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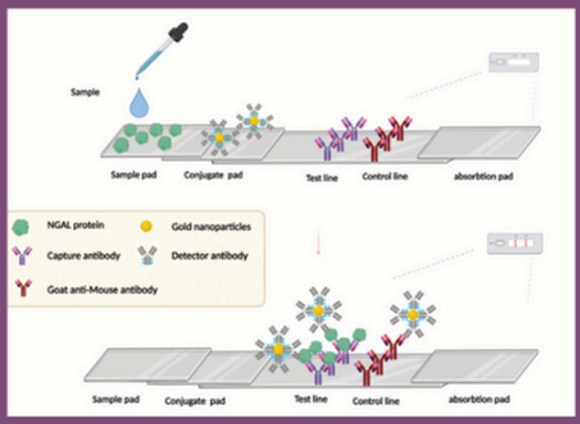

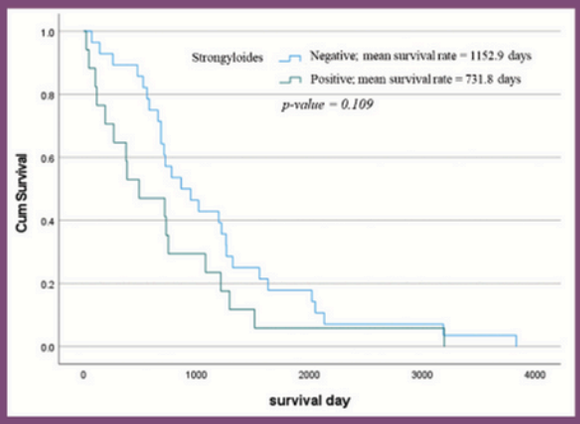

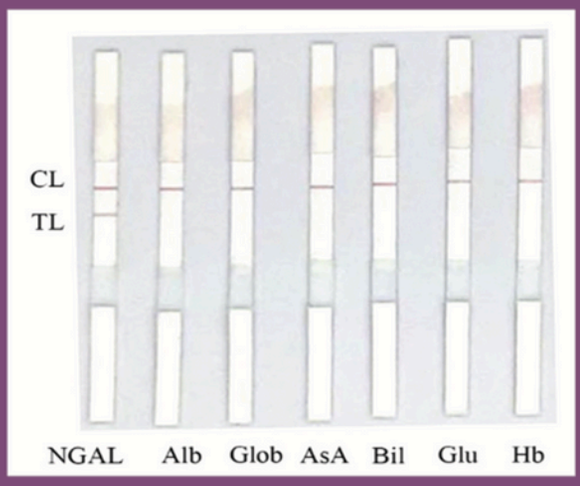

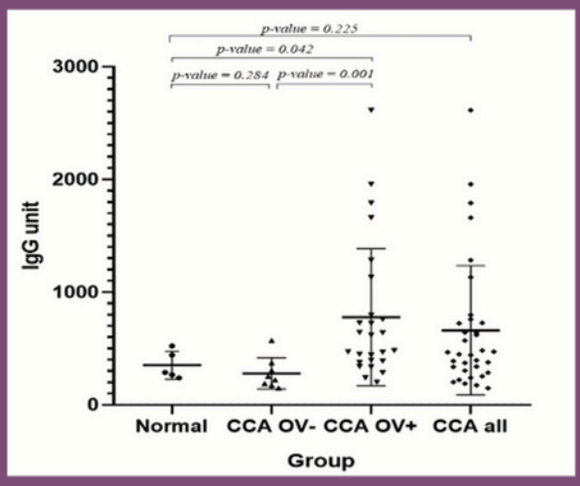

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Arpassanan Wiyanad
Sirirut Multakorn

All correspondence concerning manuscripts, editorial issues and subscription should be addressed to:
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ORIGINAL ARTICLE

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Determination of serum IgG level for *Strongyloides stercoralis* in cholangiocarcinoma

Autchasai Siriprayong[1,2,†], Supakan Amontailak[1,2,†], Phattharaphon Wongphutorn[2,3], Paiboon Sithithaworn[2,3], Kanjanaporn Taenseesaeng[1], Warakorn Pengpala[1], Gungsadan Wilaipan[1], Porntip Pinlaor[1,2], Anchalee Techasen[1,2*]

[1] Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[2] Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.
[3] Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
[†] These authors contributed equally

KEYWORDS
Stronglyloides stercoralis; Cholangiocarcinoma; IgG detection.

ABSTRACT

Strongyloides stercoralis is a helminth parasite that is predominantly endemic to the northeastern region of Thailand. Infection in people typically does not result in any noticeable symptoms. However, immunocompromised or immunodeficient patients, such as those with cancer, AIDS, or undergoing chemotherapy, are at risk of developing severe and potentially life-threatening forms of this parasite infection. Indirect enzyme-linked immunosorbent assay (ELISA) employing IgG antibodies in serum are highly sensitive for diagnosing *S. stercoralis* infection. The objective of this study was to assess the serum IgG levels of *S. stercoralis* in cholangiocarcinoma (CCA) patients and to analyze the correlation between IgG levels and clinical pathology, chemotherapy treatment status, and laboratory findings. The study found that among the 107 individuals with CCA, 34 (31.78%) tested positive for *S. stercoralis*, while 73 (68.22%) tested negative based on their serum IgG levels. Nevertheless, no statistically significant correlation was found between any of the analyzed factors, including the status of receiving chemotherapy treatment. This study indicated no significant impact of IgG antibody levels of *S. stercoralis* on the survival time of CCA patients. Additionally, no link was observed between IgG antibody levels and the severity of CCA in patients. It implies that there is no requirement for further testing on the past presence of *S. stercoralis* infection before starting chemotherapy in CCA patients. Nevertheless, this study focuses on measuring the concentration of antibodies rather than antigens. Further investigation of the antigen levels is necessary to validate the entirety of the data.

***Corresponding author:** Anchalee Techasen, PhD. Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. Email address: anchte@kku.ac.th
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Introduction

Strongyloid stercoralis belongs to the roundworm type, Nematoda phylum. Its life cycle is complete with two cycle sets including free-living cycle and parasitic cycle which enable auto-infection without a discharge[1]. Strongyloidiasis has been a major burden to public health caused by *S. stercoralis* infection in tropical and sub-tropical areas including Africa, Latin-America, Australia and Asia[2]. The highest incidence of infection belonged to the Southeast Asia where Thailand exhibited 15.9 – 28.9% of prevalence[3,4]. Khon Kaen, a province in the Northeastern part of Thailand, demonstrated a prevalence rate of 12.9% utilising the formalin-ethyl acetate concentration technique (FECT)[5,6]. Most patients were probably asymptomatic or had mild gastrointestinal symptom. Immunocompromised patients, such as those with cancer, AIDS, alcoholism, malnutrition, kidney disease, and undergoing chemotherapy, are at a higher risk of developing severe conditions. Autoinfection can lead to the dissemination of the parasite to many organs, such as the lung, trachea, heart, liver, kidney, spinal cord, and brain. This can result in inflammation, organ dysfunction, bloodstream infection, and ultimately death.

Serodiagnosis of *S. stercoralis* infection provided high sensitivity, such as indirect enzyme-linked immunosorbent assay (ELISA) using sera and extracted antigen from *S. ratti* larvae as alternative to *S. stercoralis* larvae. *S. stercoralis* larvae were difficult in antigen extraction, carrying high risk of accidental infection and ineffective cost[7]. Previous report revealed similarity between *S. ratti* and *S. stercoralis* antigen as well as specificity and sensitivity of diagnosis[8]. It was also discovered that rodent could be a source of *S. ratti* antigen production for immunological diagnosis associated strongyloidiasis in human[9]. IgG was reported to be the most common antibody in response to the antigenic surfaces of infective stage of *S. stercoralis* (Filariform larvae; L3). IgG associated with hyper-infected protection in immunocompromised host with chronic and mild symptomatic strongyloidiasis which was considered as chronic infection indicator[10].

The failure to diagnose and treat *S. stercoralis* infection may lead to the development of chronic inflammatory bowel illness, which in turn increases the risk of gastrointestinal cancer. A case of colorectal cancer with *S. stercoralis* infection was documented in a patient from Peru. Chemotherapy administration was discussed for risks and benefits after *S. stercoralis* was detected with cancer signs[11]. Individuals with immunodeficiency or those undergoing chemo-therapy are at risk of developing a life-threatening hyper-infection, which may be facilitated by chemotherapy-induced development of parasite larvae. Immunocompromised individuals, such as those undergoing chemotherapy, are at risk of developing a life-threatening hyperinfection syndrome caused by an increasing of matured parasite and consequent organ invasion[12]. Therefore, discreet decision of chemotherapy administration is highly necessary.

Cholangiocarcinoma (CCA) originates from abnormal growth of biliary mucosa cells[13]. Northeastern of Thailand was endemic area and Khon Kaen province accounted 84.6 and 36.8 per 100,000 people in respective male and female[14]. The incidence tended to decrease to 14.6 per 100,000 people by 2017[15]. CCA still be a major concern because most patients were asymptomatic in the first stage, then became noticeably symptomatic, such as weight loss, jaundice, body itch, dark urine, pale-colored stool and infected biliary system[16]. *Opisthorchis viverrini* was the causative agent of CCA in Thailand. By consuming raw fish carrying infective larvae, up to 7-time risk of CCA was reported compared to uninfected people[17]. Primary sclerosing cholangitis, chronic hepatitis virus infection, and cirrhosis were also found relating to CCA[13].

The objective of this study was to assess the serum IgG levels of *S. stercoralis* in CCA patients and to analyze the correlation between IgG levels

and clinical pathology, chemotherapy treatment status, and laboratory findings.

Materials and methods

Patient samples

Serum was obtained from patients diagnosed with CCA (n=107) and normal group (n=54). The CCA sera were collected from patients who underwent surgery at Srinagarind Hospital, Khon Kaen University, Thailand. Samples was kept at Cholangiocarcinoma Research Institute (CARI), Khon Kaen University, Thailand. Normal sera were collected from healthy volunteer from Ban Wha subdistrict, Khon Kaen, Thailand. The inclusion criteria consist of those who have had normal results from ultrasonography and have normal blood and liver function tests. All human specimens and the protocols in this study were approved by the Human Ethics Committee of Khon Kaen University, based on the ethics of human specimen experimentation of the National Research Council of Thailand (HE611196), and informed consent was obtained from each subject.

S. ratti antigen extraction

S. ratti (Filariform larva stage) was infected through epithelial injection mice. After one week, feces were collected from mice and cultured by using filter paper culture method. *S. ratti* (Filariform larva stage) were concentrated and washed by normal saline. The filariform larva was kept at -20 ºC before antigen extraction. Crude soluble antigen extract was added into phosphate buffer with anti-protease and kept in -70 ºC for 30 minutes. Then the solution was defrosted four times and the parasite was separated by using sonication and kept at -4 ºC overnight. Next, the solution was centrifuged at 15,000 g at -4 ºC for 30 minutes. The supernatant was estimated by using Bradford protein and kept at -20 ºC. This study was conducted under the approval of animal ethic by the Institutional Animal Ethical Committee, Khon Kaen University (IACUC-KKU-99/62). The procedure was performed in strict accordance with the guidelines for the Care and Use of Laboratory Animals of the National Research Council of Thailand.

Determination of anti- S. stercoralis IgG level

Indirect-ELISA was used for anti-*S. stercoralis* IgG level determination. The crude antigen of *S. ratti* was diluted to 2.5 µg/µL in coating buffer (pH 9.6) and added to 96 well MaxiSorp flat bottom plate for a duplicate in each sample 100 µl/well then incubated overnight. The plate was washed using a washing buffer and discarded. Blocking non-specific binding with 3% skim milk in 1X PBS+0.5% tween20 for two hours at room temperature. Sample was diluted in 3% skim milk with 1X PBS+0.5% tween20 and incubated for one hour at 37°C. Then the plate was washed by using a washing buffer followed by adding horseradish peroxidase-conjugated secondary antibodies and incubated for one hour at 37 ºC. The plate was washed again and added with the orthophenylene diamine hydrochloride (OPD) substrate and incubated in dark and stopped the reaction by 4M H_2SO_4 50 µl/well. The activity was observed by using an ELISA reader at the optical density (OD) of 492 nm. The OD value was calculated by using standard curve.

Standard curve for evaluating IgG unit

The standard IgG unit curve was performed from pooled positive serum as the following dilutions: 1:1000, 1:3000, 1:9000, 1:27000, 1:81000, and 1:243000. The negative serum was diluted into 1:4000 and blank were also included. The sample absorbance was measured utilizing a microplate reader (EZ Read 2000 Microplate Reader) at 492 nm against a standard graph for pooled positive serum. The result of IgG unit expressed the level of *S. stercoralis* infection.

Statistical analysis

The IgG unit level was calculated from OD result and compared to the standard curve. The cutoff values for IgG unit in serum by ELISA were determined by receiver-operating characteristic (ROC) analysis based on analysis of 40 proven-

positive and 40 proven-negative sera samples for *S. stercoralis*. Indicative performance of parameter could be distinguished by area under the curve (AUC). The cutoff was calculated for highest sensitivity and specificity which was described in our previous study[8]. The criteria for classification of *S. stercoralis* positive and negative by serum ELISA were based on the determined cutoff value. It was considered as *S. stercoralis* positive if the IgG unit ≥ 132 and negative if < 132. The correlation between of *S. stercoralis* infection and clinical pathologic parameters was investigated. The clinical pathologic parameters were analyzed using the Chi-square test, and Mann-Whitney U test. Kaplan-Meier survival analysis was used to determine the overall survival. A statistical significance was considered if *p*-value < 0.05.

Results

The level of *S. stercoralis* antibody in CCA sera

Complete data of CCA serum samples (n=107) were collected from patients diagnosed with CCA. The mean age of the patients was 60.37 years. Out of the total 107 patients, 66 (61.68%) were male and 41 (38.32%) were female. Among the 76 patients diagnosed with CCA who had available chemotherapy data, 45 of them (59.21%) had chemotherapy, as shown in table 1. For data analysis, the reference value of laboratory results from Srinagarind Hospital, Khon Kaen University, Thailand was used.

CCA serum samples were tested for the presence of anti-IgG antibody in response to *S. stercoralis* infection. A cut-off of 132 units was used to determine the detection of the IgG based on our previous study [8]. The findings demonstrated that out of 107 samples, 34 (31.78%) tested positive, while 73 (68.22%) tested negative. The statistical analysis revealed that there was no significant correlation between IgG levels and the clinical, pathological, and laboratory data, as shown in table 1.

Table 1 Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy

Variables	*S. stercoralis*			*p*-value
	N	Positive (34)	Negative (73)	
Age (year)				
<60	54	17 (50.0)	37 (50.7)	1.000
≥60	53	17 (50.0)	36 (49.3)	
Gender				
Female	41	9 (26.5)	32 (43.8)	0.081
Male	66	25 (73.5)	41 (56.2)	
Size (cm)				
<5	65	25 (73.5)	40 (54.6)	0.089
≥5	42	9 (26.5)	33 (45.2)	
Location of tumor				
Extrahepatic CCA	27	9 (26.5)	18 (24.7)	0.841
Intrahepatic CCA	80	25 (73.5)	55 (75.3)	

Table 1 Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy (Cont.)

Variables	*S. stercoralis*			*p*-value
	N	Positive (34)	Negative (73)	
Histological type				
Non-papillary	45	15 (44.1)	30 (41.1)	0.768
Papillary	62	19 (55.9)	43 (58.9)	
T stage				
0	3	1 (3.1)	2 (2.9)	
1	23	5 (15.6)	18 (25.7)	
2	29	14 (43.8)	25 (35.7)	0.676
3	28	8 (25.0)	20 (28.6)	
4	9	4 (12.5)	5 (7.1)	
Lymph node metastasis				
No	45	13 (41.9)	32 (48.5)	0.546
Yes	52	18 (58.1)	34 (51.5)	
Distance metastasis				
No	103	32 (94.1)	71 (97.3)	0.425
Yes	4	2 (5.9)	2 (2.7)	
Stage				
0	3	1 (2.9)	2 (2.7)	
1	20	4 (11.8)	16 (21.9)	
2	16	6 (17.6)	10 (13.7)	0.600
3	60	19 (55.9)	41 (56.2)	
4	8	4 (11.8)	4 (5.5)	
OV antibody				
Negative	31	8 (23.5)	23 (31.5)	0.397
Positive	76	26 (76.5)	50 (68.5)	
Chemotherapy				
No	31	9 (34.6)	22 (44.0)	0.470
Yes	45	17 (65.4)	28 (56.0)	
Liver function test				
Total protein (g/dL)				
Normal (6.6-8.7)	70	20 (60.0)	50 (70.4)	
Low (< 6.6)	25	12 (36.4)	13 (18.3)	0.076
High (> 8.7)	9	1 (3.0)	8 (11.3)	
Albumin (g/dL)				
Normal (3.5-5.2)	71	19 (59.4)	52 (73.2)	
Low (< 3.5)	29	13 (40.6)	16 (22.5)	0.104
High (> 5.2)	3	0 (0.0)	3 (4.2)	

Table 1 Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy (Cont.)

Variables	*S. stercoralis*			*p*-value
	N	Positive (34)	Negative (73)	
Globulin (g/dL)				
Normal (2.6-3.4)	33	9 (28.1)	24 (34.3)	
Low (< 2.6)	15	8 (25.0)	7 (10.0)	0.162
High (> 3.4)	54	15 (46.9)	39 (55.7)	
Direct bilirubin (mg/dL)				
Normal (0.3-1.2)	67	21 (63.6)	46 (65.7)	0.829
High (> 1.2)	36	12 (36.4)	24 (34.3)	
Alkaline phosphatase (U/L)				
Normal (40-129)	37	12 (36.4)	25 (35.2)	
Low (< 40)	8	3 (9.1)	5 (7.0)	0.948
High (> 129)	59	18 (54.5)	41 (57.7)	
Complete Blood Count				
Hemoglobin (g/dL)				
Normal (13.0-16.7)	46	15 (44.1)	31 (42.5)	
Low (< 13.0)	57	18 (52.9)	39 (53.4)	0.951
High (> 16.7)	4	1 (2.9)	3 (4.1)	
Hematocrit (%)				
Normal (40.5-50.8)	50	15 (44.1)	35 (47.9)	
Low (< 40.5)	5	18 (55.9)	35 (47.9)	0.415
High (> 50.8)	3	0 (0.0)	3 (4.1)	
WBC count (10^3/uL)				
Normal (4.6-10.60)	66	20 (60.6)	46 (63.0)	
Low (< 4.6)	4	1 (3.0)	3 (4.1)	0.928
High (> 10.60)	36	12 (36.4)	24 (32.9)	
Lymphocyte (%)				
Normal (20.1 - 44.5)	55	19 (55.9)	36 (49.3)	
Low (< 20.1)	46	15 (44.1)	31 (42.5)	0.282
High (> 44.5)	6	0 (0.0)	6 (8.2)	
Monocyte (%)				
Normal (3.4 - 9.8)	87	31 (91.2)	56 (76.7)	
Low (< 3.4)	8	2 (5.9)	6 (8.2)	0.178
High (> 9.8)	12	1 (2.9)	11 (15.1)	
Eosinophil (%)				
Normal (0.7 - 9.2)	66	16 (47.1)	50 (68.5)	
Low (< 0.7)	23	10 (29.4)	13 (17.8)	0.102
High (> 9.2)	18	8 (23.5)	10 (13.7)	

Table 1 Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy (Cont.)

Variables	*S. stercoralis*			*p*-value
	N	Positive (34)	Negative (73)	
Basophil (%)				
Normal (0.0-2.6)	103	34 (100.0)	69 (94.5)	0.164
High (> 2.6)	4	0 (0.0)	4 (5.5)	

Note: The reference range used in categorizing laboratory results derived from Srinagarind Hospital laboratory.

Abbreviation: WBC, White Blood Cell; OV antibody, antibody to *Opisthorchis viverrini*.

Survival analysis and clinicopathological data of *S. stercoralis* infection in CCA patients

The survival analysis of *S. stercoralis* infection in CCA patients showed that there was no correlation between *S. stercoralis* infection and survival time (*p*-value = 0.792). The mean survival rates for patients with positive and negative *S. stercoralis* infection were 879.2 and 906.3 days, respectively, as shown in figure 1.

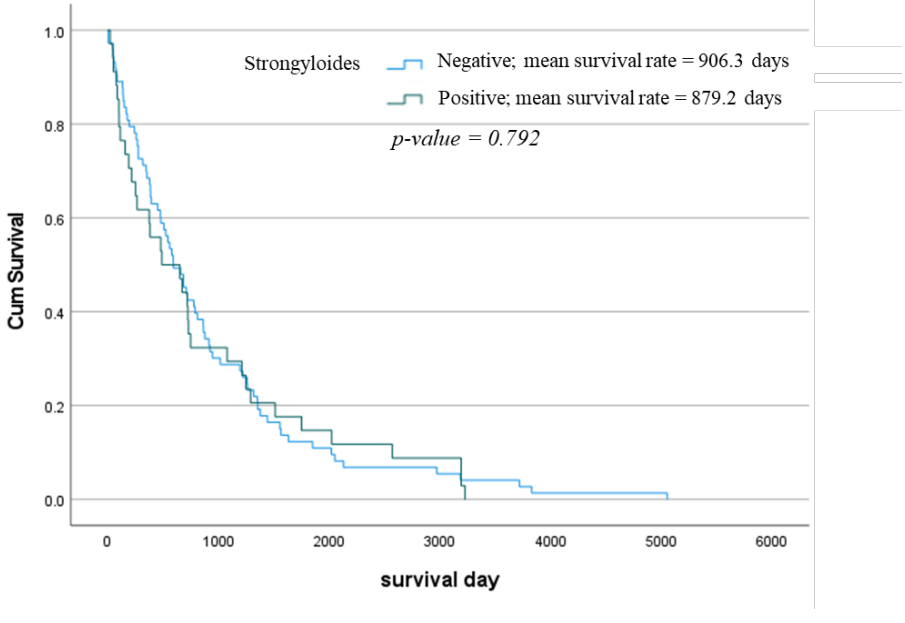

Figure 1 Comparison of *S. stercoralis* infection in CCA patients with and without a history of *S. stercoralis* infection (*p*-value = 0.792).

The relationship between CCA patients who underwent chemotherapy and their history of *S. stercoralis* infection was examined. The analysis revealed no significant link between *S. stercoralis* infection and the response to treatment, as indicated in table 1. The investigation of *S. stercoralis* infection in CCA patients revealed that there was no significant difference in survival rates between patients with and without *S. stercoralis* infection who received chemotherapy (*p*-value = 0.109). The mean survival rates for patients with and without a history of *S. stercoralis* infection were 731.8 and 1152.9 days, respectively, as shown in figure 2. Moreover, the study found no

significant difference in the 5-year survival rate of patients with CCA who received chemotherapy, regardless of whether they had a *S. stercoralis* infection or not (*p*-value = 0.173). The mean survival rates for patients with and without a history of *S. stercoralis* infection were 578.1 and 829.3 days, respectively, as shown in figure 3A. The 3-year survival rate of CCA patients who undergo chemotherapy showed no significant difference between patients with *S. stercoralis* infection and those without *S. stercoralis* infection (*p*-value = 0.399). The mean survival rates for patients with and without a history of *S. stercoralis* infection were 403.0 and 602.6 days, respectively, as shown in figure 3B.

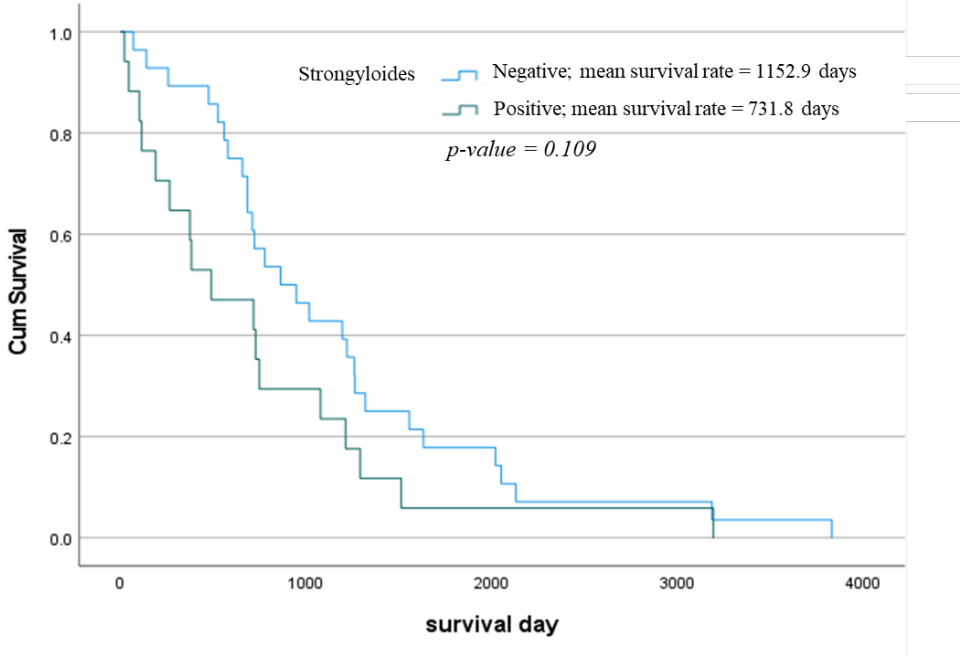

Figure 2 Comparison of *S. stercoralis* infection in CCA patients who receive chemotherapy (*p*-value = 0.109).

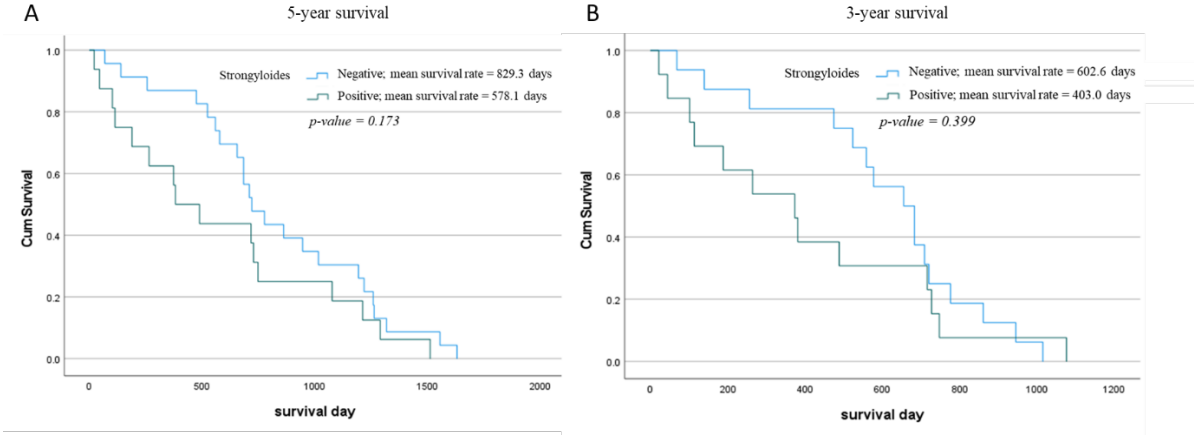

Figure 3 Comparison of *S. stercoralis* infection in CCA patients.
(A) receiving chemotherapy at 5-year survival (*p*-value = 0.173)
(B) receiving chemotherapy at 3-year survival (*p*-value = 0.399)

Comparison of anti-IgG levels in response to *S. stercoralis* infection between normal and CCA sera

IgG unit levels between CCA group and normal group were investigated and compared. CCA patients were classified by using *S. stercoralis* infection status based on and *O. viverrini* infection status. Of 107 CCA patients, positive *S. stercoralis* infection was found in 34 cases (31.78%), while negative *S. stercoralis* infection was found in 73 cases (68.22%). In CCA patients with positive *S. stercoralis* infection, twenty-six patients (34.21%) had *O. viverrini* infection and fifty patients (68.5%) had no *O. viverrini* infection. In the healthy volunteer group, positive *S. stercoralis* infection was found in 5 cases (9.25%), while negative *S. stercoralis* infection was found in 49 cases (90.75%).

Analysis of IgG unit of *S. stercoralis* infection in all groups was performed by Mann-Whitney U test and presented as mean ± SD as shown in figure 4. The mean IgG unit values of normal group were 351.8 ± 123.4. The CCA patients, with evidence of *S. stercoralis* infection, had IgG unit values of 660.8 ± 574.1. There was no significant difference between the CCA and the normal group (*p*-value = 0.225). The IgG unit values of *S. stercoralis* in CCA patients with *O. viverrini* infection (CCA OV+) and normal group exhibited significant difference (*p*-value = 0.042). Moreover, the IgG unit values of CCA OV+ and CCA OV- were highly significantly different (*p*-value < 0.001), providing mean IgG values by 778.2 ± 607.3 and 279.5 ± 119.1, respectively. No significant difference was observed in IgG unit values of the CCA OV- and the normal group (*p*-value = 0.284). The findings indicated that the IgG levels of *S. stercoralis* infection were significantly different in CCA with OV infection status but there was insignificant difference when comparing to normal. Focusing on the subgroup of CCA, the CCA OV+ had high IgG levels compared to the normal group.

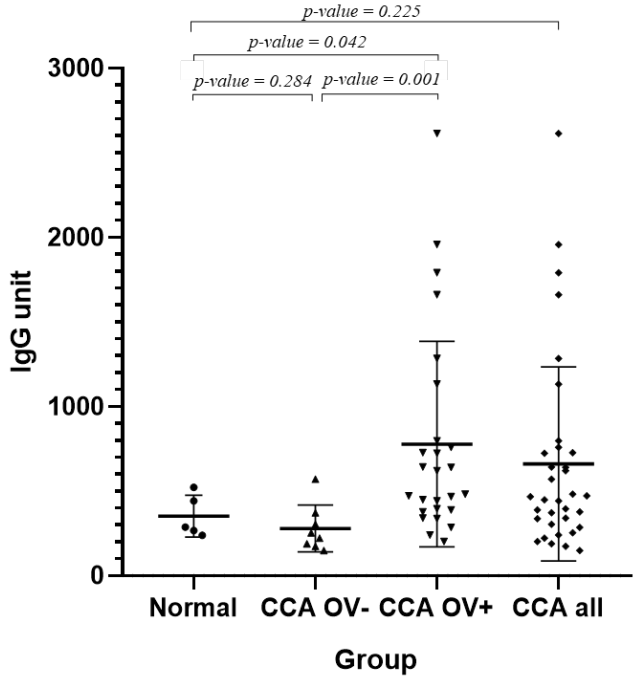

Figure 4 Comparison IgG unit in each group.
Note: The data were presented as mean ± SD. Mann-Whitney U test was used in data analysis between cholangiocarcinoma patient and healthy people.
Abbreviations: CCA, cholangiocarcinoma patient; OV+, with the presence of *O. viverrini* infection; OV-, without the presence of *O. viverrini* infection.

Discussion

Serodiagnosis of *S. stercoralis* infection provided high sensitivity, for instance, indirect enzyme-linked immunosorbent assays (ELISA) using extracted antigen from *S. ratti* larvae to detect antibody in serum. The *S. ratti* antigen used as a substitute for *S. stercoralis* due to the high cost, challenging extraction process, and potential hazard to workers if infected with *S. stercoralis*[6]. Previous report found the similarity of antigen among *S. ratti* and *S. stercoralis*, and also provided similar sensitivity and specificity in detection[7]. IgG was primarily responded to surface of filariform larvae (L3), infective stage, during *S. stercoralis* infection[9]. Thus, IgG in responsive to *S. stercoralis* infection was inspected from CCA patient serum to elucidate the association with clinicopathology, laboratory results, chemotherapy treatment status and history of *O. viverrini* infection for CCA severity analysis and survival rate of patient. In our study, the IgG levels of *S. stercoralis* were significantly elevated in individuals with CCA and *S. stercoralis* infection, particularly in the CCA OV+ group compared to the normal group. Additionally, within the CCA group, the IgG levels were higher in the CCA OV+ group compared to the CCA OV- group. An analysis of the correlation between IgG levels and clinical pathology, chemotherapy treatment status, and laboratory data revealed no observed correlation between *S. stercoralis* infection and CCA in any of the investigated parameters. It implies that there is no requirement for further testing on the past presence of *S. stercoralis* infection before starting chemotherapy in CCA patients. Another research report examined the association between *S. stercoralis* infection and patients with gastrointestinal cancer. The findings demonstrated a significantly higher occurrence of *S. stercoralis* infection in patients with gastrointestinal cancer compared to the control group (p-value < 0.05). Gastrointestinal cancer patients have a 6.7-fold chance of *S. stercoralis* infection more than the control[18]. Zueter et al[18] evaluated the correlation between the detection of *S. stercoralis* infection by real time-PCR and ELISA in cancer patients receiving chemotherapy, with or without steroid treatment, at a hospital in Malaysia. The result showed that the prevalence of *S. stercoralis* infection by using parasite-specific IgG test and IgG4 by ELISA test in cancer patients with immunocompromised were higher than the healthy control group (p-value < 0.05)[19].

Conclusion

This study indicated no significant impact of IgG antibody levels of *S. stercoralis* on the survival time of CCA patients. Additionally, no link was observed between IgG antibody levels and the severity of CCA in patients. It implies that there is no requirement for further testing on the past presence of *S. stercoralis* infection before starting chemotherapy in CCA patients. Nevertheless, this study focuses on measuring the concentration of antibodies rather than antigens. Directly detecting the presence of antigens in an infected patient could be more relevant and provide insights into the severity of CCA. It is also recommended to detect IgG4 antibodies because studies have shown that IgG4 is more specific than IgG, although it has lower sensitivity[19].

Take home messages

Detection of *S. stercoralis* infection prior to embark chemotherapy in CCA patients might not be demanded because of no significant correlation among patient's survival, CCA severity, and IgG levels of *S. stercoralis*. Detection of antigens and IgG4 could be additional evidence to fulfill this study.

Conflicts of interest

The authors declare no conflicts of interest.

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Association of musculoskeletal pain patterns of college students due to the usage of mobile phone

Andrea Mae N Aberion*, Patricia Mae J Bargamento, Neil Philip D Baronda, Jeanneth Kaye S Funcion, Jam Maica B Parejas, Erica Jean D Peñalosa, Keanna Francheska P Talon, Princess Florence N Trinchera

Department of Physical Therapy, College of Biomedical Sciences, DRTREFI.

KEYWORDS
Standardized Nordic Musculoskeletal Questionnaire; Posture; Studying; Cross-sectional study; McGill Pain Questionnaire.

ABSTRACT
Mobile phones have become widely used as online learning platforms, which provide students with flexibility to access content at a time and in a place that is convenient for them. Due to the ubiquity of mobile phones, they may be used in a variety of positions that could expose their musculoskeletal system. This study aimed to investigate if there is an association between musculoskeletal pain patterns due to the usage of mobile phones. Cross-sectional study was conducted on 778 students with individual mobile phones through self-made questionnaire that consisted of general information and a standardized Nordic Musculoskeletal Questionnaire. The study revealed that the predominant site of pain patterns was the neck (64.27%), lower back (66.2%), and upper back area (54.37%). Duration of 19- 24 hours (93.57%) and sitting position (58.87%) was associated with a higher frequency of mobile phone use. Regarding patterns of pain, fatigue (26.74%) type was predominant. There was statistically significant difference found between specific mobile phone usage behaviors and patterns of musculoskeletal pain. More specifically, study sessions were linked to upper back pain (p-value = 0.049), hips/thigh (p-value = 0.021), and neck pain (p-value = 0.021). Social media streaming was linked to neck pain (p-value = 0.041) and ankle pain (p-value= 0.035). Leisure activities and video games were linked to lower back pain (p-value = 0.048), whereas how a mobile phone is held related to wrist pain (p-value = 0.005). The posture adopted and duration of usage are strong determinants of musculoskeletal pain patterns. The study's findings indicate a strong link between musculoskeletal pain in the neck, upper back, and hips and mobile phone usage, particularly in terms of duration and posture. Understanding this relationship can help raise awareness among students, guide prevention, diagnosis, and treatment strategies.

***Corresponding author:** Andrea Mae N Aberion, Department of Physical Therapy, College of Biomedical Scienced, DRTREFT, Email address: aberion.andrea.bspt@gmail.com
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Introduction

Mobile phones have become an essential part of our lives, offering a range of mobile applications utilized for communication, education, and entertainment[1]. During the COVID-19 pandemic, students spend more time at home which results in extensive use of mobile phones, tablets, and other hand-held devices for their studies and daily usage[2]. The amount of mobile phone users, particularly among college students, has expanded dramatically in recent years[3]. Students spend an average of seven hours per day staring at their phone screens[4]. With that being said, students unknowingly adapt an awkward posture when using mobile phones leading to improper postural alignment[5]. Hence, fatigue, discomfort, and pain increases.

Musculoskeletal pain pattern refers to the temporal nature of pain and how the individual's pain changes with time. Theoretically, pain pattern involves the onset, frequency, duration, location and characteristics[6]. Furthermore, extended and repetitive use of mobile phones, along with poor ergonomics, can lead to muscle and joint issues known as musculoskeletal disorder (MSDs). These disorders may appear as pain patterns in the neck, shoulders, upper back pain, and in the hands or wrists[7]. MSDs affect not only workers in the workplace but also students who excessively use various electronic devices daily for both education and recreation[8].

Despite its importance, very limited literature exists about musculoskeletal pain patterns related with the use of mobile phones and its relationship with regards to location in the body region, frequency, duration, and posture. Therefore, the purpose of the present study is to determine the association of mobile phone use and musculoskeletal pain patterns among college students in Doña Remedios Trinidad Romualdez Educational Foundation, Inc. (DRTREFI) and Dr. Vicente Orestes Romualdez Educational Foundation (DVOREF) at Tacloban City, Leyte, Philippines.

Material and methods

The study was conducted in DRTREFI-DVOREF using a cross-sectional research design. The total number of students enrolled in DRTREFI-DVOREF is 2148, but it ended up with a sample size of 942 because 4th-year medicine was exempted due to their hectic schedule. Some students also refused to participate; therefore, the data that we gathered garnered a total of 778 out of 942. The number of respondents was calculated with Slovin's formula with a 95% margin of error to determine the sample size of the population. A stratified sampling formula was also utilized to compute the sample size per year level. Afterwards, a simple random sampling method using Microsoft Excel was used to gather random participants according to the number of sample size per program. Inclusion criteria, regardless of sex, included participants with 19 and above years of age, owns a touchscreen mobile phone, and is willing to participate in the study. A survey of 51,000 people in 32 countries found that 93% of 18 to 24-year-olds owned and used mobile phones frequently[9]. All students who had recent accident or trauma, chronic systemic illness, & previous surgery for the last six months were excluded from the study. Participants were informed about the purpose of conducting the study and informed consent was obtained from the participants. This study used two-part semi-structured questionnaire which was verified for its acceptable internal reliability with a Cronbach's alpha coefficient of 0.7187 and content validity through the method of panel experts comprising licensed physiotherapist and a professional statistician. The first part contains demographic information such as age, gender, course and year level, and participants' mobile phone use such as handedness, frequency, duration, and position. The second part was based on the standardized Nordic Musculoskeletal Questionnaire[10]. Respondents were asked to report the location of their pain, while the types of

pain experienced-such as fatigue, numbness, pins and needles, aching, cramping, and stiffness-were categorized using the McGill Pain Questionnaire[11]. Additionally, pain severity was measured using the Numerical Pain Rating Scale[12]. The approval from Eastern Visayas Health Research and Development Consortium (EVHRDC) - Ethics Review Committee with ERC Protocol No. 2023-012 was obtained on May 3, 2023.

Statistical analysis

Descriptive statistics were employed to define demographics and findings of the study which included mean values and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables. A Chi-square test was used to examine the relationship between musculoskeletal pain patterns and mobile phone usage[13]. A *p*-value < 0.05 was considered to be statistically significant. Data was analyzed using the Statistical Package for Social Sciences (SPSS version 21).

Results

Table 1 displays the succeeding discussion of the percentage distribution of the students' demographic profile. The table shows that participants were predominantly female, accounting for 65.4% of the total sample. Age distribution shows that the majority of respondents (71.85%) were between the ages of 19 and 22. Notably, first- and third-year college students made up the majority of the sample (56.42%). According to course enrollment data, BS in Medical Technology is the most represented program, accounting for 30.33% of all participants. Furthermore, a high proportion of participants identified as right-handed, accounting for 84.96% of the overall sample.

Table 1 Frequency and percentage distribution of demographic profile

Variable	Category	Frequency	Percentage
Age	19-20 years	299	38.43
	21-22 years	260	33.42
	23-24 years	99	12.72
	25-26 years	42	5.40
	27 years and above	78	10.03
Sex	Male	272	34.96
	Female	506	65.04
Year level	First year	224	28.79
	Second year	199	25.58
	Third year	215	27.63
	Fourth year	140	17.99
Course/Program	Doctor of Medicine	78	10.03
	Bachelor of Science (BS) Physical Therapy	113	14.52
	BS Criminology	16	2.06
	College of Law	88	11.31
	BS Medical Technology	236	30.33
	BS Entrepreneurship	17	2.19
	BS Biology	33	4.24
	BS Nursing	197	25.32
Handedness	Right-handed	661	84.96
	Left-handed	100	12.85
	Ambidextrous	17	2.19

Table 2 shows the distribution of duration and frequency of mobile phone use, purpose of usage, how mobile phone is held when using it, and posture adopted during usage were presented in the succeeding tables. The analysis of mobile phone usage patterns revealed interesting insights into participants' behaviors. Nearly all respondents reported using their phones continuously throughout the day, with 95% indicating 24-hour usage. Primary purposes for mobile phone usage were studying (79.18%) and internet browsing (80%), indicating a significant reliance on mobile devices for academic and informational purposes. A proportion of participants reported spending two to six days engaged with their phones, representing 79.56% of the sample. In terms of phone handling, a majority of participants employed a two-handed grip (54.24%), while others also preferred using their phones while in a seated position (58.87%).

Table 2 Distribution of mobile phone usage

Variable	Category	Frequency	Percentage
Frequency on the usage of mobile phone	Never	2	0.26
	1-2 days	258	33.16
	3-4 days	361	46.40
	5-6 days	117	15.04
	7 days	40	5.14
Duration on the usage of mobile phone	< 6 hrs	4	0.51
	7-12 hrs	15	1.93
	13-18 hrs	31	3.98
	19-24 hrs	728	93.57
How mobile phone is held	I don't hold my mobile phone	1	0.13
	Only right hand	251	32.26
	Only left hand	84	10.80
	Both hands	422	54.24
	Others	20	2.57
Posture adopted during usage	Standing	9	1.16
	Sitting	458	58.87
	Lying in front	46	5.91
	Lying on side	134	17.22
	Lying on back	131	16.84

Table 2 Distribution of mobile phone usage (Cont.)

Variable	Category	Frequency	Percentage
Purpose of usage:			
Text, call and email	I don't use it	32	4.11
	< 30 mins	372	47.81
	30 mins - < 1 hr	124	15.94
	1 hr - 2 hrs	134	17.22
	> 3 hrs	116	14.91
Studying	I don't use it	73	9.38
	< 30 mins	89	11.44
	30 mins - < 1 hr	147	18.64
	1 hr - 2 hrs	167	21.47
	> 3 hrs	302	38.82
Social Media	I don't use it	8	1.03
	< 30 mins	31	3.98
	30 mins - < 1 hr	118	15.17
	1 hr - 2 hrs	169	21.72
	> 3 hrs	452	58.10
Leisure and games	I don't use it	54	6.94
	< 30 mins	145	18.64
	30 mins - < 1 hr	119	15.30
	1 hr - 2 hrs	246	31.62
	> 3 hrs	214	27.51
Internet Browsing	I don't use it	4	0.51
	< 30 mins	162	20.82
	30 mins - < 1 hr	113	14.52
	1 hr - 2 hrs	222	28.53
	> 3 hrs	277	35.60

Table 3 presents the data with regards to musculoskeletal pain patterns which was categorized as location of pain according to their body parts (neck, shoulder, elbow, wrist/hand, upper back, lower back, one or both hips/thigh, one or both knees, and one or both ankles/feet), type of pain experienced, rating of pain severity, and relation of symptoms to mobile phone use. The examination of musculoskeletal pain patterns illuminated prevalent discomfort among participants. Lower back pain emerged as the most commonly reported issue, affecting 66.2% of respondents. This was closely followed by neck pain, experienced by 64.27% of participants, and upper back pain, affecting 54.37%. The majority of participants acknowledged experiencing physical symptoms such as stiffness and fatigue (71.72%), with a subset reporting sensation of pins, needles, and numbness (10.93%). Despite these findings, a significant proportion of participants (80.59%) reported no pain, while 19.4% of participants experienced pain ranging from 3 to 7. Furthermore, 30.9% of participants strongly agree & agree respectively that all the symptoms were related to the usage of mobile phones. Notably, there were notable correlations found between specific mobile phone usage behaviors and patterns of musculoskeletal pain. More specifically, extended

study sessions were linked to upper back pain with *p*-value = 0.049; social media streaming (*p*-value = 0.041) and studying (*p*-value = 0.021) were linked to neck pain, whereas leisure activities and video games were linked to lower back pain with *p*-value = 0.048.

Table 3 Distribution of musculoskeletal pain patterns

Variable	Category	Frequency	Percentage
Location of pain	Neck	500	64.27
	Right shoulder	135	17.35
	Left shoulder	40	5.14
	Both shoulder	222	28.53
	Right elbow	51	6.56
	Left elbow	14	1.80
	Both elbow	38	4.88
	Right wrist	199	25.58
	Left wrist	39	5.01
	Both wrist	127	16.32
	Upper back	423	54.37
	Lower back	515	66.20
	One or both hips/thigh	138	17.74
	One or both knees	115	14.78
	One or both ankles/foot	110	14.14
Types of pain	I do not have any symptoms	135	17.35
	Stiffness	182	23.39
	Fatigue	208	26.74
	Pins and needles and numbness	85	10.93
	Aching and cramping	168	21.59
Rating of pain severity	0 - 2 (no pain at all)	627	80.89
	3 - 5	147	18.89
	6 - 10	4	0.51
Do you think the pain was related to the use of your mobile device	Strongly agree	87	11.183
	Agree	154	19.794
	Neither	294	37.789
	Disagree	218	28.021
	Strongly disagree	25	3.213

Table 4 exhibits the association between the musculoskeletal pain patterns particularly the neck, wrist/hand, upper back, lower back, both hips/thighs, both knees, and both ankle/foot, and usage of mobile phone. There is a significant association between the pain experienced by the students on their neck area and mobile phone usage specifically the social media, showing a chi square value of 23.35 with *p*-value = 0.041, and studying using mobile phone with chi square value of 31.09 with a *p*-value = 0.021. Nevertheless, there is enough evidence to support the link between the wrist/hand and the way the phone is held. The chi square value is 28.2, and the *p*-value = 0.005, which is less than the significance standard of 0.05, according to the evidence. The

result further shows that there was a significant correlation between the upper back area of pain and studying using mobile device as it indicates a chi square value of 9.28 with *p*-value = 0.049. Also, there is a significant connection between lower back discomfort and leisure and mobile gaming as it indicates a chi square value of 9.58 and a *p*-value= 0.048. Lastly, there is a strong correlation between one or both ankle/foot pains experienced by the students and social media, as shown by a chi square value of 10.35 and a *p*-value = 0.035.

Table 4 Association between musculoskeletal pain patterns (location of pain) and mobile phone use

Variable	Areas	Chi-square value	*p*-value
Frequency of use	Neck	2.35	0.671
	Wrist/Hand	15.05	0.089
	Upper Back	0.97	0.807
	Lower Back	0.52	0.915
	Both Hips/Thigh	0.400	0.94
	Both Knees	2.98	0.395
	Both Ankle/Foot	2.82	0.516
Duration of use	Neck	7.4715	0.113
	Wrist/Hand	13.87	0.309
	Upper Back	0.85	0.932
	Lower Back	6.81	0.146
	Both Hips/Thigh	8.12	0.087
	Both Knees	4.06	0.398
	Both Ankle/Foot	9.39	0.052
Text, Call and Email	Neck	3.15	0.864
	Wrist/Hand	6.91	0.553
	Upper Back	4.29	0.369
	Lower Back	4.41	0.354
	Both Hips/Thigh	4.01	0.405
	Both Knees	1.99	0.738
	Both Ankle/Foot	3.83	0.43
Studying	Neck	31.09	0.021*
	Wrist/Hand	9.79	0.634
	Upper Back	9.28	0.049*
	Lower Back	2.96	0.564
	Both Hips/Thigh	11.55	0.021*
	Both Knees	0.74	0.947
	Both Ankle/Foot	5.23	0.264
Social Media	Neck	23.35	0.041*
	Wrist/Hand	9.99	0.616
	Upper Back	4.62	0.329
	Lower Back	6.21	0.184
	Both Hips/Thigh	5.79	0.215
	Both Knees	2.89	0.576
	Both Ankle/Foot	10.35	0.035*

Table 4 Association between musculoskeletal pain patterns (location of pain) and mobile phone use (Cont.)

Variable	Areas	Chi-square value	*p*-value
Leisure	Neck	3.43	0.833
	Wrist/Hand	10.65	0.489
	Upper Back	1.46	0.559
	Lower Back	9.58	0.048*
	Both Hips/Thigh	1.79	0.774
	Both Knees	3.92	0.417
	Both Ankle/Foot	3.44	0.487
Internet browsing	Neck	3.05	0.55
	Wrist/Hand	7.14	0.848
	Upper Back	2.11	0.716
	Lower Back	2.71	0.608
	Both Hips/Thigh	6.28	0.179
	Both Knees	6.65	0.156
	Both Ankle/Foot	4.14	0.388
How Mobile Phone is Held	Neck	4.48	0.345
	Wrist/Hand	28.2	0.005*
	Upper Back	3.43	0.489
	Lower Back	1.69	0.792
	Both Hips/Thigh	3.86	0.425
	Both Knees	3.43	0.489
	Both Ankle/Foot	9.22	0.056
Posture adopted during usage	Neck	2.83	0.587
	Wrist/Hand	8.97	0.706
	Upper Back	1.41	0.843
	Lower Back	12.59	0.013*
	Both Hips/Thigh	3.91	0.418
	Both Knees	4.32	0.364
	Both Ankle/Foot	2.52	0.64

Note: * significant difference (*p*-value < 0.05)

Table 5 shows the Chi Square test of musculoskeletal pain patterns (types of pain, rating of pain severity, and relation of pain experienced) and mobile phone usage. The table showed that a *p*-value range of 0.37 to 0.85 was more than the 0.05 significant threshold. The corresponding null hypothesis is accepted. However, a chi square value of 26.4 and a *p*-value = 0.049 show a strong correlation between the types of aches experienced by the students and the posture employed when using mobile phones. There is concrete evidence that the duration of using mobile devices is strongly associated with the rating of pain severity experienced by the students. Furthermore, a substantial correlation between the symptoms that the students report and the amount of time they spend using mobile devices and accessing the internet, as shown by a chi square value of 42.87 with a *p*-value = 0.049. A chi square value of 29.2 and a *p*-value = 0.023 show a high correlation between the symptoms experienced by the participants and mobile device use, particularly internet browsing.

Table 5 Chi square test of musculoskeletal pain patterns and mobile phone usage

Variable	Chi-square value	*p*-value
Types of pain		
Frequency of mobile phone use	12.38	0.415
Duration of mobile phone use	10.3	0.85
Text, call and email	11.04	0.807
Studying	20.64	0.193
Social media	12.84	0.685
Leisure	17.15	0.376
Internet browsing	17.24	0.37
How mobile phone is held	14.24	0.581
posture adopted during usage	26.4	0.049*
Rating of pain severity		
Frequency of mobile phone use	2.52	1.000
Duration of mobile phone use	40.6	0.018*
Text, call and email	26.56	0.325
Studying	27.19	0.296
Social media	9.03	0.998
Leisure	32.04	0.126
Internet browsing	29.7	0.195
How mobile phone is held	7.82	0.999
posture adopted during usage	30.9	0.157
Relation of pain experienced		
Frequency of mobile phone use	12.56	0.402
Duration of mobile phone use	42.87	0.001*
Text, call and email	10.77	0.824
Studying	12.04	0.741
Social media	20.62	0.194
Leisure	22.27	0.135
Internet browsing	29.2	0.023*
How mobile phone is held	18.77	0.281
posture adopted during usage	16.08	0.448

Note: *significant difference (*p*-value < 0.05)

Discussion

This study investigated the association of musculoskeletal pain patterns among mobile phone users on college students using an online semi-structured questionnaire. The study revealed that the most common site of pain patterns was the neck (64.27%), lower back (66.2%), and upper back area (54.37%).

The study identified that duration of mobile phone use was a critical determinant of neck pain patterns, with a strong positive correlation between musculoskeletal pain patterns and extent of mobile phone use. A duration of 19-24 hours (93.57%) rate of usage of mobile phones was found in the study, and only few from the respondents who responded 6-18 hours of usage of their mobile phones. A study showed 79% of their participants

aged between 18 and 44 use their mobile phone continuously throughout the day, with only two hours of their entire day spent without their mobile phone in hand[14]. There is also concrete evidence that the duration of using mobile devices is strongly associated with the rating of pain experienced. This signifies when mobile phone use is increased, the rating of pain experienced by the students is also increased. Primary purposes for mobile phone usage were studying (79.18%) and internet browsing (80%), indicating a significant reliance on mobile devices for academic purposes. There is an adequate evidence to support the hypothesis that using a mobile device while studying has an impact on the human body, notably on one or both hips/thighs (p-value = 0.021), and upper back area (p-value = 0.049). A study stated that mobile phone use has been associated with musculoskeletal complaints in various parts of the body including neck, upper back, lower back, and hips[15]. In the present study, sitting position (58.87%) was associated with a higher frequency of posture adopted during mobile phone use compared to standing or lying positions. A significant connection between lower back discomfort and leisure was found as it indicated a chi square value of 9.58 and a p-value = 0.048. Evidence supported that those who became acclimated to sitting while mobile gaming experienced lower back pain compared to those who did not[16]. Therefore, sitting is not directly a risk factor for low back pain, but it may become a concern if it is combined with faulty posture that contributes to low back pain.

Our study also found that the most frequent characteristic of symptoms described by participants was fatigue (26.74%), and only 17.35% of students who responded that they did not experience any symptoms. The pain severity of most participants who use mobile phones were not extreme, and results showed that 80.59% of the students responded 0-2 or no pain at all. Moreover, more than one-third (37.79%) of the participants were unsure if their symptoms were linked to mobile phone use, and only 28% disagreed that their symptoms were related to it. These results suggested that symptoms of pain patterns from participants may not be entirely related to mobile phone itself, but rather influenced by a combination of other factors. Lastly, the study did not show any association for the usage of mobile phones for shoulder, elbow, and knee pain. Nevertheless, based on these findings, it appeared that most young people were experiencing issues with their bodies.

The study had some limitations; first, the data from the study may be a subject to recall bias because students were asked to recall the pain patterns that they had experienced which might or might not be present at the time they answered the survey questionnaire. The confounding effects of factors such as the type of mobile phone, screen size, and other devices used (e.g., laptops, tablets, computers), as well as additional physical activities unrelated to mobile phone use, could not be determined in this study. This limitation may impact the comprehensiveness of the findings regarding musculoskeletal pain patterns. Future research should aim to address these variables to provide a clearer understanding of their influence on pain experiences. Moreover, future studies on musculoskeletal pain patterns may improve upon performing comprehensive pain assessment that includes a detailed health history and physical examination. Further longitudinal and experimental studies must also be conducted in order to come up with innovative ideas with objective ascertainment of the risk factors and the outcome.

Conclusion

The study indicated that there is adequate evidence to support the hypothesis that mobile phone usage has an impact on the human body. It shows that pain pattern is most likely experienced due to its posture adopted and duration, rather than the frequency. Pain patterns in the neck, upper back, and hips is highly associated with studying. Wrist pain is correlated

with how the mobile phone is held. Low back pain has also been shown to be associated with leisure and the posture adopted with usage, whereas social media streaming presents an association with neck and ankle pain. However, there is no any association for the usage of mobile phones for shoulder, elbow, and knee pain. Additionally, over one-third of participants were unsure if the symptoms they experienced were connected to mobile phone use, and only 28% disagreed that their symptoms were related to it. These results suggest that the pain patterns may not be solely due to mobile phone use but could involve other factors. Nevertheless, these findings suggest that most young people are facing physical problems. Thus, the study underscores the necessity of acknowledging the influence of mobile phone usage on musculoskeletal health, especially for college students. Including habits like exercising, taking regular breaks, and maintaining good posture could help reduce the frequency of musculoskeletal pain patterns.

Take home messages

Musculoskeletal pain pattern is a concern for students who often utilize mobile phones. Proper health evaluation for students is recommended, and effective prevention strategies and exercise techniques must be implemented to promote good musculoskeletal health.

Conflicts of interest

The authors declare no conflict of interest.

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Thai version of Family Impact of Assistive Technology Scale for Adaptive Seating (FIATS-AS-Th): Cross-cultural adaptation and preliminary reliability in children with cerebral palsy

Rumrada Inthachom[1], Saipin Prasertsukdee[1*], Stephen E. Ryan[2], Jaranit Kaewkungwal[3]

[1] Faculty of Physical Therapy, Mahidol University, Nakhonpathom, Thailand.

[2] Bloorview Research Institute, Holland Bloorview Kids, Rehabilitation Hospital and Department of Occupational Science & Occupational Therapy, University of Toronto, Toronto, Canada.

[3] Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

KEYWORDS
Adaptive seating; FIATS-AS; Children with cerebral palsy; Cross-cultural adaptation; Reliability.

ABSTRACT

Adaptive seating devices provide postural support to children with cerebral palsy who have difficulty sitting independently due to neurological impairments. They help enhance sitting stability and are useful for both children with cerebral palsy and their families. Little evidence exists on the effectiveness of adaptive seating devices from the perspective of parents or other family members who are crucial in caring for their children. The Family Impact of Assistive Technology Scale for Adaptive Seating (FIATS-AS) is a parent-reported measure of adaptive seating interventions for children. While the English version of the FIATS-AS is useful, the cross-cultural adaptation is needed to improve its utility in other cultural settings. Thus, the aims of this study were to develop a Thai version of the Family Impact of Assistive Technology Scale (FIATS-AS-Th) and estimates its internal consistency and test-retest reliability. Translation and cross-cultural adaptation of the FIATS-AS into Thai version were conducted using standardized process. Thirty primary caregivers of the children with cerebral palsy (aged 2-11 years) and Gross Motor Function Classification System levels 4-5 completed Thai version of the FIATS-AS twice at 2-week intervals to estimate internal consistency and test-retest reliability. For the internal consistency, the Cronbach's alpha was 0.84 for total scale and ranged from 0.63-0.85 for its subscales. For test-retest reliability, the intraclass correlation coefficient (ICC 3,1) for the total scores was 0.97 (95% confidence interval (CI) = 0.91 - 0.99). The ICC point estimates for subscales between 0.86-0.96 (95% CI = 0.44 - 0.99). The FIATS-AS-Th is an emerging a reliable measure of the functional impact of adaptive seating device on children with CP and their families.

***Corresponding author:** Saipin Prasertsukdee, PT, PhD. Faculty of Physical Therapy, Mahidol University, Nakhonpathom, Thailand.
Email address: saipin.pra@mahidol.ac.th
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Introduction

Cerebral palsy (CP) is described as a group of permanent disorders of the development of movement and posture due to non-progressive brain damage in early life[1].Children with CP usually present many neurological impairments that might affect their activities and social participation[2]. One of the postural control deficits in children with CP exists in sitting. Children with more functional limitations often have difficulty in sitting independently[3]. Thus, they may require external supports to accommodate some parts of the body in order to remain upright[4]. Consequentially, rehabilitation practitioners often recommend that children receive adaptive seating devices.

Adaptive seating devices are postural support equipment designed to enhance the postural alignment and stability of children with CP and other neuromotor impairments[5,6]. Adaptive seating devices have a crucial role in mitigating burden and stress of caregiver by promoting functional ability, social interaction, and autonomy[5,6]. Presently, the effectiveness of adaptive seating interventions may be judged using the International Classification of Functioning, Disability, and Health for Children and Youths (ICF-CY) as a biopsychosocial framework. Assistive technology practitioners and researchers may use this framework to conceptualize adaptive seating interventions as environmental resources that support the performance of daily activities and interactions with their peers and family members in a variety of settings. Adaptive seating devices have been useful for both children with CP and their family (e.g., improving functional activities, decreasing assistance from caregiver, and enhancing social interaction with peers)[7,8]. However, little evidence exists about the effectiveness of adaptive seating from the perspective of parents or other family members who play a key role in caring for their children[9]. Assistive technology practitioners who practice family-centred service acknowledge that parents know their children best. Thus, it makes sense that assessments of the impact of adaptive seating interventions should include the perspectives of parents to understand more about the effectiveness of adaptive seating interventions in the lives of children who are unable to sit without support and their families. Utilizing tools such as outcome measures with proven levels of reliability and validity serves to increase the accuracy and confidence in the assessment result.

The Family Impact of Assistive Technology Scale for Adaptive Seating (FIATS-AS) is a parent-reported outcome measure specifically designed to detect the functional and contextual effects of adaptive seating interventions for children aged 1 to 18 years[8]. The FIATS-AS was developed as a sound measurement scale that could be used both in clinical practice and research to detect the parent perceiving effects of adaptive seating interventions on children and families. The FIATS-AS had evidence of good face, construct, and content validities, high internal consistency (alpha = 0.94) and excellent test-retest reliability (Intraclass Correlation Coefficient: ICC for all subscales = 0.86–0.95)[10,11].

While the English version of the FIATS-AS is appropriate for use as an outcome measure in service and research, cross-cultural adaptation and estimation of key measurement properties of the FIATS-AS into other cultural settings is necessary to improve its utility. The purposes of the present study were to develop a Thai version of the FIATS-AS and estimate its preliminary internal consistency and test-retest reliability.

Materials and methods

Prior to data collection, ethical approval was obtained from the Mahidol University Central Institutional Review Board (COA. NO. 2017/155.1910). The eligible participants were enrolled after providing an informed consent. This study was divided into two phases: 1) cross-cultural adaptation of the FIATS-AS and 2) estimation of the reliabilities of the FIATS-AS-Th.

Phase 1: Cross-cultural adaptation of the FIATS-AS

Instrument and study variables

The FIATS-AS has 64 items assigned to one of the following eight subscales, including child autonomy, caregiver relief, child contentment, doing activities, effort, family & social interaction, caregiver supervision, and safety, and one non-contributing subscale, i.e., technology acceptance. The FIATS-AS uses a seven-point Likert scale (strongly agree to strongly disagree) to record a level of agreement/disagreement with each item statement[8].

Procedure

Permission for cross-cultural adaptation of the FIATS-AS was approved by the principal scale developer - Stephen E. Ryan. We used the cross-cultural adaptation process by Beaton et al. as a guideline[12]. The process comprised five steps: 1) forward translation, 2) reconciliation of items, 3) backward translation, 4) review of the forward and backward translation, and 5) cognitive interviews.

Step 1: Forward translation

The English version of the FIATS-AS was translated into Thai. This process included two native Thai speakers, who had acceptable knowledge in English and Thai languages, and experience regarding measurement development. The translators translated the FIATS-AS independently. The translators were instructed to perform the translation into Thai using simple, clear, and concise language that would be understandable to a lay person[12].

Step 2: Reconciliation of items

The two forward translations were compared and discussed together in terms of their conceptual equivalence, comprehensibility, and clarity relative to the FIATS-AS English version. The approved version was verified by the consensus agreement of the two forward translators[12].

Step 3: Backward translation

Two native English speakers, who had acceptable communication skill in both Thai and English languages, were the backward translators. The reconciled Thai translation was reversely translated into English. The backward translators did the backward translation independently and did not refer to the original source version of the English FIATS-AS[12].

Step 4. Review of the forward and backward translation

The entire forward-backward process was reviewed to provide a final forward translation. Two researchers with acceptable knowledge of both English and Thai language conducted the review procedure. One of them was the forward translator. The backward translation was compared to the original FIATS-AS and focused on conceptual differences. Then the two researchers discussed and agreed on the wording of the final questionnaire. The backward translated version of the final questionnaire was sent to the principal scale developer for review and approval to ensure that the meanings of the original items were generally retained[12].

Step 5: Cognitive interviews

Eight primary caregivers of the children with CP (Gross Motor Function Classification System (GMFCS) levels 4 or 5) who were aged 2-11 years and used adaptive seating devices completed the initial FIATS-AS-Th independently[13]. A researcher interviewed each primary caregiver independently to ask their opinions about the meaning of each item. Primary caregivers flagged items that were confusing as well as words or expressions that they found unacceptable or offensive. If more than two primary caregivers had difficulties with the same item, then the researchers reviewed and reworded these items based on the concerns raised[12].

Results

Primary caregivers indicated that they generally understood the meaning of items on the preliminary measure. Three primary caregivers identified seven items were unclear and suggested the alternatives to make them clearer. The FIATS-AS-Th was revised to incorporate these suggestions and used in phase 2 of the study.

Phase 2: Reliabilities of the FIATS-AS-Th

Sampling, study population, sample size

We recruited study participants by purposive sampling. For inclusion criteria, primary caregivers were (1) parents or family members with primary caregiving responsibilities for children with CP (GMFCS levels 4 or 5) who were aged 2-11 years and used adaptive seating devices, (2) provided ≥ 5 hours taking care of the child per day, and (3) currently living with the child for at least the past six months. The primary caregivers who did not understand Thai language by listening in case of inability in reading Thai were excluded.

About the sample size estimation of the study, thirty primary caregivers were sufficient for a hypothesized correlation of 0.7 assuming an alpha of 0.05 and power of 90%.

Procedure

A researcher instructed each participant in person how to complete the FIATS AS-Th. The primary caregivers completed the FIATS-AS-Th twice at 2-week intervals to avoid recall bias[14]. If the primary caregivers could not read or fill the questionnaire by themselves, the researcher provided assistance by reading the item aloud without interpretation.

Data analysis

The data were analysed using the IBM SPSS statistics version 22.0 software (Mahidol University license). Data from the first session that the primary caregivers completed the FIATS-AS-Th were analysed for internal consistency. Cronbach's alpha was used to evaluate the internal consistencies of the FIATS-AS-Th in each dimension. Experts recommend an alpha of more than 0.7 for internal consistency[15]. Alpha greater than 0.9 indicates possible item redundancy within subscales and subscale redundancy within the total FIATS-AS-Th[15].

Data from two sessions that the primary caregivers completed the FIATS-AS-Th were analysed for test-retest reliability. For test-retest reliabilities, data were analysed using the intraclass correlation coefficient (ICC 3,1) for the total FIATS-AS-Th and each subscale. Health measurement authorities recommend an ICC equal to or more than 0.7[15].

Results

Participant characteristics

The demographics of the primary caregivers are shown in table 1. The participants were 30 primary caregivers aged 24 to 72 years old (mean = 45.60, SD = 11.30) enrolled in this study. Most primary caregivers were mothers (67%) and had education levels below the Bachelor's degree (70%). Approximately 27% of the primary caregivers graduated in Bachelor's degree. Most primary caregivers (97%) took care of their children with CP for more than one year and more than 15 hours per day (93%). Approximately 67% of the primary caregivers reported that their perceived economic status was adequate.

Internal consistency of the FIATS-AS-Th

Internal consistency for the FIATS-AS-Th total scale and subscales are shown in table 2. Results estimate the Cronbach's alpha was 0.84 for total scale and ranged from 0.63 - 0.85 for its subscales. Two subscales (contentment and family & social interaction) had the alphas below 0.7.

Table 1 The demographics of the primary caregivers (n = 30)

Demographics variables	Frequency of response (%)
Relationship with the child	
Father	1 (3)
Mother	20 (67)
Grandmother	8 (27)
Grandfather	1 (3)
Educational level	
No formal education	2 (7)
Primary school	7 (23)
Secondary school	6 (20)
Vocational certificate	6 (20)
Bachelor degree	8 (27)
Above bachelor degree	1 (3)
Duration in taking care of the child with CP per day	
5-15 hours	2 (7)
More than 15 hours	28 (93)
Total period in taking care of the child with CP	
6 months - 1 year	1 (3)
More than 1 year	29 (97)
Perceived economic status	
Enough	20 (67)
Not enough	10 (33)

Table 2 Internal consistency for the FIATS-AS-Th total scale and subscales (n = 30)

FIATS-AS-Th	Numbers of subscales/items	Cronbach's alpha
Total scale	8*	0.84
Subscales		
Child autonomy	5	0.74
Caregiver relief	9	0.70
Child contentment	9	0.65
Doing activities	5	0.78
Parent effort	8	0.73
Family & social interaction	4	0.63
Safety	8	0.76
Caregiver supervision	7	0.70
Technology acceptance	9	0.85

Note: * Technology acceptance is an independent, non-contributing subscale of the FIATS-AS-Th.

Test-retest reliability of the FIATS-AS-Th

Test-retest reliability for the FIATS-AS-Th for the total scores and subscales are shown in table 3. The ICC 3, 1 for the total FIATS-AS-Th was 0.97 (95% CI 0.91 – 0.99). The ICC point estimates for subscales were between 0.86 - 0.96. The 95% lower and upper confidence limits for all subscales extended from 0.44 - 0.99.

Table 3 Test-retest reliability for the FIATS-AS-Th total scale and subscales (n = 30)

FIATS-AS-Th	ICC (3, 1)	95% CI
Total scale	0.97	0.91 – 0.99
Subscales		
Child autonomy	0.96	0.89 - 0.98
Caregiver relief	0.93	0.85 – 0.97
Child contentment	0.94	0.87 – 0.97
Doing activities	0.88	0.44 – 0.96
Parent effort	0.96	0.93 – 0.98
Family & social interaction	0.86	0.72 – 0.94
Safety	0.96	0.92 – 0.98
Caregiver supervision	0.96	0.92 – 0.98
Technology acceptance	0.97	0.87 – 0.99

Discussion

The aims of this study were to develop the FIATS-AS-Th and estimate its internal consistency and test-retest reliability. The participants were the primary caregivers of children with CP who cannot sit independently. We hypothesized that the FIATS-AS-Th was reliable for primary caregivers of children with CP.

The primary caregivers in both study phases were heterogeneous in view of the relationship with their children with CP, educational level, and economic status. While most were mothers, all primary caregivers were close family members who spent more than five hours each day caring for their children. Consequently, all primary caregivers were well positioned to express their views on child and family functioning.

Internal consistency indicates the extent to which items within a measurement scale measure the same construct[15]. Internal consistency in the present study was acceptable for total FIATS-AS-Th. This finding suggests that the different subscales within the FIATS-AS-Th are generally measuring a related concept and no evidence of scale redundancy is found. This is consistent with the other studies that used data collected from primary caregivers of children with CP and other disabilities to calculate internal consistency of the FIATS-AS[11,16]. Four subscales of the FIATS-AS-Th had acceptable internal consistency and two subscales had alphas marginally below the recommended threshold. Ryan et al. similarly flagged an alpha for the family & social interaction subscale that was just below 0.70[11,16]. Other researchers reported that three subscales-child contentment, family & social interaction, and child autonomy-of the Turkish version of the FIATS-AS were also below the recommended threshold[17]. All recommended that the internal consistencies of the FIATS-AS subscales continue to be monitored in future research[11,16,17].

In the present study, we found two subscales of the FIATS-AS-Th--family & social interaction, contentment--had alphas below the level recommended by measurement authorities. Possible reasons that these subscales had lower internal consistencies are that they have too few items to measure these latent constructs and have items

that do not relate well to other items assigned to the same subscale. For example, one item on the contentment subscale (My child must be with others to be content.) had very low correlations with other items on the same subscale (r = -0.087 to 0.192). Further, item ratings on the family & social interaction subscale were not well distributed. All participants rated statements on this 4-item subscale as either '6' or '7'. While While having a lower internal consistency than other subscales, it may also be less responsive to change which is an important property for an outcome measure intended to detect change following the introduction of an adaptive seating intervention. Future studies with a broader age range of children are recommended to explore further the measurement properties of the FIATS-AS-Th subscales.

Test-retest reliability is a measure of the stability obtained by repeated administration of the same questionnaire over the time[15]. Overall, the preliminary levels of test-retest reliability reported here confirm the stability of the FIATS-AS-Th. The test-retest reliability of the total FIATS-AS-Th was excellent (ICC = 0.97, 95% CI 0.91-0.99) and ICC point estimates for each of the eight subscales exceeded the recommended threshold. These results are consistent with the psychometric values reported for the original English version and the Turkish version of the FIATS-AS[11,16,17].

Interestingly, the doing activities subscale showed the greatest variation in 95% CI of test-retest reliability (0.44 – 0.96). While its point estimate ICC was high, it is possible that some children changed their ability to perform activities during the two-week retest period. Alternatively, this variation could also be due to measurement error[15]. Future studies that estimate the measurement properties of the FIATS-AS-Th should include follow-up interviews with caregivers to help interpret functional change detected by this and other subscales.

Several articles provided evidence to support the effectiveness of adaptive seating interventions for children with seating impairments[8,9,16,18,19]. This study reported on the linguistic adaption of the FIATS-AS for Thai-speaking parents and other primary caregivers. We intend to develop further the FIATS-AS-Th to be a standard outcome for assistive technology practitioners in Thailand to evaluate the role of adaptive seating interventions in the lives of children and their families. The preliminary results here confirmed that the FIATS-AS-Th has emerging levels of adequate internal consistency and test-retest reliability for use as a measure for paediatric rehabilitation services and research. Reaffirming the reliability of the FIATS-AS-Th should include recruitment of primary caregivers of older children with adaptive seating needs as well as those from diverse geographic areas to examine the effect of cultural differences in other regions of Thailand and abroad.

Limitations of the study

This study may not be representative of the whole population of the children with cerebral palsy and other childhood-onset disabilities. The scope of this study recruited only Thai-speaking primary caregivers whose young children received rehabilitation services at central region and neighbouring provinces. These results may limit the ability to generalize to older children and other regions in Thailand.

Conclusion

We provide emerging evidence that the FIATS-AS-Th has acceptable internal consistency and test-retest reliability. The FIATS-AS-Th may be considered as a candidate outcome measure for detecting the multidimensional functional impact of adaptive seating interventions on the children with cerebral palsy and their families in Thai-speaking populations.

Take home messages

The FIATS-AS-Th shows promise as reliable parent-reported questionnaire for measuring the functional impact of adaptive seating on the lives of the children with disabilities and their families in Thailand.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary

Thai version of the Family Impact of Assistive Technology Scale for Adaptive Seating (FIATS-AS-Th)

แบบสอบถามนี้สอบถามความคิดเห็นของท่านเกี่ยวกับเด็กของท่าน ชีวิตครอบครัว และอุปกรณ์เครื่องช่วย ในแบบสอบถามนี้ “เด็กของท่าน” หมายถึง เด็กผู้หญิงหรือเด็กผู้ชายที่มีอายุน้อยกว่า 19 ปี และต้องการใช้อุปกรณ์เครื่องช่วยในการทำกิจกรรมที่บ้าน ที่โรงเรียน และในชุมชน และ “อุปกรณ์เครื่องช่วย” หมายถึง อุปกรณ์ที่เด็กของท่านใช้ในบ้านของท่านตอนนี้ เช่น รถเข็น, เก้าอี้ประยุกต์

โปรดเลือกระดับของความเห็นด้วยที่ท่านเห็นด้วยกับข้อความนั้น ตัวอย่างจากข้อความแรก “ลูกของฉันปฏิสัมพันธ์กับคนอื่นในช่วงเวลาอาหาร” ถ้าท่านเห็นด้วยอย่างยิ่งกับข้อความนี้ เพราะเด็กของท่านปฏิสัมพันธ์กับคนอื่นในช่วงเวลาอาหารเสมอ ให้วงกลมตัวเลข “7” ถ้าท่านไม่เห็นด้วยอย่างยิ่งกับข้อความนี้ เพราะ เด็กของท่านไม่เคยปฏิสัมพันธ์กับคนอื่นในช่วงเวลาอาหาร ให้วงกลมตัวเลข “ 1” วงกลมตัวเลขอื่นเพียง 1 ตัวเลข ถ้าท่านเห็นด้วย หรือไม่เห็นด้วยในระดับของความเห็นด้วยอื่น แต่ละข้อความต้องการความเห็นด้วย เพียง 1 ระดับเท่านั้น

ข้อ		เห็นด้วยอย่างยิ่ง	เห็นด้วย	เห็นด้วยบางส่วน	เฉย ๆ	ไม่เห็นด้วยบางส่วน	ไม่เห็นด้วย	ไม่เห็นด้วยอย่างยิ่ง
1	ลูกของฉันปฏิสัมพันธ์กับคนอื่นในช่วงเวลาอาหาร	7	6	5	4	3	2	1
2	ฉันมีเวลาน้อยที่จะทำงานบ้านให้เสร็จ	7	6	5	4	3	2	1
3	ฉันรู้สึกปลอดภัยที่จะปล่อยลูกของฉันนั่งในห้องน้ำตามลำพัง	7	6	5	4	3	2	1
4	ลูกของฉันสามารถสื่อสารกับผู้อื่น	7	6	5	4	3	2	1
5	สมาชิกอื่น ๆ ในครอบครัวต้องช่วยฉันดูแลลูกของฉัน	7	6	5	4	3	2	1
6	ลูกของฉันอยู่กับคนอื่นได้อย่างมีความสุข	7	6	5	4	3	2	1
7	ฉันกังวลเกี่ยวกับความปลอดภัยของลูกของฉันเมื่อเขาอยู่คนเดียว	7	6	5	4	3	2	1
8	ลูกของฉันท้อแท้ง่าย	7	6	5	4	3	2	1
9	ฉันเชื่อว่าอุปกรณ์เครื่องช่วยสามารถช่วยให้ลูกของฉันเรียนรู้	7	6	5	4	3	2	1
10	ลูกของฉันอยากจะอยู่กับฉันเมื่อฉันจะออกจากห้อง	7	6	5	4	3	2	1
11	ลูกของฉันกำลังเรียนรู้ที่จะทำกิจกรรมต่าง ๆ มากขึ้นโดยไม่ต้องช่วยเหลือ	7	6	5	4	3	2	1
12	ฉันต้องพาลูกของฉันไปด้วย เมื่อฉันออกจากห้องหนึ่งไปยังอีกห้องหนึ่ง	7	6	5	4	3	2	1
13	อุปกรณ์เครื่องช่วยทำให้ลูกของฉันเล่นกับคนอื่นได้ง่ายขึ้น	7	6	5	4	3	2	1
14	คนอื่น ๆ มีความสุขเมื่อลูกของฉันสามารถร่วมกิจกรรมต่าง ๆ ในครอบครัวได้	7	6	5	4	3	2	1

ข้อ		เห็นด้วยอย่างยิ่ง	เห็นด้วย	เห็นด้วยบางส่วน	เฉย ๆ	ไม่เห็นด้วยบางส่วน	ไม่เห็นด้วย	ไม่เห็นด้วยอย่างยิ่ง
15	ฉันกังวลเกี่ยวกับความปลอดภัยของลูกของฉันขณะนั่ง	7	6	5	4	3	2	1
16	อุปกรณ์เครื่องช่วยสามารถทำให้ชีวิตของลูกของฉันง่ายขึ้น	7	6	5	4	3	2	1
17	ฉันใช้เวลานานในการทำงานบ้าน	7	6	5	4	3	2	1
18	ฉันใช้เวลามากในการดูแลลูกของฉันในแต่ละวัน	7	6	5	4	3	2	1
19	ฉันมีปัญหาในการรับมือกับสิ่งต่างๆ ที่ต้องการในการดูแลลูกของฉัน	7	6	5	4	3	2	1
20	การเฝ้าระวังลูกของฉันระหว่างวันเป็นเรื่องน่าเหนื่อย	7	6	5	4	3	2	1
21	ลูกของฉันต้องการให้ฉันช่วยจับพยุงเขาขณะเล่นกับคนอื่น	7	6	5	4	3	2	1
22	ฉันต้องการจะใช้เวลากับสมาชิกในครอบครัวคนอื่นๆ มากขึ้น	7	6	5	4	3	2	1
23	อุปกรณ์เครื่องช่วยมีบทบาทสำคัญต่อการใช้ชีวิตของลูกของฉัน	7	6	5	4	3	2	1
24	ฉันกังวลว่าการนั่งตัวตรงเป็นอันตรายต่อลูกของฉัน	7	6	5	4	3	2	1
25	ฉันต้องการความช่วยเหลือในการดูแลลูกของฉัน	7	6	5	4	3	2	1
26	มันเป็นเรื่องง่ายกว่าที่จะเล่นกับลูกของฉันเมื่อมีบางคนโอบจับเขา	7	6	5	4	3	2	1
27	ลูกของฉันสามารถเล่นเกมส์ต่างๆ ได้	7	6	5	4	3	2	1
28	ฉันรู้สึกภูมิใจเมื่อลูกของฉันสามารถใช้อุปกรณ์เครื่องช่วย	7	6	5	4	3	2	1
29	ลูกของฉันชอบที่จะรู้ว่าฉันอยู่ที่ไหน	7	6	5	4	3	2	1
30	ลูกของฉันมักจะเบื่อง่าย	7	6	5	4	3	2	1
31	ฉันหวังว่าลูกของฉันสามารถให้เวลาส่วนตัวแก่ฉันสัก 2-3 นาทีในแต่ละวัน	7	6	5	4	3	2	1
32	ฉันต้องการความช่วยเหลือในการจับลูกของฉันอยู่ในท่านั่ง	7	6	5	4	3	2	1
33	ฉันห่วงใยความปลอดภัยของลูกของฉัน เมื่อเขาอยู่ตามลำพัง	7	6	5	4	3	2	1
34	ฉันเชื่อว่าลูกของฉันควรใช้อุปกรณ์เครื่องช่วยในการทำกิจกรรมต่างๆ ในชีวิตประจำวัน	7	6	5	4	3	2	1
35	ลูกของฉันสามารถใช้มือของเขาเพื่อที่จะเล่นได้	7	6	5	4	3	2	1
36	ฉันต้องนำลูกของฉันไปด้วยเมื่อฉันไปห้องอาบน้ำ	7	6	5	4	3	2	1
37	ฉันสามารถจัดการลูกของฉันได้ด้วยตนเอง	7	6	5	4	3	2	1
38	ลูกของฉันสามารถเล่นได้โดยไม่ต้องมีใครช่วยจับเขา	7	6	5	4	3	2	1

ข้อ		เห็นด้วยอย่างยิ่ง	เห็นด้วย	เห็นด้วยบางส่วน	เฉย ๆ	ไม่เห็นด้วยบางส่วน	ไม่เห็นด้วย	ไม่เห็นด้วยอย่างยิ่ง
39	ลูกของฉันจำเป็นต้องมีสมาชิกคนหนึ่งในครอบครัวช่วยจับประคองเพื่อที่จะกินอาหารที่โต๊ะอาหาร	7	6	5	4	3	2	1
40	สมาชิกหนึ่งคนในครอบครัวจำเป็นต้องอยู่ใกล้ลูกของฉันในช่วงกลางวัน	7	6	5	4	3	2	1
41	ฉันเชื่อว่าอุปกรณ์เครื่องช่วยสามารถช่วยให้ลูกของฉันทำกิจกรรมต่าง ๆ ได้มากขึ้น	7	6	5	4	3	2	1
42	ลูกของฉันมีความมั่นใจในตนเอง	7	6	5	4	3	2	1
43	ฉันห่วงใยเมื่อลูกของฉันเล่นนอกบ้าน	7	6	5	4	3	2	1
44	ฉันคิดว่าอุปกรณ์เครื่องช่วยมีบทบาทสำคัญในชีวิตของลูกของฉัน	7	6	5	4	3	2	1
45	การจัดท่าทางให้ลูกของฉันเป็นเรื่องยาก	7	6	5	4	3	2	1
46	ลูกของฉันชอบที่จะอยู่ใกล้ฉัน	7	6	5	4	3	2	1
47	ฉันเกือบหมดแรงเมื่อสิ้นสุดแต่ละวัน	7	6	5	4	3	2	1
48	ฉันพบว่ามันง่ายที่จะเล่นกับลูกของฉัน	7	6	5	4	3	2	1
49	ฉันจำเป็นต้องทำงานบ้านให้มากขึ้น	7	6	5	4	3	2	1
50	ฉันกังวลว่าลูกของฉันจะตกจากเก้าอี้	7	6	5	4	3	2	1
51	มันเป็นเรื่องยากในการจับลูกของฉันขณะที่เขาเล่นบนพื้น	7	6	5	4	3	2	1
52	ลูกของฉันสามารถเล่นของเล่นต่าง ๆ โดยไม่ต้องช่วยเหลือ	7	6	5	4	3	2	1
53	ลูกของฉันชอบสำรวจสิ่งต่าง ๆ รอบ ๆ ตัวเขา	7	6	5	4	3	2	1
54	ลูกของชั้นสามารถอยู่กับตนเองได้	7	6	5	4	3	2	1
55	อุปกรณ์เครื่องช่วยสามารถทำให้ชีวิตครอบครัวง่ายขึ้น	7	6	5	4	3	2	1
56	ฉันใช้เวลาในการดูแลลูกของฉันมากกว่าทำสิ่งอื่น	7	6	5	4	3	2	1
57	ฉันจำเป็นต้องมีเวลาหยุดพักจากการเฝ้าดูลูกของฉันมากขึ้น	7	6	5	4	3	2	1
58	ลูกของฉันมีความสุขเมื่อฉันไม่จับเขา	7	6	5	4	3	2	1
59	ลูกของฉันจำเป็นต้องให้ฉันอยู่ใกล้ ๆ เพื่อทำกิจกรรมต่าง ๆ	7	6	5	4	3	2	1
60	ลูกของฉันรู้สึกปลอดภัยเมื่อปล่อยให้นั่งตามลำพังบนพื้น	7	6	5	4	3	2	1
61	ฉันต้องการช่วงหยุดพักจากการดูแลลูกของฉันเพิ่มขึ้น	7	6	5	4	3	2	1
62	ลูกของฉันสามารถทำกิจกรรมหนึ่ง ๆ ได้นาน	7	6	5	4	3	2	1
63	ลูกของฉันมีความสุขเมื่อเล่นคนเดียว	7	6	5	4	3	2	1
64	ลูกของฉันรู้สึกปลอดภัยเมื่อเล่นเองโดยไม่มีคนช่วย	7	6	5	4	3	2	1

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Lateral Flow Assay for NGAL Detection Using Cysteamine-Modified Gold Nanoparticles

Paweena Tunakhun[1,2], Jullasak Daduang[3], Ativit Chueaponthong[3], Tanaton Taveepok[3], Panadda Wongwichittanon[3], Nicha Charoensri[2], Oranee Srichaiyapol[4], Patcharaporn Tippayawat[2], Patcharee Boonsiri[5], Sirirat Anutrakulchai[6], Kiattawee Choowongkomon[7], Ratree Tavichakorntrakool[2], Pornsuda Maraming[2*], Sawinee Ngernpimai[4*]

[1] Biomedical Sciences, Graduate School, Khon Kaen University, Khon Kaen, Thailand.
[2] Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[3] Demonstration School of Khon Kaen University, Secondary Level (Mo Din Daeng), Khon Kaen University, Khon Kaen, Thailand.
[4] Center for Innovation and Standard for Medical Technology and Physical Therapy (CISMaP), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[5] Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
[6] Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
[7] Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand.

KEYWORDS
NGAL detection; Lateral flow assay; Cysteamine-modified gold nanoparticles; Kidney disease; Point-of-care testing.

ABSTRACT

Neutrophil gelatinase-associated lipocalin (NGAL) is a promising biomarker of chronic kidney disease (CKD). A rapid and cost-effective method is required for CKD detection. This study aimed to develop and evaluate the performance characteristics of an antibody (Ab) and a method for NGAL detection in clinical samples and assess its potential as a point-of-care test. The developed assay was highly specific for NGAL and showed no cross-reactivity with other endogenous substances. The developed lateral flow immunoassay based on cysteamine-modified gold nanoparticles exhibited a sensitivity of 12.5 ng/mL. The results showed that the dual monoclonal Ab-based sandwich is ideal for screening patients with CKD and has the potential to provide rapid and cost-effective detection of NGAL as a point-of-care testing system.

***Corresponding author:** Sawinee Ngernpimai, PhD. Centre for Innovation and Standard for Medical Technology and Physical Therapy (CISMaP), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. Email address: sawinee.ng@kkumail.com, Pornsuda Maraming, PhD. Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. Email address: pornsma@kku.ac.th
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Introduction

Chronic kidney disease (CKD) is a widespread condition and was the 12th leading cause of death worldwide in 2017[1]. Most patients with CKD die of cardiovascular disease rather than end-stage renal disease (ESRD). The United States Renal Data System reported that diabetic nephropathy is the leading cause of ESRD, accounting for 35–50% of cases[2]. The number of patients with CKD in stages 2–5 has continuously increased since 1988 due to diabetes and hypertension[3]. Disease progression is rapid in numerous patients due to poor hypertension and heavy proteinuria control[4]. Therefore, early detection can be helpful in the treatment of kidney disease and can prevent kidney disease progression to advanced or ESRD. Currently, the Kidney Disease Improving Global Outcomes (KDIGO) organization has summarized the stages of CKD based on the levels of dysfunction defined by the estimated glomerular filtration rate (stages G1–G5) and albuminuria (A1–A3)[5]. The disease is identified based on the symptoms presented by the patients for at least 3 months. However, the level of serum creatinine does not change until the loss of 50% of kidney function[6]. Therefore, new biomarkers that indicate early damage are required.

Neutrophil gelatinated associated lipocalin (NGAL) is a novel biomarker for early tubular damage detection[7]; NGAL increases within 3 h and reaches a peak at approximately 6–12 h, and is still present in the blood and urine for up to five days[8]. NGAL levels increase in the serum and urine of patients with CKD progression than those without progression. Therefore, NGAL levels can be used as a novel predictive biomarker for the progression of CKD[9].

There are several methods for detecting NGAL. Enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays are commonly used to evaluate NGAL levels[10]. However, these methods require reader equipment and specialists to interpret results. Moreover, the lateral flow strip (LFS) is an easy and rapid method that can be used for NGAL detection. LFS has the advantage of being a one-step or on-site process for rapid clinical detection, owing to its rapid analysis and simple and user-friendly operation[11]. In this study, we developed a rapid antigen test based on membrane-based immune-complex flow-through nitrocellulose principles for NGAL detection. There have been many studies on the use of LFS for NGAL detection[12,13] using different detection labels. The common detection labels that are used to bind with the detection an antibody (Ab) include gold nanoparticles (AuNPs), latex microparticles, carbon nanoparticles, luminescent particles (quantum dots and up-converting phosphor nanoparticles), and magnetic nanoparticles[14,15]. The use of luminescent colors as detection labels requires specialized equipment. Therefore, the colored particles are conjugated with the NGAL detection Ab, and the AuNPs and latex microparticles are observed in the interaction between the NGAL Ab and NGAL with the naked eye.

AuNPs are used as the detection label because of their unique optical properties that allow for distinct colorimetric changes that can be easily observed without specialized equipment[16]. Additionally, AuNPs exhibit high biocompatibility, low toxicity, and exceptional stability, making them ideal candidates for medical diagnostic applications[17]. The ease of functionalization further enhances the sensitivity and specificity of the assay[18].

This study explored the use of cysteamine, a small molecule containing both an amino and a thiol group[19], which was utilized as a modifying agent for AuNPs. Cysteamine offered unique benefits for nanoparticle stabilization and functionalization of LFS[20]. Specifically, this study investigated the use of cysteamine-AuNPs as novel conjugates for NGAL detection in LFS. The distinctive properties of cysteamine enhance nanoparticle stability and promote effective conjugation with biomolecules, which can facilitate improved signal generation. This approach aimed

to leverage the advantages of cysteamine to develop a sensitive and reliable platform for NGAL detection in clinical diagnostics.

Materials and methods

Materials

C-terminal polyhistidine-tagged recombinant human lipocalin-2 (NGAL) was purchased from Sino Biological Inc. (Beijing, China). Mouse monoclonal anti-human NGAL Ab for use as a capture and detector Ab was purchased from Fitzgerald Industries International (North Acton, USA), and goat anti-mouse Ab as a control line (CL) was purchased from MyBioSource (San Diego, CA, USA). Globulin, bilirubin, hemoglobin, albumin, bovine serum albumin (BSA), chloroauric acid ($HAuCl_4$.3H2O), cysteamine hydrochloride, sodium borohydride, and human serum albumin (HSA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glucose and ascorbic acid were purchased from KemAus (Cloisters Cherrybrook, Australia).

Methods

Ethics statement

All clinical samples were collected from the Clinical Chemistry Unit of the Srinagarind Hospital, Khon Kaen University. This study was approved by the Ethics Committee of Khon Kean University (HE641124).

Participant Selection and Urine Sample Collection

Urine samples were obtained from 10 individuals, comprising five individuals without CKD and five patients with CKD; all individuals were aged >18 years. The CKD group included patients diagnosed with CKD following the KDIGO clinical practice guidelines at Srinagarind Hospital, Khon Kaen University, Thailand, between May 2023 and June 2023. Blood creatinine levels and glomerular filtration rate (GFR) were assessed to confirm CKD, defined by a GFR of ≤60 mL/min. Patients with acute kidney injury were excluded from the study. The non-CKD group consisted of five participants without CKD. Patients with CKD and non-CKD controls underwent detailed clinical evaluation and history recording. All participants were thoroughly informed of the study and provided written informed consent before enrollment. Random midstream urine samples from both groups were collected at the Clinical Chemistry Unit of Srinagarind Hospital, Khon Kaen University.

Preparation of Cysteamine-AuNPs and conjugation with detector Ab

We modified an established protocol to synthesize stable cationic AuNPs[21], achieving a uniform size range of 10–20 nm. The synthesis started by placing 40 mL of an aqueous $HAuCl_4$ solution (1.4 mM) into a clean flask at room temperature. While stirring vigorously, 400 µL of an aqueous cysteamine hydrochloride solution (213 mM) was slowly added. After 20 minutes, 40 µL of freshly prepared sodium borohydride ($NaBH_4$, 10 mM) was introduced into the mixture, while maintaining vigorous stirring. The next 8–10 minutes were critical for precise nanoparticle formation, as indicated by the development of a deep wine-red color. The resulting AuNPs exhibited a bright red hue in solution and adhered to the glass flask, likely owing to the interactions between anionic silicate ions and cationic particles.

Conjugation of cysteamine-AuNPs with the detector Ab was performed by first preparing a dispersion of cysteamine-AuNPs at an optical density of 1, measured across the wavelength range of 400–800 nm, adjusted with deionized water (DI). To this dispersion, 12 µL of detector Ab at a concentration of 0.5 mg/mL was added to 0.8 mL of the cysteamine-AuNP solution. The mixture was then incubated at room temperature on a rotator for 15 minutes to allow the Ab to effectively bind to the AuNPs. After this incubation, 90 µL of 10% BSA was added to the mixture to achieve a final concentration of 1% BSA, and the samples were incubated for an additional 10 minutes on a rotator to prevent non-specific binding. The samples were subsequently centrifuged at 6,000 g for 20 minutes to pellet the

detector Ab-AuNP conjugates. The supernatant was removed, and the pellets were resuspended in 200 µL of a suspension solution containing 10 mM phosphate buffer, 5% sucrose, 1% BSA, and 0.25% Tween 20, and the pH was adjusted to 7.4. The cysteamine-AuNPs and conjugate solutions were then characterized by UV-Vis spectroscopy.

Assembly of LFS strip

Notably, 1 mg/mL and 2 mg/mL of test lines (TL) (monoclonal mouse anti-human NGAL Ab [capture Ab]) and 1 mg/mL of CL (goat anti-mouse Ab) were dispensed using KinBio Platform dispenser (Shanghai KinbioTech.Co., Ltd. (Shanghai, China)) with different conditions in these factors including speed, X-axis (vertical position), Y-axis (horizontal position), and Z-axis (position of dispensing tip and platform height). The optimal conditions for dispensing were as follows: speed, 50 mm/s; X, 18; Y, 26, Z, 16 for nitrocellulose membrane (CN140). After dispensing, the membranes were dried at 37ºC for 1 h.

For the conjugate pad, 5 µL of the conjugate solution from each method was dropped onto the conjugate pad and dried at 37ºC for 1 h. For LFS assembly, the strip test comprised five parts: conjugate pad (GF33 treated), nitrocellulose membrane (CN140, Sartorius Stedim Biotech SA, Goettingen, Germany), backing card, absorbent pad (CF5), and sample pad (GR470, obtained from Global Life Sciences Solutions USA LLC) as shown in figure 1.

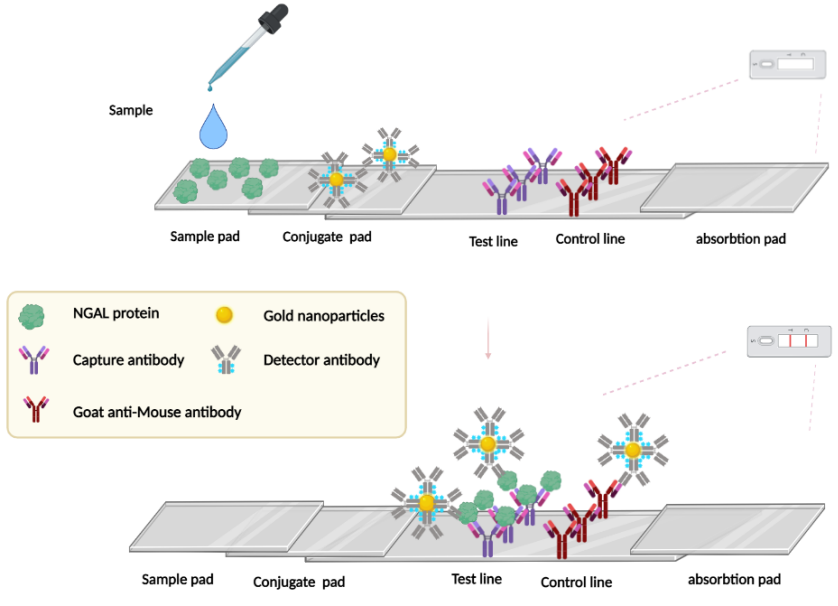

Figure 1 Schematic diagram of the lateral flow strip (LFS) for neutrophil gelatinated associated lipocalin (NGAL) detection (created with BioRender.com).

Note: LFS was divided into five parts: the sample pad, conjugate pad, nitrocellulose membrane (test, capture, control line (CL), and goat anti-mouse Ab), backing card, and absorbent pad.

Visual detection limit (vLOD) of the LFS

The different concentrations of the NGAL protein were diluted with phosphate buffer saline (pH 7.4) containing 0.5% BSA, and were tested with LFS from 0 to 100 ng/mL. Subsequently, 10 µL of each sample was loaded onto the sample pad. Subsequently, 110 µL of the running buffer was added. If the sample contains NGAL, NGAL would bind with the Ab at the conjugate pad. After migration, the sandwich format would occur in this area because NGAL is captured with the capture Ab, after which the excess Ab binds with the goat

anti-mouse Ab. Consequently, a purple color is observed in the TL and CL, indicating a positive result. Color intensity varies with the amount of NGAL. If a purple color is not observed in the CL, the LFS is invalid.

Specificity of LFS

To determine the specificity of LFS, endogenous substrates were tested, including 10 mg/dL albumin, 10 mg/dL globulin, 40 mg/dL ascorbic acid, 4 mg/dL bilirubin, 500 mg/dL glucose, and 10 mg/dL hemoglobin compared with standard NGAL protein 12.5 ng/mL as a positive control.
Clinical sample testing and analysis

To evaluate the effectiveness of the developed LFS for NGAL detection, 10 µL of urine from each of the five samples per group (CKD and non-CKD) was tested. After loading 10 µL of each sample onto the sample pad, 110 µL of running buffer was added.

Results

Characterization of cysteamine-AuNPs by UV-Vis spectrophotometry

The synthesized cysteamine-AuNPs and cysteamine-AuNP-conjugated Ab were characterized by UV-Vis spectrophotometry. The maximum absorption peaks of the cysteamine-AuNPs and the conjugates were at 516 nm and 526 nm, respectively. The results showed a peak shift from 522 to 525 nm, indicating conjugation (Figure 2).

vLOD of the LFS

Different concentrations of NGAL, ranging from 0 to 100 ng/mL, were spiked into urine samples and tested using the LFS. The vLOD, defined as the lowest concentration of NGAL that produced a visible TL, was 12.5 ng/mL (Figure 3).

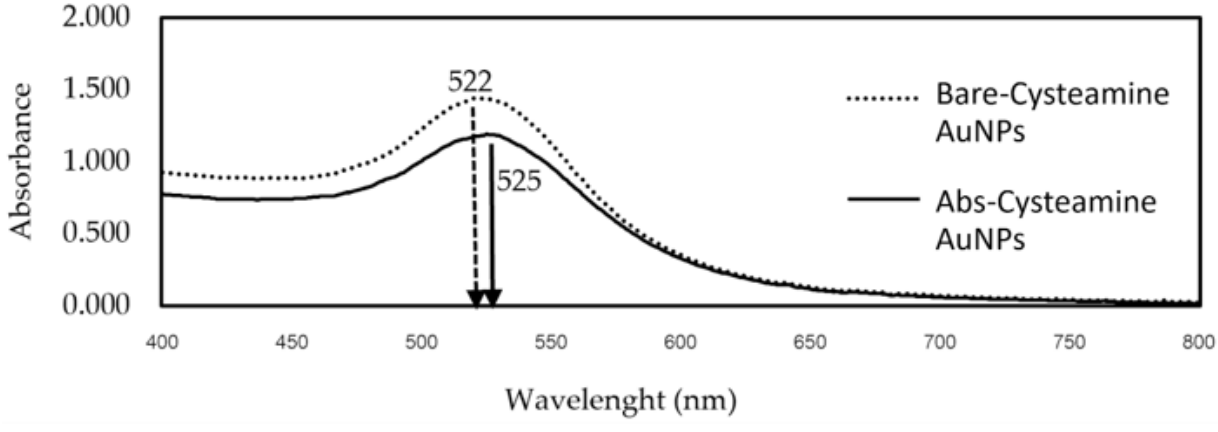

Figure 2 UV-Vis Spectrophotometry analysis of bare-cysteamine gold nanoparticles (Bare-Cysteamine AuNPs) and antibody-conjugated cysteamine AuNPs (Ab-Cysteamine AuNPs).

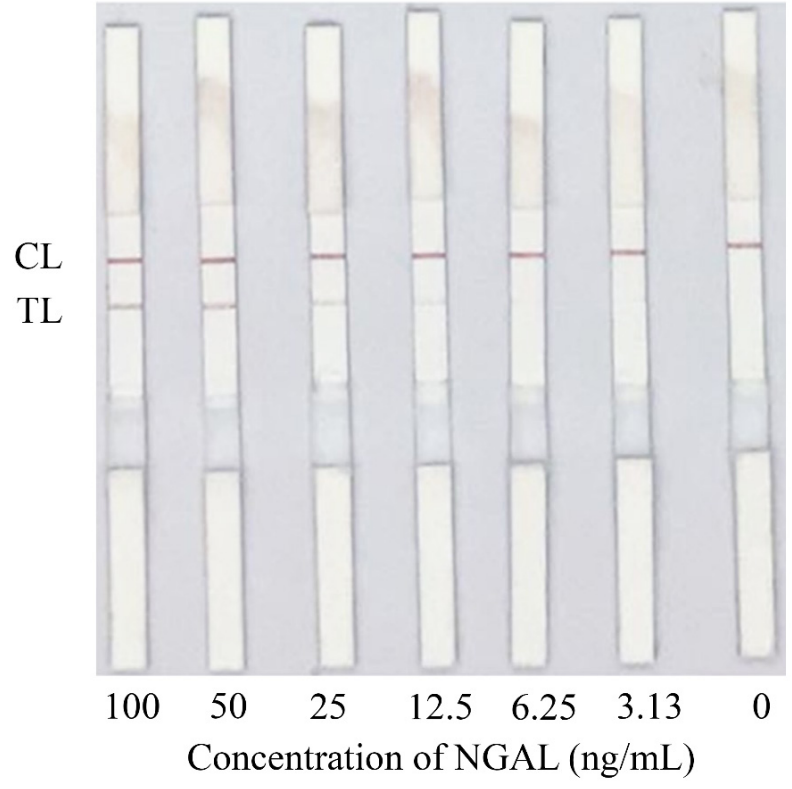

Figure 3 Visual limit of detection (vLOD) of the NGAL LFS.
Note: Representative images of LFS showing TL intensity at various NGAL concentrations (0–100 ng/mL) spiked into urine samples.

Specificity of LFS

To determine the specificity of the developed LFS for NGAL detection, a urine sample spiked with NGAL protein (12.5 ng/mL) along with ascorbic acid (40 mg/dL), bilirubin (4 mg/dL), hemoglobin (10 mg/dL), glucose (500 mg/dL), albumin (10 mg/dL), and globulin (10 mg/dL) was tested. Only samples spiked with NGAL produced visible CL and TL. These findings indicated that the developed LFS was highly specific to NGAL (Figure 4).

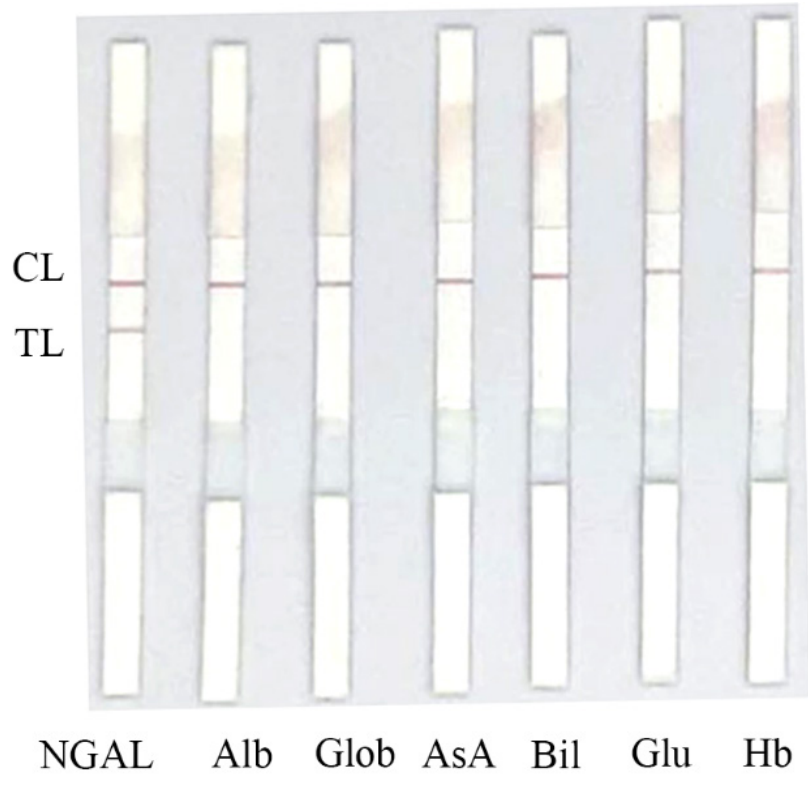

Figure 4 Specificity of the developed LFS.
Note: The specificity of the LFS was tested by exposing it to various substances, including NGAL (100 ng/mL), albumin (Alb, 10 mg/dL), globulin (Glob, 10 mg/dL), ascorbic acid (AsA, 40 mg/dL), bilirubin (Bil, 4 mg/dL), glucose (Glu, 500 mg/dL), and hemoglobin (Hb, 10 mg/dL).

Clinical sample testing and analysis

Five urine samples from each group (CKD and non-CKD) were tested to assess the performance of the developed LFS in detecting NGAL. The results were compared with those obtained using ELISA. The LFS accurately detected NGAL in all five CKD samples, with a sensitivity of 100%, and correctly identified all five non-CKD samples as negative, resulting in a specificity of 100% (Figure 5). These results indicated that the Ab-based LFS was highly accurate for NGAL detection in clinical urine samples.

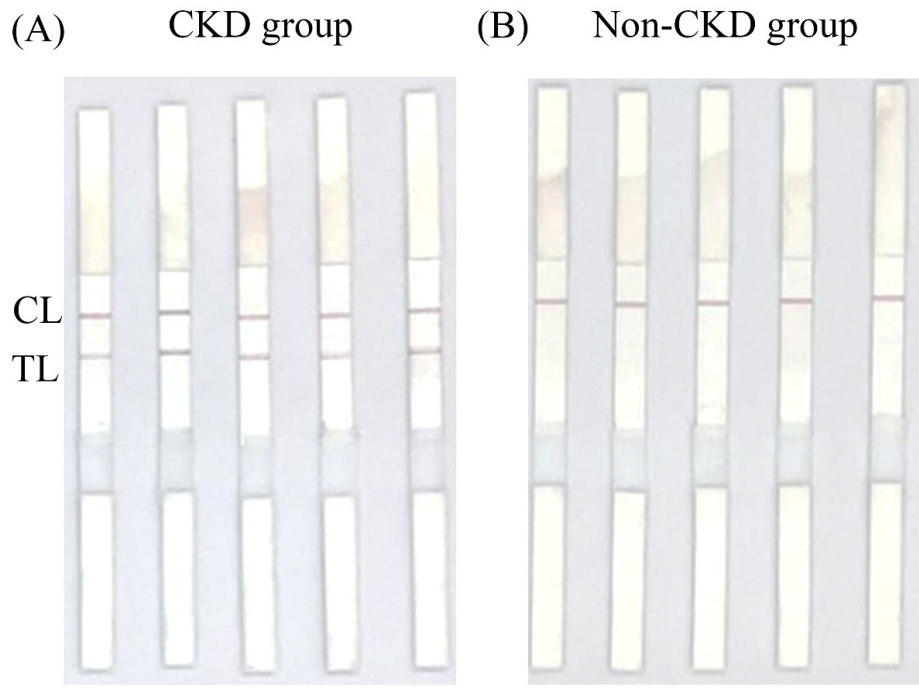

Figure 5 Clinical validation of the developed NGAL LFS using urine samples.
(A) Representative LFS results for CKD
(B) representative LFS results for non-CKD samples

Discussion

The development of a rapid and highly specific LFS for NGAL detection represents a significant advancement in the early diagnosis and management of CKD. This study demonstrated that the cysteamine-modified AuNPs used in our LFS assay provided a reliable and sensitive platform for NGAL detection, showing high sensitivity (12.5 ng/mL) and specificity without cross-reactivity with other endogenous substances, such as albumin, hemoglobin, glucose, and bilirubin. The ability of this assay to differentiate between CKD and non-CKD urine samples with 100% accuracy underscored its potential as a valuable tool for clinical diagnostics. Our findings suggested that the NGAL LFS assay could be a critical component of point-of-care testing for CKD. Early detection of kidney dysfunction allows for timely intervention, which can significantly slow the progression of CKD to ESRD. Traditional biomarkers of kidney function, such as serum creatinine, do not indicate kidney damage until a substantial portion of renal function is lost[22]. In contrast, NGAL is a sensitive biomarker that increases rapidly after kidney injury, making it a suitable candidate for early detection of CKD.

Although prior methods, such as LFS using fluorescent detection systems like europium and up-converting nanoparticles (UCP), demonstrated lower limits of detection of 0.36 ng/mL for europium[13] and 7.68 ng/mL for UCP[12], these methods necessitate the use of specialized fluorescent readers. However, the need for a fluorescent reader limits their practical applications in resource-limited settings. In contrast, the NGAL LFS assay developed in this study allowed for detection through naked-eye observation, eliminating the need for advanced equipment, thus enhancing its suitability for point-of-care testing.

The use of cysteamine-AuNPs in the assay improves the effectiveness of the nanoparticle conjugation with antibodies[23], which is crucial for achieving high sensitivity and specificity. Cysteamine allows for strong binding with antibodies and minimal non-specific interactions[24], which are crucial for accurate diagnostics. This innovative approach to nanoparticle functionalization can be applied to other biomarkers and diseases, thereby potentially broadening the scope and utility of LFS-based diagnostics. Given the global burden of CKD, particularly in low-resource settings, the NGAL LFS offers a promising solution for rapid on-site testing. Its ease of use, low cost, and rapid turnaround time make it suitable for deployment in remote or underserved areas where access to advanced laboratory facilities is limited. Furthermore, the assay does not require specialized equipment or extensive training[25], which can facilitate its widespread adoption and frequent monitoring of at-risk populations. Although the results are promising, further studies are necessary to validate the assay performance across diverse populations and clinical settings. Large-scale clinical trials could provide more comprehensive data on the diagnostic accuracy, reliability, and potential limitations of this assay. Additionally, investigating the long-term benefits of regular NGAL monitoring using the LFS in patients with CKD could provide valuable insights into its role in improving patient outcomes. Future research should explore the application of LFS technology to other biomarkers indicative of different diseases, thereby expanding its diagnostic utility. Enhancing the multiplexing capabilities of the assay could allow simultaneous detection of multiple biomarkers, further increasing its clinical relevance and application.

Conclusion

The developed NGAL LFS represents a promising point-of-care diagnostic tool for rapid and accurate detection of NGAL in urine samples. Its high sensitivity and specificity suggest that the LFS could be an asset in the early detection and monitoring of kidney dysfunction, particularly in resource-limited settings, where rapid on-site testing is crucial. The ability of this assay to correctly differentiate between CKD and non-CKD samples demonstrates its potential clinical utility. Further large-scale clinical studies are warranted to establish its effectiveness in diverse patient populations and clinical settings.

Take home messages

We developed a simple and rapid urine test strip to accurately detect a kidney marker (NGAL). This test can help identify kidney problems early, potentially improving patient outcomes. These are particularly promising for use in areas with limited access to advanced medical facilities.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Effectiveness of iron supplementation and education in preventing iron deficiency anemia among frequent blood donors with low hemoglobin levels

Poonsup Sripara, Namrin Boonmawongsa, Boonsong Benjangkaprasert, Thanet Thanukarn, Weerasak Mahama, Ronnarit Boonyarat*

Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

KEYWORDS
Donor care;
Iron deficiency anemia;
Donor anemia.

ABSTRACT

Frequent blood donation can lead to iron deficiency anemia (IDA), especially in donors with low baseline hemoglobin (Hb) levels. This study aimed to evaluate the effectiveness of iron supplementation (ferrous fumarate) and donor education in preventing and managing IDA in frequent blood donors with low Hb levels, with particular attention to age and gender-specific responses. Frequent blood donors (more than two donations in the same 12-month period) with Hb levels below 13.00 g/dL (male) or 12.50 g/dL (female) received ferrous fumarate supplementation (200 mg twice daily) along with education on iron deficiency and compliance. Hb levels were measured pre-and post-intervention at 2-3 weeks. The study included 47 donors (76.60% female, 23.40% male), aged 20-64 years. After excluding donors with ferritin >100 ng/mL to focus on iron-deficient donors, 31 donors were analyzed. The median pre-intervention Hb was 12.10 g/dL, which increased to 13.20 g/dL post-intervention. In iron-deficient donors, younger donors (20-39 years, n=18) showed significantly higher median Hb increment compared to older donors (40-65 years, n=13) (2.00 vs 1.20 g/dL, *p*-value = 0.02). While not reaching statistical significance, younger donors demonstrated a trend toward higher overall response rates (94.44% vs 84.62%, *p*-value = 0.36) and significant response rates (83.33% vs 53.85%, *p*-value = 0.07). Female donors (n=26) showed higher response rates (92.31% vs 80.00% in males, *p*-value = 0.58) and greater Hb improvement (1.50 vs 1.20 g/dL, *p*-value = 0.81). Baseline ferritin levels were comparable between age groups (28.50 vs 39.00 ng/mL, *p*-value = 0.81), suggesting age-related differences in treatment response were independent of initial iron status. Iron supplementation with ferrous fumarate, combined with donor education, effectively improved Hb levels in frequent blood donors with iron deficiency, with younger donors showing particularly favorable responses. These findings suggest the benefit of age-specific approaches in managing iron deficiency among blood donors.

***Corresponding author:** Ronnarit Boonyarat, MD, Dip.Thai Board of Hematology, Dip. Thai Board of Transfusion Medicine Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. Email address: ronnbo@kku.ac.th
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Introduction

Blood donation is a fundamental aspect of healthcare systems worldwide as it provides the indispensable blood products necessary for treating and caring for patients in need. However, the repeated nature of blood donation can lead to substantial iron depletion, necessitating effective strategies to replenish these vital stores among donors, particularly those who donate frequently and are at heightened risk for iron deficiency and anemia[1-3]. Iron deficiency anemia in blood donors not only compromises the health and well-being of the donors but also affects the quality and safety of the donated blood.

Frequent blood donors are especially vulnerable to iron deficiency due to the regular loss of blood, which depletes their iron stores. Research indicates that repeated blood donations without adequate recovery time can lower Hb and ferritin levels considerably, leading to iron deficiency anemia[1,4]. Female donors face an even greater risk of iron deficiency due to additional factors such as menstrual blood loss[1,2,5], underscoring the need for targeted interventions to preserve adequate iron levels and prevent anemia in this group. Iron deficiency anemia can manifest as symptoms such as fatigue, weakness, and decreased physical performance, which may deter donors from continuing their donations[1]. Furthermore, low Hb levels can result in donors being deferred from making donations, thereby diminishing the available blood supply and adversely impacting patients who rely on these vital blood products. As such, addressing iron deficiency in blood donors is crucial for safeguarding donor health and ensuring the sustainability of blood donation programs.

Iron supplementation is widely acknowledged as an effective measure for replenishing iron stores and improving Hb levels in individuals with iron deficiency anemia. Providing iron supplements to frequent donors can mitigate the risk of developing iron deficiency anemia and promote a healthier donor population, enhancing the overall safety and quality of donated blood products[1,3,6-8]. Ferrous fumarate, a commonly used iron supplement containing approximately 66 mg of elemental iron per 200 mg tablet, has been shown to raise iron levels when administered consistently and effectively[9].

Implementing routine ferritin testing in blood donation centers could further aid in identifying individuals at risk of iron deficiency, allowing for timely interventions that reinforce donor health and the quality of blood donations over time[2,4]. Educating donors about the dangers of iron deficiency and the significance of iron supplementation is vital for fostering compliance with supplementation regimens. Informed donors are more likely to adhere to these protocols, leading to improved health outcomes and sustained participation in blood donation programs[1,7,10,11]. Therefore, integrating iron supplementation with comprehensive donor education represents a promising approach to addressing IDA in frequent blood donors.

This study was conducted at the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University. The center is committed to providing high-quality blood products and services and is critical in ensuring patients' continuous supply of safe blood. It focuses on donor health and safety, regularly monitoring Hb and ferritin levels and providing targeted iron supplementation strategies tailored to donor needs. Despite existing evidence on iron supplementation, more data are needed on the combined effect of iron supplementation and education in this population.

The study aims to evaluate the effectiveness of ferrous fumarate supplementation, donor education, and follow-up Hb monitoring in addressing iron deficiency anemia among frequent blood donors.

Materials and methods

This prospective cohort study was conducted at the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Thailand.

The study population consisted of blood donors who had donated more than twice in a year and had Hb levels below the established thresholds. The inclusion criteria were based on the Thai Red Cross Society guidelines, which define anemia as Hb levels below 13.00 g/dL for male donors and below 12.50 g/dL for female donors, as determined by point-of-care Hb testing[12]. This group of donors is at high risk of iron deficiency anemia[1-3,6,13,14]. Donors with known hematological disorders unrelated to iron deficiency were excluded from the study.

Intervention

Participants meeting the inclusion criteria were invited to participate in the study. Consenting donors received 200 mg of ferrous fumarate supplements twice daily. They were also provided educational materials on iron deficiency, the importance of maintaining adequate iron levels, and the need for adherence to the supplementation regimen. The academic component included informational pamphlets, one-on-one counseling sessions, and follow-up phone calls to encourage compliance with the supplementation plan. Follow-up appointments were scheduled 2-3 weeks after the start of the intervention. Donors were considered to have responded positively if their Hb levels increased by more than 0.50 g/dL within 2-3 weeks[9]. The study collected the following measurements to enable a comprehensive assessment of each donor's iron status before and after the intervention:

- Gender, age, baseline Hb, ferritin, and complete blood count (including red blood cell count, hematocrit, and related parameters)
- Hb levels measured at the start of the study and one month later
- Ferritin levels measured at the start to assess baseline iron stores

Sample Size Calculation

The sample size was determined to assess whether the response rate in our study population differed from the previously reported rate of 58.10%[5]. A margin of error of 15.00% was considered clinically significant. Using a 95.00% confidence level, the sample size was calculated using a single proportion formula with a minimum of 42 participants. This ensured that the study was adequately powered to detect meaningful differences in response rates.

The study adhered to rigorous ethical standards, with approval from the Center for Ethics in Human Research, Khon Kaen University (reference number HE661201), and written consent from all participants. Confidentiality was ensured by securing data and removing identifiable information. Statistical analyses were conducted using appropriate methods for non-normal distributions; median values were analyzed, and the Wilcoxon signed-rank test was employed to assess changes in Hb levels. Descriptive statistics summarized ferritin levels, and Spearman's rank correlation was used to evaluate the relationship between initial ferritin levels and Hb improvement.

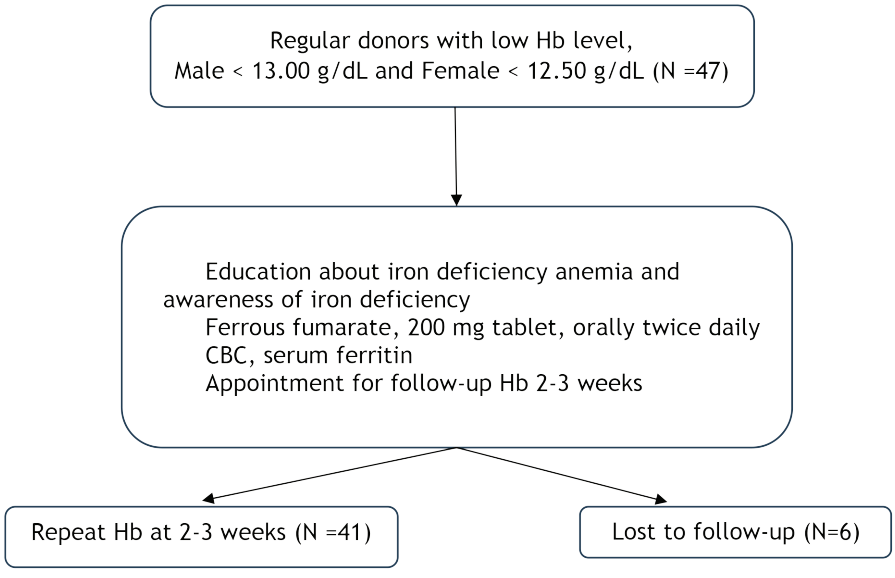

Figure 1 Consort diagram representing a flowchart of regular donors with low Hb levels and follow-up outcomes.

Results

The study population consisted of 47 regular blood donors from the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Thailand. As summarized in table 1, the participants included 36 females and 11 males, ranging in age from 20 to 64 years.

Table 1 Baseline characteristics

Characteristic	Value
Total donors	47
Female	36 (76.60%)
Male	11 (23.40%)
Age range (years)	20 - 64
Median pre-intervention Hb (g/dL)	12.10
IQR pre-intervention Hb (g/dL)	11.50-12.30
Median ferritin level (ng/mL)	42.00
IQR ferritin level (ng/mL)	24.00 - 96.00

Abbreviation: Hb, hemoglobin; IQR, interquartile range; g/dL, grams per deciliter; ng/mL, nanograms per milliliter.

Hemoglobin levels

The study assessed the participants' Hb levels before and after the intervention, which involved providing ferrous fumarate supplements and educating the donors. The median baseline Hb level was 12.10 g/dL, which increased significantly to 13.20 g/dL following the intervention. The median improvement in Hb was 1.2 g/dL, indicating substantial enhancement. Moreover, the overall response rate, defined as an increase in Hb greater

than 0.50 g/dL within 2-3 weeks, was 87.80%. Iron deficiency and response by ferritin level

The effectiveness of iron supplementation varied based on the donors' ferritin levels, as summarized in table 2. Donors with ferritin levels below 30 ng/mL exhibited the highest median Hb increment of 1.30 g/dL, indicating a strong response to iron supplementation. Those with ferritin levels between 30-50 ng/mL and 50-100 ng/mL showed moderate improvement, with median Hb increments of 0.80 g/dL and 0.90 g/dL, respectively. In contrast, donors with ferritin levels exceeding 100 ng/mL had the lowest median Hb increment of 0.20 g/dL, suggesting a more variable and less robust response to the iron supplementation.

Table 2 Iron deficiency and response by ferritin level

Ferritin level (ng/ml)	Number of donors (41)	Median Hb increment	*p*-value
< 30	10	1.30	< 0.001
30-50	7	0.80	
50-100	13	0.90	
>100	11	0.20	

Abbreviation: Hb, hemoglobin; ng/mL, nanograms per milliliter.

Response by gender

In the subgroup analysis of iron-deficient donors (ferritin ≤100 ng/mL, n=31), we examined gender-specific responses to iron supplementation. Female donors (n=26) demonstrated a higher median Hb increment (1.50 g/dL) compared to male donors (n=5, 1.20 g/dL, *p*-value = 0.81), although this difference did not reach statistical significance. Similarly, while females showed higher overall response rates (92.31% vs 80.00%, *p*-value = 0.58) and a greater proportion achieving significant response (Hb increment ≥1 g/dL: 73.08% vs 60.00%, *p*-value = 0.64), these differences were not statistically significant. Baseline ferritin levels differed between genders (females: 33.50 ng/mL vs males: 15.00 ng/mL, *p*-value = 0.78), but this difference also lacked statistical significance.

Response by age group in iron-deficient donors

Analysis of iron-deficient donors (ferritin ≤100 ng/mL) revealed critical age-related differences in treatment response, as shown in table 3. Younger donors (20-39 years) demonstrated a significantly higher median Hb increment of 2.00 g/dL compared to 1.20 g/dL in older donors (40-65 years) (*p*-value = 0.02). While examining other response parameters in table 3, we observed that although not reaching statistical significance, there was a consistent trend toward better outcomes in the younger group. The overall response rate was notably higher in younger donors (94.44% vs 84.62%, *p*-value = 0.36). Similarly, the proportion of donors achieving significant response (Hb increment ≥1 g/dL) was substantially higher in the younger group (83.33% vs 53.85%, *p*-value = 0.07). Despite not all reaching statistical significance, these consistent trends across different measures of response suggest a biological pattern of better iron utilization in younger donors. As shown in table 3, baseline median ferritin levels were comparable between the age groups (28.50 vs. 39.00 ng/mL, *p*-value = 0.81), indicating that the observed differences in response were likely due to age-related factors rather than initial iron status. This observation aligns with known age-related changes in iron metabolism and absorption efficiency, even though the sample size may have limited the statistical power to detect significant differences in some parameters

Table 3 Response to iron supplementation by age group in iron deficient donors (ferritin ≤100 ng/mL)

Characteristic	20-39 years	≥ 40 years	*p*-value
Number of donors	18	13	
Median Hb increment (g/dL)†	2.00	1.20	0.02
Response rate (%)*	94.44	84.62	0.36
Significant response (increase Hb ≥ 1 g/dL) (%)	83.33	53.85	0.07
Median ferritin level (ng/mL)	28.50	39.00	0.81

Note: *Response rate defined as Hb increment > 0.50 g/dL within 2-3 weeks.
Abbreviations: Hb, hemoglobin; g/dL, grams per deciliter; ng/mL, nanograms per milliliter.

Discussion

The findings of this study provide important insights into the management of iron deficiency in regular blood donors. A combination of Hb monitoring and targeted iron supplementation showed significant improvements in Hb levels, particularly among female donors, who showed greater susceptibility to iron deficiency. The high response rate (87.70%) further emphasizes the effectiveness of this approach. Regularly monitoring blood donors' Hb levels is crucial for the timely identification and management of iron deficiency. Conducting pre-donation Hb screening can enable early detection of donors at risk of iron deficiency anemia, facilitating prompt intervention. Equipping blood donation centers with point-of-care Hb testing devices and training staff to interpret the results can enhance the efficiency of this screening process.

Targeted iron supplementation based on Hb levels and clinical symptoms is effective[6,15-17]. Administering ferrous fumarate (200 mg twice daily) to donors with Hb levels below 13.00 g/dL for males and 12.50 g/dL for females or those exhibiting symptoms of iron deficiency ensures that iron levels are restored adequately. Educating donors on the importance of adherence to the supplementation regimen and managing side effects is essential for maximizing the benefits of this treatment[7]. Previous research has demonstrated that such targeted supplementation significantly improves Hb levels in blood donors[2,4-8,14,18]. The higher response rate among female donors underscores the importance of addressing gender-specific factors in iron management.

Although previous studies have consistently demonstrated higher rates of iron deficiency in female donors[2,19,20], our subgroup analysis of donors with ferritin ≤100 ng/mL showed no significant gender differences in treatment response. While we observed trends favoring female donors in both Hb improvement and response rates, these differences were not statistically significant, possibly due to our limited male sample size. This finding suggests that once iron deficiency is established, the response to iron supplementation may be more influenced by individual factors than gender. These factors include baseline ferritin levels, which varied notably between our gender groups (females: 33.50 ng/mL vs males: 15.00 ng/mL), adherence to supplementation, and the presence of underlying conditions affecting iron metabolism[20]. Therefore, our results support an individualized approach to iron supplementation in blood donors, focusing on these modifiable factors rather than gender-specific protocols for optimal IDA management.

Age-related response patterns and mechanisms

Analysis of iron-deficient donors (ferritin ≤100 ng/mL) revealed significant age-related differences in treatment response. Younger donors (20-39 years) achieved a significantly higher median Hb increment compared to older donors (40-65 years) (2.00 vs 1.20 g/dL,

p-value = 0.02). This marked difference in response, observed despite comparable baseline ferritin levels between age groups (28.50 vs 39.00 ng/mL, *p*-value = 0.81), suggests that age independently affects iron supplementation efficacy. The superior response in younger donors was further supported by their higher significant response rates (83.33% vs 53.85%, *p*-value = 0.07), although this difference approached but did not reach statistical significance.

These age-related differences in treatment response can be attributed to several physiological mechanisms. First, younger donors' superior response likely reflects more efficient gastrointestinal iron absorption, as aging is associated with reduced gastric acid secretion and alterations in intestinal mucosa that may impair iron absorption[21]. Additionally, the capacity to utilize absorbed iron for erythropoiesis appears more efficient in younger donors, independent of iron stores[21-24]like myelodysplastic syndrome (MDS. These age-related changes in both iron absorption and utilization suggest the need for age-adjusted supplementation strategies. Our findings indicate that donors over 40 years may require modified approaches, such as higher doses or alternative iron formulations, to achieve optimal response. Future studies should focus on developing and validating age-specific protocols to optimize iron replacement in blood donors.

Donor education and counseling play a pivotal role in the success of iron supplementation programs. Providing comprehensive information on iron deficiency, its symptoms, and the importance of maintaining healthy iron levels helps donors understand the significance of adherence to supplementation regimens. Personalized counseling sessions to discuss the test results, benefits of iron supplementation, and strategies for managing side effects can address individual concerns and encourage proactive management of iron levels. Similar approaches in other studies have been shown to enhance donor compliance and improve outcomes[6-8,10].

In cases where donors do not respond to iron supplementation, a comprehensive assessment is necessary. Additional tests, such as ferritin and transferrin saturation (TSAT), can provide a more accurate evaluation of iron status, helping to identify underlying causes of non-response[2,4,14,25-27]. Considering other potential causes of anemia, such as chronic disease or thalassemia, and adjusting treatment accordingly ensures that donors receive appropriate and targeted care. Studies have highlighted the benefits of using ferritin and TSAT testing to differentiate between iron deficiency and other conditions[4,28-30].

Monitoring and evaluation are critical for continuously improving iron deficiency management strategies. Scheduling follow-up Hb tests and clinical evaluations at regular intervals helps track the effectiveness of supplementation and overall donor health. Regular data analysis on donor Hb levels, supplementation adherence, and outcomes can provide valuable insights into the effectiveness of the implemented strategies and identifying areas for improvement. This approach aligns with findings from other research that underscore the importance of continuous monitoring and data analysis in donor management programs. Compared to other studies, the significant improvements in Hb levels and high response rate in this study are consistent with the findings from similar research[2,3,8,18]. Our approach of using Hb levels and clinical symptoms for therapeutic diagnosis of iron deficiency, rather than relying solely on ferritin testing, has proven practical and cost-effective, particularly in resource-limited settings[3,6,9,18,28,31].

The findings of this study on the effectiveness of iron supplementation with ferrous fumarate in frequent blood donors with low Hb levels are consistent with previous studies, such as those by Cable et al[2] and Mast et al[7], which demonstrated significant improvements in Hb levels following supplementation. However, our study adds new insights by integrating an educational component, which enhances compliance and maximizes the

clinical benefits of supplementation. In this study, the combination of strategies resulted in a higher response rate than those reported in earlier studies, suggesting that a comprehensive approach may be more effective in managing iron deficiency among frequent donors.

Our stratification of donors based on ferritin levels provided a further understanding of how baseline iron stores influence the response to supplementation. Similar to the findings of Bialkowski et al[3], donors with lower ferritin levels exhibited greater improvements in Hb, reinforcing the need for targeted interventions. Our study also highlights the importance of tailoring supplementation strategies to individual donor risk profiles, which could inform future guidelines for blood donation centers.

These new insights underline the potential benefits of integrating supplementation and education programs in routine donor management, particularly in settings with limited resources. Future research should explore the long-term sustainability of these interventions as well as their impact on donor retention and blood supply adequacy. Our findings highlight the importance of age and gender-specific considerations in blood donor iron supplementation programs. Young donors (20-29 years) showed optimal response to standard protocols (response rate 100%, Hb improvement 1.83 g/dL), while older donors required more intensive monitoring and alternative supplementation strategies due to lower response rates and higher baseline ferritin levels. Female donors demonstrated better overall response (90.00% vs 81.80% in males), suggesting the benefit of gender-specific approaches. These demographic variations indicate that personalized supplementation strategies, along with targeted education programs, could enhance the effectiveness of iron repletion in blood donors. Future research should focus on optimizing these demographically-tailored interventions and their impact on long-term donor retention.

Conclusion

This study demonstrates that targeted iron supplementation with ferrous fumarate, combined with donor education, effectively improves Hb levels among frequent blood donors with iron deficiency (ferritin≤100 ng/mL). Our findings revealed that age, rather than gender, may be a more crucial factor in treatment response. Younger donors (20-39 years) showed significantly better Hb improvement compared to older donors (40-65 years), while gender differences in response were not statistically significant in our iron-deficient cohort.

Given these findings, blood donation centers should consider implementing regular Hb monitoring and individualized iron supplementation based primarily on age and individual factors rather than gender-specific protocols. Particular attention should be paid to donors over 40 years who demonstrated lower response rates, potentially requiring modified supplementation strategies. Education programs should focus on promoting adherence to supplementation regimens and addressing individual risk factors that might affect treatment response.

Future research should focus on validating age-specific supplementation protocols and investigating the mechanisms underlying age-related differences in iron supplement response. Additionally, larger studies with more balanced gender distribution are needed to definitively assess the role of gender in iron supplementation response. These efforts will help optimize donor care and ensure a sustainable blood supply.

Take home messages

Regular iron supplementation and education significantly improve Hb levels in frequent blood donors with low Hb, with particularly strong responses among women and younger donors. Ensuring proper iron intake and awareness, with consideration for gender and age-specific needs, can help maintain donor health and a sustainable blood supply. Regular monitoring and demographically- tailored interventions are essential for preventing donor iron deficiency and anemia.

Conflicts of Interest

The authors declare no conflict of interest.

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