging software are the more tremendous the quality of the polygonal mesh in these reconstructed 3D skull images can obtain.

Recognition result

Figure 5 shows the number of 100 people, 31 males and 69 females, who answered a survey paper. 11% of people can truly match all pictures between 3D real face images and 3D facial images reconstruction, 5% can match 3 of 4 faces, 34% can match 2 of 4 faces, 28% can match just one face and 22% cannot match all of the face images.

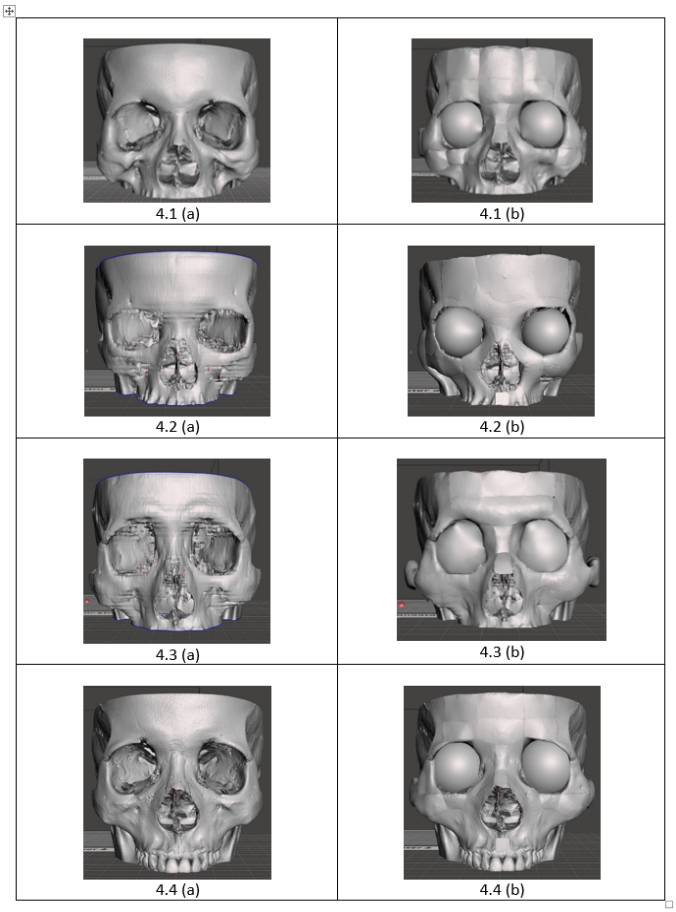

Figure 4. *3D facial image reconstruction; 4.1(a)-4.4(a) show the original 3D skull matrix whereas figures 4.1(b)-4.4(b) show the correspondent skull images after the facial reconstruction process.*

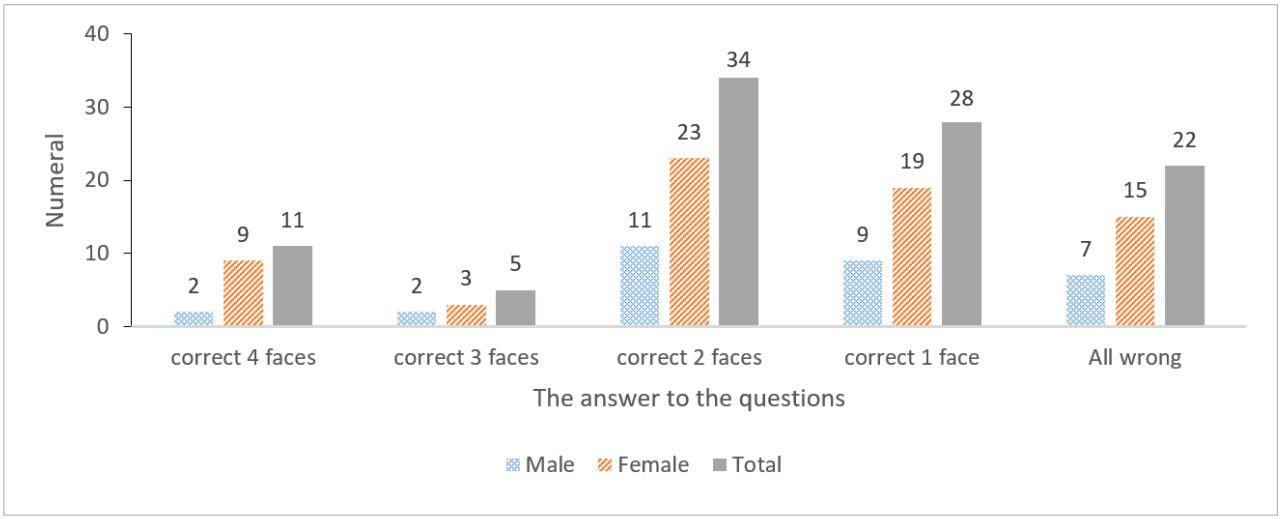

Figure 5. *The result of the survey.*

Discussion

According to the results of this study, the authors compared the facial soft tissue thickness values obtained with the results by other 2 researchers collecting the data from Thai people. Both studies focused on different parts of Thailand, the Northeast region, and the Central region, respectively.[12,15] When comparing with both works, it is worth noting the facial soft tissue thickness in males and female at rhinion and mid-philtrum. Similar to the work of Puavaranukroh who studied in the Central Region of Thailand and revealed his result that males in a normal BMI category showed facial soft tissue thickness thicker than females at the end of nasal (rhinion), as well as at mid-philtrum.[12] However, the results showed difference in the female patients. In this study, it was shown that there was no statistically significant difference which revealed the female was greater than male while Puavaranukroh showed that female soft tissue thickness is greater than male at lateral orbit. Compared to Puavaranukroh and our work, the study done in the Northeast has shown different results with regard to the soft tissue thickness. Sirisin showed that the average of soft tissue thickness in some landmarks of the males was higher than those of the females at nasospinale, right and left area, anterior nasal spine (this landmark was very nearly with mid-philtrum) and nasion.[15] In reverse, the females displayed higher thickness than the males at right ectomolare, left and right gonion, left and right jugale, left and right ectoconchion, and right orbitale superius (supra orbital). However, it is not easy to compare this study and other studies because of the variation in anatomical facial landmarks in each work. It is possible to compare the central values, mean and median, and some of the landmarks which were a repeated measurement point and with no statistically significant differences between the three works. However, it is unlikely to assume that the thickness values from this work can be generalized to create a facial image reconstruction for all regions of Thailand.

Conclusion

The result of the survey paper proves that some people are able to recognize real faces from a 3D facial image reconstruction showing just only skin on the skull without other facial organs such as eyes, nose, and lips. It is believed that further studies on facial soft tissue sampling and other facial organ studies on such facial organs in Thai people might be helpful to create a huge database for rebuilding most satisfying result of facial reconstruction. This database could, in turn, be effective to increase the number of people recognizing real faces.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

We would like to express our sincere appreciation to all staff in Forensic Science Program at the Graduate School of Chiang Mai University. Our deepest gratitude also goes to all technical staff at Faculty of Associated Medical Sciences, Chiang Mai University, for their continuing technical support throughout our study.

References

[1] Wilkinson C. Computerized forensic facial reconstruction: a review of current systems. Forensic Sci Med Pathol 2005; 1(3): 173-77.

[2] Cavanagh D, Steyn M. Facial reconstruction: soft tissue thickness values for South African black females. Forensic Sci Int 2011; 206: 215.e1-7.

[3] Ayoub F, Saadeh M, Rouhana G, Haddad R. Midsagittal facial soft tissue thickness norms in an adult Mediterranean population. Forensic Sci Int 2019; 294: 217.e1-7.

[4] Wang J, Zhao X, Mi C, Raza I. The study on facial soft tissue thickness using Han population in Xinjiang. Forensic Sci Int 2016; 266: 585.e1-5.

[5] Panenková P, Beňuš R, Masnicová S, Obertová Z, Grunt J. Facial soft tissue thicknesses of the mid-face Slovak population. Forensic Sci Int 2012; 220(1-3): 293.e1-6.

[6] Tedeschi-Oliveira SV, Melani RF, de Almeida NH, de Paiva LA. Facial soft tissue thickness of Brazilian adults. Forensic Sci Int 2009; 193(1-3): 127.e1-7.

[7] Shui W, Zhou M, Deng Q, Wu Z, Ji Y, Li K, He T, Jiang H. Densely calculated facial soft tissue thickness for craniofacial reconstruction in Chinese adults. Forensic Sci Int 2016; 266: 573.e1-12.

[8] Dong Y, Huang L, Feng Z, Bai S, Wu G, Zhao Y. Influence of sex and body mass index on facial soft tissue thickness measurements of the northern Chinese adult population. Forensic Sci Int 2012; 222(1-3): 396.e1-7.

[9] Prieels F, Hirsch S, Hering P. Holographic topometry for a dense visualization of soft tissue for facial reconstruction. Forensic Sci Med Pathol 2009; 5(1): 11-16.

[10] Sipahioğlu S, Ulubay H, Diren HB. Midline facial soft tissue thickness database of Turkish population: MRI study. Forensic Sci Int 2012; 219(1-3): 282. e1-8.

[11] Verzé L. History of facial reconstruction. Acta Biomed 2009; 80(1): 5-12.

[12] Puavaranukroh P and Srettabunjong S. Facial Soft Tissue Thickness in Thai Adults Cadavers, Vajira Med J 2010; 54(3): 267-79 [in Thai].

[13] Suazo GIC, Cantín LM, Zavando MDA, Perez RFJ, Torres MSR. Comparisons in soft-tissue thicknesses on the human face in fresh and embalmed corpses using needle puncture method. Int J Morphol 2008; 26(1): 165-69.

[14] Fourie Z, Damstra J, Gerrits PO, Ren Y. Accuracy and reliability of facial soft tissue depth measurements using cone beam computer tomography. Forensic Sci Int 2010; 199(1-3): 9-14.

[15] Sirisin J. Exploratory study of facial soft tissue thickness in North Eastern Thai adults [Thesis]. Graduate school: Khon Kaen University; 2011 [in Thai].

[16] Chung JH, Chen HT, Hsu WY, Huang GS, Shaw KP. A CT-scan database for the facial soft tissue thickness of Taiwan adults. Forensic Sci Int 2015; 253: 132. e1-11.

[17] Tilotta F, Richard F, Glaunès J, Berar M, Gey S, Verdeille S, et al. Construction and analysis of a head CT-scan database for craniofacial reconstruction. Forensic Sci Int 2009; 191(1-3): 112.e1-12.

[18] Wilkinson C, Rynn C, Peters H, Taister M, Kau CH, Richmond S. A blind accuracy assessment of computer-modeled forensic facial reconstruction using computed tomography data from live subjects. Forensic Sci Med Pathol 2006; 2(3): 179-87.

Journal of Associated Medical Sciences 2021; 54 (2): 29-34

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

A preliminary study of myofascial release technique effect on the range of hip flexion, knee flexion, and ankle dorsiflexion motion at affected lower extremity in individuals with chronic stroke

Sataporn Intanon **Peanchai Khamwong*** **Sothida Nantakool**

Department of Physical Therapy, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai Province, Thailand.

ARTICLE INFO

Article history:
Received 16 April 2020
Accepted as revised 1 April 2021
Available online 12 April 2021

Keywords:
Chronic stroke, muscle flexibility, myofascial release, soft tissue contracture.

ABSTRACT

Background: Muscle contractures lead to many problems (i.e., reduced joint range of motion (ROM) and decreased soft tissue extensibility) and may consequently lead to deformities and loss of function in individuals with chronic stroke. Myofascial release (MFR) technique has been recognized as a therapy option for improving the soft tissue extensibility and increasing joint ROM in other population. However, no study has investigated effects of the MFR technique on lower limb muscle flexibility in individuals with chronic stroke.

Objectives: This preliminary study aimed to investigate the effect of myofascial release (MFR) technique at the superficial back line on ROM of hip flexion, knee flexion, and ankle dorsiflexion changes in the affected side of lower extremity in individuals with chronic stroke.

Materials and methods: Fifteen individuals with chronic stroke who complained stiffness of the affected lower extremity while walking and met all inclusion criteria were enrolled in the study. The MFR technique was applied on the superficial back line (plantar fascia, achilles tendon, gastrocnemius muscle and hamstrings muscle) in the affected side of lower extremity, 10 minutes per area, 3 times per week for 4 weeks (12 times) by a physical therapist. ROM of hip flexion, knee flexion and ankle dorsiflexion were measured at pre-intervention, immediate-intervention, and 4 weeks after intervention using a goniometer by a blinded assessor. One-way repeated measure analysis of variance was used to compute data.

Results: The ROM of hip flexion, knee flexion, and ankle dorsiflexion were significantly greater at immediate-intervention and 4 weeks after intervention as compared to baseline (p<0.05).

Conclusion: This preliminary study summarized that the MFR technique could increase ROM of hip flexion, knee flexion and ankle dorsiflexion in the affected side of lower extremity in individuals with chronic stroke. The MFR technique may be used as an alternative option combined with general training program for stroke rehabilitation.

* **Corresponding author.**
Author's Address: *Department of Physical Therapy, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai Province, Thailand.*
** **E-mail address:** peanchai.k@cmu.ac.th
doi: 10.14456/jams.2021.14
E-ISSN: 2539-6056

Introduction

Sensory and motor impairments, including weakness for voluntary movement, spasticity, and poor coordination are causes of long term disability in individuals with stroke.[1] The sensorimotor impairments contribute to further impairments including increased pain, increased muscle tone, decreased range of motion, and shortened muscle length, which lead to reduced function in individuals with chronic stroke.[2, 3] Immobilized muscle in a shortened position causes negative altering in a muscular structure, known as muscle contractures (such as "spastic myopathy").[4] The muscle contractures are characterized by decreased muscle fiber length, shortened muscle connective tissue, and increased muscle stiffness.[5] These altered structures affect reductions of joint range of motion (ROM) and soft tissue extensibility, leading to deformities and loss of function (e.g., walking, standing and activity of daily living).[3, 4] Various Physical Therapy techniques used to improve soft tissue contracture in individuals with chronic stroke includes hot pack, ultrasound, neuromuscular electrical stimulation, stretching, mobilization, and myofascial release technique (MFR).[6] A previous systematic review by Wilke et al.[7] has suggested that myofascial release (MFR) technique at superficial back line (plantar fascia, achilles tendon, gastrocnemius muscle and hamstrings muscle) increases muscle flexibility. This technique might improve muscle properties, function ability and activity in daily living in patients with chronic stroke due to myofascial chain change.

The MFR technique is a manual technique of soft tissue releasing and muscle stretching to maintain muscle length, decrease pain, and increase muscle flexibility, soft tissue flexibility, and joint ROM.[6, 8] This technique requires an external force used for decreasing fibrous tissue adhesive in the muscles, low load, and long duration used to stretch the myofascial complex in order to restore an optimal length, resulting in decreased pain and improved function.[9] The MFR has been reported that it has beneficial effect on dilatating skin capillary and changing skin temperature.[10] Moreover, there are some changes in joint biomechanics and increase in muscle flexibility,[10] and decrease in fascial adhesion.[11]

Accumulating evidence have investigated an effect of MFR technique in various populations. A previous study investigating effect of self MFR at hamstrings muscle using a foam roller found significant increase in joint ROM and muscle flexibility but not in a muscle power in athletes.[12] Silva et al.[13] investigated an acute effect of MFR in individuals with total knee arthroplasty and reported a significant increase of knee flexion. A pilot study by Park and Hwang[14] showed a beneficial effect of MFR with a tennis ball on improving in balance performance in patients with chronic stroke who had a spastic on lower extremity muscle flexibility. However, an evidence demonstrating an effect of MFR technique in individuals with chronic stroke on increasing joint ROM and muscle flexibility on lower extremity due to soft tissue contracture is scarce. Therefore, this preliminary study aimed to determine effect of MFR technique at superficial back line on ROM of hip flexion, knee flexion, and ankle dorsiflexion at the affected lower extremity in individuals with chronic stroke.

Materials and methods

Participants

Fifteen individuals with chronic stroke who complained stiffness (difficulty in moving leg up to clear the floor during walking) in the affected lower extremity while walking was enrolled in this study.

Inclusion criteria consisted of (a) a diagnosis of infarction or hemorrhage stroke more than 6 months post-onset, (b) a Modified Ashworth Score (MAS) greater than 1 of affected lower extremity, (c) independent to walk with or without an assistive device, (d) no orthopedic problems at the lower extremities that would affect gait such as total knee or total hip arthroplasty in affected side, fracture in affected lower extremity. Exclusion criteria were (a) a stroke more than one hemisphere, (b) the repeated stroke, (c) a flaccid tone of lower extremity muscle, (d) premorbid problems that would affect patterns.

Sample size was estimated from a pilot study using a ROM of hip flexion (mean±SD). With 80% power, 5% type I error, a sample size of 12 was required to detect a difference between pre- and post-intervention. To allow 20% drop out, a total sample size of 15 was required for this study.

Participants who agreed to participate in this study were asked to sign informed consent before conducting the study. Ethical approval number 431/2019 for the study was granted by the Faculty of Associated Medical Science, Chiang Mai University, Thailand.

Measurements

ROMs of hip flexion, knee flexion, and ankle dorsiflexion in each participant were measured using goniometer by a blinded assessor. Degree of passive and active movements of all three actions was assessed during supine lying position. On each action, a maximum value among three trials was recorded. ROMs were measured before, immediate (immediately at the first end) and 4 weeks after (immediately at the last end) intervention. The assessor was assessed test-retest reliability before study. Degrees of passive and active movements of the hip flexion, knee flexion, and ankle dorsiflexion in five individuals with chronic stroke were measured. The test-retest reliability was calculated using by ICC model (3, 1).

Hip flexion

Fulcrum of goniometer was placed on a greater trochanter of a femur bone, stationary arm was positioned along lateral line of body, and moveable arm was placed on the side of the femur bone toward lateral epicondyle of the femur bone. For passive movement performed at the affected side, an assessor moved the knee to chest until the end of movement or limit by pain. For active movement, participants were instructed to perform action same as the passive movement with their backs straight.[15] In this study, the test-retest reliability was 0.98 in passive and active movement.

Knee flexion

Fulcrum of goniometer was placed on lateral epicondyle of femur, stationary arm was paralleled to the femur bone

pointed to greater trochanter, and moveable arm was paralleled to the fibular bone pointed to the lateral malleolus. For passive movement of knee flexion, assistance slipped foot of the participants on the bed towards buttock until the end of movement or limit by pain. For active movement of knee flexion, the participants were commanded to perform action same as the passive movement.[16] The test-retest reliability of passive and active movements were 0.95 and 0.99, respectively.

Ankle dorsiflexion

To measure the ROM of ankle dorsiflexion, fulcrum of goniometer was placed on the lateral malleolus, stationary arm was put along the fibular bone, and moveable arm was paralleled the alignment of the fifth metatarsal bone. For measurement of passive movement, plantar of the affected side of each participant was moved up, in which the calf muscle was not too much stretched for preventing reflex from the ankle joint. The participants were then commanded to move their plantar in the affected side up for measuring an active movement. During the active movement, knee flexion during moving plantar up should be warned.[17] The test-retest reliability of passive and active movements was 0.87 and 0.80, respectively.

Intervention

All the participants were treated using MFR technique by a physical therapist who has an experience in the MFR technique. The participants were asked to perform a prone position prior to apply the MFR technique. Dominant hand of the physical therapist was then placed on the area of superficial back line (i.e., hamstring, gastro soleus muscle and plantar fascia) at the affected side of lower extremity, with a gentle force as per the participants tolerance, slow movement along transverse plane of each muscle fiber[9, 10, 12] (Figure 1). In each time, the hand force was applied almost the same. The MFR technique was applied for 10 minutes per area, 3 times per week for 4 weeks (12 times).[14, 18]

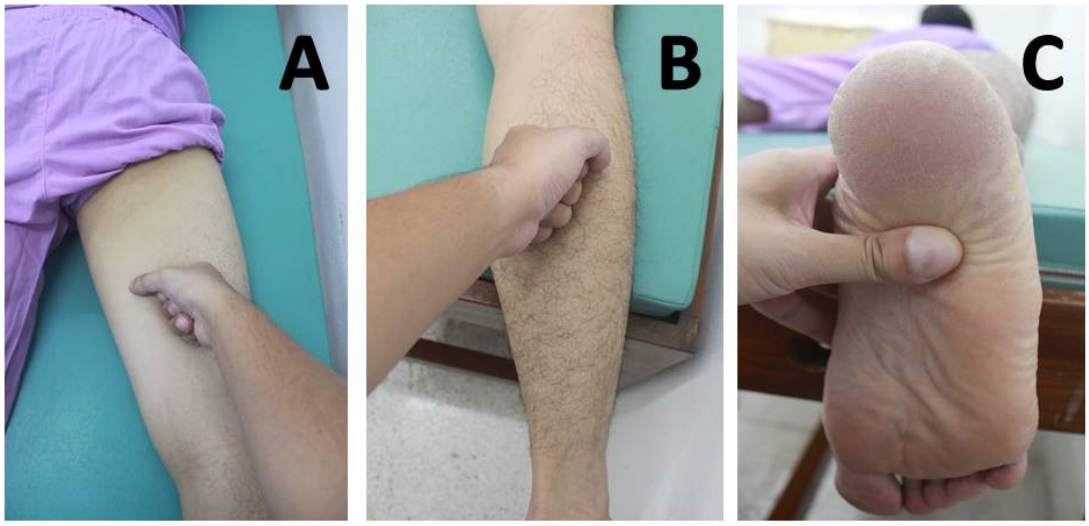

Figure 1. *MFR technique on superficial back line of the effected lower extremity. Note: A: hamstrings muscle, B: gastrocnemius muscle and achilles tendon, C: plantar fascia.*

Statistical analysis

SPSS version 19.0 was administered for statistical analyses. Shapiro-Wilk test was used to analyze the data distribution. One-way repeated measure analysis of variance (ANOVA) was used to analyze differences of ROM of hip flexion, knee flexion, and ankle dorsiflexion among three time points (i.e., before, immediate, and 4 weeks after intervention). The level of statistical significance was set at $p<0.05$.

Results

Fifteen individuals with chronic stroke (9 males), who had a mean age of 48.20 (SD=5.38) years, a mean body weight of 70.05 (SD=14.60) kg., height of 161.27 (SD=5.70) cm, and body mass index of 26.76 (SD=4.30) kg/m^2, were presented in this study. An average time of stroke onset was 34.27 (SD=5.17) months. There were 10 participants of hemorrhagic stroke and 5 infarction strokes. Five participants were affected with right side while the other 10 participants affected with left side. Eight participants could walk independently, whereas 7 participants could walk with a tripod cane. Underlying diseases was including two hypertension, two diabetes mellitus and one dyslipidemia. There were 7 participants who had a Modified Ashworth Score (MAS) score of "1+" (slight increase in muscle tone, manifested by a catch followed by minimal resistance through the remainder of the range of motion but the affected part is easily moved) and 8 participants with MAS score of "2" (more marked increase in muscle tone through most of the range of movement, but affected part easily moved).[19] All characteristics of participants were demonstrated in Table 1.

The results showed that ROMs of hip flexion, knee flexion, and ankle dorsiflexion during passive and active movements were significantly higher at immediate and 4 weeks after intervention when compared to their baselines as showed in Table 2 ($p<0.05$).

Table 1 Characteristics of the participants.

Characteristics	Mean±SD
Gender (male : female)	9:6
Age (year)	48.20±5.38
Height (cm)	161.27±5.70
Weight (kg)	70.05±14.60
Body mass index; BMI (kg/m^2)	26.76±4.30
Time of stroke onset (month)	34.27±5.17
Modified Ashworth Score (MAS)	
Score of 1+	7
Score of 2	8
Underlying disease	
Hypertension (n)	2
Diabetes mellitus (n)	2
Dyslipidemia (n)	1
Type of stroke	
Hemorrhage (n)	10
Infarction (n)	5
Affected side	
Right (n)	5
Left (n)	10
Using assistive device during walking	
No (n)	8
Tripod cane (n)	7

Note: *Data are expressed as mean±SD, otherwise as indicated, SD: standard deviation, n: number.*

Table 2 Mean ROM between pre-test, immediate and post 4 weeks of MFR intervention of passive and active movements.

	Range of Motion (ROM) (°)			*p* value
	Pre-test	Immediate	Post-test	
Passive movement				
Hip flexion (°)*	113.87±11.22	120.27±9.53^b	122.13±9.57^a	0.000
Knee flexion (°)*	136.40±5.15	141.13±5.04^b	143.73±6.7^a	0.000
Ankle dorsiflexion (°)*	29.87±2.45	35.33±2.38^b	37.07±2.60^a	0.000
Active movement				
Hip flexion (°)*	103.20±10.76	108.87±10.63^b	112.80±9.44^a	0.000
Knee flexion (°)*	120.67±8.92	126.67±8.52^b	129.40±8.32^a	0.000
Ankle dorsiflexion (°)*	0.53±0.64	1.93±1.58^b	1.33±0.97^a	0.003

Note: *^{a}significant level between pre and post-test of ROM after using MFR intervention ($p<0.05$), ^{b}significant between pre and immediate test of ROM after using MFR intervention ($p<0.05$), *mean±SD, °degrees, SD: standard deviation.*

Discussion

The aim of this preliminary study was to determine the effect of MFR technique on ROM of hip flexion, knee flexion and ankle dorsiflexion changing at the affected side of lower extremity in individuals with chronic stroke. Findings of this study demonstrated significant improvements of the ROMs of hip flexion, knee flexion and ankle dorsiflexion during passive and active movements after immediate and 4 weeks after using MFR technique. These findings are in line with the previous studies reporting beneficial effects of MFR technique on improved lower extremity functions in several populations.[12, 13] Silva *et al.*[13] investigated the effect of MFR in individuals with post-op total knee arthroplasty found a significant increase of knee flexion. In addition,

Kalichman and David[12] reported that self MFR using a foam roller at hamstring muscle could increase joint ROM and muscle flexibility in athletes. These increased joint ROMs may be explained by altering in muscle flexibility responsible for therapeutic pressure of MFR applied.[7]

Some proposed mechanisms attributed to the muscle flexibility changes involve with interfibrous tissue change muscle spindle and Golgi Tendon Organ (GTO) responses. During MFR technique, pressure applied might contribute to a reduction of interfibrous tissue adhesion and increase of temperature at the interfibrous tissue, which might lead to enhanced muscle flexibility.[9, 12] Additionally, rapidly released GTO and continuously facilitated stretch reflex might be occurred due to stress pressure performed on tendon, which might reduce muscle contraction and consequently increased muscle flexibility and increased ROM.[20] The MFR technique is an applying of external force combined with manual traction and prolonged assisted stretching maneuvers for breaking up fibrous tissue adhesive in muscle. This technique also requires low load and long duration which contributes to stretch on the myofascial complex in order to restore an optimal length, consequently improving muscle flexibility and function.[8, 9] MFR is transferred to other connective tissue structures for establishing a balance between the tension and the compressive force of the joint.[21] Luomala *et al*.[22] suggested that movement dysfunction may be induced by the modifications of connective tissue, which generates disarrangement of the surrounding fascia's structure, compromising the sliding system between layers. Furthermore, gentle pressure with light weight increase flows in extracellular matrix and interstitial fluids,[23] and elongate viscoelastic fascia,[14] resulting in flexible muscular structures.

Few limitations in this study should be concerned. Firstly, an effectiveness of the MFR technique on outcomes might be limited due to lack of control group compared. Secondly, this study investigated only one outcome aspects impaired in individuals with chronic stroke. Further study should confirm the effectiveness of the MFR technique on function such as balance and walking ability in this population. Lastly, force level applied during the MFR technique was difficult to regulate. We suggest for further study to evaluate force level applied objectively by asking a pain score during applied force or patient tolerance level.

Conclusion

The preliminary study concluded that MFR technique applied on superficial back line had significantly immediate effects and 4 weeks after the intervention effects on improved ROMs of hip flexion, knee flexion, and ankle dorsiflexion in the affected side of lower extremity in individuals with chronic stroke. The MFR technique may be used as an alternative treatment combined with general training program for stroke rehabilitation.

Acknowledgments

This study was granted by the Department of Physical Therapy, Faculty of Associated Medical Science and the Graduate school, Chiang Mai University. The authors wish to thank all the patients to associate in this study, the physical therapists and the researcher's assistance who come to help and make the study completed. Thank you for 4th floor community "karee takhli"

References

[1] Lee SSM, Spear S, Rymer WZ. Quantifying changes in material properties of stroke-impaired muscle. Clin Biomech. 2015; 30(3): 269-75. doi: 10.1016/j.clinbiomech.2015.01.004.

[2] Kuo C-L, Hu G-C. Post-stroke spasticity: a review of epidemiology, pathophysiology, and treatments. Int J Gerontol. 2018; 12(4): 280-4. doi: 10.1016/j.ijge.2018.05.005.

[3] Ward AB. A literature review of the pathophysiology and onset of post-stroke spasticity. Eur J Neurol. 2012; 19(1): 21-7. doi: 10.1111/j.1468-1331.2011.03448.x.

[4] Lecharte T, Gross R, Nordez A, Le Sant G. Effect of chronic stretching interventions on the mechanical properties of muscles in patients with stroke: a systematic review. Ann Phys Rehabil Med. 2020; 63(3): 222-9. doi: 10.1016/j.rehab.2019.12.003.

[5] Wood KS, Daluiski A. Management of joint contractures in the spastic upper extremity. Hand Clin. 2018; 34(4): 517-28. doi: 10.1016/j.hcl.2018.06.011.

[6] Konin JG, Jessee B. 6 - Range of motion and flexibility. In: Andrews JR, Harrelson GL, Wilk KE, editors. Physical rehabilitation of the injured athlete 4th Ed. Philadelphia: W.B. Saunders; 2012: 74-88.

[7] Wilke J, Krause F, Vogt L, Banzer W. What is evidence-based about myofascial chains: a systematic review. Arch Phys Med Rehabil. 2016; 97(3): 454-61. doi: 10.1016/j.apmr.2015.07.023.

[8] Meltzer KR, Cao TV, Schad JF, King H, Stoll ST, Standley PR. In vitro modeling of repetitive motion injury and myofascial release. J Bodyw Mov Ther. 2010; 14(2): 162-71. doi: 10.1016/j.jbmt.2010.01.002.

[9] Ajimsha MS, Al-Mudahka NR, Al-Madzhar JA. Effectiveness of myofascial release: systematic review of randomized controlled trials. J Bodyw Mov Ther. 2015; 19(1): 102-12. doi: 10.1016/j.jbmt.2014.06.001.

[10] Kain J, Martorello L, Swanson E, Sego S. Comparison of an indirect tri-planar myofascial release (MFR) technique and a hot pack for increasing range of motion. J Bodyw Mov Ther. 2011; 15(1): 63-7. doi: 10.1016/j.jbmt.2009.12.002.

[11] Liptan G, Mist S, Wright C, Arzt A, Jones KD. A pilot study of myofascial release therapy compared to swedish massage in fibromyalgia. J Bodyw Mov Ther. 2013; 17(3): 365-70. doi: 10.1016/j.jbmt.2012.11.010.

[12] Kalichman L, David CB. Effect of self-myofascial release on myofascial pain, muscle flexibility, and strength: a narrative review. J Bodyw Mov Ther. 2017; 21(2): 446-51. doi: 10.1016/j.jbmt.2016.11.006.

[13] Silva DCCMe, de Andrade Alexandre DJ, Silva JG. Immediate effect of myofascial release on range of motion, pain and biceps and rectus femoris muscle activity after total knee replacement. J Bodyw Mov Ther. 2018; 22(4): 930-6. doi: 10.1016/j.jbmt.2017.12.003.

[14] Park D-J, Hwang Y-I. A pilot study of balance performance benefit of myofascial release, with a tennis ball, in chronic stroke patients. J Bodyw Mov Ther. 2016; 20(1): 98-103. doi: 10.1016/j.jbmt.2015.06.009.

[15] Nussbaumer S, Leunig M, F Glatthorn J, Stauffacher S, Gerber H, Maffiuletti N. Validity and test-retest reliability of manual goniometers for measuring passive hip range of motion in femoroacetabular impingement patients. BMC Muscoskel Disord. 2010; 11(1): 194-205. doi: 10.1186/1471-2474-11-194.

[16] Santos CMd, Ferreira G, Malacco PL, Sabino GS, Moraes GFdS, Felício DC. Intra and inter examiner reliability and measurement error of goniometer and digital inclinometer use. Rev Bras Med Esporte. 2012; 18: 38-41. doi: 10.1590/S1517-86922012000100008.

[17] Keating JL, Parks C, Mackenzie M. Measurements of ankle dorsiflexion in stroke subjects obtained using standardised dorsiflexion force. Aust J Physiother. 2000; 46(3): 203-13. doi: 10.1016/s0004-9514(14)60329-9.

[18] Ghasemi E, Khademi-Kalantari K, Khalkhali-Zavieh M, Rezasoltani A, Ghasemi M, Baghban AA, et al. The effect of functional stretching exercises on neural and mechanical properties of the spastic medial gastrocnemius muscle in patients with chronic stroke: a randomized controlled trial. J Stroke Cerebrovasc Dis. 2018; 27(7): 1733-42. doi: 10.1016/j.jstrokecerebrovasdis.2018.01.024.

[19] Blackburn M, van Vliet P, Mockett SP. Reliability of measurements obtained with the modified ashworth scale in the lower extremities of people with stroke. Phys Ther. 2002; 82(1): 25-34. doi: 10.1093/ptj/82.1.25

[20] Stark C EW. Myofascial release. In: Somers D, editor. Principle of mannual sports medicine. Philadelphia: Lippincott Williams & Wilkins; 2005.

[21] Grieve R, Cranston A, Henderson A, John R, Malone G, Mayall C. The immediate effect of triceps surae myofascial trigger point therapy on restricted active ankle joint dorsiflexion in recreational runners: a crossover randomised controlled trial. J Bodyw Mov Ther. 2013; 17(4): 453-61. doi: 10.1016/j.jbmt.2013.02.001.

[22] Luomala T, Pihlman M, Heiskanen J, Stecco C. Case study: could ultrasound and elastography visualized densified areas inside the deep fascia? J Bodyw Mov Ther. 2014; 18(3): 462-8. doi: 10.1016/j.jbmt.2013.11.020.

[23] Reddy NP, Palmieri V, Cochran GV. Subcutaneous interstitial fluid pressure during external loading. Am J Physiol Regul Integr Comp Physiol. 1981; 240(5): 327-9. doi: 10.1152/ajpregu.1981.240.5.R327.

Journal of Associated Medical Sciences 2021; 54 (2): 35-41

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Associations between urinary excretion of cadmium with alpha-1 microglobulin and microalbuminuria: a cross-sectional study in northwestern Thai population

Sujitra Sikaphan[1] **Ratchaneekorn Boonthum**[2] **Siriwan Leudang**[1] **Sittiporn Parnmen**[1*]

[1]*Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.*
[2]*Regional Medical Sciences Center 2, Department of Medical Sciences, Ministry of Public Health, Phitsanulok Province, Thailand.*

ARTICLE INFO

Article history:
Received 29 January 2021
Accepted as revised 15 April 2021
Available online 18 April 2021

Keywords:
Alpha-1 microglobulin, microalbuminuria, urinary cadmium

ABSTRACT

Background: In Thailand, a high Cadmium (Cd) levels were observed in the northwestern population, especially in Mae Sot District, Tak Province. However, the association between urinary cadmium level and changes in renal biomarkers after long-term expose has not been studied, especially in case of low-level Cd exposures.

Objectives: The main focus of this study was to investigate the associations between urinary cadmium levels and renal biomarkers in the northwestern Thai population.

Materials and methods: A total of 817 persons (males=309, females=508) living in Cd-polluted area of Mae Sot district were participated in this study. The urinary cadmium level was analyzed using graphite furnace atomic absorption spectrometry (GFAAS). Two renal biochemical markers were selected, namely urinary alpha-1-microglobulin and microalbuminuria.

Results and Conclusion: The geometric mean concentration for urinary cadmium was 3.67(±3.08) µg/gm creatinine and 4.83(±3.82) µg/gm creatinine in adult males and females, respectively. Based on correlation analysis, the urinary cadmium level was positively correlated with age, years of residence, microalbuminuria and urinary alpha-1 microglobulin in both male and female. While a significant inverse correlation was found between the urinary cadmium level and BMI. Linear regression analysis yielded that alpha-1-microglobulin and microalbuminuria were significantly increased with increasing of urinary cadmium levels. Interestingly, the prevalence of increased alpha-1 microglobulin was higher than the prevalence of increased microalbuminuria in subjects with low urinary cadmium levels. The findings of this study indicate that urinary alpha-1 microglobulin showed a best of biomarker for monitoring the low-level of Cd exposure in both populations.

Introduction

Cadmium (Cd) pollution has become an issue of public health concern and associated with nephrotoxic effects as well as noncommunicable diseases such as cardiovascular disease, diabetes mellitus.[1-3] Primary sources of Cd exposure are natural components (soils, air, water), cigarette smoke, dietary intake and anthropogenic activities from industrial production such as batteries, plastic and synthetic materials.[4-6] Continuous exposure to Cd has been linked to bioaccumulation in living organisms and potential adverse health effects in human especially people living in contaminated areas.[5,7,8] In the northwest of Thailand, the cadmium contaminated areas were located in Mae Sot District, Tak Province. Major contamination source is believed to be a zinc mine nearby two creeks of Mae Tao and Mae Ku which had been operated more than 20 years.[9] As a result of this mining activities, increasing cadmium levels have been recorded in the areas by heavy metal monitoring scheme.[10] Several studies have shown a degree of cadmium contaminated from food

* **Corresponding author.**
Author's Address: *Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.*
** **E-mail address:** sittiporn.p@dmsc.mail.go.th
doi: 10.14456/jams.2021.15
E-ISSN: 2539-6056

chain to local population living in the areas.[7-9,11,12]

Cadmium toxicity refers to the level of tubular dysfunction, which is diagnosed by increased urinary excretion of low molecular weight proteins such as alpha-1 microglobulin (α_1-MG), beta-2 microglobulin (β_2-MG), retinol-binding protein (RBP), metallothionine (MT), microalbuminuria and kidney injury molecule-1(KIM-1).[5, 7, 11-14] These biomarkers can reflect the early changes and the late stage of renal function. Using the German Commission on Human Biological Monitoring (HBM) recommended two different reference values for Cd in urine for the general population based on toxicology and epidemiology studies, HBM I (2 µg/gm creatinine) and HBM II (5 µg/gm creatinine).[15] The concentrations below the lower HBM I level are not considered to be a risk for the general population, while concentrations above HBM II indicate an increased risk of adverse health effects in susceptible individuals of the general population.[15]

In the present study, we investigated the relationships between two renal biomarkers, namely alpha-1 microglobulin and microalbuminuria with urinary excretion of cadmium from 817 subjects living around the cadmium contaminated area of Mae Sot District, Tak Province. Based on the cut-off values, the level of microalbuminuria below 20 mg/L indicate normal albuminuria whereas level of urinary alpha-1 microglobulin above 15 mg/gm creatinine is assumed tubular dysfunction.[16, 17]

Materials and methods

Study subject and study site

The study was conducted in northwest Thai population at Tak Province. A total of 817 subjects were recruited including 309 males and 508 females. This study was approved by the Ethical Review Committee for Research in Human Subjects at Department of Medical Sciences, Ministry of Public Health, Thailand (Protocol No. 2/2014). All participants signed a written informed consent form. Demographics information, health behavior, education and occupation were administered.

Urine sample collection and urinary cadmium determination

Urine samples were collected from all subjects. Urine volume was measured and 30-50 mL was collected in 100 mL polypropylene tube before being stores at 4-8 °C for alpha-1 microglobulin and –20°C for creatinine as well as urinary Cd until analysis.

Biochemical markers and urinary Cd analyses

Analysis of the urine samples were performed at Toxicology center, National Institute of Health (Department of Medical Sciences, Ministry of Public Health). Using an automated analyzer (Beckman coulter, AU 480 chemistry System), the following urinary analyses were analyzed, according to manufacturer's instructions: creatinine according to the kinetic Jaffe method (compensated, rate blanked), microalbuminuria measured by turbidimetry and urinary alpha-1 microglobulin based on immunological agglutination.

Urinary cadmium levels were analyzed using graphite furnace atomic absorption spectrometry (GFAAS) (Perkin Elmer, Analyst 600). Urine sample and standard were diluted 1: 5 in modifier (0.1% triton x-100, 0.2% ammonium dihydrogen phosphate and 0.2% magnesium nitrate). The electrodeless discharge lamp (EDL) was used with the 228.8 nm wavelength cadmium line.

Data and statistical analysis

Statistical analysis were carried out on all 817 urine samples. Values below the limit of quantitation (LOQ) were treated as half of this limit, i.e. 0.25 µg/gm creatinine. Pearson's correlation coefficient was used to measures the statistical association between the two variables. Linear regression analysis was performed to determine the relationships between urinary Cd and two renal biomarkers as well as demographic parameters. The statistical analysis was analyzed using SPSS Statistics (version 27.0.1.0, IBM). Reference values used for urinary cadmium were published by the German Commission on Human Biological Monitoring.[15] In adults >25 years old, urinary cadmium concentrations below the lower HBM I level (2 µg/gm creatinine) are not considered to be a risk of advance health effects, whereas concentrations above HBM II (5 µg/gm creatinine) are increased risk of adverse health effects in susceptible individuals in the general population.[15] For two renal biomarkers, the cut-off values microalbuminuria levels above 20 mg/L indicate abnormal albuminuria and the cut-off values of urinary alpha-1 microglobulin above 15 mg/gm creatinine was assumed tubular dysfunction.[16]

Results

Descriptive profile

Characteristics of the study population are summarized in Table 1. A total of 817 subjects (309 males, 508 females) were included for statistical analysis. Subjects were in their mid-50s and the mean years of residence was 54 years (SD 16, with ranges of 3 to 91 years) in males and 53 years (SD 14, with ranges of 3 to 83 years) in females. Geometric mean level of urinary cadmium was 3.67±3.08 µg/gm creatinine in males and 4.83±3.82 µg/gm creatinine in females. The urinary-Cd levels ranged from 0.25 to 21.53 in male and 0.25 to 24.00 µg/gm creatinine in female participants. Twenty male and 22 female participants had urinary Cd levels below the LOQ. GM concentration of microalbuminuria was 21.55 mg/L (SD 45.03, with ranges of 0.45-281.64 mg/L) in males and 17.95 mg/L (SD 39.94 with ranges of 0.45-281.64 mg/L) in females. GM concentration of alpha-1 microglobulin was 25.23 mg/gm creatinine (SD 27.11, with ranges of 1.87-258.94 mg/gm creatinine) in males and 24.51 mg/gm creatinine (SD 36.42 with ranges of 2.09-425.79 mg/gm creatinine) in females.

Table 1 Characteristics of the study population.

Gender	Characteristics/Parameters	Mean±SD (min-max)
Males (n=309)	Age (years)	58±11 (35-91)
	Body mass index (kg/m^2)	24±4 (15-38)
	Years of residence (years)	54±16 (3-91)
	Urinary-Cd (µg/gm creatinine)	3.67±3.08 (0.25-21.53)
	microalbuminuria (mg/L)	21.55±45.03 (0.45-281.64)
	alpha-1microglobulin (mg/g creatinine)	25.23±27.11 (1.87-258.94)
Females (n=508)	Age (years)	56±11 (35-84)
	Body mass index (kg/m^2)	24±4 (14-38)
	Years of residence (years)	53±14 (3-83)
	Urinary-Cd (µg/gm creatinine)	4.83±3.82 (0.25-24.00)
	microalbuminuria (mg/L)	17.95±39.94 (0.45-281.64)
	alpha-1 microglobulin (mg/gm creatinine)	24.51±36.42 (2.09-425.79)

Urinary Cd, microalbuminuria and urinary alpha-1 microglobulin among study subjects

Standard reference urinary cadmium values of HBM I and HBM II were exceeded among the male participants by 134 (44%) and 75 (24%) males respectively, whereas among the female participants, by 213(42%) and 188 (37%) females respectively (Table 2). The normal cut-off microalbuminuria values (<20 mg/L) were exceeded among the male participants by 62 (20%) males and the female participants by 92 (18%) females. While the normal cut-off alpha-1 microglobulin values (<15 mg/gm creatinine) were exceeded among the male participants by 180 (58%) males and the female participants by 235 (46%) females. Subjects were classified into three groups with low-exposure (urinary-Cd level below 2 µg/gm creatinine), middle-exposure (urinary-Cd level between 2-5 µg/gm creatinine) and high-exposure (urinary-Cd level >5 µg/gm creatinine) (Table 2). Increasing urinary alpha-1 microglobulin levels were detected in both male and female populations exposed to lower cadmium levels (<2 µg/gm creatinine). While increasing microalbuminuria levels were cross-sectionally associated with increased urinary cadmium levels in both populations.

Table 2 Urinary Cd levels by the degree of microalbuminuria and urinary alpha-1 microglobulin among study subjects.

Gender	Urinary Cd levels (µg/g creatinine)	N (%)	N (%) with microalbuminuria >20 mg/L	N (%) with alpha-1 microglobulin >15 mg/gm creatinine
Males	<2	100 (32%)	13 (13%)	49 (49%)
	2-5	134 (44%)	28 (21%)	81 (60%)
	>5	75 (24%)	21(28%)	50 (67%)
Females	<2	107 (21%)	7 (7%)	43 (40%)
	2-5	213 (42%)	37 (17%)	86 (40%)
	>5	188 (37%)	48 (26%)	106 (56%)

Correlation and linear regression analysis of the determinant parameters

Correlation analysis between urinary-Cd and renal biochemical makers are summarized in Table 3. The urinary cadmium level was positively correlated with age (males: 0.184 p<0.001 & females: 0.226, p<0.001), and years of residence (males: 0.228 p<0.001& females: 0.243, p<0.001), microalbuminuria (males: 0.274, p<0.001 & females: 0.174, p<0.001) and alpha-1 microglobulin (males: 0.133, p<0.05 & females: 0.175, p<0.001) concentrations. Furthermore, the urinary cadmium levels revealed a significant inverse correlation with BMI (males: -0.161 p<0.05 & females: -0.207, p<0.001).

The regression analysis yielded that alpha-1 microglobulin and microalbuminuria significantly increased with increasing urinary cadmium levels (Table 4). Moreover, two demographic parameters (age & years of residence) significantly increased with increasing of two renal biomarkers. Interestingly, the BMI was significantly associated with alpha-1 microglobulin levels in an inverse manner (Table 4).

Table 3 Pearson correlation coefficient analysis between urinary-Cd and parameters.

Parameters	Correlations	Male	Female
Age (years)	*r*	0.184**	0.226**
	Significant (2-tailed)	<0.001	<0.001
BMI (kg/m^2)	*r*	-0.161*	-0.207**
	Significant (2-tailed)	0.004	<0.001
Years of residence (years)	*r*	0.228**	0.243**
	Significant (2-tailed)	<0.001	<0.001
microalbuminuria (mg/L)	*r*	0.274**	0.174**
	Significant (2-tailed)	<0.001	<0.001
alpha-1 microglobulin (mg/gm creatinine)	*r*	0.133*	0.175**
	Significant (2-tailed)	0.020	<0.001

** Correlation is significant at 0.05 level **Correlation is significant at 0.01 level.*

Table 4 Linear regression analysis of study parameters and renal markers.

Gender	Independent variables	alpha-1microglobulin (mg/g creatinine)		microalbuminuria (mg/l)	
		β	*p* value	β	*p* value
Males	Urinary-Cd (µg/gm creatinine)	1.185 (adjusted r^2 =0.015)	<0.05	4.007 (adjusted r^2 =0.072)	<0.001
	Age (years)	0.663 (adjusted r^2 =0.069)	<0.001	0.933 (adjusted r^2 =0.049)	<0.001
	BMI (kg/m^2)	-1.176 (adjusted r^2 =0.026)	<0.001	-0.049 (adjusted r^2 =-0.003)	0.941
	Years of residence (years)	0.430 (adjusted r^2 =0.062)	<0.001	0.607 (adjusted r^2 =0.044)	<0.001
Females	Urinary-Cd (µg/g creatinine)	1.664 (adjusted r^2 =0.029)	<0.001	1.816 (adjusted r^2 =0.028)	<0.001
	Age (years)	1.032 (adjusted r^2 =0.091)	<0.001	0.628 (adjusted r^2 =0.027)	<0.001
	BMI (kg/m^2)	-1.547 (adjusted r^2 =0.025)	<0.001	-0.354 (adjusted r^2 =-0.001)	0.445
	Years of residence (years)	0.638 (adjusted r^2 =0.062)	<0.001	0.382 (adjusted r^2 =0.017)	<0.05

Discussion

Cadmium (Cd) is one of serious health and environment problems in several countries. Due to the toxicant is non-biodegradable with a long biological half-life approximately 10 to 30 years.[3,18] Generally, the biomonitoring of Cd exposure in human can be assessed in urine for long-term exposure and in blood for a short period of time or recently exposed.[19] The survey of Cd-contamination in Thailand has been reported by several authors.[6-10, 14] The highest mean level of urinary cadmium was recorded in the subjects from northern (Mae Sot, Tak Province) and followed by the subjects from northeastern and central part of the country.[5] Swaddiwudhipong and colleagues was surveyed 7,697 subjects in Mae Sot District, Tak Province.[9] They reported that 4.9% of subjects had Cd levels between 5 to 10 µg/gm creatinine and 2.5% of subjects showed a Cd levels more than 10 µg/gm creatinine.[9]

In present study, we conducted a cross-sectional study among general population of Tak Province which is historically exposed to cadmium. Two biomarkers were used to assess the expression of renal function, namely alpha-1 microglobulin and microalbuminuria. The alpha-1 microglobulin is a low molecular weight protein that can pass through the glomerulus.[16] Increasing of the alpha-1 microglobulin in urine indicate early renal tubular dysfunction.[15, 16, 18] In term of microalbuminuria is described as the urinary albumin excretion of 20 to 200 mg/L.[17] The urinary albumin excretion below 20 mg/L define as normoalbuminuria while the value exceed 200 mg/L define as macroalbuminuria indicating an end-stage renal impairment within 10 to 20 years.[17] Based on this property, alpha-1 microglobulin and microalbuminuria have been studied as a renal biomarker in various diseases such as diabetes mellitus, hypertension and heavy metal intoxication.[1, 3, 16, 18]

Of the 817 subjects surveyed, the total geometric mean level of urinary cadmium from males did exceed regulatory limits of the Commission of Human Biological Monitoring.[15] Amongst the total number of 309 males, 209 (68%) exceeded the HBM I Cd value of 2 µg/gm creatinine, whereas 401 females exceeded HBM I (79%). Moreover, the geometric mean urinary Cd level in both subjects (males: 3.67±3.08 µg/gm creatinine & females: 4.83±3.82 µg/g creatinine) were higher than the levels found in previous study.[9]

Increasing of urinary cadmium with age, years of residence, microalbuminuria and urinary alpha-1 microglobulin were similar observed in both male and female participants. A significant inverse correlation was found between the urinary cadmium levels and BMI. Previous studies have demonstrated that the levels of urinary cadmium decreased with increasing of BMI. However, their correlation is still inadequate.[20, 21]

In the model testing for the association between the urinary cadmium levels and two renal biomarkers, yielded that alpha-1-microglobulin and microalbuminuria were significantly increased with increasing of urinary cadmium levels. Interestingly, the prevalence of increased alpha-1 microglobulin was higher than the prevalence of increased microalbuminuria in subjects with urinary cadmium concentrations below 2 µg/gm creatinine. According to HBM I level, the concentrations below the lower HBM I level are not considered to be a risk for the general population.[15] This result was consistent with the studies conducted by

Järup et al.[22] They suggested that the development of renal tubular damage associated with low-level cadmium exposures.[22] While the microalbuminuria levels appeared to be significant association with the moderately (>2-5 µg/gm creatinine) to high (>5 µg/gm creatinine) levels of urinary cadmium.

Conclusion

The findings of this study indicate that most 610 subjects did exceed the standard of the Commission of Human Biological Monitoring of the HMB I level. The urinary cadmium level showed a significant correlation with age, years of residence, microalbuminuria and urinary alpha-1 microglobulin in both male and female. Moreover, this study indicated that the cadmium exposure at levels below 2.0 µg/gm creatinine may produce measurable changes in renal biomarker of urinary alpha-1 microglobulin. Therefore, we suggested that the urinary alpha-1microglobulin can be used for monitoring the early stages of Cd exposure.

Acknowledgments

This work was financially supported by the Department of Medical Sciences, Ministry of Public Health (Thailand). The authors wish to thank Miss Punthip Teeyapant, Ms. Panisa Getngern and Toxicology center staffs for their useful suggestions.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Gallagher CM, Meliker JR. Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. Environ Health Perspect 2010; 118(12): 1676–84.

[2] Tellez-Plaza M, Jones M, Dominguez Lucas A, Guallar E, Navas-Acien A. Cadmium exposure and clinical cardiovascular disease: a systematic review. Curr Atheroscler Rep 2013; 15: 356.

[3] Anetor JI, Uche CZ, Ayita EB, Adedapo SK, Adeleye JO, Anetor GO, et al. Cadmium level, glycemic control, and indices of renal function in treated type II diabetics: implications for polluted environments. Front public Health 2016; 4: 114.

[4] Pinot F, Kreps S, Bachelet M, Hainaut P, Bakonyi M, Polla B. Cadmium in the environment: sources, mechanisms of biotoxicity, and biomarkers. Rev Environ Health 2000; 15: 299–323.

[5] Apinan R, Satarug S, Ruengweerayut R, Tassaneeyakul W, Na-bangchang K. Cadmium exposure in Thai populations from central, northern and northeastern Thailand and the effects of food consumption on cadmium levels. Southeast Asian J Trop Med Public Health 2009; 40: 177–86.

[6] Chunhabundit R. Cadmium exposure and potential health risk from foods in contaminated area, Thailand. Toxicol Res 2016; 32(1): 65–72.

[7] Boonprasert K, Kongjam P, Limpatanachote P, Ruengweerayut R, Na-Bangchang K. Urinary and blood cadmium levels in relation to types of food and water intake and smoking status in a Thai population residing in cadmium-contaminated areas in Mae Sot. Southeast Asian J Trop Med Public Health 2011; 42(6): 1521–30.

[8] Sriprachote A, Kanyawongha P, Ochiai K, Matoh T. Current situation of cadmium-polluted paddy soil, rice and soybean in the Mae Sot District, Tak Province, Thailand. Soil Sci Plant Nutr 2012; 58(3): 349–59.

[9] Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, Krintratun S, Padungtod C. Cadmium-exposed population in Mae Sot District, Tak Province: 1. Prevalence of high urinary cadmium levels in the adults. J Med Assoc Thai 2007; 90(1): 143–8.

[10] Pollution Control Department. Cadmium contamination in Mae Tao Creek, Mae Sot District, Tak Province. Bangkok, Thailand: Ministry of Natural Resources and Environment; 2004.

[11] Honda R, Swaddiwudhipong W, Nishijo M, Mahasakpan P, Teeyakasem W, Ruangyuttikarn W, et al. Cadmium induced renal dysfunction among residents of rice farming area downstream from a zinc-mineralized belt in Thailand. Toxicol Lett 2010; 198(1): 26–32.

[12] Swaddiwudhipong W, Limpatanachote P, Nishijo M, Honda R, Mahasakpan P, Krintratun S. Cadmium-exposed population in Mae Sot district, Tak province: 3. Associations between urinary cadmium and renal dysfunction, hypertension, diabetes, and urinary stones. J Med Assoc Thai 2010; 93(2): 231–8.

[13] Zhang Y, Wang P, Liang X, Tan CS, Tan J, Wang J, et al. Associations between urinary excretion of cadmium and renal biomarkers in nonsmoking females: A cross-sectional study in rural areas of south China. Int J Environ Res Public Health 2015; 12(10): 11988–2001.

[14] Satarug S, Swaddiwudhipong W, Ruangyuttikarn W, Nishijo M, Ruiz P. Modeling cadmium exposures in low- and high-exposure areas in Thailand. Environ Health Perspect 2013; 121(5): 531–6.

[15] Jakubowski M, Trzcinka-Ochocka M. Biological monitoring of exposure: trends and key developments. J Occup Health 2005; 47(1): 22–48.

[16] Hong C-Y, Hughes K, Chia K-S, Ng V, Ling S-L. Urinary α1-microglobulin as a marker of nephropathy in type 2 diabetic asian subjects in Singapore. Diabetes Care 2003; 26(2): 338–42.

[17] Chavan VU, Sayyed A, Durgawale PP, Sontakke A V, Nilakhe SD. Practical aspects of calculation, expression and interpretation of urine albumin measurement. Natl J Integr Res Med 2011; 2: 29–34.

[18] Pennemans V, De Winter LM, Munters E, Nawrot TS, Van Kerkhove E, Rigo J-M, et al. The association between urinary kidney injury molecule 1 and urinary cadmium in elderly during long-term, low-dose cadmium exposure: a pilot study. Environ Health 2011; 10:77.

[19] Adams S V, Newcomb PA. Cadmium blood and urine concentrations as measures of exposure: NHANES 1999-2010. J Expo Sci Environ Epidemiol 2014; 24(2): 163–70.

[20.] Padilla MA, Elobeid M, Ruden DM, Allison DB. An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02. Int J Environ Res Public Health 2010; 7(9): 3332–47.

[21] Tinkov AA, Filippini T, Ajsuvakova OP, Aaseth J, Gluhcheva YG, Ivanova JM, et al. The role of cadmium in obesity and diabetes. Sci Total Environ 2017; 601–602: 741–55.

[22] Järup L, Hellström L, Alfvén T, Carlsson MD, Grubb A, Persson B, et al. Low level exposure to cadmium and early kidney damage: the OSCAR study. Occup Environ Med 2000; 57(10): 668–72.

Journal of Associated Medical Sciences 2021; 54 (2): 42-50

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Cytotoxic and antiproliferative effects of crude ethanolic extract from *Piper betle* leaves on leukemic cell lines

Methee Rungrojsakul[1*] Siriporn Okonogi[2] Pawaret Panyajai[3] Songyot Anuchapreeda[3*]

[1]*College of Alternative Medicine, Chandrakasem Rajabhat University, Bangkok, Thailand.*
[2]*Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.*
[3]*Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.*

ARTICLE INFO

Article history:
Received 11 January 2021
Accepted as revised 15 April 2021
Available online 21 April 2021

Keywords:
Cytotoxicity, antiproliferation, Piper betle, Wilms' tumor 1, leukemia

ABSTRACT

Background: Leukemia is a group of malignant diseases characterized by the uncontrolled proliferation of abnormal white blood cells in the bone marrow and peripheral blood. These abnormal cells interfere with normal cell growth and development. Nowadays, chemotherapy is the most effective treatment for leukemia but it causes side effects for patients at the same time. To decrease the side effects, medicinal and edible plants are a choice for leukemia treatment. *Piper betle* (betel) leaves are a choice of interest because it is a common material in the recipes of Thai folk medicine.

Objectives: This work aims to investigate cytotoxic and antiproliferative activities of crude ethanolic betel leave extract in leukemic cells.

Materials and methods: The cytotoxic activity and WT1 protein levels were determined using MTT assay and Western blotting, respectively. Hydroxychavecol content in the crude extract was determined using HPLC.

Results: Crude ethanolic betel leave extract had the highest cytotoxicity effects against K562, Molt4, HL60, and U937 cells with IC_{50} values of 36.2±1.1, 19.7±1.6, 28.9±5.6, and 17.3±0.7 µg/mL, respectively. Moreover, it could decrease WT1 protein level and total cell number in a time- and dose-dependent manner compared to vehicle control. Hydroxychavecol was the main compound in crude ethanolic extract following HPLC determination.

Conclusion: Crude ethanolic betel leave extract had high anti-cancer activity *via* WT1 protein suppression in K562 cells.

Introduction

Piper betle, commonly known as betel leaf, is a plant part of the family of Piperaceae which has been used as Thai folk medicine. It is also used in tropical Asia and East Indies. Betel leaves were often chewed together with a little quicklime and areca nut, especially by ancient people. The betel leaves contain carotenes, ascorbic acid, and phenolic compounds. The phenolic compounds of this plant are chavicol,[1] chavibetol, chavibetol acetate,[2] and eugenol.[3] Hydroxychavicol has been reported as the main compound (39.31%), which was determined by GC-MS from crude aqueous extract.[4] In addition, though, in the essential oil extract, the eugenol was reported to be the main compound with a percentage of 36.2 by GC-MS.[5] Betel leaf extract exhibit antioxidative, anti-inflammatory,[6] antimutagenic,[1] antibacterial, antifungal, and antiproliferative activities.[7] Furthermore, the anticancer activity of betel leave extract was reported using aqueous extract (boiling fresh leaves in distilled water) and showed antiproliferative activity in KB cells.[7] However,

* **Corresponding author.**
Author's Address: *College of Alternative Medicine, Chandrakasem Rajabhat University, Bangkok, Thailand.*
** **E-mail address:** mathewhor@hotmail.com, songyot.ancuh@cmu.ac.th
doi: 10.14456/jams.2021.16
E-ISSN: 2539-6056

antileukemic cell reports are limited. The Wilms' tumor 1 or WT1, is a protein encoded by the *WT1* gene which has been used as a leukemic cell biological marker to identify prognosis in leukemia patients. Low levels of WT1 protein expression have been found in normal blood cells, and in contrast, WT1 protein have been found overexpressed in leukemic blood cells.[8] Recently, many reports showed that wild-type WT1 is expressed in the majority of Wilms' tumors in addition to a variety of cancers including breast cancer,[9, 10] lung cancer,[11] bone and soft-tissue sarcoma,[12] head and neck squamous cell carcinoma,[13] thyroid,[14] colon,[15] esophageal,[16] pancreatic ductal cancer,[17] and human primary leukemia,[18] and has been implicated as an oncogene in these tumors.[19] *WT1* gene expression has been reported to be suppressed by curcumin), the active compound from turmeric,[20-22] crude kaffir lime leave extract,[23] and crude mangosteen extract.[24] In this study, the anticancer activity of betel leaf extract was examined. The WT1 protein was used as a biological tool to determine the inhibitory mechanism of K562 cells for leukemic cell proliferation.

Materials and methods

Plant material

The betel (*Piper betle* L.) leaf was collected from the Faculty of Pharmacy, Chiang Mai University, Chiang Mai Province, Thailand. A voucher specimen, No. 008612, was deposited at an herbarium, the Northern Research Center for medicinal plants, Faculty of Pharmacy, Chiang Mai University, Thailand. The leave material was washed and chopped into small pieces and then dried by circulating dry air in an oven at 50°C. The materials were then soaked in 95% ethanol and placed in an ultrasonic bath for 15 min, filtered by filter paper No. 1 with 90 mm diameter and concentrated by evaporation under reduced pressure with a rotary evaporator at 50°C.

Cells and cell culture conditions

K562 (chronic myelocytic), Molt4 (human lymphoblastic), HL60 (human promyelocytic), and U937 (human monocytic) leukemic cell lines were cultured in RPMI-1640 medium containing 10% fetal calf serum, 1 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin (Invitrogen™ Life, Carlsbad, CA, USA). KB-3-1 (human cervical carcinoma) and Caco-2 (human colorectal adenocarcinoma) cells were cultured in Eagle's Minimum Essential Medium (EMEM) and supplemented with 10% fetal bovine serum (FBS) (Invitrogen™ CA, USA), 100 Units/mL penicillin and 100 µg/mL streptomycin. All cell lines will be incubated in humidified 95%, 5% CO_2, and atmosphere at 37°C.

MTT cytotoxicity assay

Cytotoxicity of crude ethanolic betel leave extract was evaluated using MTT assay. Cells (1.0×10^4 cells/well) were cultured in a 96-well plate containing 100 µL medium prior to crude extracts at 37°C for 24 hrs. Afterward, 100 µL of fresh medium containing crude ethanolic betel leaf extract at various concentrations (3-100µg/mL) were added into each well and further incubated for another 48 hrs. After removal of 100 µL medium, 15 µL of MTT dye solution (Sigma-Aldrich, St Louis, MO, USA) at 5 mg/mL was added, and the plate was incubated at 37°C for 4 hrs. Then, aqueous solution was removed, and 200 µL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured using AccuReader™ microplate reader (Metertech-Inc, Taipei, Taiwan) at 545 nm. Metabolic activity of each well was determined and compared to untreated cells (vehicle control). High optical density readings corresponded to a high intensity of dye color, that is, to many viable cells able to metabolize MTT salts. The following formula calculated the fractional absorbance:

$$\% \text{ Cell viability} = \frac{\text{Mean absorbance in test well}}{\text{Mean absorbance in vehicle control well}} \times 100$$

Average cell survival obtained from triplicate determinations at each concentration was plotted as a dose-response curve. The 50% inhibitory concentration (IC_{50}) values of the betel leave and other plant extracts were determined as the lowest concentration, which reduced cell growth by 50% in treated culture, compared to vehicle control. The IC_{50} values were mean±standard error of the mean (SEM) and their activities.

Cell proliferation assay

Total cell numbers were counted using trypan blue exclusion method. Live cells have intact membranes and can exclude trypan blue dye, whereas dead cells with compromised membranes are stained by the trypan blue dye solution. A cell suspension and 0.2% trypan blue were mixed, and viable (unstained) and dead (stained) cells were counted using a hemacytometer. Percentage of viable cells was then calculated.

Protein extraction and Western blotting

Total protein extracts from treated cells were prepared using RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.5 mM EDTA, 0.1% SDS, and protease inhibitor cocktail). Protein concentration was measured by Folin-Lowry method. Proteins were separated using 12% SDS polyacrylamide gel electrophoresis. Analysis for WT1 protein detection were performed using primary rabbit polyclonal anti-WT1 (Santa Cruz Biotechnology, CA, USA) in the ratio of 1:100, followed by a treatment of HRP-conjugated goat anti-rabbit IgG (Promega, Madison, WI, USA) in a 1:20,000 dilution. The GAPDH protein was probed by primary rabbit polyclonal anti-GAPDH (Santa Cruz Biotechnology, CA, USA) in the ratio of 1:1,000, followed by treatment with HRP-conjugated goat anti-rabbit IgG in a 1:20,000 dilution. The proteins were visualized using the Luminata™ Forte Western HRP Substrate (Merck Millipore Corporation, Billerica, MA, USA) and quantified by the ChemiDoc™ XRS (Bio-Rad, Hercules, CA, USA).

Analysis of crude ethanolic extracts using high performance liquid chromatography (HPLC)

Portions of 0.5-100 mg of samples were dissolved in 1 mL of ethanol for HPLC analysis. The solution was filtered through a 0.22 µm filter (nylon syringe filter, Filtrex, USA). The sample was analyzed by a Dionex ICS-3000 HPLC system with a PDI-100 photodiode array detector. A C18 column (150×4.6 mm, 5 µm particle size, Nucleodur®) with a guard column was used. For elution of the constituents, two solvents denoted as A and B were applied. Solution A was 70%

methanol, whereas B was 30% deionized water with an isocratic program for 20 min. The flow rate was 0.7 mL/min at room temperature, and the injection volume of the extract was 20 µL. The retention times and UV spectra of significant peaks were recorded.

Statistical analysis

All data were expressed as the mean±standard deviation (SD) or mean±standard error of the mean (SEM) from triplicate samples of three independent experiments. Statistical differences between the means were determined using one-way ANOVA. The differences were considered significant when the probability value obtained was less than 0.05 (p<0.05).

Results

Cytotoxicity of crude ethanolic betel leave extract on leukemic and other cancer cell lines

As shown in Table 1 and Figure 1, crude ethanolic extract of betel leave was treated with various concentrations (3-100 µg/mL) for 48 hrs in K562, Molt4, HL60, U937, KB-3-1, and Caco-2 cell lines. Crude ethanolic of betel leave extract exhibited the strongest cytotoxicity in the U937 cells with an IC_{50} value of 17.3±0.7 µg/mL. When compared to that of its leave extract, the result showed the lower values with IC_{50} values of 36.2±1.1, 19.7±1.6, 28.9±5.6, and 40.2±7.7 µg/mL in K562, Molt4, HL60, and Caco-2 cells, respectively. However, the betel leave extract did not affect KB-3-1 cells (IC_{50} >100 µg/mL).

Table 1 Inhibitory concentration (IC_{50}) of crude ethanolic extracts from Thai traditional plants.

Crude ethanolic extract	Cytotoxicity IC_{50} (µg/mL)					
	K562	Molt4	HL60	U937	KB-3-1	Caco-2
Betel leaves	36.2±1.1	19.7±1.6	28.9±5.6	17.3±0.7	>100	40.2±7.7

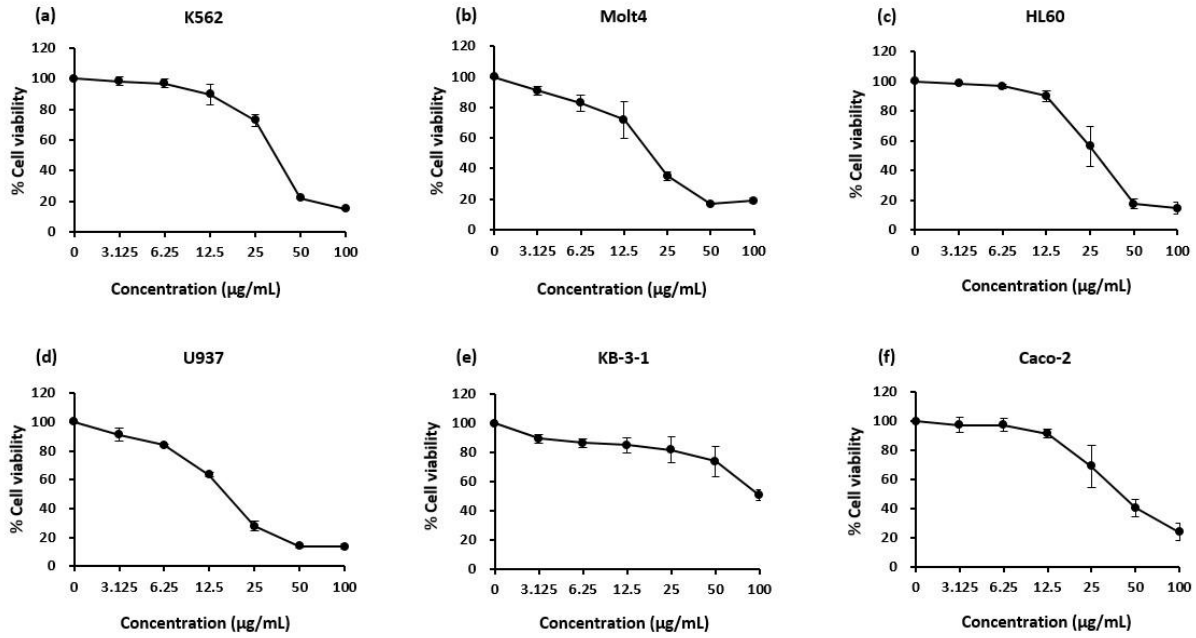

Figure 1. *Inhibitory concentration (IC_{50}) of crude ethanolic betel leaf extract in (a) K562, (b) Molt4, (c) HL60, (d) U937, (e) KB-3-1, and (f) Caco-2 cell lines.*

Inhibitory effects of crude ethanolic betel leaf extract on WT1 protein levels and total cell number in K562 cells

In this experiment, activity of crude ethanolic betel leaf extract on WT1 protein expression was examined using the IC_{20} value (20 µg/mL) in time and dose responses in K562 cells using Western blotting. In this study, K562 cells was used as a WT1 expressing cell model while HL60 and U937 cells do not express WT1 protein.[22] Molt4 cells express lower level of WT1 than K562 cells.[22] Furthermore, there is no report of WT1 expression in KB-3-1 and Caco-2 cell lines. Different time points (12, 24, and 48 hrs) of crude ethanolic betel leave extract were confirmed their cytotoxicity by MTT assay. The result showed no cytotoxicity at 20 µg/mL at 12, 24, and 48 hrs (Figure 2A). Following crude ethanolic betel leave extract treatment, WT1 protein expression levels were decreased by 42.9, 29.6, and 12.7%, respectively, as compared to vehicle control (Figures 2B, and 2C). The effect of various concentrations of this crude ethanolic extract decreased WT1 protein levels by 14, 23.2, and 37.3% in response to 15, 20, and 25 µg/mL, respectively (Figures 3A and 3B). Moreover, the different time points could significantly decrease the total cell number at 48 hrs by 57.6% in K562 cells (Figure 4). Following various concentrations of crude ethanolic extract treatments, the total cell number was significantly decreased at 12 hrs by 16.7, 23.2, and 34.3%, in response to 15, 20, and 25 µg/mL, respectively, as compared to vehicle control (Figure 5).

Hydroxychavicol content of betel leave extract using HPLC

HPLC chromatogram of crude ethanolic betel leaf extracts showed 5 main peaks at retention times of 1.98, 2.80, 3.36, 4.11, and 5.43 min (Figure 6). At 4.11 and 5.43 min, area under the peak curve was 74.54 and 18.67%, respectively. Retention time of standard hydroxychavicol was 4.10 min, and area percentage under the graph was 87.98%, which was consistent with the time peak of crude ethanolic betel leaf extract curve that was shown. It is possible that retention time at during 4.11 min of ethanolic leaf extract from betel leaves is hydroxychavicol.

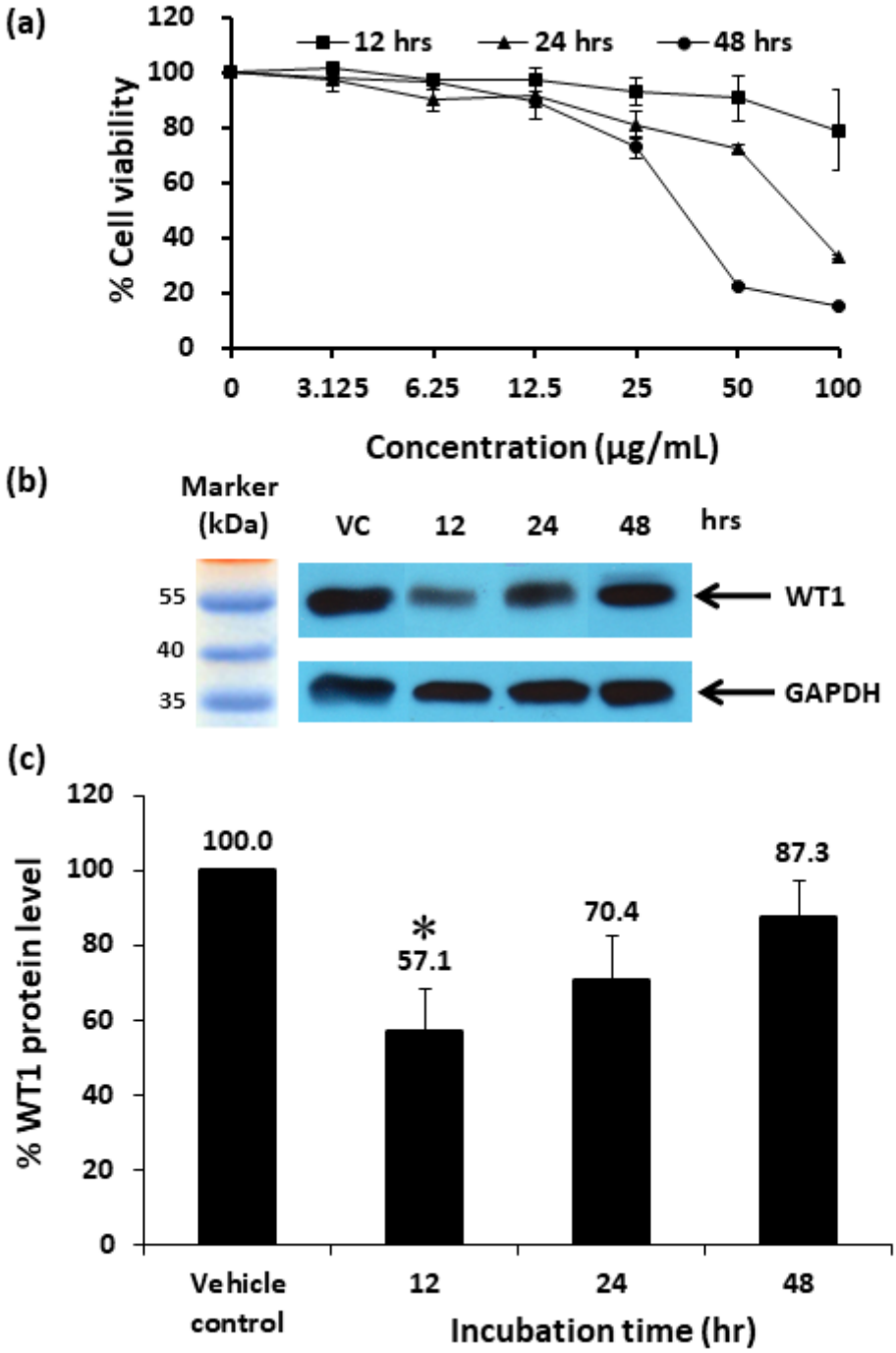

Figure 2. *Times course of measuring crude ethanolic betel leaf extract effect on WT1 protein levels in K562 cells using Western blotting. (a) Effects of cytotoxicity in K562 cells at 12, 24, and 48 hrs. Cells were treated with 20 µg/mL of crude ethanolic betel leave extract for different time points (12, 24, and 48 hrs). (b) Levels of WT1 protein expression were assessed by immunoblotting; GAPDH was used as a loading control. (c) Densitometry was used to quantitate the protein levels and graph as the percentage of vehicle control (0.02% DMSO alone without the crude ethanolic betel leave extracts in the culture medium). Data is reported as the mean value±SEM of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p<0.05$).*

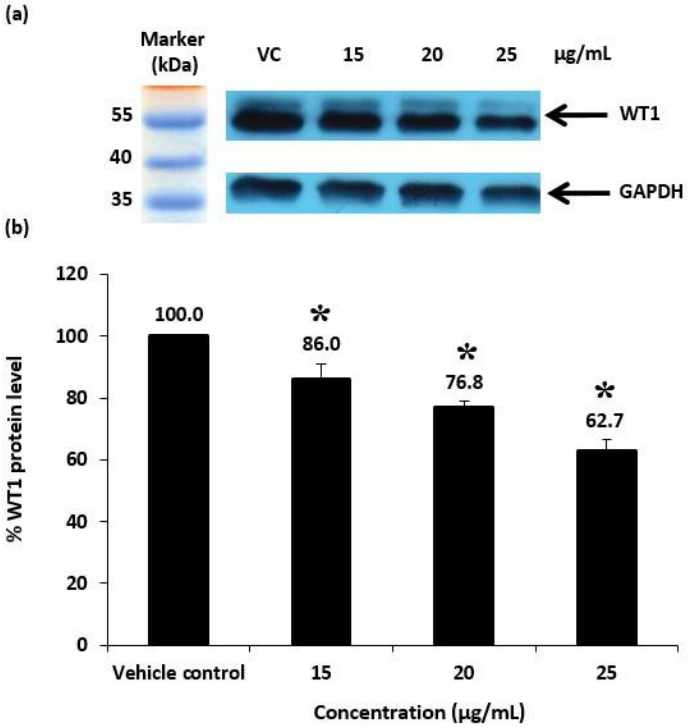

Figure 3. *Effect of various concentrations of crude ethanolic betel leaf extract on WT1 protein levels in K562 cells using Western blotting. Cells were treated with different concentrations of crude ethanolic betel leave extract (15, 20, and 25 µg/mL) for 12 hrs. (a) The levels of WT1 protein expression were assessed by immunoblotting; GAPDH was used as a loading control. (b) Densitometry was used to quantitate the protein levels and graph as the percentage of vehicle control (0.02% DMSO alone without the crude ethanolic betel leave extracts in the culture medium). Data is reported as the mean value±SEM of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control (p<0.05).*

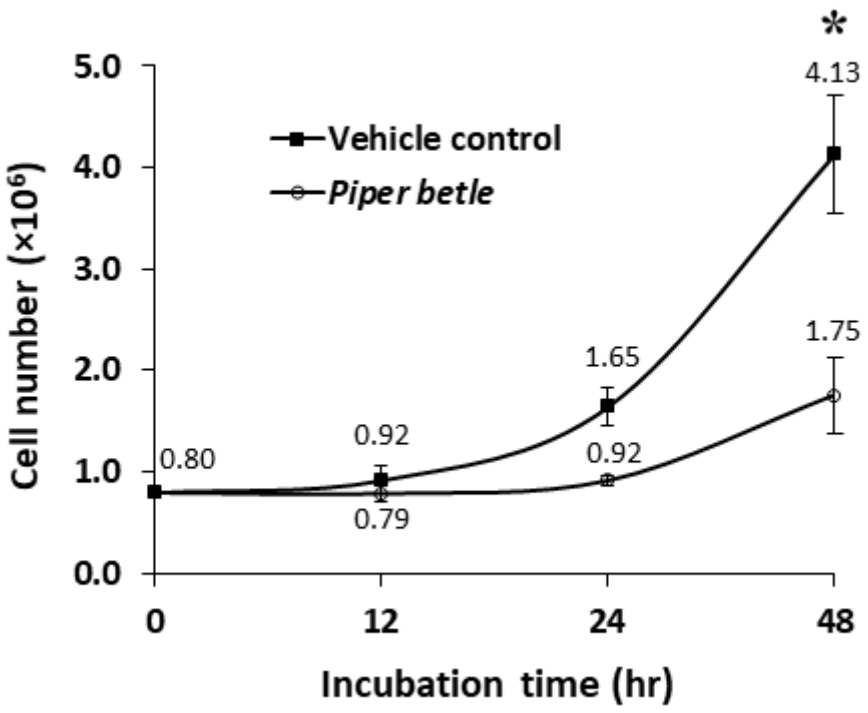

Figure 4. *Total cell number of K562 cells following 20 µg/mL crude ethanolic betel leaf extract treatment during 12-48 hrs. Asterisks (*) denote values that were significantly different from vehicle control (p<0.05) in the same period.*

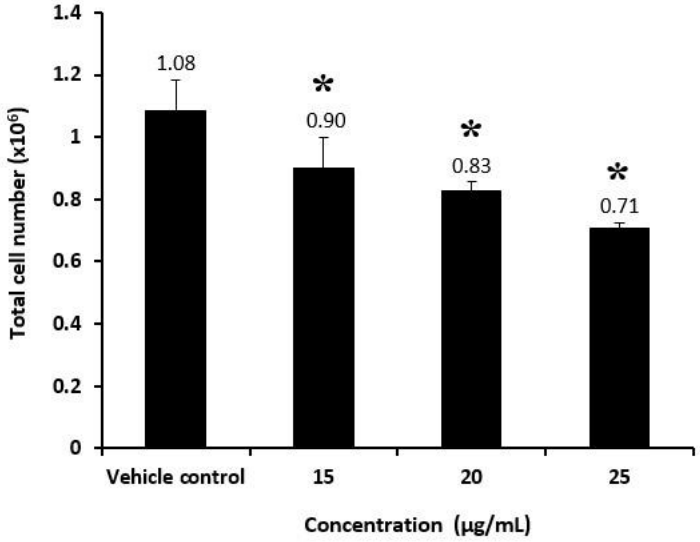

Figure 5. *Total cell number of K562 cells following various concentrations of ethanolic betel leaf extract treatments for 12 hrs. Asterisks (*) denote significant differences from vehicle control (p<0.05).*

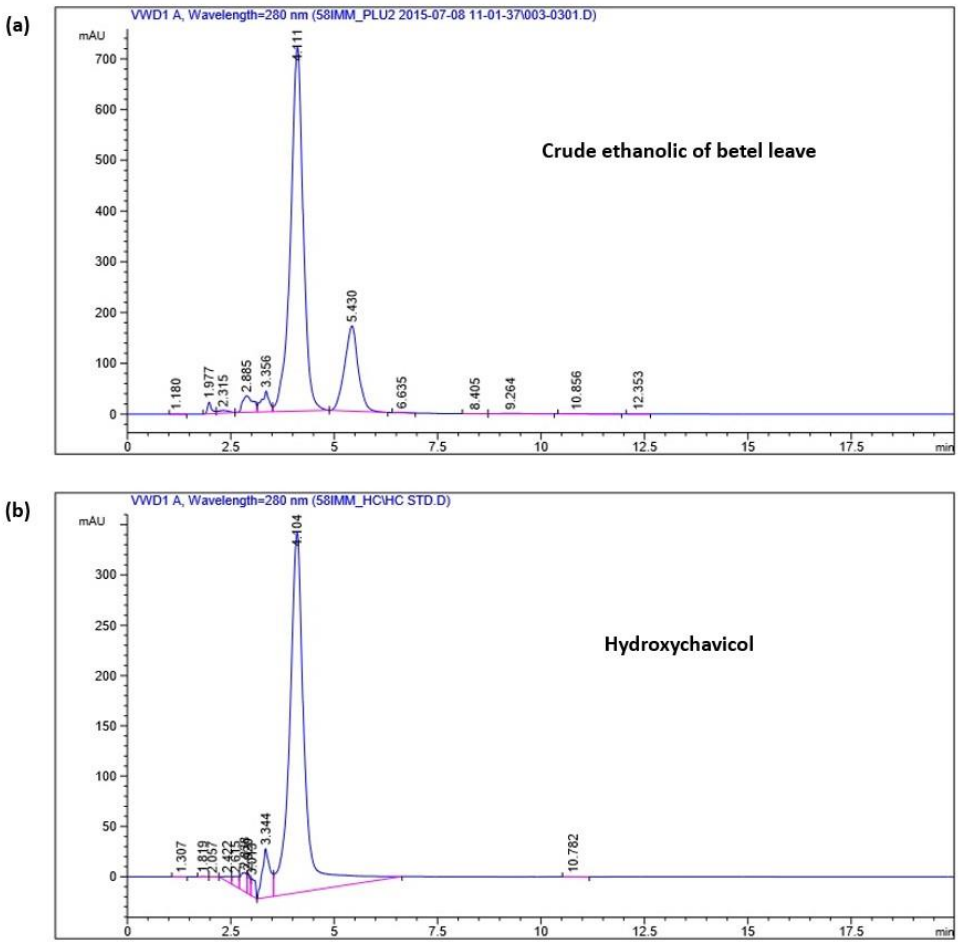

Figure 6. *HPLC chromatogram of the crude ethanolic betel leaf extracts.*

Discussion

Betel (*Piper betle* L.) leaf was selected to study the biological activities because it demonstrated the best activity of anticancer property. This study is the first report of cytotoxic activity of crude ethanolic betel leaf extract in human leukemic cells. It had the strongest activity against human leukemia cell lines. The best activity was shown in U937 cells (IC_{50} value of 17.3±0.7 µg/mL). There was a cytotoxic report of essential oil from betel leaf extract in P388 murine leukemia cell line by MTT assay. The result indicated that essential oil from betel leaf extract exhibited the IC_{50} of over 0.6 mg/mL.[25] Our result was also in line with the effect of kaffir lime leaf extract on U937 cells with IC_{50} of 19.8±1.0 µg/mL. When compared its effect to curcumin in U937 cells, the IC_{50} value after curcumin treatment was 8.7 µg/mL.[26] The cytotoxic effects of crude betel leave extract in both K562 and Molt4 cells were less than that of U937 cells due to the reason for lower levels of WT1 protein in U937 cells.[22] WT1 protein is the transcription factor that promotes cell proliferation in leukemic cells. Thus, high levels of WT1 protein can induce a high rate of cell proliferation in both K562 and Molt4 cells. Furthermore, difference in cell phenotype is one factor that causes differences in cell sensitivities. According to the absence report of the cytotoxic data of crude ethanolic betel leaf extract in human leukemic cells, the cytotoxicity effects of crude ethanolic betel leaf extract in other types of cancer cells have been reported. Crude *Piper betle* leaf extract showed the cytotoxicity with IC_{50} of 0.136 µg/mL at 48 hrs by MTS assay in the HeLa cervical cancer cell line, while *Piper fragile* Benth showed higher cytotoxicity (IC_{50} value of 0.005 µg/mL) than the *Piper betle* leaf extract.[27] However, aqueous *Piper betle* leaf extracts did not show activity in HeLa cells (IC_{50} >100 µg/mL) using neutral red cytotoxicity assay.[7] Cytotoxicities of crude ethanolic betel leaf extracts in U937, K562, Molt4, and HL60 cells were less than vincristine ($9x10^{-5}$, $8x10^{-3}$, $3.9x10^{-4}$, $6.3x10^{-4}$ µg/mL, respectively) in the previous report by $1.92x10^{5}$-, $4.53x10^{3}$-, $7.41x10^{4}$-, and $3.13x10^{4}$-fold, respectively.[28] IC_{50} values of doxorubicin (0.8 ± 0.06 µg/mL) and idarubicin (0.41±0.04 µg/mL) were less than crude ethanolic betel leaf extract in K562 cells by 45.25- and 88.29-fold, respectively.[29]

In this study, cervical carcinoma cell line (KB-3-1) and colon cancer cell line (Caco-2) were analyzed to compare differences amongst the cell types. The crude ethanolic betel leaf extract did not affect the KB-3-1 cells (IC_{50} value >100 µg/mL), while Caco-2 cells had the IC_{50} value of 40.2±7.7 µg/mL. Moreover, aqueous betel leaf extract showed cytotoxicity in human nasopharyngeal epidermoid carcinoma cells (KB) with the IC_{50} value of 29.5±0.3 µg/mL by using neutral red cytotoxicity assay[7] but essential oil extract of *Piper betle* leaf exhibited no antiproliferative activity inhuman mouth epidermal carcinoma cells (KB) and murine leukemia cells (P388) by MTT assay.[25] The aqueous extract of crude betel leaf extract exhibited cytotoxic effect in human epidermoid larynx carcinoma cells (Hep-2).[30] Betel leaf extract inhibited human ductal breast epithelial tumor (T47D) cell proliferation with an IC_{50} value of 55.2 µg/mL using MTS assay.[31] On the opposite, ethyl acetate and hexane extracts showed dose-dependent inhibitory effects on breast cancer (MCF-7) cells with IC_{50} values of 65±0.0 and 163.3±2.89 µg/mL, respectively.[32]

Here WT1 protein was used as a biological marker of leukemic cell proliferation,[8] and as a tool for determining the effect of crude ethanolic betel leaf extract on leukemia cell proliferation. Normally, it has been used to predict leukemia progression. Overexpression of *WT1* gene is important in leukemogenesis because both the RNA and the protein levels of WT1 increase 1,000-10,000 folds in leukemia cells.[8, 33] This suggests it may play an important role in oncogenesis. Pure curcumin extracted from turmeric, a spice grown in Asia's tropical regions, has been shown to decrease WT1 mRNA and WT1 protein levels in human leukemic cell lines.[20-22] *Piper betle* was the herb that presented the cytotoxicity values comparable to other cancer cell lines. This is the first report to show that betel leaves obtain their activity from its bioactive compounds to suppress *WT1* gene expression. Results from Western blotting indicated that the crude betel leaf extract was efficient decreasing levels of WT1 protein in a time- and dose-dependent manner. The maximum value of inhibition (37.3%) was shown at the dose of 25 µg/mL for 12 hrs. The active compound of crude ethanolic betel leaf extract will be further studied. However, previous reports have shown the main compound from betel leaf was hydroxychavicol, which was characterized by GC-MS.[4] Moreover, chavicol was found in crude ethanolic betel leaf extract by HPLC before partitioning into the oil.[34] Their HPLC results showed the same pattern when compared to our results. The biological activity of hydroxychavicol was recently found to induce apoptosis in chronic myelocytic leukemia (CML) cells expressing wild type and mutated bcr/abl with imatinib resistance phenotype.[35] It was also found to induce cell cycle arrest and cell apoptosis of oral KB carcinoma cells.[36] Thus, the active compound from betel leaves will be further studied to elucidate its activity on inhibitory mechanism of the WT1 signaling pathway. However, it has also been reported WT1 signaling involved in protein kinase C alpha (PKCα) and the c-Jun N terminal kinase (JNK) signaling pathway.[37]

Conclusion

Crude ethanolic from *Piper betle* leaf extract had cytotoxic response in leukemic cells. This is the first report to show the inhibitory effect of crude ethanolic from betel leaf via WT1 protein suppression in K562 leukemic cells. Moreover, crude ethanolic extract decreased total cell number in a time- and dose-dependent manner. It is a source of hydroxychavicol, the main active compound product displaying potent inhibitory activity on leukemic cell proliferation, and a promising candidate as a naturally occurring antileukemic drug in the future.

Conflict of interest

There are no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

The authors would like to extend their gratitude for the grants received from Chandrakasem Rajabhat University.

References

[1] Amonkar A.J., Nagabhushan M., D' Souza A.V., Bhide S.V. "Hydroxychavicol: a new phenolic antimutagen from betel leaf." Food and Chemical Toxicology 1986; 24(12): 1321-4.

[2] Rimando A. M., Han B. H., Park J. H. and Cantoria M. C. Studies on the Constituents of Philippine Piper betel Leaves. Archives of Pharmacal Research 1986; 9(2): 93-7.

[3] Nagabhushan M., Amonkar A.J, Nair U.J., D' Souza A.V., Bhide S.V. Hydroxychavicol: a New Anti-Nitrosating Phenolic Compound from Betel Leaf. Mutagenesis 1989; 4(3): 200-4.

[4] Nalina T. Rahim Z.H.A. The crude aqueous extract of Piper betle L. and its antibacterial effect towards Streptococcus mutans. American Journal of Biotechnology and Biochemistry 2007; 3(1): 10-5.

[5] Row L.C.R., Ho J.C. The antimicrobial activity, mosquito lavrvicidal activity, antioxidant property and tyrosinase inhibition of Piper betle. Journal of the Chinese Chemical Society 2009; 56: 653-8.

[6] Pin K.Y., Chuah A.L., Rashih A.A., Mazura M.P., Fadzureena J., Vimala S. and Rasadah M.A. Antioxidant and anti-inflammatory activities of extracts of betel leaves (Piper betle) from solvents with different polarities. J Trop For Sci 2010; 22(4): 448-55.

[7] Fathilah A.R., Sujata R., Norhanom A.W., Adenan M.I. Antiproliferative activity of aqueous extract of Piper betle L. and Psidium guajava L. on KB and HeLa cell lines. Journal of Medicinal Plants Research 2010; 4(11): 987-90.

[8] Inoue K., Sugiyama H., Ogawa H., Nakagawa M., Yamagami T., Miwa H., et al. WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood 1994; 84(9): 3071-9.

[9] Loeb D.M., Evron E, Patel CB, Sharma PM, Niranjan B, Buluwela L, et al. Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. Cancer Res 2001; 61: 921-5.

[10] Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, et al. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. Clin Cancer Res 2002; 8(5): 1167-71.

[11] Oji Y, Miyoshi S, Maeda H, Hayashi S, Tamaki H, Nakatsuka S, et al. Overexpression of the Wilms' tumor gene WT1 in de novo lung cancers. Int J Cancer 2002; 100(3): 297-303.

[12] Ueda T, Oji Y, Naka N, Nakano Y, Takahashi E, Koga S, et al. Overexpression of the Wilms' tumor gene WT1 in human bone and soft-tissue sarcomas. Cancer Sci 2003; 94(3): 271-6.

[13] Oji Y, Inohara H, Nakazawa M, Nakano Y, Akahani S, Nakatsuka S, et al. Overexpression of the Wilms'tumor gene WT1 in head and neck squamous cell carcinoma. Cancer Sci 2003; 94 (6): 523-9.

[14] Oji Y, Miyoshi Y, Koga S, Nakano Y, Ando A, Nakatsuka S, et al. Overexpression of the Wilms' tumor gene WT1 in primary thyroid cancer. Cancer Sci 2003; 94(7): 606-11.

[15] Oji Y, Yamaboto H, Nomura M, Nakano Y, Ikeba A, Nakatsuka S et al. Overexression of the Wilms tumor gene WT1 in colorectal adenocarcinoma. Cancer Sci 2003; 94(8): 712-7.

[16] Oji Y, Yano M, Nakano Y, Abeno S, Nakatsuka S, Ikeba A. et al. Overexpression of the Wilms' tumor gene WT1 in esophageal cancer. Anticancer Res 2004; 24(5B): 3103-8.

[17] Oji Y, Nakamori S, Fujikawa M, Nakatsuka S, Yokota A, Tatsumi N, et al. Overexpression of the Wilms' tumor gene WT1 in pancreatic ductal adenocarcinoma. Cancer Sci 2004; 95(7): 583-7.

[18] Seidl C, Siehl J, Ganser A, Hofmann WK, Fischer M, Kirchmaier CM, et al. Platelet glycoprotein expression in patients with myelodysplastic syndrome. Thromb Res 2000; 100(1): 27-34.

[19] Sugiyama H. Wilms' tumor gene WT1: Its oncogenic function and clinical application. Int J Hematol 2001; 73: 177-87.

[20] Anuchaprreda S., Limtrakul P., Thanarattanakorn P., Sittipreechacharn S., Chanarat P. Inhibitory effect of curcumin on WT1 gene expression in patient leukemic cells. Arch Parm Res 2006; 29(1): 80-7.

[21] Anuchaprreda S., Thanarattanakorn P., Sittipreechacharn S., Chanarat P. Limtrakul P. Curcumin inhibits WT1 gene expression in human leukemic K562 cells. Acta Pharmacologica Sinica 2006; 27(3): 360-6.

[22] Anuchapreeda S., Tima S., Duangrat C., Limtrakul P. Effect of pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin on WT1 gene expression in leukemic cell lines. Cancer Chemother Pharmacol 2008; 62: 585-94.

[23] Chueahongthong F., Ampasavate C., Okonogi S., Tima S., Anuchapreeda S. Cytotoxic effects of crude kaffir lime (Citrus hystrix, DC.) leaf fractional extracts on leukemic cell lines. Journal of Medicinal Plants Research 2011; 5(14): 3097-105.

[24] Semsri S., Ampasavate C., Okonogi S., Srikamchum M., Anuchapreeda S. Effect of Mangosteen peel fraction extracts on WT1 gene and WT1 protein expression in leukemic cell lines. Songkla Med J 2009; 27(5): 389-403.

[25] Manosroi J., Dhumtanom P., Manosroi A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Letters 2006; 235: 114-20.

[26] Anuchapreeda S., Sadjapong W., Duangrat C., Limtrakul P. The cytotoxic effect of curcumin, demethoxycurcumin and bisdemethoxycurcumin purified from turmeric powder on leukemic cell lines. Bull Chiang Mai Assoc Med Sci 2006; 39(1): 60-71.

[27] Widowati W., Wijaya L.Wargasetia T.L., Bachtiar I., Yellianty Y., Laksmitawati D.R. Antioxidant, anticancer, and apoptosis-inducing effects of Piper extracts in HeLa cells. Journal of Experimental and Integrative Medicine 2013; 3(3): 225-30.

[28] Ampasavate C, Ogonoki S, Anuchapreeda S. Cytotoxicity of extracts from fruit plants against leukemic cell lines. Afr J Pharm Pharmacol 2010; 4(1): 013-21.

[29] Anuchapreeda S, Fah Chueahongthong F, Viriyaadhammaa N, Panyajai P, Anzawa R, Tima S, Ampasavate C, Saiai A, Rungrojsakul M, Usuki T, Okonogi S. Antileukemic Cell Proliferation of Active Compounds from Kaffir Lime (Citrus hystrix) Leaves. Molecules 2020; 25(6):1300; https://doi.org/10.3390/molecules25061300

[30] Chaurasia S., Kulkarni G.T., Shetty L.N. Phytochemical studies and in vitro cytotoxicity screening of Piper betle leaf (PBL) extract. Middle-East J.Sci.Res 2010; 6(5): 532-6.

[31] Widowati W., Mozef T., Risdian C., Ratnawati H., Tjahjani S., Sandra F. The comparison of antioxidative and proliferation inhibitor properties of Piper betle L., Catharanthus roseus [L] G.Don, Dendrophtoe petandra L., Curcuma manga Val. Extracts on T47D cancer cell line. Journal of Biochemistry and Bioinformatics 2011; 1(2): 022-8.

[32] Abrahim N.N., Kanthimathi M.S., Abdul-Aziz A. Piper betle shows antioxidant activities, inhibits MCF-7 cell proliferation and increases activities of catalase and superoxide dismutase. Research article from BioMed Central 2012; 11 p.

[33] Menssen H.D., Renkl H.J., Rodeck U., Maurer J., Notter M., Schwartz S., Reinhardt R., Thiel E. Presence of Wilms' tumor gene (WT1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. Leukemia 1995; 9: 1060-7.

[34] Maisuthisakul P. Phenolic antioixidants from betel leaf (Piper betel Linn.) extract obtained with different solvents and extraction time. UTCC Journal 2008; 28(2): 52-64.

[35] Chakraborty J.B., Mahato S.K., Joshi K., Shinde V., Rakshit S., Biswas N., et al. Hydroxychavicol, a Piper betle leaf component, induces apoptosis of CML cells through mitochondrial reactive oxygen species-dependent JNK and endothelial nitric oxide synthase activation and overrides imatinib resistance. Cancer Science 2011; 103(1): 88-99.

[36] Chang M.C., Uang B.J., Wu H.L., Lee J.J., Hahn L.J., Jeng J.H., Inducing the cell cycle arrest and apoptosis of oral KB carcinoma cells by hydroxychavicol: roles of glutathione and reactive oxygen species. Br J Pharmacol 2002; 135: 619-30.

[37] Semsri S., Krig S.R., Kotelawala L., Sweeney C.A., Anuchapreeda S. Inhibitory mechanism of pure curcumin on Wilms' tumor1 (WT1) gene expression through the PKCα signaling pathway in leukemic K562 cells. FEBS Letters 2011; 585: 2235-42.

Instructions for Authors

Instructions for Authors

Original article/thesis can be submitted through the on-line system via website https://www.tci-thaijo.org/index.php/bulletinAMS/

General Principles

Journal of Associated Medical Sciences is a scientific journal of the Faculty of Associated Medical Sciences, Chiang Mai University. The articles submitted to the journal that are relevant to any of all aspects of Medical Technology, Physical Therapy, Occupational Therapy, Radiologic Technology, Communication Disorders, and other aspects related to the health sciences are welcome. Before publication, the articles will go through a system of assessment and acceptance by at least three experts who are specialized in the relevant discipline. All manuscripts submitted to Journal of Associated Medical Sciences should not have been previously published or under consideration for publication elsewhere. All publications are protected by the Journal of Associated Medical Sciences' copyright.

Manuscript categories

1. **Review articles** must not exceed 20 journal pages (not more than 5,000 words), including 6 tables/figures, and references (maximum 75, recent and relevant).
2. **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).
3. **Short communications** including technical reports, notes, and letter to editor must not exceed 5 journal pages (not more than 1,500 words), including 2 tables/figures, and references (maximum 10, recent and relevant).

Manuscript files

To submit your manuscript, you will need the following files:

1. A Title page file with the names of all authors and corresponding authors*
2. Main document file with abstract, keywords, main text and references
3. Figure files
4. Table files
5. Any extra files such as Supplemental files or Author Biographical notes

Manuscript Format

1. **Language**: English, Caribri 10 for text and 7 for all symbols. PLEASE be informed that the Journal only accept the submission of English manuscript.
2. **Format**: One-side printing, double spacing. Use standard program and fonts and, add page and line number for all pages.
3. **A Title page**: Include article title, names of all authors and co-authors, name of the corresponding author and acknowledgements. Prepare according to following contents;
   - *Title of the article*: Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulate where possible.
   - *Author names and affiliation*: Where the family name may be ambiguous (e.g. a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with superscript number immediately after author's name and in front of appropriate address. Provide the full postal address of each affiliation, including the province, country and, if available, the e-mail address of each author.
   - *Corresponding author*: Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication, ensure that telephone and fax numbers (with postal area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.
   - *Acknowledgements*: Acknowledgements will be collated in a separate section at the end of the article before the references in the stage of copyediting. Please, therefore, include them on the title page, List here those individuals who provided help during the research (e.g. providing language help, writing assistance or proof reading the article, etc.)
4. **Main article structure**: The manuscripts should be arranged in the following headings: Title, Abstract, Introduction, Materials and Methods, Results, Discussion and Conclusion, and Reference. Prepare according to following contents;
   - *Abstract*: Not exceeding 400 words, abstract must be structured with below headings in separated paragraph:
     - Background,
     - Objectives,
     - Materials and methods,
     - Results,
     - Conclusion, and
     - Keywords (3-5 keywords should be included)
   - *Introduction*: State the objectives of work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.
   - *Materials and Methods*: Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference, only relevant modifications should be described. Ensure that each table, graph, or figure is referred in the text. According to the policy of ethical approval, authors must state the ethical approval code and conduct informed consent for human subject research (If any) and for animal research, authors must include a statement or text describing the experimental procedures that affirms all appropriate measures (if any) in this section.
   - *Results*: Results should be clear and concise. Present the new results of the study such as tables and figures mentioned in the main body of the article and numbered in the order in which they appear in the text or discussion.
   - *Discussion*: This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion

section is often appropriate. Avoid extensive citations and discussion of published literature.
- *Conclusion*: The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a "Discussion" or "Results and Discussion".
- *Conflict of interes*t: All authors must declare any financial and personal relationship with other people or organization that could inappropriately influence (bias) their work. If there is no interest to declare, then please state this: "The authors declare no conflict of interest".
- *Ethic approval*: Ethic clearance for research involving human and animal subjects.
- *References*: Vancouver's style.

5. **Artwork Requirements**
   - Each table, graph and figure should be self-explanatory and should present new information rather than duplicating what is in the text. Prepare one page per each and submit separately as supplementary file(s).
   - Save the figures as high resolution JPEC or TIFF files.

Note: Permission to reprint table(s) and/or figure(s) from other sources must be obtained from the original publishers and authors and submitted with the typescript.

Ensuring a blind peer review

To ensure the integrity of the double-blinded peer-review for submission to this journal, every effort should be made to prevent the identities of the authors and reviewers from being known to each other. The authors of the document have deleted their names from the main text, with "Author" and year used in the references and footnotes, instead of the authors' name, article title, etc. After the journal was accepted, the name of authors and affiliation and the name of the corresponding author must be included into the document and re-submitted in the copyediting stage.

Proof correction

The Proofs of final paper approved for publication are to be returned by email to the researcher before publication.

Page charge

No page charge.

References Format

1. References using the Vancouver referencing style (see example below).
2. In-text citation: Indicate references by number(s) in the order of appearance in the text with superscript format. Reference numbers are to be placed immediately after the punctuation (with no spacing). The actual authors can be referred to, but the reference number(s) must always be given. When multiple references are cited at a given place in the text, use a hyphen to join the first and last numbers that are inclusive. Use commas (with no spacing) to separate non-inclusive numbers in a multiple citation e.g. (2-5,7,10). Do not use a hyphen if there are no citation numbers in between inclusive statement e.g. (1-2). Use instead (1,2).
3. References list: number the references (numbers in square brackets) in the list must be in the order in which they are mentioned in the text. In case of references source from non-English language, translate the title to English and retain "in Thai" in the parentheses.
4. Please note that if references are not cited in order the manuscript may be returned for amendment before it is passed on to the Editor for review.

Examples of References list

Multiple Authors: List up to the first 6 authors/editors, and use "et al." for any additional authors.

Journal Articles (print): In case of reference source contains DOI, retain doi: at the end of reference. Vancouver Style does not use the full journal name, only the commonly-used abbreviation: "Physical Therapy" is cited as "Phys Ther". As an option, if a journal carries continuous pagination throughout a volume (as many medical journals do) the month and/or issue number may be omitted. Allow one space after semi-colon and colon and end each reference with full stop after page number.

- 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.
- 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.
- 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.
- 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): 104-109. doi: 10.1055/s-0040-1713398.
- 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).

Book / Chapter in an Edited Book References

PLEASE be informed that references of books and chapter in edited book should not be include in the research article, but others manuscript categories.

- Grove SK, Cipher DJ. Statistics for Nursing Research: A Workbook for Evidence-Based Practice. 3rd Ed. St. Louis, Missouri: Elsevier; 2019.
- 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

- Dehkharghani S, editor. Stroke [Internet]. 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.
- 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 [Internet]. 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

- Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [Dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.
- 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].

Conference Proceedings

- 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.
- 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.

Government Organization Document

- Australian Government, Department of Health. Physical activity and exercise guidelines for all Australian. 2021 [updated 2021 May 7; cited 15 Jul 2021]. Available from: https://www.health.gov.au/health-topics/physical-activity-and-exercise/physical-activity-and-exercise-guidelines-for-all-australians.
- Department of Health. Situation survey on policy and implementation of physical activity promotion in schools for first year 2005. (in Thai). Nonthaburi: Ministry of Public Health; 2005.
- Department of Local Administration, Ministry of Interior Affairs. Standard of Sports Promotion. (in Thai). Bangkok. 2015:7–9.
- World Health Organization. WHO guidelines on physical activity and sedentary behaviour. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

Journal History

Established in 1968

- 1968-2016 As the Bulletin of Chiang Mai Associated Medical Sciences
  - Vol1, No1 - Vol.49, No3
- 2017, the Journal of Associated Medical Sciences
  - Vol.50, No1 and forward.

Journal Sponsorship Publisher

Faculty of Associated Medical Sciences, Chiang Mai University

Sponsors

Faculty of Associated Medical Sciences, Chiang Mai University

Sources of support

Faculty of Associated Medical Sciences, Chiang Mai University