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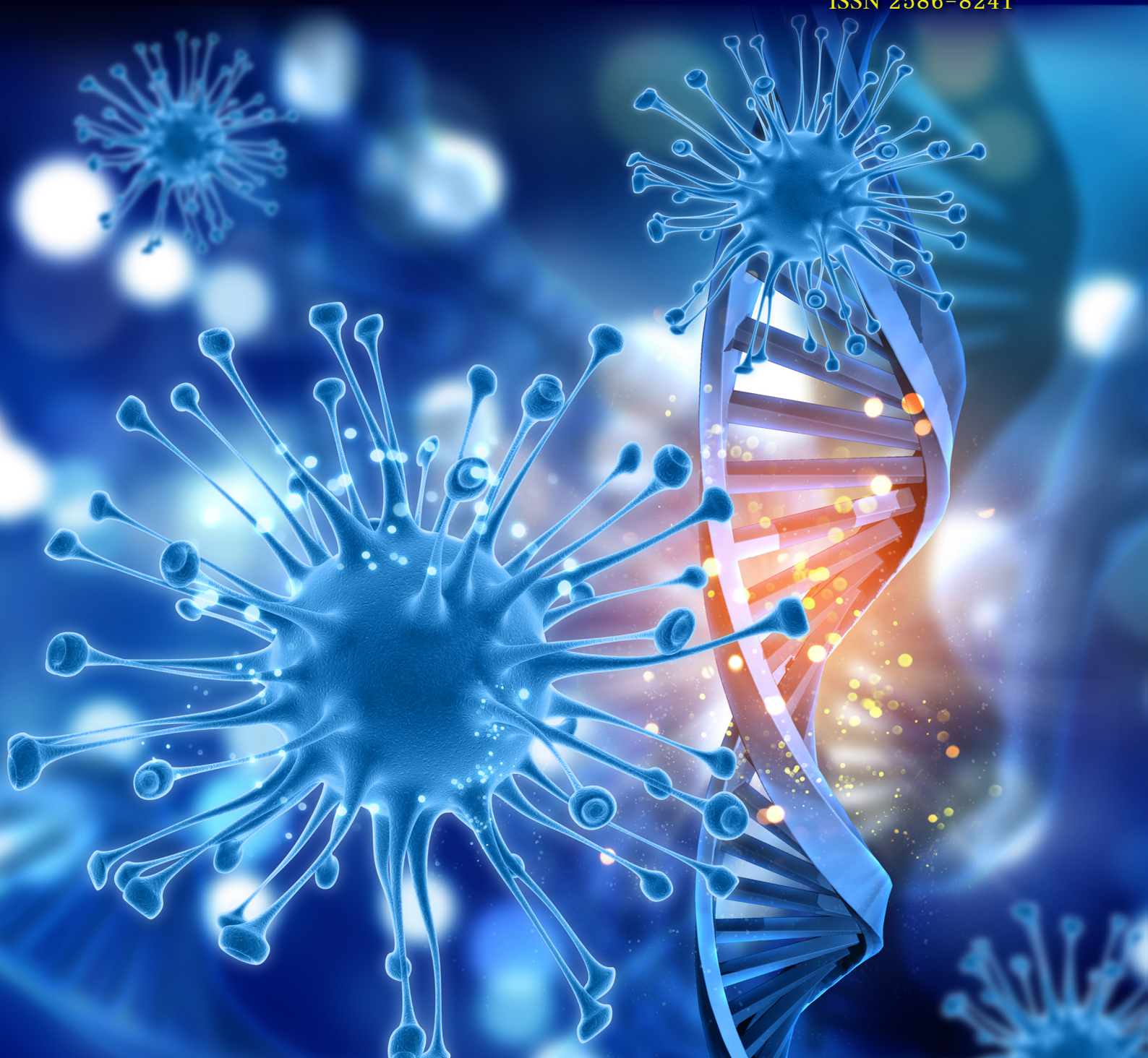

Introduction

The Journal of Southeast Asian Medical Research is a peer-reviewed journal with printing every 6 months. The main goal of this collaboration project is to distribute new knowledge in medical sciences to medical communities and scientists, as well as encouraging scientific collaborations within Southeast Asia and also other nations around the world. The journal publishes original research in the medical sciences: clinical and basic. We welcome original articles from across the world. The editorial board consists of international experts in various fields of medicine, ranging from internal medicine to a variety of surgeries. The full text of the journal is available online at http://www.jseamed.org

It is our aim to publish the most up-to-date and useful research information in medical sciences. In Southeast Asia, there are some unique problems in health care and diseases, such as tropical diseases, and it is crucial that health professionals can access, share and exchange knowledge promptly. In this region, there is still a gap of knowledge in health sciences that needs to be closed by scientific research, which we are hoping to close after this collaboration project. We hope that the journal will fulfill the objectives and will provide benefit to all, both medical practitioners and researchers alike.

Editorial board

JOURNAL OF SOUTHEAST ASIAN MEDICAL RESEARCH

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A.Original Article

EFFECT OF PULMONARY HYPERTENSION ON INTRADIALYTIC HYPOTENSION AMONG PATIENTS WITH END STAGE RENAL DISEASE

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ABSTRACT

Background: Intradialytic hypotension (IDH) is an important problem in end stage renal disease (ESRD). Therefore, this study aimed to assess the effect of pulmonary hypertension (PHT) on IDH among patients with ESRD using transthoracic echocardiography.

Methods: In this prospective etiognostic study, transthoracic echocardiography was performed among patients with ESRD in Burapha University Hospital, Thailand. The hemodialytic flow chart data of patients in the hemodialysis unit was collected to ascertain whether these patients presented IDH. The baseline clinical hemodialysis profiles and echocardiographic findings were analyzed using univariate predictors of IDH. Multivariate risk regression was used to identify independent predictors of IDH.

Results: A total of 35 patients with ESRD were enrolled between June 2020 and March 2021. Of these, 16 had PHT (45.7%). The incidence of IDH was 48.5%. All patients exhibited a normal left ventricular ejection fraction. No significant difference was observed of RVSP between frequent-IDH group and occasional-IDH group (45.33 ± 11.62 mmHg and 41.06 ± 13.78 mmHg, respectively, p=0.401). Using univariate analysis, being female, left ventricular mass index, left ventricular ejection fraction and PHT were significantly associated with IDH. No factors were indicated related to IDH occurrence using multivariate analysis. Nevertheless, female patients with ESRD presenting PHT illustrated a tendency to have IDH. This was evidenced by the risk ratio of being female and patients with PHT being 3.13 (95% CI: 0.74-13.30) and 2.18 (95% CI: 0.34-7.06), respectively.

Conclusion: Patients with ESRD presenting PHT showed a higher tendency of developing IDH during hemodialysis than patients with ESRD without PHT. The difference however was statistically insignificant.

Keywords: End stage renal disease, Intradialytic hypotension, Pulmonary hypertension

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Introduction

Intradialytic hypotension (IDH) is one of the complications associated with dialysis procedures, occurring at about 20 to 48%[1-3] of patients with end stage renal disease (ESRD). The condition is important in clinical settings because it could lead to even more serious complications such as an increase of mortality rate[4, 5] and myocardial ischemia from decreasing coronary blood flow.[6]

IDH condition has been hypothesized to cause a lower cardiac output. In fact, both left-sided and right-sided cardiac outputs are closely related. When the right-sided cardiac output is lower, whatever the cause, low blood pressure will occur. Therefore, hemodialytic sessions involving a removal of body fluid from blood vessels, resulting in a decreasing preload in the right ventricle, should be particularly cautioned because it may reduce the right-sided cardiac output. Special attention should be paid to the occurrence of associated conditions such as pulmonary hypertension (PHT).

Approximately 26 to 66% of patients with ESRD(7-11) already being treated by hemodialysis present PHT and are correlated with cardiovascular deaths.[9] Also, PHT is a strong independent predictor of mortality among patients undergoing hemodialysis.[12]

No related studies have significantly documented the relationship between PHT and IDH. Therefore, this study aimed to assess the effect of PHT on IDH among patients with ESRD using transthoracic echocardiography.

Methods

Study design and population

This constituted a prospective etiognostic study that performed echocardiography among patients with ESRD in Burapha University Hospital, Thailand between June 2020 and December 2020. After that, the hemodialysis flow chart data of patients in the hemodialysis unit was collected to ascertain whether these patients presented IDH. The protocol was approved for ethics considerations by the BUU Ethics Institutional Review Board (HS021/2563). All patients provided written informed consent.

The sample size was calculated by comparing the mean of two independent groups. A related study[13] indicated that on average, patients with ESRD receiving hemodialysis exhibited pulmonary artery systolic pressure about 33.9±10.6 mmHg. Moreover, experts suggested that patients with ESRD and IDH should have higher pulmonary artery systolic pressure than patients with ESRD without IDH. The difference was expected to be about 10 mmHg, which is a value predicting the emergence of poor long term survival.[13] Thus, patients with ESRD and IDH are expected to have pulmonary artery systolic pressure equal to 43.9 mmHg. The sample size was calculated to include 48 patients with ESRD in the study (24 patients in each group).

Patients with IDH under the condition that systolic blood pressure decreased by 20 mmHg or more, the mean arterial pressure decreased by more than 10 mmHg or blood pressure was lower than 100/60 with associated symptoms of hypotension including yawning, cramps, nausea, vomiting, tweaking and dizziness.[14] Patients in the frequent-IDH group needed to be particularly treated during the hemodialysis sessions. Examples of treatments included temporarily stopping an ultrafiltration (UF) operation, providing inotropic agents or saline.[15] Patients with frequent-IDH in this study exhibited the aforementioned condition more than three times during a three-month study, whereas patients with occasional-IDH showed the condition less than three times or none during the same timeframe. The hemodialysis chart of patients was reviewed to collect data about 1) general information including age, sex, body mass index (BMI), underlying diseases, causes of chronic renal failure, medicines used, and laboratory results and 2) cardiovascular disease-related information including previous HF hospitalization, coronary artery disease, history of pulmonary hypertension and history of syncope and 3) kidney dialysis related information including weekly frequency of kidney dialysis, length of time of each kidney dialysis, mean net UF weekly, position of catheter, pre/post HD weight, and pre/post blood pressure and heart rate. The inclusion criteria included patients (1) aged more than 18 years (2) having

treatment of hemodialysis for six months or more, (3) receiving hemodialysis at least twice weekly and at least four hours each session, (4) without history of catheter related infection and (5) without history of abnormal bleeding in the last month. The exclusion criteria included (1) BMI of 40 kg/m2 or more, (2) catheter-related infection in the last month, (3) bleeding disorder in the last month, (4) inadequate hemodialysis defined by losing HD twice in the last month, (5) significant left sided valvular heart disease (any aortic/ mitral stenosis), (6) HIV positive status and (7) pregnancy.

Hemodialysis

All patients with ESRD received dialysis two to three times weekly for four hours each session.

Transthoracic echocardiography

Echocardiography was performed before hemodialysis to collect important parameters including left ventricular ejection fraction (LVEF), left ventricular mass index (LVMi), pattern of left ventricular geometry, LA volume index, diastolic function, E/e', right atrial pressure, tricuspid regurgitation velocity, right ventricular systolic pressure (RVSP), mean pulmonary arterial pressure (mean PAP) using Abbas's formula and right ventricular systolic function (tricuspid annular plane systolic excursion and peak velocity of tricuspid annulus). Echocardiogram was performed by a cardiologist in Burapha Hospital. We used the American Society of Echocardiography guidelines and recommendations to assess and measure these parameters. The cardiologist performed all examinations using a diagnostic ultrasound system (Philips EPIQ CVx with a Philips X5-1 MHz Phased Array Probe).

Statistical Analysis

Subject characteristics were described using descriptive statistics, including frequency and percentage for categorical variables. Continuous variables were reported as means, standard deviation of normally distributed variables and median, minimum and maximum of normally distributed variables. Factors to predict IDH used the independent t-test for continuous variables and categorical variables used the Fisher exact test. Variables found to be significant in the univariate analysis, were entered in a multivariate risk regression analysis (backward elimination). For all tests performed, a two-tailed $p < 0.05$ was considered statistically significant.

Results

The data of 40 patients with ESRD were collected. Three patients were excluded from the study because parameter values using an echo-cardiogram were unable to be collected. Two patients with severe valvular heart disease were excluded. Finally, a total of 35 patients with ESRD were analyzed. Of these, 16 had PHT (45.7%), and incidence of IDH was 48.6%. General information of patients in both groups are shown in **Table 1** and hemodialysis profiles are shown in **Table 2.**

All patients exhibited normal LVEF and right ventricular systolic function (tricuspid annular plane systolic excursion and peak velocity of tricuspid annulus). Most patients presented Grade I diastolic function and concentric hypertrophy of the left ventricular geometric pattern. The echocardiographic data are shown in **Table 3**.

The primary research outcome was that a relationship between PHT and IDH was insignificant. When the dataset was subjected to univariate analysis, variables with a significant correlation with IDH were being female, LVMi, LVEF and PHT. The RVSP of the frequent-IDH group and the occasional-IDH group appeared to be 45.33 ± 11.6224.86 ± 8.16 mmHg, respectively (p=0.234).

Table 1. Clinical characteristics of frequent-IDH and occasional-IDH groups

Characteristic	Frequent-IDH (n=17)	Occasional-IDH (n=18)	*p*-value
Female, n (%)	14 (73.7)	5 (26.3)	0.002
Age (years, mean ± sd)	68.82 ± 12.51	66.11 ± 16.35	0.567
Body weight (kg, mean ± sd)	59.18 ± 16.14	60.78 ± 12.94	0.747
Body mass index (kg/m^2, mean ± sd)	24.32 ± 5.8	23.61 ± 4.74	0.691
Underlying disease, n (%)			
Hypertension	11 (45.8)	13 (54.2)	0.725
Coronary artery disease	2 (28.6)	5 (71.4)	0.402
Stroke	2 (66.7)	1 (33.3)	0.603
Diabetes mellitus	4 (43.8)	9 (56.2)	0.738
Dyslipidemia	15 (60.0)	10 (40.0)	0.06
Previous heart failure	1 (20.0)	4 (80.0)	0.338
Etiology of ESRD, n (%)			0.49
Diabetes mellitus	8 (44.4)	10 (55.6)	
HTN (HT nephrosclerosis)	5 (62.5)	3 (37.5)	
Glomerulonephritis	0	1 (100.0)	
Unknown	4 (50.0)	4 (50.0)	
Number of Anti HTN drugs (mean ± SD)	1.88 ± 1.22	2.23 ± 1.31	0.433
Nitrate, n (%)	0	2 (100.0)	0.486
Beta blocker, n (%)	4 (33.3)	8 (66.7)	0.289
CCB, n (%)	10 (47.6)	11 (52.4)	0.582
ACEI, n (%)	1 (50.0)	1 (50.0)	0.743
ARB, n (%)	5 (50.0)	5 (50.0)	0.604
Diuretic, n (%)	11 (47.8)	12 (52.2)	0.592
Hydralazine, n (%)	1 (25.0)	3 (75.0)	0.603
Laboratory investigation			
Hb (mg/dL, mean ± SD)	9.45 ± 1.54	9.76 ± 1.39	0.545
Albumin (mg/dL, mean ± SD)	3.8 ± 0.36	3.77 ± 0.31	0.77
Total calcium (mg/dL, mean ± SD)	8.85 ± 0.98	8.92 ± 0.8	0.813
Phosphate (mg/dL, mean ± SD)	4.68 ± 1.51	4.32 ± 0.93	0.415
PTH level (pg/mL, mean ± SD)	262.11 ± 242.11	284.73 ± 287.04	0.811

Values presented as mean±SD or n (%), *p*-values corresponded to independent-t test and Fisher's exact test.

ESRD: end stage renal disease, anti HTN drugs: antihypertensive drugs, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin II receptor blockers, CCB: calcium channel blockers, Hb: hemoglobin, PTH: parathyroid hormone

Table 2. Hemodialysis profiles of frequent-IDH and occasional-IDH groups

Hemodialysis profile	Frequent-IDH group (n=17)	Occasional-IDH group (n=18)	*p*-value
IDH symptoms, n (%)			0.198
None	7 (31.8)	15 (68.2)	
Fainting	4(66.6)	2 (34.4)	
Dyspnea	1 (100.0)	0	
chest pain	1 (100.0)	0	
abdominal pain	1 (100.0)	0	
muscle cramping	2 (66.7)	1 (33.3)	
back pain	1 (100.0)	0	
Frequency of dialysis, n (%)			0.315
2 sessions/week	9 (42.9)	12 (57.1)	
3 sessions/week	8 (57.1)	6 (42.9)	
Mean Net UF, (ml, mean ± sd)	2535.29 ± 829.11	2742.22 ± 1283.69	0.577
BFR, (ml/min, mean ± sd)	329.41 ± 25.36	327.78 ± 30.78	0.865
Dialysis membrane			0.591
HDF 80	9 (60.0)	6 (40.0)	
HDF 100	2 (25.0)	6 (75.0)	
Elisio 170	3 (42.9)	4 (57.1)	
Elisio 190	1 (50.0)	1 (50.0)	
Elisio 210	2 (66.7)	1 (33.3)	
PreHD SBP, (mmHg, mean ± SD)	141.06 ± 27.88	131.28 ± 24.48	0.274
PreHD DBP, (mmHg, mean ± SD)	64.24 ± 17.49	61.89 ± 15.13	0.673
PreHD HR, (bpm mean ± SD)	76.76 ± 15.83	78.06 ± 17.13	0.819
Position of catheters, n (%)			0.67
AVF	10 (47.6)	11 (52.4)	
AVG	1 (33.3)	2 (66.7)	
Perm cath	4 (44.4)	5 (55.6)	
DLC	2 (100.0)	0	

Values presented as mean±SD or n (%), *p*-values corresponded to independent-t test and Fisher's exact test.

Mean net UF: mean net ultrafiltration, BFR: blood flow rate, PreHD SBP: prehemodialysis systolic blood pressure, PreHD DBP: prehemodialyisis diastolic blood pressure, PreHD HR: prehemodialysis heart rate, AVF: arteriovenous fistula, AVG: arteriovenous graft, DLC: double lumen catheter.

These variables and other variables that were likely to be related to the occurrence of IDH including diabetes, coronary artery disease, albumin level, presystolic blood pressure, prediastolic blood pressure, LVMi and LVEF were then analyzed using multivariable analysis. Results showed that no factors were related to IDH occurrence. Nevertheless, female patients and patients with PHT illustrated a tendency to have IDH. This was evidenced by the risk ratio of being female and patients with PHT at 3.13 (95% CI= 0.74-13.30) and 2.18 (95% CI= 0.34-7.06), respectively. multivariate risk regression analysis to determine factors associated with IDH is shown in **Table 4.**

Table 3. Echocardiographic data between frequent-IDH and occasional-IDH groups

Echocardiographic findings	Frequent-IDH group (n=17) (mean ± SD)	Occasional-IDH group (n=18) (mean ± SD)	*p*-value
LVDd (mm)	44.07 ± 5.8	41.47 ± 5.77	0.193
LV mass index (g/m^2)	128.48 ± 26.52	110.22 ± 21.69	0.032
RWT	0.62 ± 0.14	0.69 ± 0.14	0.173
LV geometry, n (%)			0.125
concentric remodeling	2 (22.2)	7 (77.8)	
concentric hypertrophy	14 (56.0)	11 (44.0)	
LVEF (%)	64.08 ± 11.19	72.87 ± 5.89	0.006
Diastolic function, n (%)			0.429
normal	1 (25.0)	3 (75.0)	
grade I	13 (54.8)	11 (45.8)	
grade II	2 (33.3)	4 (66.7)	
grade III	1 (100.0)	0	
E/e’	17.83 ± 7.01	15.15 ± 6.32	0.243
LA volume index (mL/sqm)	47.38 ± 13.85	50.12 ± 14.92	0.579
TAPSE (mm)	25.42 ± 3.86	25.29 ± 4.78	0.931
Peak velocity of tricuspid annulus (cm/sec)	12.7 ± 2.51	11.99 ±1.62	0.326
mean PAP (mmHg)	28.28 ± 6.94	24.86 ± 8.16	0.234
RVSP (mmHg)	45.33 ± 11.62	41.06 ± 13.78	0.401
PHT, n (%)	11 (68.8)	5 (31.3)	0.044
RAP (mmHg)	8.54 ± 1.82	8.71 ± 2.12	0.788
CO (mL/min)	5.31 ± 2.25	4.32 ± 0.98	0.100

Values presented as mean±SD or n (%), *p*-values corresponded to independent-t test and Fisher’s exact test.

LVDd: left ventricular dimension in diastole, LV mass index: left ventricular mass index, RWT: relative wall thickness, LV geometry: left ventricular geometry, LVEF: left ventricular ejection fraction, LA volume index: left atrial volume index, TAPSE: tricuspid annular plane systolic excursion, Mean PAP: mean pulmonary arterial pressure RVSP: right ventricular systolic pressure, PHT: pulmonary hypertension, RAP: right atrial pressure, CO: cardiac output.

Discussion

IDH is a serious and frequent complication of chronic hemodialysis. IDH is the end result of the interaction between ultrafiltration rates, cardiac output and arteriolar tone.[16] As mentioned previously, we hypothesized that the occurrence of IDH among patients undergoing chronic hemodialysis was related to reduced cardiac output and PHT. When developing IDH, the human body exhibits the following adapted mechanisms.[17] First, interstitial fluid moves to blood vessels to refill the blood volume. Second, increased sympathetic outflow to arteriolar vasoconstriction and increased peripheral vascular resistance, helps to maintain BP. Finally, the heart contracts more, causing the rising heart rate to increase cardiac output and raise BP. When all of these mechanisms fail, IDH eventually strikes. Decreased cardiac output plays a key role in the patho-physiology of IDH. Cardiac output depends on preload, afterload, heart rate and contractility. Changes in preload, determined mainly by

Table 4. Multivariate regression analysis to determine factors associated with intradialytic hypotension

Prognostic factor	Frequent-IDH (n=17)	Occasional-IDH (n=18)	Risk Ratio	Adjusted Risk Ratio (95% CI)	*p*-value
PHT, n (%)	11 (68.8)	5 (31.3)	2.18	1.56 (0.34-7.06)	0.565
Sex, n (%)					
male	4 (23.5)	13 (76.5)	1		0.120
female	14 (73.7)	5 (26.3)	3.13	3.14 (0.74-13.30)	
DM, n (%)	8 (47.1)	9 (52.9)	0.89	0.49 (0.13-1.92)	0.308
CAD, n (%)	3 (37.5)	5 (62.5)	0.70	0.81 (0.16-4.18)	0.799
Albumin, (mean±SD)	3.85±0.41	3.77±0.31	1.39	0.53(0.06-4.30)	0.528
PreSBP (mean± SD)	75.0±21.97	114.5±20.63	1.0	0.98 (0.94-1.04)	0.666
PreDBP (mean± SD)	42.17±13.36	59.67±13.91	1.0	0.98 (0.94-1.04)	0.538
LVMi, (mean± SD)	128.48±26.52	110.22±21.69	1.01	1.0 (0.97-1.03)	0.798
LVEF, (mean± SD)	64.08±11.19	72.87±5.89	0.96	0.97 (0.91-1.04)	0.427

PHT: pulmonary hypertension, DM: diabetes mellitus, CAD: coronary artery disease, PreSBP: presystolic blood pressure, PreDBP: prediastolic blood pressure, LVMi: left ventricular mass index, LVEF: left ventricular ejection fraction

intravascular volume, seem to play a major role in the development of IDH.[16] Therefore during our hemodialysis sessions, intravascular fluid has to be removed. Patients presenting pulmonary hypertension may experience induced IDH due to decreasing cardiac output.

multivariate risk regression revealed that such a relationship does not exist. However, the chance that patients with ESRD and PHT condition (indicated by their RVSP being equal or greater than >50 mmHg or their mean PAP by Abbas's formula being equal or greater than >25 mmHg) will develop IDH 2.18 times that of patients without PHT. The pathogenesis of PHT in this population remains poorly understood. Reported associations include arteriovenous fistulae, cardiac dysfunction, fluid overload, bone mineral disorder and non-biocompatible dialysis membranes.[18]

This study also illustrated that female patients had a higher chance of developing IDH than male patients. In particular, our multivariate risk regression analysis indicated that the chance of female patients with IDH was 3.13 times than that of male patients. This finding was consistent with several studies. For example, Andras Tisler et al.[19] found that being female was a statistically significant factor of IDH occurrence. Similarly, Johanna et al.[20] examining the prevalence of IDH among patients with conventional hemodialysis, found that four determining factors of IDH included diabetes, high interdialytic weight gain, being female, and low body weight. Studies of Johanna Kuipers et al.[21] and Orofino L et al.[22] also found similar results, that female patients with low body weight increases the chance of having IDH even further. This could be explained in that females in general have lower body weight and, consequently, have a higher UF rate (mL/h/kg body weight) during hemodialysis for a similar interdialytic weight gain than males.[20]

Patients with frequent IDH exhibit higher LVMi than patients with occasional IDH indicating that these patients have left ventricular hypertrophy (LVH). For patients with chronic renal failure, the prevalence of LVH increases progressively as renal function deteriorates. The development of LVH results from coronary hypoperfusion,

myocardial stunning and renin-angiotensin-aldosterone system dysregulation. Finally, LVH actively contributes to IDH occurrence, through the induction of LV stiffening, myocardial ischemia and arrhythmia.[23]

Barberato et al.[24] observed that patients experiencing ESRD with LVEF less than 50% and LA volume index greater than 35 mL/m2 indicated diastolic dysfunction, which is a factor that can significantly predict the occurrence of IDH. This study found that both groups of patients (frequent-IDH and occasional-IDH) exhibit normal LVEF, i.e., greater than 60%, and normal cardiac output measured by an echocardiogram. Therefore, high LA volume index will not necessarily cause IDH during chronic hemodialysis.

Apart from patients with ESRD, patients with PHT must be treated with noncardiac operations. This patient group represented an important risk factor for increased perioperative morbidity and mortality. In other words, these patients illustrated a significantly increased risk for hemodynamic instability.[25] Thus, physicians have to give special attention to these patients by monitoring closely, optimizing systemic BP, oxygenating and ventilating, avoiding exacerbating factors, and using vasopressors and pulmonary vasodilators whenever necessary as essential elements of management.[26]

One limitation of our study was the small sample size; only 40 patients could be enrolled during the study period. This restriction affected the multivariate risk regression analysis in that its revealed risk of IDH factors was unclear. Although female patients and patients with PHT showed a higher chance of IDH occurrence than their counterparts, the difference was not statistically significant.

Conclusion

Patients with ESRD and PHT measured by RVSP or mean PAP during echocardiography showed a higher tendency of developing IDH during hemodialysis than patients with ESRD without PHT. Therefore, patients with ESRD who were diagnosed having PHT showed clinical significance so that physicians had to closely monitor the possible occurrence of hypotension during hemodialysis.

Conflict of interest

The authors declare they have no conflict of interest.

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USE OF TENSION BAND WIRING TECHNIQUE IN PROXIMAL ULNAR FRACTURES: A FINITE ELEMENT BIOMECHANICAL ANALYSIS

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Abstract

Background: Tension band wiring is considered the standard treatment for olecranon fracture. A recent study proved that it can be used for the fracture as distal to the coronoid process.

Objective: The study aimed to investigate whether tension band wiring can be used in proximal ulnar fracture fixation up to and distal to the coronoid process.

Methods: Models of simple proximal ulnar fracture including 4 intraarticular and 2 extraarticular fractures were created. Fixation was completed using tension band wiring technique, and biomechanical responses were evaluated using finite element analysis. After a physiologic load was applied, the fracture displacement, von Mises stress, and stiffness were recorded.

Results: All fracture models were able to withstand the load of daily activities with a maximum displacement of 50% of the articular surface. In addition, the von Mises stress was the highest in the middle articular fracture. The mean transcortical K-wire tension band wiring stiffness of the intra-articular and extra-articular fractures was 1144.89 N/mm and 1231.45 N/mm, respectively.

Conclusion: Tension band wiring is another option to treat proximal ulnar fractures with the ability to withstand immediate postoperative load.

Keywords: Tension band wiring, Olecranon fracture, Proximal ulnar fracture, Biomechanical study, Finite element analysis

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Introduction

Proximal ulnar fracture is one of the most common upper extremity fractures, for which many surgical options have been proposed. To date, even though many studies have concluded no evidence of any perfect surgical treatment option exists for this fracture. [1-3]

A simple displaced olecranon fracture, Mayo classification type IIa (AO/OTA 2U1B1), is the most common fracture reported among olecranon fractures. (4-8) Plate fixation is increasingly used for olecranon and proximal ulnar fracture fixation. It provides greater compressive force across the fracture site, more stable fixation, lower second operation rate compared with tension band wiring (TBW), and good clinical outcomes. Nevertheless, several drawbacks exist, namely, a higher rate of infection than in TBW, and a cost as high as twice that of TBW.[7, 9-12]

Currently, TBW is still considered the surgical technique of choice for simple olecranon fractures. It possesses the benefits of being reproducible, less invasive and yields good clinical results. The most important problem is implant migration and loosening, leading to a second operation.[4, 13-15] TBW was once believed to create dynamic compression across the fracture site from the distraction force of the triceps muscle. Hence, TBW has been suggested to be used in cases with no more than 50% of articular involvement. Recently, many biomechanical studies have shown results against this principle, and authors have concluded that with the static component of the stabilization, TBW can be used for the fixation of simple olecranon fractures regardless of articular involvement.[1, 4, 16]

Although TBW has been shown to have many advantages, to our knowledge, no study has been conducted to determine the biomechanical response distal to the coronoid processes TBW can be used. Thus, this study was designed to investigate the biomechanical response of TBW using finite element analysis under physiological conditions in fractures up to and distal to the coronoid process. We hypothesized that TBW could be an effective technique for the treatment of such fractures.

Methods

This study was conducted under the Police General Hospital Ethics Committee (No. 15/2563). Informed consent was obtained from the subjects involved in the study.

Finite element modeling

The ulnar bone model was created using computed tomography (CT) of the right forearm of a healthy 40-year-old man after obtaining informed consent. The CT images from Digital Imaging and Communications in Medicine (DICOM) were imported into Mimics 10.01 (Materialize, Leuven, Belgium) to create a 3D ulnar bone geometry. The file was then transferred to PowerSHAPE 2016 (Delcam Plc, Birmingham, UK) to create a computer-aided design (CAD) model suitable for meshing. After creating the complete ulnar model, the fractures were reproduced in six models designed by dividing the olecranon in four parts with equal ranges (from the coronoid tip to the tip of the olecranon). The other two were made once and twice of the same range distal to the tip of the olecranon. All fractures were reproduced in the true axial plane **(Figure 1).**

The TBW was created using two 1.6 mm K-wires and 1 mm cerclage wire (18-gauge) as models, reflecting the surgical recommendations of the AO Foundation **(Figure 2)**.[17] As for the interaction of the K-wire system, the frictional values between the K-wire and cortical bone, cortical bone and cancellous bone were 0.5 and 0.3, respectively.[18, 19]

Convergence test and model validation

The Ulnar finite element models were meshed with 1, 2, and 3 mm element sizes using quadratic tetrahedral elements (Solid 92) in ANSYS 15.0 (Ansys Inc., Canonsburg, PN, USA). The cancellous and cortical bones were considered isotropic, linear and elastic, with elastic moduli of 1.3 GPa and 17 GPa, respectively.The Poisson's ratio was set to 0.3. [20, 21] A surface-to-surface gluing contact parameter was inserted at the interface of the cortical and cancellous bones, to prevent movement between the meshes of these regions at the interface. The elastic

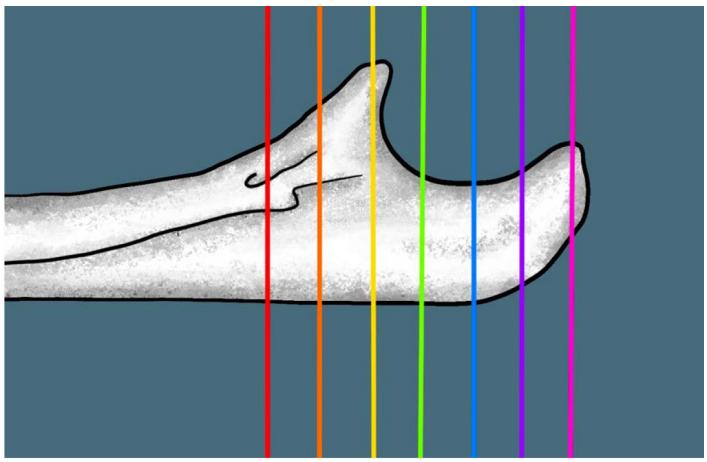

Figure 1. Fracture locations: purple, blue, green and yellow lines were simulated as intra-articular fractures, and orange and red lines were simulated as extra-articular fractures. The olecranon process to the coronoid process represents 100% articular surface. Intra-articular fracture patterns were divided in four parts [25% (purple), 50% (blue), 75% (green) and 100% (yellow) articular surfaces]. For extra-articular fracture, we measured the 25% articular surface (orange) and 50% of articular surfaces (red) distal to the coronoid process.

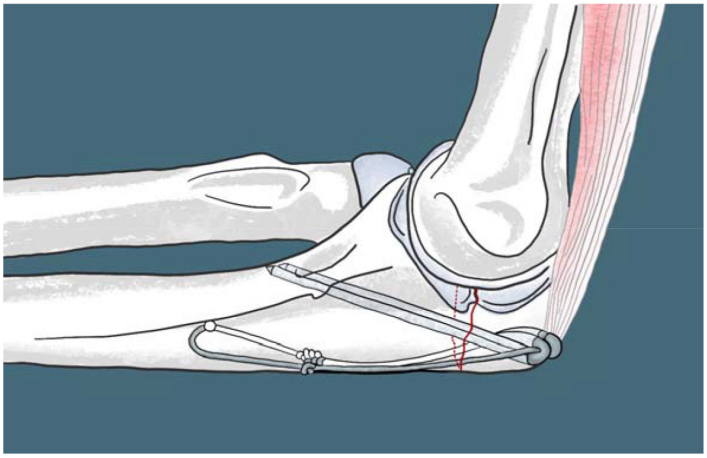

Figure 2. The tension band wiring was created using two 1.6 mm K-wires and an 18-gauge cerclage wire as recommended by AO Foundation's surgical guidelines.[17]

modulus and Poisson's ratio of stainless steel were 210 GPa and 0.3, respectively. [20, 22, 23] The figure-of-eight loop was placed close to the bone, as the TBW became more stable while turning over the adjacent bone surface.[24] For extra-articular fracture, the entry and the exit points of the K-wire were located at the tip of the olecranon and 2.7 cm distal to the fracture site, respectively.

The distal end of the ulnar model was fixed, and the traction force of 150 N was applied at the tip of the olecranon in all fracture patterns. The maximum displacement, maximum strain and maximum von Mises stress value at the fracture gap were evaluated for convergence in all models. The tolerance level was set within 5%.

The model was validated by comparison with the results from a cadaveric study. The authors found that a mean force of 490 N created a displacement of 2 mm.[13] To simulate the setting, the model of the olecranon fracture with 50% articular involvement was fixed with TBW under the boundary condition and the material properties used in the convergence test. These studies applied the force of 484 N to create a displacement of 2 mm indicating that the ulnar model had a response similar to that of the human ulnar bone under the same conditions.

Finite element analysis

To investigate the biomechanical responses from the TBW in simple olecranon fractures under the simulation of the magnitudes and directions of physiologic loads during active elbow joint movement of daily activities, six finite element models with different fracture locations (four intra-articular and two extra-articular) were fixed with TBW. An axial force of 150 N to imitate the triceps tendon was applied at the tip of the olecranon at one movement cycle.(13) The biomechanical properties of the implants, the von Mises stress value and displacement of the fracture gap were recorded.

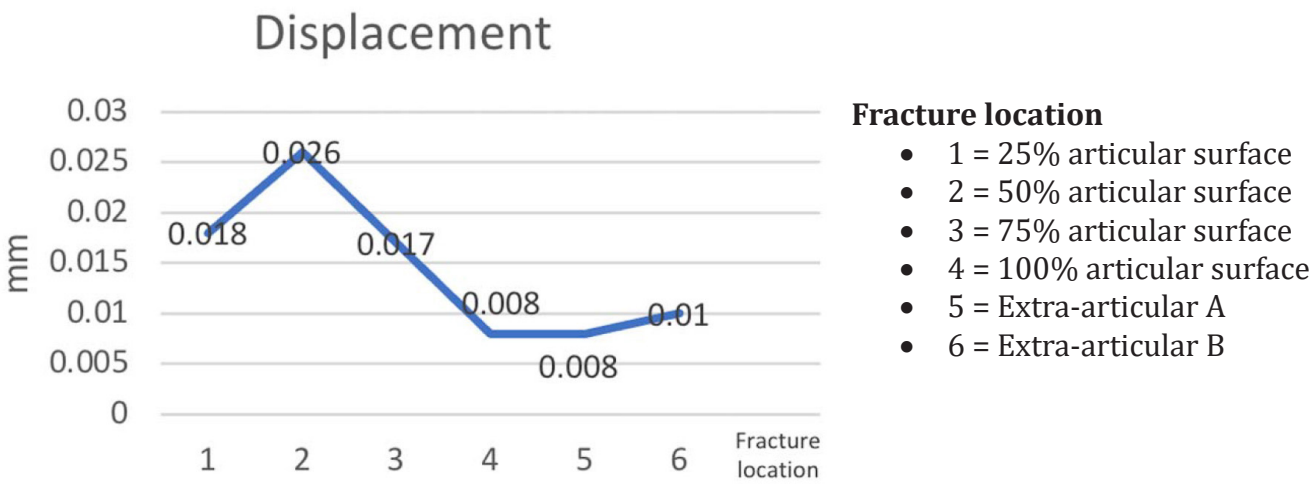

Figure 3. The displacement of the fracture gap after physiologic loading

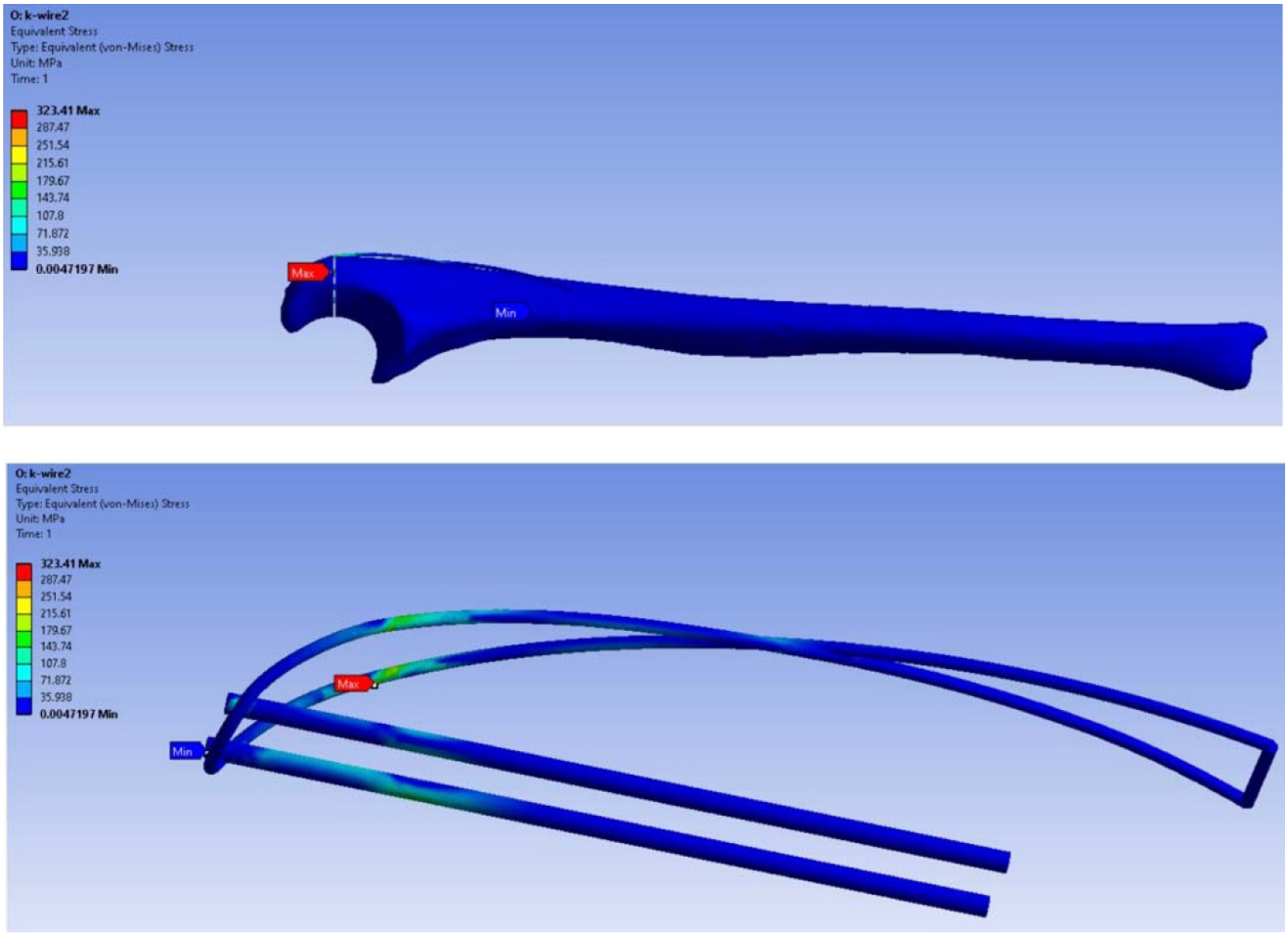

Figure 4. The distribution of the von Mises stress of 50% articular surface fracture pattern

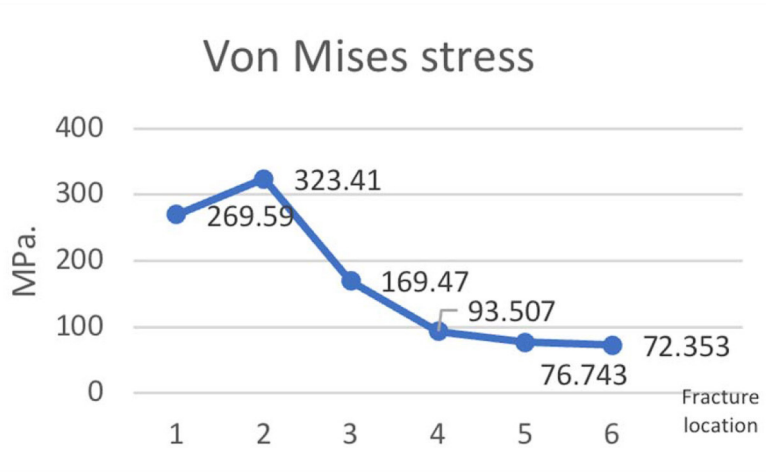

Figure 5. The von Mises stress of each model after physiologic loading

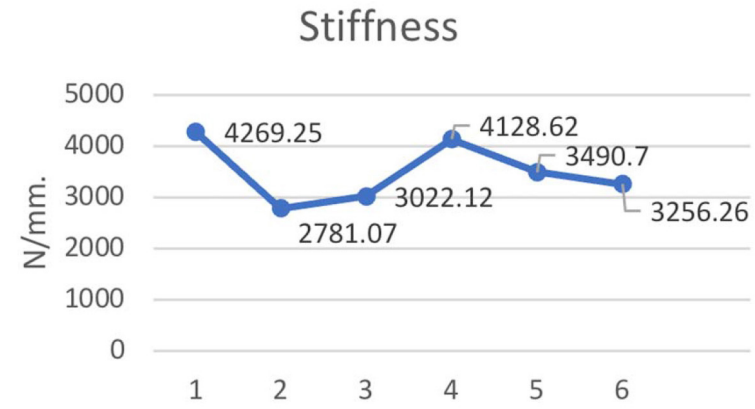

Figure 6. Stiffness of each model after physiologic loading

Results

Displacement

All fracture models, either intra- or extra-articular locations, were able to tolerate the load with insignificant displacement **(Figure 3).**

Von-Mises stress

The maximum stress was found in the model with a fracture located in the middle of the olecranon **(Figures 4 and 5).** The mean von Mises stress of intra-articular fracture fixation was 213.99 MPa, while that of extra-articular fixation was 74.55 MPa **(Figure 5).**

Stiffness

The mean stiffness of the intra-articular and extra-articular fracture fixation was 1144.89 N/mm and 1231.45 N/mm, respectively **(Figure 6).**

Discussion

In proximal (extra-articular) ulnar fractures, plate and screw fixation is the mainstay for operative treatment.[25] It provides stability that can withstand the force of daily life activities and allows early motion. However, some drawbacks result, including high implant costs and implant prominence. TBW is the most commonly performed procedure for simple olecranon fracture fixation, being reproducible, cost-effective, exhibiting a low implant prominence, and good clinical outcomes. Currently, the only known drawback is that a second operation is frequently required.[10-12] However, this statement might be questionable because a multicenter study indicated that the implant removal rate in proximal ulnar fractures did not differ between plate fixation and TBW (64.5% vs. 63.6%).[26] TBW was originally thought to rely on dynamic compression from active

movement of the elbow, but a biomechanical study proved that TBW only possesses static properties and can be used for simple olecranon fractures as distal as the coronoid tip with the same stability. (13) To our knowledge, no related study has evaluated the use of TBW in simple proximal ulnar fractures distal from the tip of the coronoid.

This study was designed using finite element analysis to evaluate the biomechanical responses of TBW fixation of intra-articular to extra-articular proximal ulnar fractures from the force of daily activities; finite element analysis is commonly used as an analytic tool to study biomechanical responses. Its cost effective and variable parameters can be adjusted in a more controllable manner. [27, 28]

Our results showed that the fracture displacement and von Mises stress had the maximum value in the 50% articular model. This result was similar to that of the study by Hammond et al.13) This could be explained by the position of the fracture that was directly aligned under the humerus, which acted as a wedge when the force from the triceps was applied. Nevertheless, the maximum displacement was 0.026 mm indicated that the TBW system was able to withstand the load of daily life activities immediately after the surgery. Thus, encouraging patients to start early range of motion to prevent stiffness is reasonable, especially in extra-articular fractures where the acceptable alignment is much greater than in intra-articular fractures. [29] The current results showed that the more distal the location of the fracture, created less stress of the implant. This could be explained by the increase in the working distance between the fixation point and the fracture site, decreasing construct stiffness and stress and allowing more motion at the fracture gap.[30-33]

The results of this study suggest that TBW is not only useful in treating simple olecranon fractures regardless of location, but also feasible in treating simple isolated extra-articular proximal ulnar fractures without any associated injuries such as radial head or ligamentous injuries. Results also suggest that patients with fractures managed by TBW should be encouraged to perform early motion because TBW has sufficient strength to withstand the immediate load of daily motion.

This study encountered several limitations. Although finite element analysis is considered one of the most widely used methods in biomechanical studies, it still lacks a physiological environment and body reaction. We did not perform endurance tests, which may cause problems such as pin loosening during clinical use. Further studies are needed to validate and extend our results for practical and clinical use.

Conclusion

TBW is a reproducible procedure. This agrees with the use of TBW for simple olecranon fractures at any fracture location. Our finite element study's results suggest that TBW is able to withstand the immediate force required for daily life activities, even distal to the coronoid level. In addition, results suggest that caution must be taken when the fracture is located in the middle of the olecranon. Further studies are needed to evaluate the clinical use of TBW.

Acknowledgment

We sincerely thank the volunteers for providing the ulnar model from their CT scans.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of interest: The authors declare they have no conflict of interest.

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CUT OFF VALUE OF GOOD PRONOSTIC FACTOR OUTCOMES IN LARGE TERRITORY ISCHEMIC STROKE UNDERGOING EARLY DECOMPRESSIVE CRANIECTOMY

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ABSTRACT

Background: Decompressive craniectomy (DC) significantly reduces mortality in large territory ischemic strokes that develop intractable cerebral edema. However, evidence for functional benefit remains sparse and contradictory.

Objective: This study aimed to assess cut-off value for predictor outcomes of early DC.

Methods: We conducted a prospective, observational cohort study from December 2016 to June 2021. Patients were screened for ischemic stroke involving the middle cerebral, internal carotid artery or both using the National Institutes of Health Stroke Scale score. All patients underwent DC. Multivariate analysis was performed for an array of clinical variables in relation to functional outcomes according to the modified Rankin Scale (mRS) and Pearson's correlation coefficient analysis. Clinical outcome was assessed after 3- and 6-month follow-up.

Results: In total, 243 patients were included in this study. Age ≤71 years (AUC=0.955, p <0.001 accuracy 89.7%), onset to DC ≤9 hours (AUC=0.824, p <0.001 accuracy 78.8%), volume of infarction ≤155 cm^3 (AUC=0.939, p <0.001 accuracy 93.6%) and the Alberta Stroke Program Early CT Score or ASPECT score ≥6 (AUC = 1, p <0.001 accuracy 100%) were significantly associated with good clinical outcomes in early DC (mRS 0 to 3).

Conclusion: Among patients with large territory ischemic strokes undergoing early DC, age ≤71 years, onset to DC ≤9 hours, volume of infarction ≤155 cm^3 and ASPECT score ≥6 was significantly associated with good clinical outcomes. All prognostic factors in early DC correlated well with functional outcomes at 6 months which could be used to predict outcome, and consider clinical indications and informed post-
operative complications among patients with large territory ischemic stroke.

Keywords: Intractable cerebral edema, Large-territory ischemic strokes, Decompressive craniectomy, Modified Rankin Scale, Outcome predictor

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Introduction

Patients presenting large-territory ischemic strokes may develop intractable cerebral edema putting them at risk of herniation and death. Patients may have severe neurological deficits, with hemiplegia, head and gaze deviation towards the side of the infarction and deterioration of consciousness. Brain edema may subsequently be associated with transtentorial brain herniation and death. In a number of clinical trials survival rates of 67 to 84% have been reported among patients with malignant middle cerebral artery (MCA) infarction treated with decompressive craniectomy (DC) compared with 20 to 30% among conservatively treated patients. Related described series have observed fatality rates of about 80%, and most survivors were left severely disabled.[1, 2] Unfortunately, medical management for malignant MCA infarction is generally ineffective, necessitating a surgical approach for its relief.[3] A pooled analysis of individual patient data of the three randomized controlled trials showed that surgical decompression reduced the risk of death or disability, defined as modified Rankin Score (mRS) ≥3.[4] Although DC was shown to significantly reduce mortality among patients compared with medical therapy alone, concern has been expressed that life is preserved at the potentially unacceptable cost of marked functional disability.[5]

Large territory ischemic stroke refers to a cerebral infarction involving more than one half to two thirds of MCA territory.[7,8] Among large territory ischemic stroke cases, those with early clinical deterioration or involvement of complete MCA territory were frequently tagged as malignant large infarction (MLI).[9] DC for MLI was scarcely performed before 2000 mainly because it might just increase survival with overwhelming neurological impairment and handicap. However, increased interest in DC has been shown since the early 21st century as a result of mortality reduction as well as a chance of outcome improvement.[10-16] Although various prognostic factors affecting the outcome after DC have been identified, the strength of association has not been well-established. Most published data has been obtained from western populations where long-term stroke rehabilitation facilities exist and stroke units are well-established.

Another crucial factor for DC in MLI is timing of surgery, performed within 24 to 48 hours that presents a benefit associated with early DC. Early DC markedly reduced mortality and the volume of infarcted brain tissue. This study aimed to assess cut off value of good prognostic factor outcomes in large territory ischemic strokes undergoing early DC.

Methods

A prospective, observational cohort study was conducted between December 2016 and June 2021 at the Department of Neurology and Neurosurgery, Phramongkutklao Hospital. The study was approved by the Institutional Review Board, Royal Thai Army Medical Department (approval no. R102h/59). Research followed Council for International Organization of Medical Science Guidelines 2012 and Good Clinical Practice of International Conference on Harmonization statement no. IRBRTA 1731/2559.

The sample size estimation revealed at least 243 patients were required for this study. Written informed consent was obtained from all patients. Patients with life-threatening malignant MCA infarction indicated to undergo DC on the basis of clinical assessment using National Institute of Health Stroke Scale (NIHSS), Glasgow coma scale (GCS) and neuroimaging using computed tomography (CT) were prospectively enrolled. Patients who died within 24 hours of presentation, those with dilated and fixed pupils at presentation, GCS <6, mRS ≥3 before the current stroke and known metabolic cause for altered sensorium were excluded to reduce the risk of bias.

The diagnosis of stroke was clinically established and confirmed by neuroimaging [noncontrast CT head]. Details of demographics including age, sex, address, contact number, body mass index (BMI, kg/m^2), detailed history of event, presenting symptoms and signs, risk factors for stroke, blood pressure, GCS and NIHSS score, laboratory parameters, imaging findings (type of stroke, arterial territory involved, the Alberta Stroke Program Early CT Score (ASPECT score), volume of infarction (cm^3) and midline shift), onset to DC (hour), intracranial pressure (ICP)

at operative field and postoperative events such as surgical site infection and hydrocephalus were noted. The Western Aphasia Battery was used to record the severity of aphasia. Aphasia quotient was calculated by kerrtesz formula; a score ≤93.8 was taken as cut-off for defining aphasia.[6] DC in our patients consisted of creating a large fronto-parieto-temporal free bone flap (at least 12 cm) and duraplasty. No intervention on brain tissue was performed. Details of DC including time of onset of symptoms to DC, duration of surgery, blood loss, postoperative complications and intensive care unit (ICU) stay were recorded. Course of hospital stay including stroke recurrence, death and status at discharge was noted. Clinical status at the time of discharge was measured using GCS, Glasgow Coma Outcome Scale (GCOS), NIHSS, modified Barthel Index and mRS score. Outcome on follow-up was assessed using mRS during Out-patient Department visits in the stroke clinic at 3 and 6 months. Aphasia, using the Western Aphasia Battery, was tested on follow-up visits at the Stroke Clinic. The mRS of ≤3 was taken as a good outcome. We also analyzed the results using mRS of ≤3 as a good outcome.

Participants

A total of 243 patients admitted in the Medical Stroke Unit and Surgical ICU, Phramongkutklao Hospital, Bangkok, Thailand from December 2016 to June 2021 was monitored for invasive arterial blood pressure, peripheral O_2 saturation, and electrocardiogram. All patients were measured for oxygenation, arterial blood pressure and glucose. The inclusion criteria were age >18 years, diagnosis of large territory ischemic stroke involving ≥two thirds of the MCA territory on cranial CT or magnetic resonance imaging (MRI) within 48 hours after symptoms onset (the score of NIHSS item 1a reflecting consciousness needed to be ≥1), DC was operated within 48 hours of onset. The exclusion criteria were large volume hemorrhagic transformation, malignant herniation, severe coagulopathy, severe infection, patient refusal of treatment, patients who died within 24 hours of presentation, dilated and fixed pupils at presentation, GCS <6, mRS ≥3 before the current stroke and known metabolic cause for altered sensorium.

Standard medical therapy

All patients were admitted to a Stroke Unit or ICU, Department of Neurology and Neurosurgery. The patient's head was kept elevated at 30°. All patients were kept in a mild fluid restriction state with 1800 mL of daily fluid in the first week. Intravenous antihypertensive agents were administered when blood pressure was higher than 220/120 mmHg. Body temperature was kept below 38°C and blood glucose level was maintained <180 mg/dL. Endotracheal intubation was performed to maintain adequate tissue oxygenation among patients with clinical deterioration or signs of respiratory insufficiency. Hyperventilation was used only in an emergency with the target level of $PaCO_2$ of 30 to 35 mmHg. Osmotherapy with mannitol or glycerol launched when evidence of mass effect was observed. Mannitol was administered at the dosage of 0.25 to 0.5 g/kg body weight bolus. During osmotherapy, blood osmolarity was maintained at approximately 300 to 320 mOsm/L. Oxygenation, blood pressure and glucose level was sustained at appropriate levels. Early enteral nutrition was provided. Pneumonia and deep venous thrombosis were monitored and well treated.

Surgical treatment

Early DC was carried out within 48 hours of onset. This consisted of a craniectomy with dimensions of at least 12 cm in the anteroposterior and 10 cm in the superoinferior direction which was sufficiently large to match the infarcted area. Additional temporal bone removal was performed so that the floor of the middle cerebral fossa could be fully explored and decompressed. The dura was opened and an augmented patch was inserted to further relieve the high intracranial pressure. Those surgical survivors received a secondary operation of cranioplasty three months after DC.

Outcome assessment

Outcome was assessed with mRS at 3 and 6 months follow-up and was first dichotomized as good (mRS 0 to 3) and poor (mRS 4 to 6) to compare and contrast survivors' functional outcome in early DC.

Hemodynamic monitoring

Radial arterial and central venous catheters were linked to a bedside monitor on one side and to a specific transducer (Philips Intellivue Philips MX600, USA) for blood pressure and central venous pressure monitoring. When patients had unstable hemodynamic values of cardiac output and stroke volume were estimated using pulse contour analysis (EV1000 clinical platform, Edwards advanced hemodynamic monitoring tools for an integrated Edwards Critical Care System, USA).

Statistical analysis

Statistical analysis was performed using SPSS, Version 23.0. The primary outcome was the cut off value of good prognostic factors in large territory ischemic stroke undergoing early DC while the secondary outcome was clinical variables in relationship to functional outcomes according to mRS. Results were expressed as mean ± SD when data were normally distributed or median and interquartile range (IQR) if not. A *p*-value less than 0.05 was considered statistically significant. Multivariate analysis was performed using an array of the cut off values in relationship to functional outcomes according to the mRS and Pearson's correlation coefficient analysis. Clinical outcomes were assessed at 3- and 6-month follow-up. For the cut off value of good prognostic factor, the Area Under the Curve (AUC) or Receiver Operating Characteristics (ROC) curve were used representing the degree or separable measurement, then a graphic plot was drawn to illustrate the diagnostic ability of a binary classifier system as its discrimination threshold varied in these predictive factors. The higher the AUC, the better the model was at distinguishing between patients presenting good and poor outcomes. The value of AUC near 1

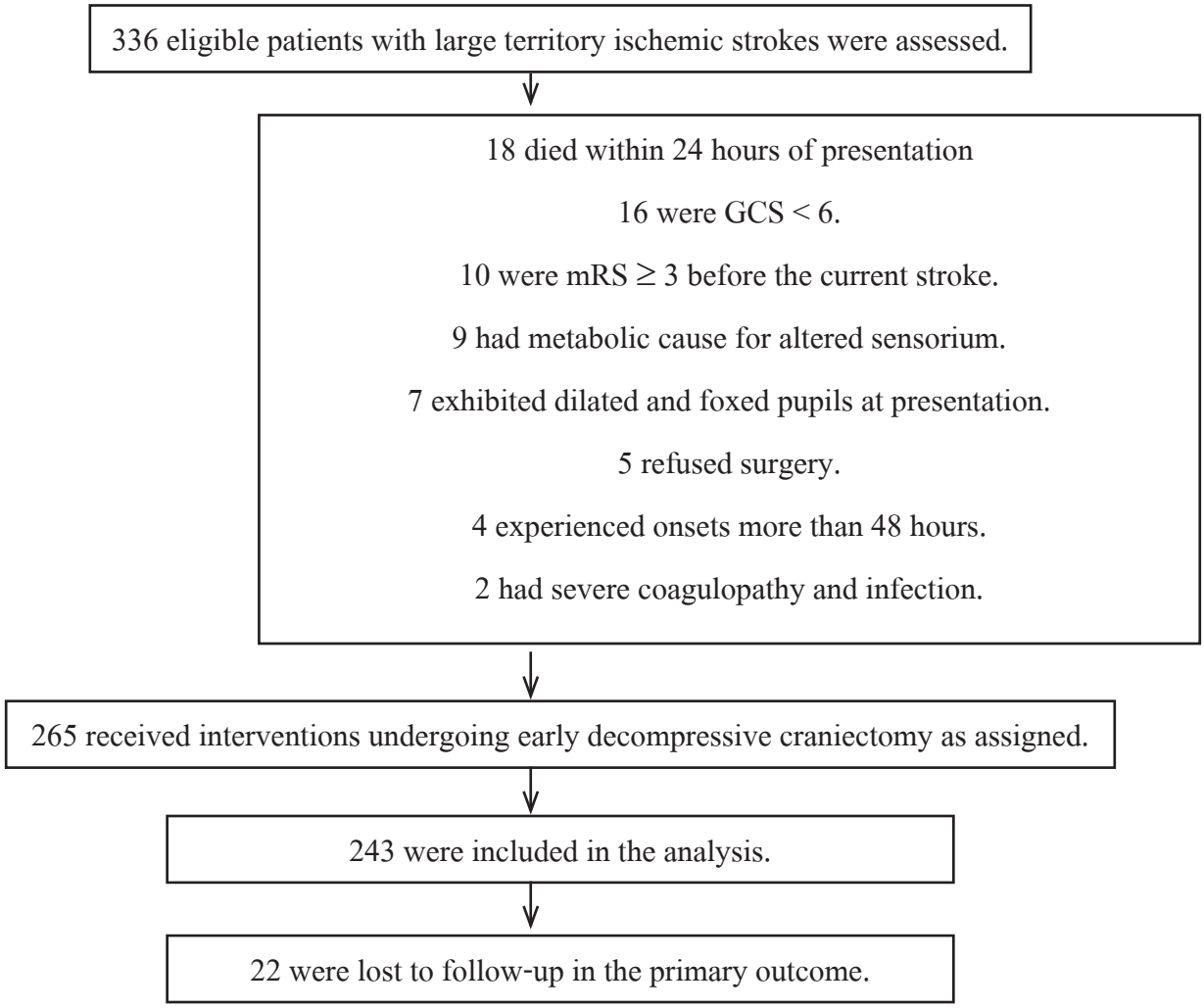

Figure 1. Flow chart of patient enrollment and analysis

showed a good separable measurement while AUC near 0 showed the worst separable measurement.

Results

Patients' characteristics

During the study period, 243 patients with large territory ischemic stroke undergoing early DC were included in data analysis. Most patients were male (80.7%) with average age of 65 years. The most frequent coexisting disease was hypertension (88%). Of these, 153 (63%) patients had left territory stroke while 90 (37%) patients had right territory stroke. Among these, mean range of NIHSS was 21 and GCS at the time of admission was 9 (9.5 ± 3). Mean onset to DC from time of onset of symptoms was 10 hours (10.41 ± 5.96). Mean ICP at the operative field was 19 mmHg (19.32 ± 8.6). Mean volume of infarction was 135 cm3 (135.19 ± 51.1), and mean ASPECT score was 5 (5.77 ± 2.41). Six patients (2.5%) had postoperative surgical site infections while 23 (9.5%) patients revealed postoperative hydrocephalus (Table 1). Outcome was assessed using GCOS, NIHSS, Modified Barthel Index at 3- and 6-month follow-up as shown in **Table 2**.

Table 1. Demographic data of 243 patients with large territory ischemic strokes undergoing early DC

Variables	N = 243
Male, n (%)	196 (80.7%)
Age (yr)	64.82 ± 14.81
Body mass index, BMI (kg/m^2)	29.14 ± 6.2
Diabetes mellitus	186 (76.5%)
Hypertension	214 (88.1%)
Tobacco use	126 (51.9%)
Old cerebrovascular accident	87 (35.8%)
History of ipsilateral TIA	22 (9.1%)
Hypercholesteralemia	167 (68.7%)
History of angina pectoris	87 (35.8%)
Coronary artery disease	116 (47.7%)
Atrial fibrillation	116 (47.7%)
Peripheral arterial occlusive disease	48 (19.8%)
Position of large-territory infarction	
left	153 (63%)
Right	90 (37%)
bilateral	0 (0%)
Onset to DC (hr)	10.41 ± 5.96
GCS at admission	9.5 ± 3
NIHSS at admission	21.44 ± 9.64
ICP at operative field	19.32 ± 8.6
Volume of infarction(cm$^{3)}$	135.19 ± 51.1
ASPECT score	5.77 ± 2.41
Post-operative surgical site infection	6 (2.5%)
Post-operative hydrocephalus	23 (9.5%)

Value presented as mean ± SD

Table 2. Outcome was assessed with GCOS, NIHSS, Modified Barthel Index at 3- and 6-month follow-up in early DC

Variables	Baseline	3 Month	6 Month
GCOS			
Death	7 (2.9%)	7 (2.9%)	7 (2.9%)
Persistent vegetative state	90 (37%)	52 (21.4%)	52 (21.4%)
Severe disability	85 (35%)	54 (22.2%)	54 (22.2%)
Moderate disability	61 (25.1%)	110 (45.3%)	13 (5.3%)
Good recovery	0	20 (8.2%)	117 (48.1%)
NIHSS	21.95 ± 8.89	19.51 ± 9.46	18.47 ± 9.58
Modified Barthel Index	20 (0, 60)	61 (20, 90)	90 (30, 95)
No symptoms	0	0	0
No significant disability	0	0	0
Slight disability	0	60 (24.7%)	113 (46.5%)
Moderate disability	4 (1.6%)	68 (28%)	16 (6.6%)
Moderate to severe disability	85 (35%)	4 (1.6%)	1 (0.4%)
Severe disability	147 (60.5%)	104 (42.8%)	94 (38.7%)
Death	7 (2.9%)	7 (2.9%)	19 (7.8%)

Values presented as frequency (%), interquartile range (IQR) and mean ± SD

Table 3. Correlation between postoperative hydrocephalus and clinical outcome (mRS 0 to 3) at 6-month follow-up in early DC

	Good outcome (n=156)	Poor outcome (n=87)	*p-value*
Hydrocephalus	12 (9.6%)	11 (9.3%)	0.941
No Hydrocephalus	113 (90.4%)	107 (90.7%)	

Values presented as frequency (%), *p-value* corresponds to Pearson's correlation or chi-square test

Clinical outcomes

Correlation between postoperative hydrocephalus and clinical outcome (mRS 0 to 3) at 6-month follow-up showed no significant differences between good and poor outcomes. Postoperative hydrocephalus did not affect clinical outcomes (**Table 3**).

Cut off value for good clinical outcomes in early DC (mRS 0 to 3) (**Figure 2**)

This study showed that age ≤71 year was significantly associated with good clinical outcome in early DC (mRS 0 to 3) (AUC = 0.955, p <0.001 accuracy 89.7%) (**Figure 2A**). Onset to DC ≤9 hours was also significantly associated with good clinical outcome in early DC (mRS 0 to 3) (AUC = 0.824, p <0.001 accuracy 78.8% (**Figure 2B**). Volume of infarction ≤155 cm^3 was significantly associated with good clinical outcome in early DC (mRS 0 to 3) AUC = 0.939, p<0.001 accuracy 93.6%. (**Figure 2C**). Additionally, ASPECT score ≥6 was significantly associated with good clinical outcome in early DC (mRS 0 to 3) (AUC = 1, p <0.001 accuracy 100%) (**Figure 2D**).

Discussion

In this study, management of patients with large territory ischemic stroke remained a challenge. DC could relieve the mass effect resulting from infarcted brain tissue, preventing brain herniation and death. Postoperative hydrocephalus did not

Figure 2. Cut off value for good clinical outcome in early DC (mRS 0 to 3)

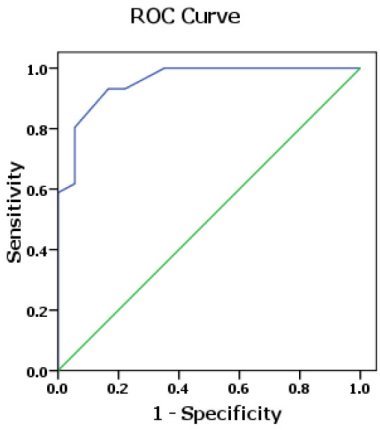

Figure 2A.
ROC Area = 0.955, p <0.001

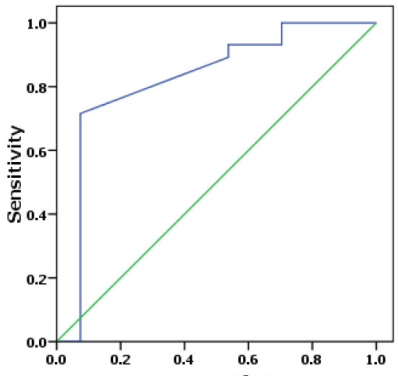

Figure 2B.
ROC Area = 0.824, p <0.001

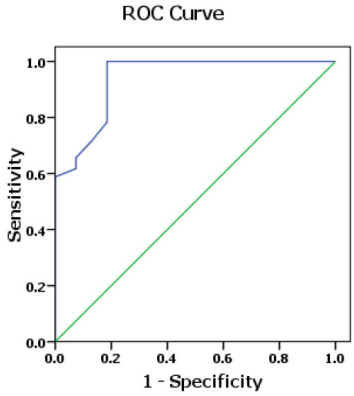

Figure 2C.
ROC Area = 0.939, p <0.001

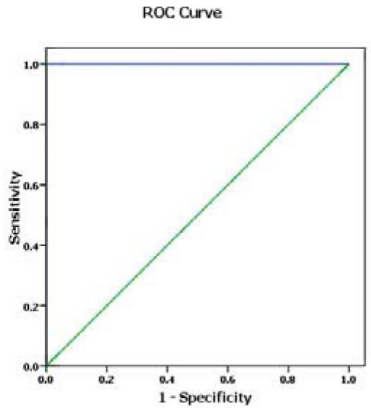

Figure 2D.
ROC Area = 1, p <0.001

Figure 2A. Age cut off value for good clinical outcome in early DC (mRS 0 to 3)
Figure 2B. Onset to DC (hr) cut off value for good clinical outcome in early DC (mRS 0 to 3) (set accuracy more than 70%)
Figure 2C. Volume of infarction (cm^3) cut off value for good clinical outcome in early DC (mRS 0 to 3)
Figure 2D. ASPECT score cut off value for good clinical outcome in early DC (mRS 0 to 3)

Table 4. Multivariate analysis cut off value in relationship to functional outcome at 6-month follow-up (mRS 0 to 6)

	Beta coefficient	**Standard Error (95%CI)**	***p-value***
(Constant)	1.425	0.265	<0.001*
Onset to DC (hr) ≤ 9 hrs	-0.068	0.13	0.6
ASPECT score ≥ 6	3.766	0.236	<0.001*
Volume of infarction ≤ 155 cm^3	0.718	0.198	<0.001*
Age ≤ 71 yrs	0.274	0.159	0.086

Value presented as mean ± SD or n (%), p-value corresponds to Pearson's correlation. Multivariate analyses were performed for an array of clinical variables in relation to functional outcome at 6-month follow-up. Linear regression was performed for an array of clinical variables in relation to functional outcomes.

affect clinical outcome. Age ≤71 years, onset to DC ≤9 hours, volume of infarction ≤155 cm^3 and ASPECT score ≥6 were significantly associated with good clinical outcome in early DC (mRS 0 to 3). Three randomized controlled trials; HAMLET[17] DECIMAL[14] and DESTINY[12] compared decompressive surgery plus medical treatment with medical treatment alone among patients with large territory ischemic stroke. The present study showed similar mortality benefits and better functional outcomes (mRS ≤3) at the end of one year when compared with those studies mentioned earlier. Inclusion of patients with less severe stroke to the study when compared with related studies could have resulted in better functional outcomes. A recent Cochrane review concluded that surgical decompression lowered the risk of death or severe disability (defined as mRS ≥4) in selected patients at 60 years or younger with a large territory ischemic stroke and cerebral edema. [18] This meta-analysis involving 134 patients, 60 years or younger suggested that surgical decompression reduced the risk of death at the end of follow-up (OR = 0.19, 95% CI = 0.09-0.37) and the risk of death or disability defined as mRS ≥4 at 12 months (OR = 0.26, 95% CI = 0.13-0.51). Death or disability defined as mRS >3 at the end of follow-up did not differ between the treatment arms (OR = 0.56, 95% CI = 0.27-1.15). Because all trials had to stop early, the possibility of an overestimation of the effect size was expressed. A review of 13 uncontrolled studies of 138 patients, older than 50 years constituted a strong predictor of poor functional outcome after surgical decompression. However, timing of the operation, side of the infarction and involvement of other vascular territories did not affect the outcome.[19] In one recent uncontrolled study, only 6 (8%) of 72 patients older than 60 years had a favorable outcome after surgery when compared with 77 (54%) of 143 younger patients.[20]

In this study, we used high accuracy for each cut off value of prognostic factors affecting good outcome in early DC and also calculated the sensitivity, specificity, positive predictive value, negative predictive value, accuracy. Then, the AUC or ROC was plotted representing the degree or separable measurement. Our results showed that age ≤71 years was a cut off value as a predictor of surgery outcome. The impact of age on outcome has not been well studied in malignant MCA infarction. Poor functional outcome and increased mortality were observed among older patients undergoing hemicraniectomy.[21-24] Moreover, the potential for recovery of function after stroke generally declines steeply after the age of 60 years. [25] Foerch et al. reported that age was the only factor affecting functional outcomes.[26] Walz et al. agreed that the outcome was related to age.[23] Uhl et al. evaluated 188 patients undergoing decompressive craniectomy and found that those older than 50 years had higher mortality and poorer outcomes.[27] Age was the most important pretreatment prognostic factor. Thus, age could be the most important factor in deciding which patients should undergo DC.

Timing of surgery could be another crucial factor for DC among patients with malignant MCA infarction. Animal and clinical studies have provided evidence that a benefit was associated with early DC. In a rat MCA occlusion model, early DC markedly reduced mortality and reduced the volume of infarcted brain tissue.[28] Schwab et al. found that decompression within 24 hours of ictus (early DC) was associated with a lower mortality rate (16%) and a mean Modified Barthel Index (BI) score of 68.8.[29] In their series, 26 of 31 (84%) patients had a BI ≥60. Similar results were also reported in a related study.[30] Decompression within 6 hours of ictus was associated with a 8.3% mortality rate and a mean modified BI score of 70.0, compared with a 36.7% mortality rate. Further, a mean BI score of 52.8 was reported among patients in whom decompression was carried out after 6 hours of ictus. Our study as well as others showed that onset to DC ≤9 hours was significantly associated with good clinical outcomes in early DC.

Although some studies have found that clinical signs of herniation were not associated with functional outcome among patients with malignant MCA infarction undergoing hemicraniectomy,[26, 28] anisocoria indicating herniation leads to mesencephalic ischemia and links to a worse prognosis. Better outcomes can be expected

from early treatment before the clinical signs of herniation appear. In our study, poor functional outcome was associated with the presence of clinical signs of herniation before treatment. Thus, we excluded patients who had signs of herniation from the study. Our suggestion for DC was to be performed within 6 hours of ictus, and before the clinical signs of herniation appeared among patients with malignant MCA infarction. This means that time of operation rather than early operation was likely more important to obtain a better outcome. Theoretically, when strong evidence supports the very high risk of subsequent clinical deterioration, early surgery before clinical worsening would be the ideal surgical timing. Several clinical studies have shown several promising parameters in the early prediction of malignant MCA infarction, i.e., lesion volume >145 cm^3 in diffusion weighted imaging of MRI and a complete MCA territory perfusion deficit shown on MR angiography. [31, 32] In this study, we found that volume of infarction ≤155 cm^3 was significantly associated with good clinical outcomes in early DC. Involvement of more than one vascular territory (internal carotid artery infarction) and hemispheric infarction of the dominant hemisphere are often cited as prognostic indicators of poor function outcome and contraindications to decompression. In this study, ASPECT score ≥6 was significantly associated with good clinical outcome in early DC (mRS 0 to 3).

This study encountered several limitations. First, outcome assessment at 3 and 6 months might be insufficient to understand the true benefit of early DC that constituted a lifesaving surgery. At least one year follow-up is recommended to assess functional benefits. Second, the study was conducted at a single center. Finally, many patients undergoing early DC were excluded from the study.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interests:

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

Acknowledgements

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ZERO-PROFILE DEVICE IMPLANTATION IN ANTERIOR CERVICAL DISCECTOMY AND FUSION: A SINGLE INSTITUTE EXPERIENCE

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Abstract

Background: A surgical procedure, anterior cervical discectomy and fusion (ACDF), is used for neural decompression in degenerative cervical disk disease and cervical spondylosis. A new type of cervical interbody cage, the Zero-profile device (ZPD), has been developed which could reduce postoperative complications among patients.

Objectives: The study aimed to examine the effect of ZPD on clinical outcomes and cervical spine alignment of enrolled patients at 1-year follow-up in the management of ADCF.

Methods: This study retrospectively evaluated the clinical and radiographic outcomes using the Zero-profile device (ZPD) in the anterior cervical discectomy and fusion (ACDF). All patients who underwent ACDF at Vajira Hospital between May 2017 and June 2021 were included in this study. Radiographic images obtained from picture archiving and communication systems (PACS) were used to evaluate the device-level Cobb angle (DLCA), segmental Cobb angle (SCA), global Cobb angle (GCA), sagittal vertical axis (SVA), and intervertebral disk height. The Japanese Orthopaedic Association (JOA) scores and visual analog scale (VAS) were obtained from the patients' medical records. The preoperative DLCA, SCA, GCA, SVA, and intervertebral disk height measurements were compared with the postoperative measurements at 1 year.

Results: A total of 31 patients (45 disks) who underwent ACDF with the ZPD were included in this study. A significant improvement was found in JOA, VAS, DLCA, SCA, GCA, SVA, and intervertebral disk height after ACDF with ZPD ($p<0.001$). Immediate postoperative dysphagia occurred in two patients (6.5%), which resolved after 3 months. No subsidence was reported at 1-year follow-up. Age, BMI as well as the preoperative cervical alignment did not affect outcomes in this study.

Conclusion: The use of the ZPD in ACDF improved clinical and radiographic outcomes in the correction of cervical spine alignment, and minimized postoperative complications of dysphagia. No device-related failure occurred, and favorable outcomes persisted at 1-year follow-up.

Keywords: Alignment, Anterior cervical discectomy and fusion, Zero-profile device

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Introduction

Anterior cervical discectomy and fusion (ACDF) is a surgical procedure for neural decompression in degenerative cervical disk disease and cervical spondylosis and is performed using an anterior approach. After the discectomy, the surgeon inserts an autologous bone graft or traditional interbody cage and plate structure to restore the height of the intervertebral space, reconstructs the cervical spine curvature and maintains cervical spine stability. Reported complications of this procedure include dysphagia, odynophagia and hoarseness. Further, cage subsidence can lead to implant failure or adjacent segment pathology. A new type of cervical interbody cage, the Zero-profile device (ZPD), has been developed. The ZPD comprises an interbody polyetheretherketone (PEEK) cage and an internal implant with locking screws. The characteristics of PEEK are well suited to ACDF because it has elastic properties that closely match cortical bone.[1] The ZPD cervical interbody cage is held with screws inserted into the adjacent vertebral bodies, fixing the implant in the intervertebral disc space after discectomy. No cervical plate is attached between the anterior surface of the vertebral body and esophagus or any prevertebral soft tissue with the ZPD, which may lead to a reduction in postoperative dysphagia.[2]

Furthermore, the shape of the ZPD is lordotic and is similar to the normal anatomical alignment of the cervical spine. Many studies have indicated that cervical kyphosis alone without cord compression increases longitudinal cord tension and intramedullary pressure. This has been shown to cause neuronal loss and demyelination in animal models.[3, 4] The loss of lordosis and the development of kyphosis can cause neurological deterioration and should be avoided.[5] Koeppen et al. reported a study of 102 patients with cervical spondylosis myelopathy involving 219 fused levels with a traditional lordotic PEEK cage. They demonstrated that kyphosis was associated with pre- and postoperative neck pain.[6] The hypothesis of this study constituted whether the ZPD may improve cervical sagittal alignment. Thus, this study aimed to examine the effect of the ZPD on clinical outcomes and cervical spine alignment of enrolled patients at 1-year follow-up in the management of ADCF.

Methods

This study was approved by the Ethics Committee of Vajira Hospital (045/64). All patients who undergoing ACDF at the Neurosurgical Department of Vajira Hospital between May 2017 and June 2021 were enrolled. The indications for ACDF were cervical spine injuries, cervical spondylosis, cervical spondylodiscitis, and ossification of the posterior longitudinal ligament of the cervical spine. Patients with no record of the cage type in the operation notes, with no pre- or postoperative imaging, or incomplete follow-up data were excluded. A total of 31 patients undergoing ACDF with the ZPD implantation in 45 disk levels were included in this study.

The pre-operative visual analog scale (VAS) for neck pain and Japanese Orthopaedic Association (JOA) score were assessed, and a cervical spine X-ray and spine MRI were performed. General anesthesia was performed without neuromuscular blockers or agents that affected neuromuscular monitoring. Intra-operative neuromuscular monitoring was performed to evaluate somatosensory-evoked potentials (SSEPs) and transcranial motor-evoked potentials (tcMEP). Electromyography (EMG) was also performed. A train of four twitches was used at the common peroneal nerve, and a response rate ≥75% was required before recording the EMG.

Among all patients, surgery was performed by an anterior cervical approach using the Smith-Robinson technique. The surgeons comprised two experienced spinal neurosurgeons with more than five years' experience and a cervical retractor was applied for distraction. Discectomy, removal of osteophytes with a high speed drill and Kerrison rongeurs and opening of the posterior longitudinal ligament was performed in all cases under a microscope. After preparation of the fusion bed, interbody fusion was performed with the ZPD. All the ZPDs, filled with bone graft substitute, were 17 mm wide, 14 mm long, and exhibited a 7˚lordotic taper. Multiple implant heights

accommodated the varied patient anatomy. Robust implants with 3 screws, 4 mm in diameter, were inserted using a freehand technique. The angled instruments were designed to work perpendicular to the spine. The correct screw angle and trajectory were automatically achieved when the screwdrivers were seated properly within the screw hole. The length of the screws was measured from the anterior portion of the implant to the total distance reached posteriorly. The trajectory of the screws was at about a 40-degree angle to the superior and inferior surfaces of the ZPD. A soft collar was applied for six weeks after surgery among all patients.

Postoperative evaluation and radiographic outcomes

All patients underwent a physical examination and radiography of the cervical spine at 1, 3, 6 and 12 months after surgery. The X-rays were reviewed, and the operator and another neurosurgeon measured the parameters using a single measurement before and after surgery at 12 months using picture archiving and communication systems (PACS) measurement features. The sagittal alignment was assessed using a device-level Cobb angle (DLCA) at each operative level, segmental Cobb angle (SCA), global Cobb angle (GCA) and C2 to 7 sagittal vertical axis (SVA). Pre- and postoperative imaging at one year were compared. DLCA was measured by drawing lines parallel to the inferior endplate of the upper operative vertebral level and the inferior endplate of the lower operative vertebral level. SCA was measured by drawing lines parallel to the inferior endplate of the uppermost operative vertebral level and the inferior endplate of the lowermost operative vertebral level. Perpendicular lines were then drawn from each of the above two lines, and the angle of intersection constituted the SCA (**Figure 1**). GCA was measured by drawing lines parallel to the inferior endplate of C2 and the inferior endplate of C7. Perpendicular lines were drawn from each of the lines, and the angle of intersection was the GCA (**Figure 2**). C2 to 7 SVA comprised the distance in mm measured between a plumb line dropped from the centroid of C2, and another plumb line dropped from the posterosuperior aspect of the C7 vertebral body (**Figure 3**). Pre- and postoperative (at one-year follow-up) ventral and reduced dorsal segmental height were measured and compared. A height reduction of more than 3 mm ventrally or dorsally was defined as subsidence.[7] Fusion was primarily assessed using cervical X-rays and bridging trabecular bone between the endplates. The absence of a radiolucent gap between the endplate and graft was evidence for osseous union. When it remained unclear, flexion and extension cervical views showing <1 mm of motion between the spinous processes confirmed fusion. Operative time, blood loss, and complications were recorded. Dysphagia was evaluated according to the Bazaz grading system.[8]

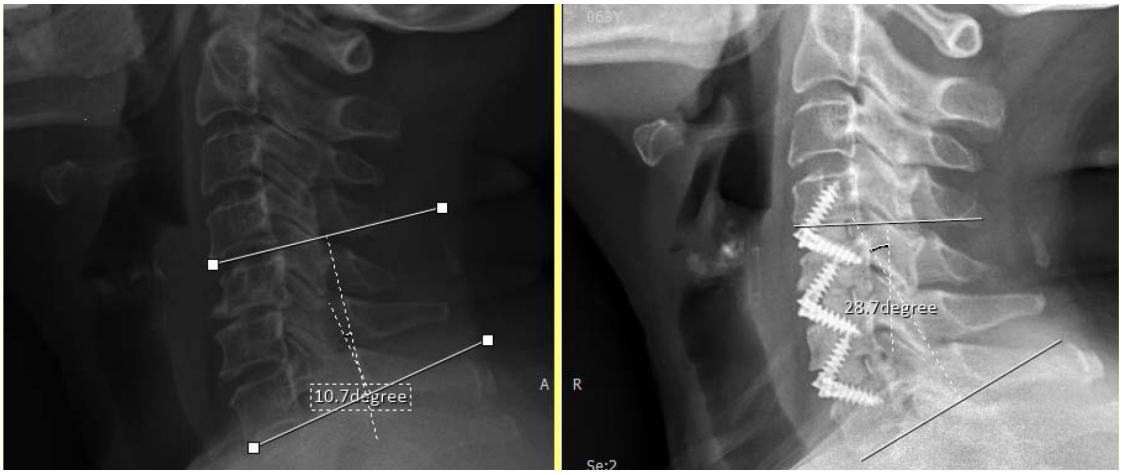

Figure 1. Segmental Cobb angle (SCA) was measured by drawing lines parallel to the inferior endplate of the uppermost operative vertebral level and the inferior endplate of the lowermost operative vertebral level. Perpendicular lines were then drawn from each of the above two lines, and the angle of intersection constituted the SCA. Pre- and postoperative imaging at one year were compared.

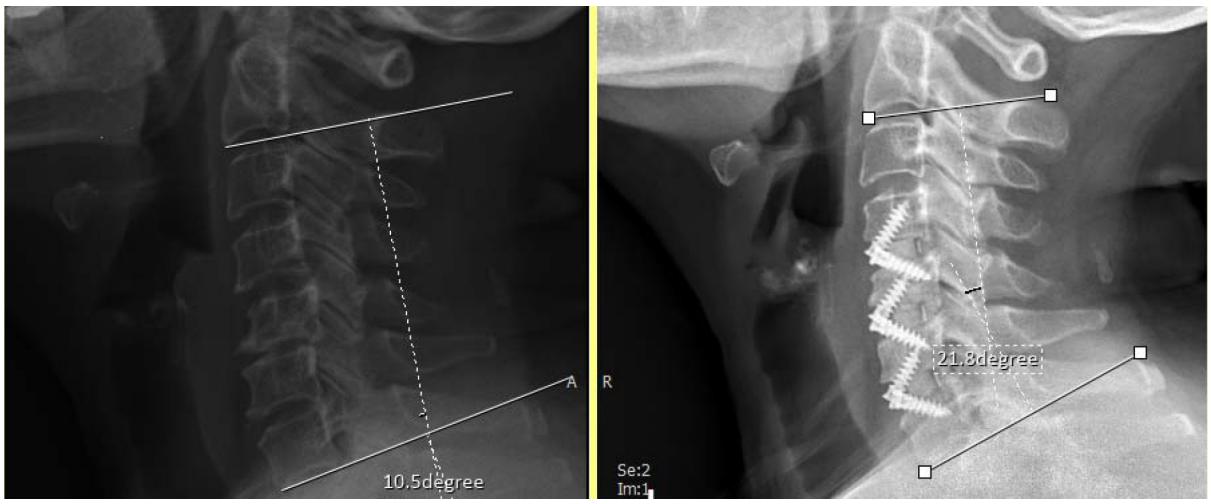

Figure 2. GCA was measured by drawing lines parallel to the inferior endplate of C2 and the inferior endplate of C7. Perpendicular lines were drawn from each of the lines, and the angle of intersection comprised the GCA. Pre- and postoperative imaging at one year were compared.

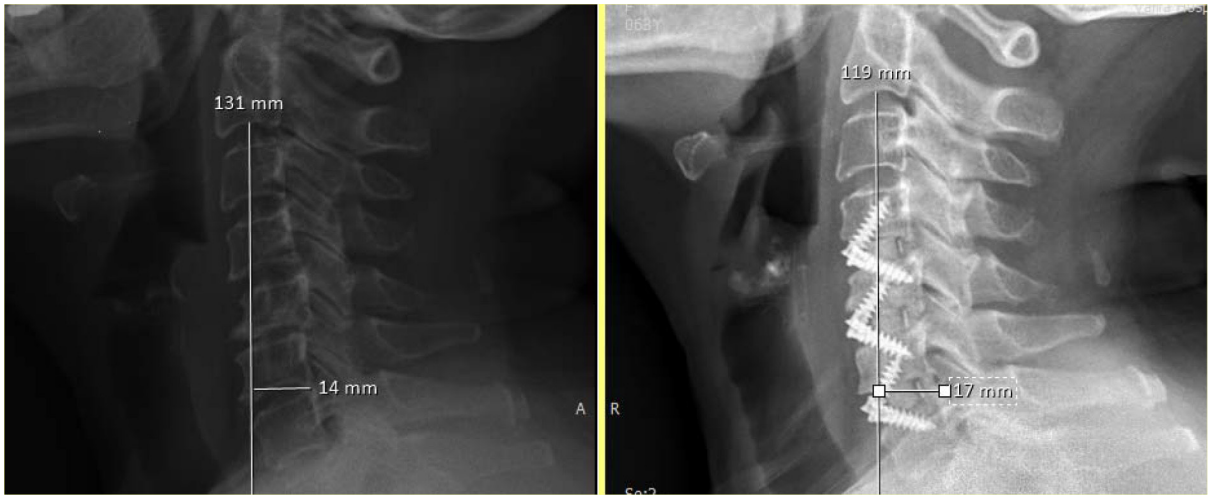

Figure 3. C2 to 7 SVA is the distance in mm measured between a plumb line dropped from the center of C2, and another plumb line dropped from the posterosuperior aspect of the C7 vertebral body. Pre- and postoperative imaging at one year were compared.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences Software, Version 22 for Windows (SPSS Inc., Chicago, IL, USA). The t-test was used to analyze continuous quantitative variables. Pre-and post-operative cervical parameters were compared by ANOVA and to calculate the p-value. A *p*-value <0.050 was considered statistically significant.

Results

Patient and treatment characteristics

In total, 31 patients were initially included, and 45 fused intervertebral discs were analyzed. The demographic data of the patients are presented in **Table 1.** Of these, 17 males and 14 females had a median age of 57 (25 to 84) years. The most common indications for ACDF were spondylosis (64.5%) and trauma (32.3%). The preoperative curvature of the cervical spine was kyphosis among 12 patients (38.7%) and lordosis among 19 patients (61.3%). Most patients underwent single level ACDF (61.3%), and the most common fused level was C5/6 (67.7%). The mean operative time was 202 minutes, and the mean operative blood loss was 96 mL. No patients had pre-existing dysphagia or gastro-esophageal reflux disease before surgery. No postoperative neurological

deficits, infections, or hematomas were observed. Immediate postoperative dysphagia occurred in two patients (6.5%), which resolved within three months. None of the patients presented postoperative dysphagia beyond three months. The two cases that had immediate postoperative dysphagia had been operated on at level C5/6 and C6/7 and had a BMI >27.5 kg/m2. No cage subsidence was found at one-year follow-up.

Table 1. Patient and treatment characteristic

Characteristic	Patients (n=31)
Sex	
Male	17 (54.8)
Female	14 (45.2)
Age, years	57 (26.0-84.0)
Diagnosis	
Trauma	10 (32.3)
Spondylosis	20 (64.5)
Infection	1 (3.2)
Curvature of cervical spine	
Lordosis	19 (61.3)
Kyphosis	12 (38.7)
Operation time (minutes)	202 (95.0-480.0)
Blood loss (milliliter)	96 (10.0-680.0)
Length of stay (day)	8 (3.0-25.0)
Fused level	
C3/4	6 (19.4)
C4/5	8 (25.8)
C5/6	21 (67.7)
C6/7	10 (32.3)
Number of fused level	
1	19 (61.3)
2	10 (32.3)
3	2 (6.5)
Post-operative complications	
Dysphagia	2 (6.5)
Hoarseness	2 (6.5)
None	27 (87.1)

Values are presented as number (%) or median (range)

Table 2. Comparison of clinical and radiographic features

Variable	Pre-operative	Post-operative	Change	*p*-value
VAS neck	5.8 (1.5)	0 (0.3)	5.7 (1.5)	<0.001
JOA	3.7 (2.6)	8.8 (4.4)	5.1 (4.2)	<0.001
DLCA, degree	12.6 (3.6)	15.1 (3.3)	2.5 (1.3)	< 0.001
SCA, degree	4.8 (3.4)	10.9 (4.8)	6.1 (4.3)	<0.001
GCA, degree	11.8 (7.6)	18.1 (6.8)	6.3 (7.1)	<0.001
C2-7 SVA, cm	1.8 (1.1)	2.7 (1.1)	0.9 (1.3)	<0.001
Intervertebral disc height, cm	4.0 (1.4)	4.9 (1.7)	0.9 (0.7)	<0.001

Values are presented as mean (SD)
VAS=Visual analogue scale for neck pain, JOA=the Japanese Orthopaedic Association score, DLCA=device level Cobb angle, SCA=segmental Cobb angle, GCA=global Cobb angle, SVA=sagittal vertical axis, cm=centimeter

Table 3. Subgroup analysis by change of pre-operative alignment

Variable	Initial lordosis		Initial kyphosis		*p*-value
	Means (SD)	No.	Means (SD)	No.	
Δ DLCA, degree	5.8 (4.1)	15	4.7 (4.1)	30	0.424
Δ SCA, degree	6.0 (4.3)	11	6.1 (4.3)	20	0.911
Δ GCA, degree	3.0 (5.8)	11	8.1 (7.3)	20	0.055
Δ C2-7 SVA, cm	2.6 (1.4)	11	0.8 (1.2)	20	0.430
Δ Intervertebral disc height, cm	0.9 (0.6)	11	1.0 (0.7)	20	0.637

Values are presented as mean (SD).
Abbreviations: Δ=change in, DLCA=device level Cobb angle, SCA=segmental Cobb angle, GCA=global Cobb angle, SVA=sagittal vertical axis, cm=centimeter

Comparison of clinical and radiographic features

The mean changes for patients undergoing ACDF with the ZPD were documented. The mean VAS for neck pain across the cohort was 5.8 (±1.5) pre-operatively and 0 (±0.3) postoperatively, improving by a mean of 5.7 (±1.5), which was considered statistically significant. The mean JOA across the cohort was 12.6 (± 3.6) pre-operatively and 15.1 (± 3.3) postoperatively, improving by a statistically significant mean of 2.5 (±1.3). The mean parameters including DLCA, SGA GCA and SVA improved toward lordosis after surgery, which were considered statistically significant. The mean fused level intervertebral disk height was 4.0 (± 1.4 mm) pre-operatively and 4.9 (±1.7 mm) at one-year follow-up, demonstrating a significant increase between pre-operative and postoperative measurements **(Table 2).**

Table 4. Subgroup analysis by change of age

Variable	Age ≤ 60		Age>60		*p*-value
	Means (SD)	No.	Means (SD)	No.	
Δ DLCA, degree	5.4 (4.6)	27	4.7 (3.7)	19	0.543
Δ SCA, degree	5.7 (4.5)	20	6.8 (3.8)	11	0.429
Δ GCA, degree	6.7 (8.1)	20	5.7 (8.1)	11	0.984
Δ C2-7 SVA, cm	0.7 (1.0)	20	1.1 (1.6)	11	0.598
Δ Intervertebral disc height, cm	0.9 (0.7)	20	1.0 (0.7)	11	0.329

Values are presented as mean (SD).
Abbreviations: Δ=change in, DLCA=device level Cobb angle, SCA=segmental Cobb angle, GCA=global Cobb angle, SVA=sagittal vertical axis, cm=centimeter

Table 5. Subgroup analysis by change BMI

Variable	BMI (< 27.5)		BMI (> 27.5)		*p*-value
	Change	No.	Change	No.	
Δ DLCA, degree	5.2 (4.2)	34	4.1 (3.6)	11	0.439
Δ SCA, degree	6.2 (4.4)	23	6.6 (3.8)	8	0.839
Δ GCA, degree	7.2 (6.4)	23	5.4 (5.5)	8	0.119
Δ C2-7 SVA, cm	1.0 (1.3)	23	0.8 (1.2)	8	0.718
Δ Intervertebral disc height, cm	0.9 (0.7)	23	1.1 (0.8)	8	0.390

Values are presented as mean (SD).
Abbreviations: Δ=change in, DLCA=device level Cobb angle, SCA=segmental Cobb angle, GCA=global Cobb angle, SVA=sagittal vertical axis, cm=centimeter

Table 6. Subgroup analysis by change of level of discectomy

Variable	1		More than 1		*p*-value
	Change	No.	Change	No.	
Δ DLCA, degree	4.6 (2.2)	19	3.2 (2.7)	26	0.391
Δ SCA, degree	5.7 (4.6)	19	6.6 (3.8)	12	0.429
Δ GCA, degree	6.9 (8.1)	19	5.4 (5.5)	12	0.984
Δ C2-7 SVA, cm	1.0 (1.3)	19	0.8 (1.2)	12	0.598
Δ Intervertebral disc height, cm	0.8 (0.6)	19	1.1 (0.8)	12	0.329

Values are presented as mean (SD).
Abbreviations: Δ=change in, DLCA=device level Cobb angle, SCA=segmental Cobb angle, GCA=global Cobb angle, SVA=sagittal vertical axis, cm=centimeter

The results were analyzed based on pre-operative alignment in which 11 patients had cervical lordosis, and 20 had cervical kyphosis. In patients with any cervical alignment, the SA parameters were improved toward lordosis, but was not statistically significant **(Table 3)**. In **Table 4**, the JOA score and the changes in DLCA, SCA, GCA, SVA, and fused level inter-vertebral disk height were compared between patients older than 60 years and those 60 years and younger. No statistically significant differences were found between the two age groups regarding the clinical outcome, cervical alignment parameters, and fused level intervertebral disk height for patients undergoing ACDF with lordotic PEEK cages.

Table 5 shows the subgroup analysis by BMI. Every subgroup of BMI exhibited improving JOA score, changes in DLCA, SCA, GCA, SVA, and fused level intervertebral disk height but they were not statistically significance. Single or multilevel ACDF indicated no statistically significant difference in clinical or radiographic outcomes **(Table 6)**.

Discussion

According to in vitro biomechanical studies, the ZPD provides comparable biomechanical stability to that of the traditional interbody cage and plate structure.[9, 10] Clinical studies have also indicated that the ZPD is safe and efficient, even in multilevel cases.[11, 12] Cervical spine alignment is one of the most important influences on clinical outcomes. According to a meta-analysis, ACDF with ZPD significantly improved post-operative GCA and curvature of the cervical spine. It has been reported that a single-level ACDF might not substantially impact cervical sagittal alignment.[13] This study found no statistically significant differences between clinical or radiographic outcomes for single or multilevel ACDF (**Table 6**). Patients presenting single-level ACDF may increase lordosis due to lordotic PEEK cage and obtain better posture after surgery. For multilevel ACDF, even more than one cage has been implanted; however, postoperative lordosis changes are likely to decrease due to stiffness of neck in a long term. In contrast, with previous cohorts, the author found that ACDF with ZPD, regardless of the number of discectomy levels, could significantly improve sagittal alignment. This study also found that ACDF with ZPD improved the JOA score and demonstrated a statistically significant restoration of cervical lordosis alignment. Furthermore, among patients with pre-operative cervical kyphosis or lordosis, the use of a ZPD significantly increased the sagittal alignment parameters in both groups. The author considers that patients who had any alignment of the preoperative cervical curve were likely to benefit from using the ZPD during ADCF. The shape of the ZPD is 7˚ lordotic, which may restore the lordotic neck curvature of these patients. In our study, all parameters of sagittal alignment were improved toward lordosis after surgery.

ACDF is a common operative treatment for cervical disk pathology. It has been generally accepted that using an anterior cervical plate construct after interbody cage implantation promotes successful fusion.[14-16] However, this procedure can result in various complications. The overall reported incidence of dysphagia after anterior cervical spine surgery varied from 2 to 60%.[17-19] Furthermore, according to many studies, ACDF with an anterior cervical plate can lead to postoperative dysphagia in 2.0 to 67.0% of patients in the early postoperative period.[8] Most of these symptoms disappear within three months of surgery. The incidence of chronic dysphagia after ACDF is between 3.0 and 21.0%. [20] The pathologic mechanism of postoperative dysphagia remains unknown, but may be associated with direct impingement of the implant on the posterior esophagus.[21] Only two patients (6.5%) reported postoperative dysphagia in this cohort, which resolved within three months. The author considers that ACDF with ZPD could minimize the postoperative dysphagia. The ZPD has advantages in this regard because it can be implanted completely inside the decompressed intervertebral disc space and fixed with integrated screws without an anterior cervical plate which could irritate the esophagus.

According to many studies, the age and BMI of patients may affect the outcome of ACDF. Di Capua et al. retrospectively investigated 20,563

patients between 2010 and 2014. They found that elderly patients 61 years old and over had an increased risk of pulmonary and cardiac complications, venous thromboembolism, infection, and unplanned readmission.[22] Omidi et al. demonstrated that ACDF is more effective at improving disability among patients over 45 years of age.[23] Chotai et al. reported that patients older than 65 years have a slightly higher cost use ratio than younger patient groups, and surgery in the older cohort had significantly improved pain, disability, and quality of life outcomes.[24] Obesity is an increasing global public health issue. The prevalence of obesity (BMI ≥25 kg/m^2) among adults in Thailand increased from 13.0% among men and 23.2% among women in 1991 to 22.4 and 34.3%, respectively, in 2004. [25] Obese patients had a greater potential for complications such as intra-operative durotomy, dysphagia, neurological, cardiopulmonary, and hematologic complications following ACDF procedures.[26] Basques et al. reported that obesity did not impact clinical outcomes but affected cervical sagittal alignment and adjacent segment degeneration in a short term follow-up after ACDF.[27] In the current study, although the two cases with postoperative dysphagia were classified as obese, after multivariate analysis, the author found the relationship without statistical significance. Furthermore, the current study found no age-related effects in the clinical outcomes, complications, cervical sagittal alignment parameters, or the fused level intervertebral disk height in ACDF with the ZPD. This indicated that patients can benefit from the ZPD regardless of age and BMI. Age is a nonmodifiable risk factor; and therefore, presents a fixed source of postoperative risk following elective ACDF. The author considers that the outcome of ACDF may be improved by intra-operative neuromonitoring, the use of a microscope during surgery, and the influence of experienced surgeons. This issue may be worth further study.

Cage subsidence is common after ACDF and can lead to deterioration of long term function. It causes loss of the correction of the segmental angle and the Cobb angle from C2–7. Criteria for evaluating subsidence remain insufficient. The most common method is to measure the postoperative reduction in the heights of the ventral and dorsal segments between the two fused vertebral bodies. A decrease in total intervertebral disc height of 2 mm or more is significant subsi dence. Subsidence of less than 2 mm is acceptable. The incidence of cage subsidence with interbody PEEK cages ranges from 0.0 to 18.0% in the literature.[5, 28] Lee et al. reported that the subsidence rate of a ZPD (58.6%) was higher than that of an interbody PEEK cage with plate (38.5%), indicating that plate fixation can prevent subsidence by supporting the anterior disk height.[29] In contrast, Scholz et al. reported that cage subsidence was not observed in cases with a ZPD, although their follow-up was only six months. [30] In this cohort, no subsidence was reported at one-year follow-up. The reason for this difference may be that performing ACDF with a plate involves flattening the anterior vertebral surface of the cervical spine by resecting osteophytes to enable the plate to fit closely. Inserting the ZPD does not require this, and in addition to a secure and rigid screw fixation, it probably contributes to preserving the anterior bony support and reducing subsidence. The author considers that using the ZPD in ACDF may not result in subsidence after surgery; however, a larger sample size and longer follow-up period are needed to confirm this. The occurrence of cage subsidence may be related to several factors, including the size and position of the cage, bone density, and the contact surface ratio of the cage.

Several limitations, encountered in this study, need to be acknowledged. It constituted a retrospective study that did not compare the ZPD with other types of cervical cage. Furthermore, the length of follow-up was short so that the incidence of late complications such as subsidence, adjacent segment pathology, or device-related failure could not be assessed. In addition, the variation of diseases in this study was quite diverse. The vertebral body might not have responded best regarding the trauma and infection and could have affected the result analysis. Moreover, only a small number of available patients from a single institute were included.

Conclusion

The use of the ZPD in ACDF surgery improved both clinical and radiographic outcomes by correcting cervical spine alignment and minimizing the postoperative complication of dysphagia. No device-related failure was observed, and favorable outcomes persisted at one-year follow-up. Age, BMI, and pre-operative cervical alignment did not affect outcomes in this study.

Conflicts of Interest

The author has no conflicts of interest to declare.

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OUTCOME OF USING PLATELET, PLASMA AND GROWTH FACTORS AS AN ORTHOBIOLOGIC DERIVATIVE TO AVOID INVASIVE SURGICAL PROCEDURES FOR TREATING KNEE OSTEOARTHRITIS AMONG ELDERLY PATIENTS

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Abstract

Background: The application of platelet, plasma and growth factors (PP&GF) is an intra-articular orthobiologic intervention that has been proven to be safe, having less systemic complications compared with conventional treatments and could constitute an option for treating elderly patients with knee osteoarthritis (OA). However, an intermediate result of using PP&GF has yet to be well established.

Objectives: This study aimed to report the survival analysis of 24-month follow-up treatment using PP&GF among elderly patients with knee OA as primary outcome. The secondary outcomes were functional improvement in terms of international knee documentation committee (IKDC) score, Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) score and visual analog scale (VAS) pain score.

Methods: A prospective cohort study was performed among patients with knee OA (Kellgren and Lawrence (KL) grade I-IV), aged more than 65 years who did not respond to conservative treatments. All patients received intra-articular PP&GF treatment and were followed up to 24 months. Primary outcome was recorded as any surgical treatment at any time point post-PP&GF injection. Secondary outcomes including IKDC, WOMAC and VAS pain score were also assessed.

Results: A total of 184 participants were enrolled in this study. The overall survival rate of patients not undergoing any surgical procedures during 24-month follow-up was 87.50%±2.44%. The mean IKDC, WOMAC and VAS pain scores were 39.59±0.58, 55.9±1.09 and 6.63±0.13, respectively at baseline while those at 24-month follow-up were 46.77±0.81, 38.32±1.33 and 4.92±0.13, respectively. The mean platelet concentrations before and after centrifugation were $1.85x10^5$ cells/μL ($1.20\text{-}3.36x10^5$ cells/μL) and $1.4x10^6$ cells/μL ($5.80x10^5\text{-}3.5\ x10^6$) cells/μL, respectively, which showed final PP&GF products contained 6-10 times higher platelet concentration than those in the peripheral blood.

Conclusion: Intra-articular injection of PP&GF is a potential treatment for severe knee OA especially among elderly patients. This method provided 87.50% survivorship from surgical intervention at 24-month follow-up. Most patients improved both pain and functional outcomes. We propose that the optimal preparation technique for PP&GF is the key step for improving patients' clinical outcomes and regaining their quality of lives.

Keywords: Orthobiologics, Plasma, Platelet, Growth factor, Knee osteoarthritis

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Introduction

Knee osteoarthritis (OA) is a degenerative health condition impacting nationwide health care systems and health socioeconomic costs and affecting daily life activities of individuals.[1] Patients with knee OA are usually treated in a stepwise pattern starting from patient education, oral medication, physical therapy, intra-articular injection of anti-inflammatory drugs or visco-supplementation agents and surgery. However, conventional modalities have been proven to produce some disadvantages. Oral glucosamine supplement was proven to have insufficient evidence of effectiveness.[2] Bruyere et al. found that 6.3% of patients receiving glucosamine supplement underwent total knee replacement after 8-year follow-up, compared with 14.5% in the placebo group.[3] Intra-articular steroid injection may have short term effect and negatively affects the cartilage.[2, 4] Regarding injecting viscosupplementation agents, Boutefnouchet et al. reported a 67% survival rate at 5 years for patients treated with viscosupplement for knee OA.[5] Patients undergoing total knee arthroplasty (TKA) may need revision surgery due to infection or mechanical loosening over time. [6]

Studies showed that the number of TKAs is rising. One study estimated the amount of TKA in the US alone was predicted to be 1,272,000, 1,921,000 and 3,416,000 in 2025, 2030 and 2040 compared with 688,000 TKAs in 2009.[7] These high number of operations would become problematic considering socio-economic costs.

One of the most vulnerable groups to develop knee OA is advanced age patients. Most have multiple co-morbidities and take multiple medications. For these people, receiving nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief significantly increased the risk of gastro-intestinal and cardiovascular events and drug interactions including anticoagulants which in turn might affect coagulation cascades.[8] Moreover, some patients might also be categorized as high risk to develop peri-operative myocardial infection or death. Undergoing surgical operation would be a major concern. Treating elderly patients with these modalities might be unsuitable.

Recently, orthobiologic agents including platelet-rich products have gained popularity for treating patients with knee OA due to their biosafety, simplicity and clinical effectiveness. The component of platelets, plasma and growth factors (PP&GF) constitute one of the platelet-rich products adjusted for platelet and fibrin concentrations, leukocyte population and proper activator status. PP&GF initiates the repair processes, modulates inflammation, transports growth factors and attracts medical signaling cells (MSCs).[4, 9] PP&GF differs from platelet rich plasma (PRP) in terms of reproducing substantial amounts of platelet concentration. The PP&GF system provides an average of 6 times higher platelet concentration than baseline value.[10] With this high concentration of platelets in platelet-rich derivative products, large amount of proteins and growth factors are released from platelet alpha granules and act directly to create great healing potential. Up until today, no major adverse events have been reported from PP&GF use.[2, 10] However, the intermediate outcome of PP&GF use in knee OA has not been established. This study aimed to report primary outcome in terms of survival analysis of 24-month follow-up treatment using PP&GF especially among elderly patients with knee OA. Secondary outcomes aimed to assess functional outcomes in terms of international knee documentation committee (IKDC) score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score as well as visual analog scale (VAS) pain score.

Methods

From February 2018 to June 2019, a prospective cohort study was conducted after the internal review board and hospital ethics committee approval at the Biomedical Technology Research and Development Centre, Police General Hospital, Bangkok, Thailand. Calculation of sample size was performed based on a related study [11] with an alpha of 0.05 and a power of 80%. The required sample size was 524 knees divided to 424 knees in Kellgren and Lawrence (KL) grade I to III and 100 knees in KL grade IV groups.

Patients with knee pain visiting the outpatient Orthopaedic Clinic were evaluated clinically and radiographically for their eligibility. Those meeting the following criteria were included in the study: patients aged more than 65 years diagnosed with knee OA classified as KL grade I to IV, those who failed conservative treatments including no response to oral medications for at least six months, physiotherapy for at least three sessions, intra-articular injection of steroid or hyaluronic acid at least one dose, hemoglobin (Hb) levels >11 g/dL, and platelet count >80,000 cells/μL.

The exclusion criteria included patients receiving a diagnosis of meniscal or knee ligament injury, inflammatory arthritis including uncontrolled bleeding disorder, those presenting radiographic deformity of the tibiofemoral angle more than 5 degrees and those having history of using NSAIDs within 5 days of blood drawn or taking anticoagulant or anti-aggregate drugs.

In this study, a total number of 186 patients met the criteria. Patients were informed about the study protocol and signed written consent forms (**Figure 1**).

PP&GF preparation

A 30-ml peripheral blood sample for single knee injection (60 mL for bilateral knees) was collected from each patient. The first 20 mL of blood was separated in 2 centrifuge tubes (red) (PP&GF, Bangkok, Thailand) with 10 mL for each tube to mix with acid citrate dextrose anticoagulant. The blood was centrifuged using the ALPAS centrifugation machine (Bangkok, Thailand) at 250g for 6 minutes. After the first spin, the blood was separated in three components: red blood cells on the bottom layer, a buffy coat in the middle layer and platelet-containing plasma at the top layer. The upper two layers were gently aspirated and transferred to a new tube (yellow) and centrifuged again at 1000g for 10 minutes. After the second spin, the platelet-poor supernatant plasma was gently aspirated for removal. One milliliter of residual leukocyte-rich PP&GF was collected for complete blood count. The remaining PP&GF was aspirated using a 5-mL sterile injection syringe for intra-articular injection. The remaining 10 mL of peripheral blood was transferred to the last tube (green) and was centrifuged once with 250g for 6 min to produce platelet-rich fibrin and used as a natural activator (**Figure 2**).

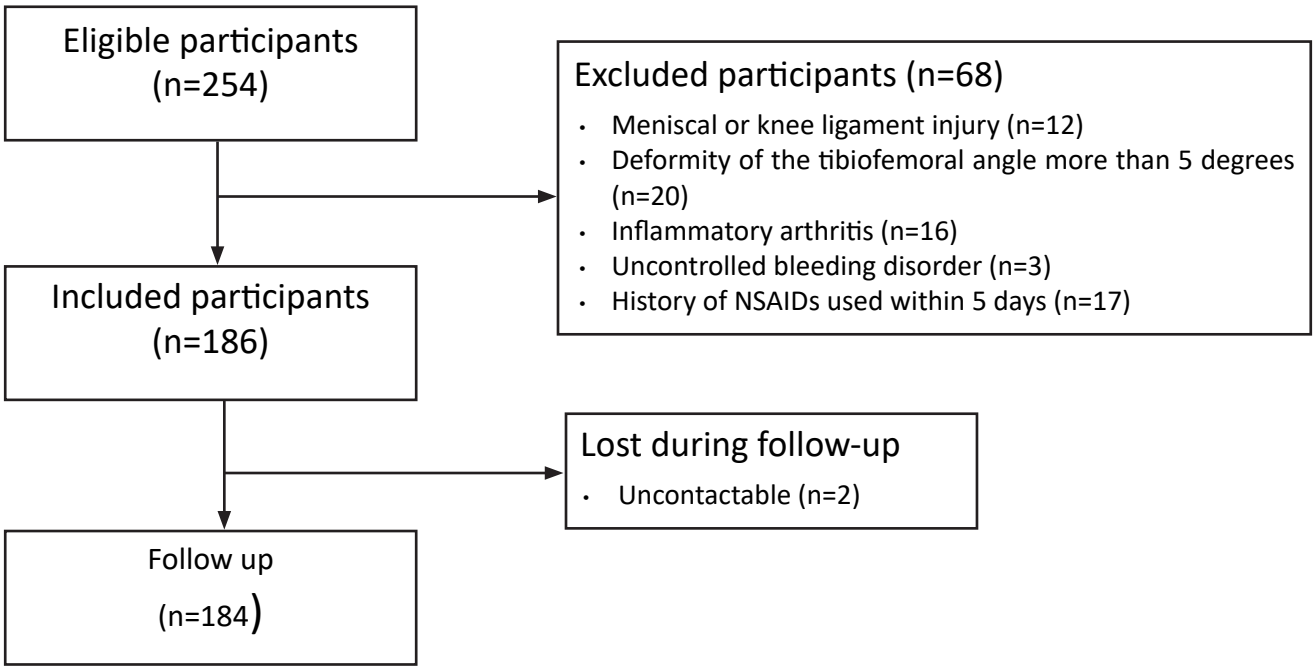

Figure 1. Flow chart of patient enrollment

Figure 2. Platelet, plasma and growth factors (PP&GF) tubes: the red tube is used for the first spinning process. The yellow tube is used for the second spinning process. The green tube is used for platelet-rich fibrin preparation.

Injection protocol

All injections were administered by a well-trained team of orthopedic staff and residents. The patient was placed in the supine position with knee flexed 90 degrees. The injection site was marked at the anteromedial joint space, and an antiseptic agent was applied to the skin. The analgesic agent infiltrated the skin and subcutaneous tissues surrounding the injection site using a 25G needle. Air-test was performed with a 18G needle to confirm the intra-articular placement of the needle. PP&GF biologic agents were delivered to the joint space using a 18G needle. The knee was immediately extended after the biologic agent was delivered, and the patient was allowed to walk in full weight bearing after injection.

Rehabilitation protocol

The patient was instructed to perform fixed arc quadriceps exercise two days after injection. The patient sat on a chair with their legs extended forward for 100 seconds on each side. This exercise was recommended to perform three to five times daily.

Follow-up assessment

Patients were assessed at 2, 4, 6, 12, 18 and 24-month posttreatment follow-up. Primary outcome was recorded as any surgical treatment performed by any surgeon encountered by participants (either arthroscopic knee surgery, unicondylar arthroplasty or TKA) at any time point post PP&GF injection. Secondary outcomes, including IKDC, WOMAC and VAS pain scores, were also assessed.

Statistical analysis

Continuous data were elaborated in mean and standard deviation, and discrete data were elaborated in percentage and proportion. A box plot was used to demonstrate the tendency of collected data. Survival analysis, using monthly interval units, determined the event rate. The time at risk comprised the number of months that the participants were followed up with an end point of 24 months. Right truncation, left truncation, right censoring and left censoring protocol were used. Kaplan-Meier curve was used to evaluate the primary outcome as the percentage of survival at each time point. The difference of survival function was determined using the log-rank test for equality of survivor function. A p-value <0.05 was considered statistically significant. Stata Software, Version 16.0 (Stata Corp, TX, USA) was used for statistical analysis.

Table 1. Characteristics of patients categorized based on KL classification

	KL I	KL II	KL III	KL IV	Total
Amount (knees)	3	67	41	73	184
Age (Mean±SD)	66.67±1.2	73.36±0.66	72.15±0.8	73.5±0.66	73.03±5.45
Sex (Female:Male)	1:2	38:29	31:10	62:11	132:52
BMI (Mean±SD)	27.33±3.53	26.06±0.65	25.05±0.77	25.95±0.6	25.8±5.17
Site (Left:Right)	2:1	33:34	18:23	31:42	84:100

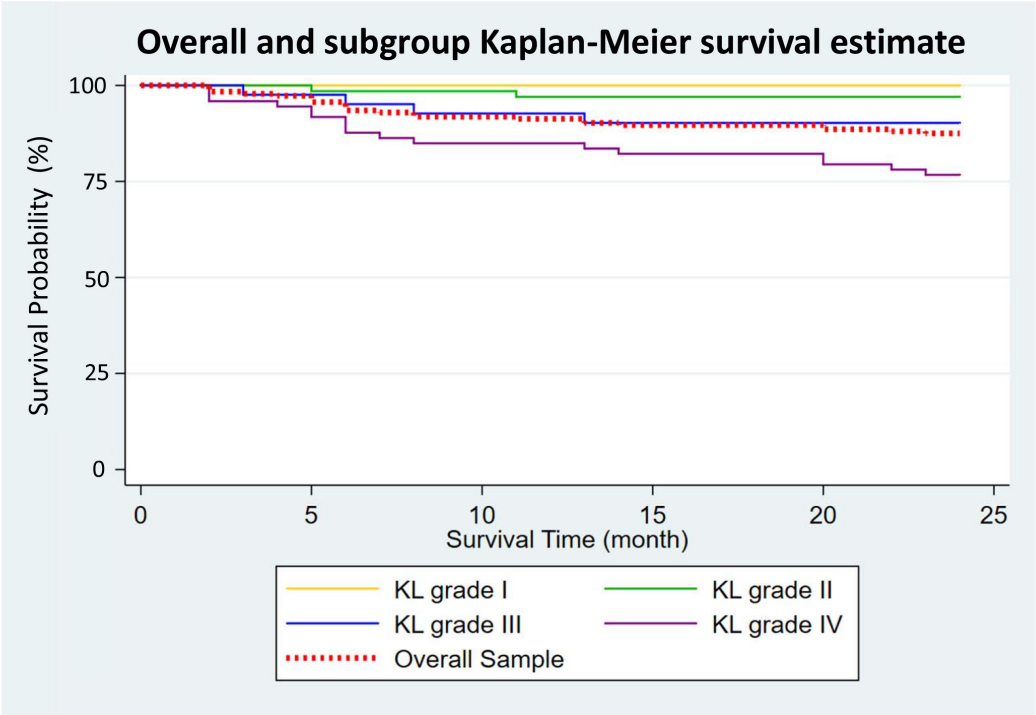

Figure 3. Kaplan-Meier curve showing overall and subgroup analysis of survival estimate rate of participants with different severity of knee OA not undergoing any surgical intervention after intra-articular PP&GF treatment. The red dot line represents overall samples. The yellow line represents KL grade I subgroup. The green line represents KL grade II subgroup. Blue line represents the KL grade III subgroup. Purple line represents the KL grade IV subgroup.

Results

A total number of 186 participants were included. Of these, 2 participants were lost to follow up, leaving 184 participants completing the study. Characteristics of patients are demonstrated in **Table 1**.

The overall survival rate of knees not undergoing any surgical procedures during the 24-month follow-up was 87.50 ± 2.44%. Subgroup analysis for survival rate was performed according to severity grading (KL classification) (**Figure 3**). The log rank test was used to analyze the difference of survival rates between subgroups. The survival rates at 24-month follow-up revealed significant differences between knees with KL I to III and KL grade IV, i.e., 94.59% ± 2.15 and 76.71% ± 4.95, respectively (p = 0.0003). The survival rates at 24-month follow-up also significant differed between knees without subluxation and knees with subluxation, i.e., 90.12% ± 2.34 and 68.18% ± 9.93, respectively (p = 0.0028). The survival rates at 24-month follow-up of knees with KL-IV statistically differed; however, no significance was found between knees without

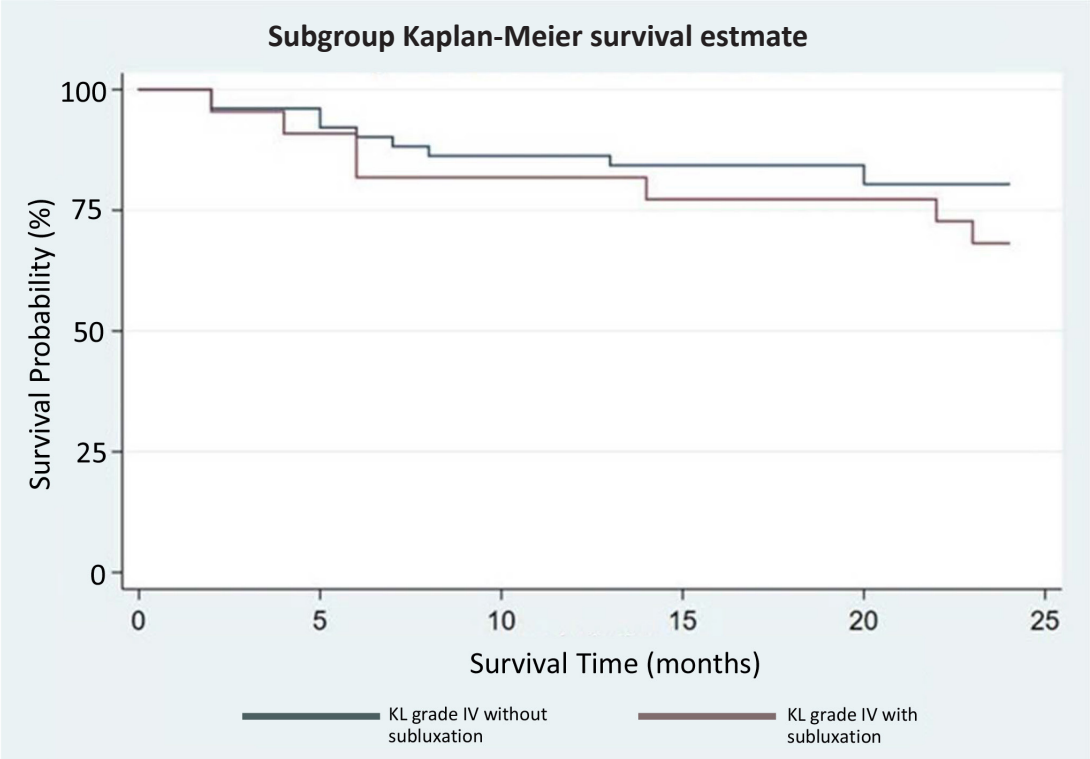

Figure 4. Kaplan-Meier curve showing subgroup analysis of survival estimate rate of participants in knee KL IV with different subluxation profiles not undergoing any surgical intervention after intra-articular PP&GF treatment. The dark green line represents the knee in KL grade IV without subluxation subgroup. The brown line represents the knee in KL grade IV with subluxation subgroup.

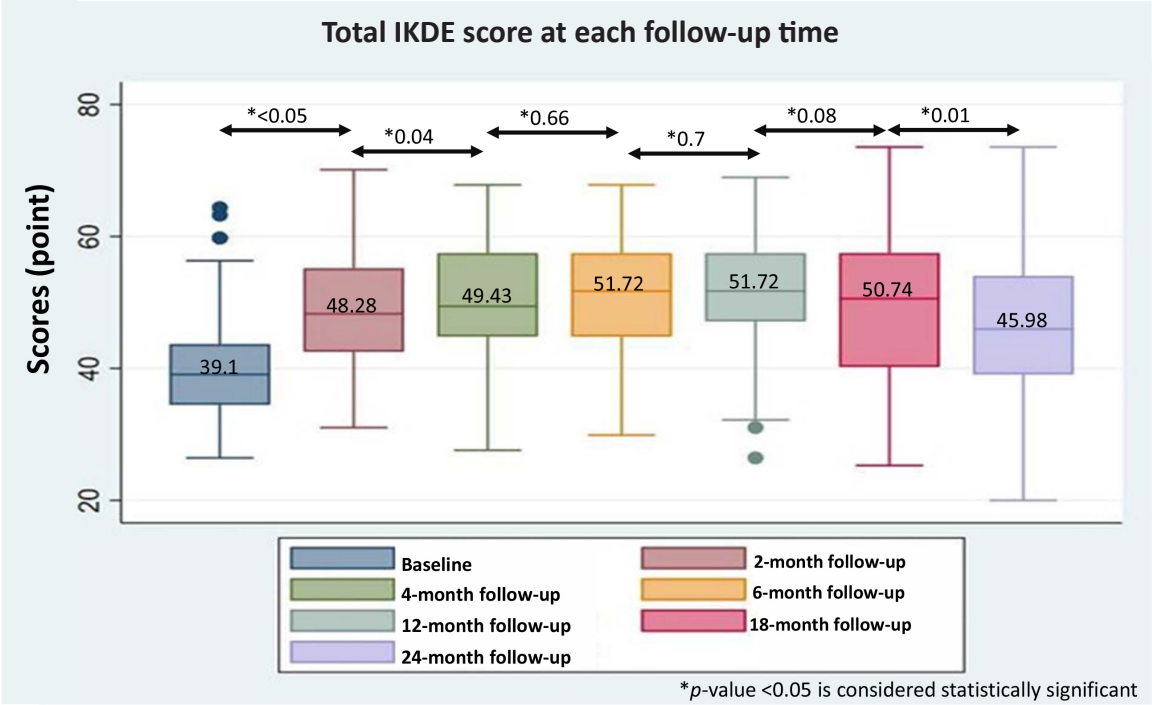

Figure 5. Box plot showing total IKDC score at different follow-up times. The asterisk (*) represents *p*-value of the test of difference between mean score at each follow-up time. The blue box represents score at baseline. The red box represents score at 2-month follow-up. The green box represents score at 4-month follow-up. The orange box represents score at 6-month follow-up. The mint-green box represents score at 12-month follow-up. The pink box represents score at 18-month follow-up. The purple box represents score at 24-month follow-up.

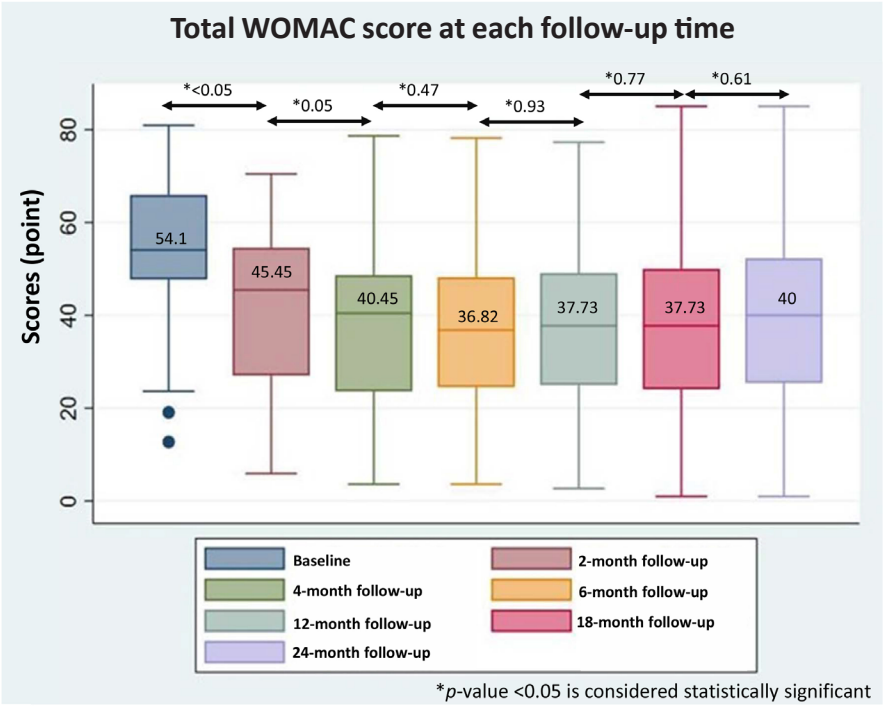

Figure 6. Box plot showing total WOMAC score at different follow-up times. The asterisk (*) represents *p*-value of the test of difference between mean score at each follow-up time. The blue box represents score at baseline. The red box represents score at 2-month follow-up. The green box represents score at 4-month follow-up. The orange box represents score at 6-month follow-up. The mint-green box represents score at 12-month follow-up. The pink box represents score at 18-month follow-up. The purple box represents score at 24-month follow-up.

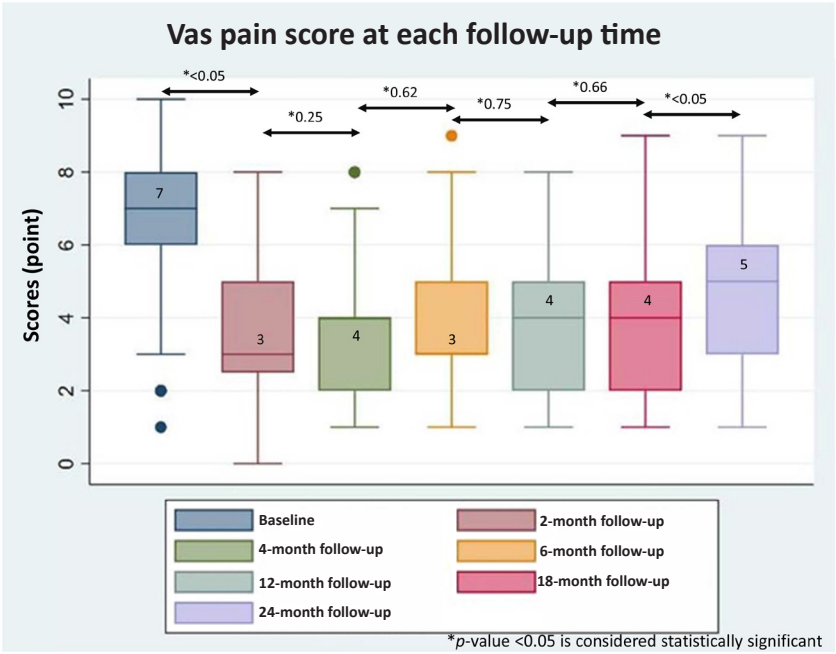

Figure 7. Box plot showing VAS pain scores at different follow-up times. The X axis represents time while the Y axis represents VAS pain scores in point unit. The asterisk (*) represents *p*-value of the test of difference between the mean scores at each follow-up time. The blue box represents the baseline score. The red box represents score at 2-month follow-up. The green box represents score at 4-month follow-up. The orange box represents score at 6-month follow-up. The mint-green box represents score at 12-month follow-up. The pink box represents score at 18-month follow-up. The purple box represents score at 24-month follow-up.

subluxation and knees with subluxation, i.e., 80.39% ± 5.56 and 68.18% ± 9.93, respectively (p= 0.2762) (**Figure 4**).

Regarding total IKDC score, mean IKDC score at 24-month follow-up was 46.77 ±0.81 compared with 39.59 ± 0.58 at baseline. Significant differences were observed between the mean score at baseline and 2-month follow-up, between 2- and 4-month follow-up and between 18- and 24-month follow-up (p <0.05, 0.04, 0.01, respectively). No significant difference of mean scores was observed between each period from 4- to 18-month follow-up (**Figure 5**). IKDC scores, recorded among different follow-up periods were also categorized using KL classification as shown in **Table 2**.

Regarding total WOMAC score, mean WOMAC score at 24-month follow-up was 38.32 ± 1.33 compared with 55.9 ± 1.09 at baseline. A significant difference of mean score was observed between baseline and 2-month follow-up (p <0.05). No significant difference of mean score was observed between each period from 2- to 24-month follow-up (**Figure 6**). WOMAC scores among different follow-ups were also categorized using KL classification shown in **Table 3**.

Regarding VAS pain score, mean VAS pain score was 4.92 ± 0.13 at 24-month follow-up compared with 6.63 ± 0.13 at baseline. Significant differences were noted of mean score between the baseline and at 2-month follow-up (p <0.05) and between 18- and 24-month follow-up (p <0.05). No significant difference was found of the mean score between each period from 4- to 18-month follow-up (**Figure 7**). VAS pain scores during different follow-ups were also categorized using KL classification as shown in **Table 4.**

The mean platelet concentrations before and after centrifugation were $1.85x10^5$ cells/μL ($1.20\text{-}3.36x10^5$ cells/μL) and $1.4x10^6$ cells/μL ($5.80x10^5$-$3.5\ x10^6$) cells/μL, respectively, showing that final PP&GF product increased platelet concentration 6 to 10 times higher than those in peripheral blood samples.

Table 2. Mean IKDC scores recorded among different follow-up periods categorized using KL classification

	KL I	KL II	KL III	KL IV	Total
Baseline	48.28±8.07	40.30±0.94	40.26±1.22	38.58±0.92	39.59±0.58
2-month follow-up	55.56±7.75	48.70±1.00	49.40±1.25	49.02±1.05	49.00±0.63
24-month follow-up	47.24±9.10	45.21±1.22	43.06±1.70	50.34±1.29	46.77±0.81

Table 3. Mean WOMAC score at different follow-ups categorized by KL classification

	KL I	KL II	KL III	KL IV	Total
Baseline	44.85±16.24	53.17±1.81	56.26±2.24	58.47±1.64	55.90±1.09
2-month follow-up	29.39±12.71	41.97±1.95	39.94±2.33	41.24±1.98	41.22±1.18
24-month follow-up	26.52±13.21	41.95±2.10	42.74±2.62	32.42±2.11	38.32±1.33

Table 4. Mean VAS pain scores recorded among different follow-ups categorized using KL classification

	KL I	KL II	KL III	KL IV	Total
Baseline	5.33±2.33	6.19±0.23	6.82±0.28	6.93±0.19	6.63±0.13
2-month follow-up	3±1.53	3.45±0.20	3.97±0.30	3.88±0.22	3.74±0.13
24-month follow-up	5.67±1.45	5.05±0.22	5.53±0.26	4.43±0.19	4.92±0.13

Discussion

In this study, the results showed significantly different survival rates between those with the knee in KL I to III including KL IV with PP&GF treatment. These findings imply an advantage of early intervention in OA knee treatment contributing to reduced surgical interventions. We also found that the non-subluxated knee had a significantly higher survival rate when compared with the knee with subluxation; this implied that mechanical parameters should also be considered along with biologic profiles of knee pathology to select appropriate treatment options. Thus, we proposed that severity of knee OA and subluxation profile are two identifiable negative predictive factors for failure of biologic intervention. However, due to using a small sample size in this study, significant differences of survival rate were not found between the knee with and without subluxation in the KL IV group.

Platelet-rich products entered the spotlight of orthopedic surgeons due to their potential in wound healing and tissue regeneration processes. Once properly activated, alpha granules in platelets released large pool of proteins and growth factors. These growth factors included platelet-derived growth factor (PDGF) promoting mitogen for connective tissue cells, transforming growth factor-β (TGF-β) stimulating osteoprogenitor cells to proliferate and halting the process at later stage of cell differentiation and mineralization. Moreover, insulin-like growth factor (IGF-1) promotes the late stage differentiation and activity of osteoblasts, and vascular endothelial growth factor (VEGF) induces endothelial cell proliferation and migration.[12] These molecular findings explained why PP&GF is suitable to treat knee OA. In this study, the authors chose PP&GF to treat knee OA because it exerts an action on OA at the accurate sites of pathology including chondrocyte, synovium and synovial fluid via its chondrogenic effects, stem cell migration and healing cascade while other traditional treatments mainly focused on eliminating pain. Gobbi et al. studied the effectiveness of intra-articular platelet-rich product injections among patients with symptomatic knee OA and revealed significant improvement in decreasing pain, improving quality of life and returning to their daily life activities after 6 and 12 months.[13] In 2013, Turajane et al. combined intra-articular autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with hyaluronic acid (HA) together with arthroscopic micro-drilling mesenchymal cell stimulation (MSC)[14, 15]; this pilot study highlighted future research to focus on combining different orthobiologics products to maximize the effective use of autologous biomaterials.

Regarding pain reducing mechanisms of platelet-rich derivatives, Lee et al. investigated cannabinoid receptor gene expression in PRP-containing hydrogels used for articular cartilage defects. Cannabinoid receptors CB1 and CB2 have been reported to directly correlate with analgesic effects in animal arthritis models. The result showed that PRP-containing hydrogels produced an increased expression of cannabinoid receptors compared with PRP-free hydrogels.[16] Sundman et al. also found that PRP decreased the concentration of tumor necrosis factor (TNF)-α in synovium and cartilage culture medium. TNF-α is recognized as a pro-inflammatory cytokine initiating neuropathic pain pathways in human disease states.[17]

Regarding functional improvement among patients with OA treated with PRP, Sundman et al. also reported that PRP significantly decreased matrix metalloproteinase-13 (MMP-13) expression in synoviocyte culture media. MMP-13 has been recognized as the cartilage matrix degradation of the synovial membrane contributing to disruption of nutrition, removal of waste products, shock absorption, lubrication mechanisms of the synovial membrane which in turn are responsible for functional outcome of patients.[17]

According to the results of secondary outcomes, a remarkable trend in outcome was observed. The scores were significantly improved at 2-month follow-up after treatment. Comparing the scores at each subsequent follow-up, most were not significantly changed. However, scores once again dropped statistically at 18- and 24-month follow-up. The difference in tendency of outcomes between the 18- and 24-month follow-up was also observed by Filardo et al.[18]

With these trends, we could imply that PP&GF exerted its highest function at 2 to 18 months lasting until 24 months. Thereby, we suggested that a single dose of PP&GF was sufficient to treat knee OA and patients initially responding to intra-articular PP&GF treatment should receive a repeated dose between 12 and 18 months after the first injection to maintain high efficacy of the treatment. Vilchez-Cavazos et al. also performed a meta-analysis to compare the clinical effectiveness of single and multiple injections of platelet-rich derivatives to treat patients with knee OA. The results showed that a single injection was as effective as multiple injections in reducing pain. Multiple injections were more effective in joint functionality than a single injection at 6 months. [19]

Regarding safety issues, in our recent study, after 24-month follow-up of intra-articular PP&GF injections, neither local nor systemic adverse effects were reported. The data correlated with a systematic review of 29 articles by Laver et al. [20] studying platelet-rich product use for OA revealing no adverse effects of intra-articular PRP injections. This emphasized the intermediate term safety profile of intra-articular PP&GF injections and supported the results of our related study on short term safety profiles. [11]

Until now, different preparation techniques and components of platelet-rich products have yielded different patient's outcome. [21] The strength of this study was the protocol for intra-articular PP&GF injection including strict patient selection criteria, reproducible PP&GF preparation technique and using an accurate biologic treatment delivery method. This study comprised a unique and precise method that minimized the heterogeneity of the treatment and uniformly intervened with patients. In this study, the PP&GF preparation technique provided 6 to 10 times higher platelet concentration when compared with those in peripheral blood. We adopted the air-test technique when performing intra-articular injection among all patients receiving PP&GF treatment to ensure that the biologic product was delivered to a correct position in the knee joint.

This study encountered several limitations. First, a small sample size affected the process to prove significant differences among subgroup analysis of the knee with KL IV. Second, the placebo effect of an intra-articular injection could have contributed to positive responses of patients. Nonetheless, Vannabouathong et al. performed a meta-analysis and found that intra-articular PRP had the greatest effect estimates among many treatment modalities when accounting for the intra-articular placebo effect. [22] Third, no information of treatment with repeated dose of the intra-articular PP&GF was reported in this study because we found the trend of secondary outcome after the study ended. Fourth, the primary outcome as decisions of patients to received surgery involved confounders which could have led to bias in this study. Fifth, exploring biochemical profiles of growth factors and protein components of PP&GF was not performed in this study. Lastly, analysis of blood samples other than platelet concentration before and after centrifugation was not performed. Thus, future research focusing on biochemical profiles of leukocyte and red blood cell components is recommended.

Conclusion

An intra-articular injection of PP&GF is a potential treatment for the severe knee OA especially among elderly patients, providing survivorship from surgical intervention at 24 months for 87.50% of patients. Most patients improved in pain and functional outcomes. We propose that the optimal preparation technique for PP&GF is the key step for improving clinical outcomes and quality of life.

Funding

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PSYCHOLOGICAL IMPACT AND COPING AMONG MEDICAL STUDENTS IN PHRAMONGKUTKLAO COLLEGE OF MEDICINE DURING THE COVID-19 PANDEMIC

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Abstract

Background: The COVID-19 pandemic has impacted on medical education and other areas of life causing psychological distress.

Objectives: The study aimed to assess psychological impact and coping and to identify factors associated psychological impact among medical students.

Methods: An online cross-sectional study was conducted at Phramongkutklao College of Medicine (PCM) in April 2021. All medical students were invited to complete a standardized online questionnaire for demographics data, impact of COVID-19, coping, fear of illness and virus evaluation (FIVE). The Depression Anxiety Stress Scales (DASS-21) Thai version was used to assess the psychological impact. The factors associated with depression, anxiety and stress were analyzed using logistic regression analysis.

Results: In total, 256 medical students completed the questionnaire. Of these, 54.7% reported having psychological impact, 43.4% depression, 36.7% anxiety and 29.7% stress. Depression was associated with preclinical students (adjusted OR(AOR) =3.03, 95% confidence interval (95% CI) =1.54-5.97), sleep problem (AOR =2.20, 95% CI=1.16-4.16) and extreme deterioration of family income (AOR =7.27, 95% CI=1.81-29.29). Anxiety was associated with preclinical students (AOR =3.20, 95% CI=1.52-6.72), COVID-19 like symptoms (AOR =2.93, 95% CI=1.26-6.83), slight problems adjusting to new learning methods (AOR =6.11, 95% CI=1.54-24.24) and extreme deterioration of family income (AOR =8.29, 95% CI=1.44-47.59). Stress was associated with preclinical students (AOR =3.84, 95% CI=1.75-8.40), COVID-19-like symptoms (AOR =3.54, 95% CI=1.53-8.19), and no confidence in COVID-19 policy of PCM (AOR =2.3, 95% CI=1.06-4.98). A positive correlation between Fear of Illness and Virus Evaluation (FIVE) and psychological impact was observed (r=0.449; p<0.001). Common coping activities were the use of social media, video chats and exercise.

Conclusion: A high prevalence of psychological impact was observed among medical students during the COVID-19 pandemic. To establish psychological support and resilience, training is needed to improve mental wellbeing and prepare medical students to cope with unprecedented situations.

Keywords: Psychological impact, Covid-19, Coping, Medical students, Phramongklao College of medicine (PCM)

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic started in December 2019 in China and then spread rapidly worldwide. [1] Since May 2021, 153,738,171 cases of COVID-19 have been confirmed globally, including 3,217,281 deaths, as reported by the World Health Organization (WHO).[2] In Thailand at that time, 74,900 cases of COVID-19 including 318 deaths were confirmed. [3]

The COVID-19 pandemic has caused public panic and deterioration in mental wellbeing. Medical healthcare workers are particularly at risk of psychological distress. [4] Moreover, medical students have higher rates of mental illness, such as anxiety and depression than the general population[5-7] due to social isolation, lack of vaccinations at the beginning, having sudden transition to synchronized online/virtual learning as well as impacts to other areas of life. [8-9]

Many global medical educational studies have been conducted to assess the psychological impact among medical students during the COVID-19 pandemic.[10-13] A study from China reported 24.9% of medical students experienced anxiety symptoms due to the impact of daily living, having relatives or acquaintances infected with COVID-19 and delays in academic activities.[10] In Pakistan, 75.8% of medical students were afraid of becoming infected during rotations while 73.9% did not believe that their institutions could handle the situation.[11] Final year medical students in the UK experienced stress about the OSCE examination and the transition from being students to doctors.[12] In Australia, 68% of Australian medical students reported deterioration in mental wellbeing. The common coping activities were the use of video chats, social media, exercise and hobbies.[13]

In Thailand, from 1996 to 1997, mental health surveys of medical students were conducted at Srinakharinwirot University where 24.63% reported severe stress resulting in mental health problems. Associated factors related to mental problems included the academic year of students; second year students had the highest number of stress episodes due to financial issues, accommodation problems, extra-curricular activities and relationships with parents, teachers and friends.[14] Studies concerning the mental health of the medical students at Prince of Songkla University used a questionnaire (Thai GHQ-12) indicating that 29.1% were at risk for mental health problems.[15] In 2008, a study focused on the prevalence and sources of stress among medical students. Phramongkutklao College of Medicine reported that 31.94% had stress condition; 20.60% of the respondents had mild stress while 11.34% had significant stress levels affecting their daily lives. [16]

Even though research has been published about assessing the psychological impact of medical students during the COVID-19 pandemic in many countries, studies regarding this topic in Thailand remain limited. This study aimed to assess the psychological impact, investigate activities that medical students used to cope during the COVID-19 pandemic and identify factors associated with psychological impacts among medical students at Phramongkutklao College of Medicine, the only military medical school in Thailand providing a unique curriculum for military medicine expertise.

Methods

Study designs and setting

The protocol of this study was reviewed and approved by the Royal Thai Army Medical Department Institutional Review Board (approval number R008q/64_Ex). The sample size calculation required for this study was 181 participants. An online cross-sectional study was conducted at Phramongkutklao College of Medicine from 1 to 14 April 2021 during the first two weeks of the third wave of the COVID 19 outbreak in Thailand. An electronic survey was conducted including informed consent in Google forms and the survey link was distributed through Line and Facebook applications. During the survey, medical curriculum was modified and shifted to a combination of face-to-face and online/ virtual learning activities.

Participants

Participants were enrolled from 490 second to sixth year medical students, Phramongkutklao

College of Medicine, agreeing to participate to the study and able to respond to online questionnaire. Answers could only be taken and submitted once to avoid replicated responses. Participants with severe medical or psychiatric illness, diagnosed with Covid-19 were excluded.

Measures

General information questionnaire

The general information questionnaire consisted of 30 items that the researcher created to collect demographic data, determine health status and learning platforms, information related to COVID-19, i.e., its impact, level of exposure to the disease and relevant coping activities. The questionnaire was examined by three psychiatrists who were experts in child and adolescent health including an investigator who pilot-tested 30 people to examine the reliability of the questionnaire having a Cronbach's alpha coefficient of 0.86.

Fear of Illness and Virus Evaluation (FIVE)-Adult Report Form

The FIVE form was created by Prof. Jill Ehrenreich-May from Miami University.[17-18] The scale consisting of 35 items also includes child, parent, and adult forms. The adult form was used in this study. Participants rated the scores from 1 to 4 based on the frequency of each item. The scale consisted of 4 parts: fear about contamination and illness (9 items), fear about social distancing (10 items), behaviors related to illness and fear of virus (14 items) and impact of illness and fear of virus (2 items). Permission was obtained and the survey was translated to Thai by three psychiatrists of whom two were experts in child and adolescent health. The investigator tested for reliability by pretesting 30 people using the questionnaire for which a Cronbach's alpha coefficient was obtained at 0.90.

Depression anxiety stress scale (DASS-21)

The DASS-21 is a set of three self-reported scales designed to measure the emotional states of depression, anxiety, and stress. The scale consists of 21 items. Participants rated each item starting from 0 to 3. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, and lack of interest/ involvement as well as anhedonia and inertia. The anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety and subjective experience of anxious affect. The stress scale is sensitive to levels of chronic non-specific arousal. The DASS-21 Thai obtained an acceptable internal consistency ($\alpha = 0.75$). [19] In this study, permission was obtained from those who prepared the Thai forms.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp., released 2015). Demographic data were determined using descriptive statistics. Categorical data were presented as numbers and percentages, while continuous data were presented as mean and standard deviation (SD). The prevalence of psychological impact was determined using descriptive statistics and reported as a percentage with a 95% confidence interval (95% CI). The chi-square test was used to compare categorical data. Multivariable regression analysis was performed to identify factors associated with psychological impact (depression, anxiety and stress). Adjusted odds ratio (AOR) from the multivariate analysis was presented with corresponding 95% CI, and statistical significance was set at $p < 0.05$. Pearson's (r) correlation analysis was used to evaluate the linear relation between Fear of Illness and Virus Evaluation (FIVE) and psychological impact (DASS-21).

Results

Demographic data

A total of 256 medical students participated in the study with a 52.24% response rate. Of these, 142 (55.5%) were males and 128 (50%) were preclinical students. More than one half of the participants (55.1%) reported sleep problems. Most participants were at no risk of exposure to virus infection (86.3%), 19.1% had COVID-19-like symptoms, and 67.6% did not believe that the institution could handle the situation (**Table1**).

Table 1. General characteristics of medical students

Characteristic	Frequency N (%)
Sex	
Male	142 (55.5)
Female	114 (44.5)
Age	Mean 22 (±1.57 SD)
Year of Course	
Clinical students (MD 4-6)	128 (50.0)
Preclinical students (MD 2-3)	128 (50.0)
Underlying medical diseases	50 (19.5)
A visit to psychiatric clinic/ psychiatrist/ psychologist	22 (8.6)
Family history of psychiatric illness	22 (8.6)
Sleep problems	104 (40.6)
Average hours of sleep per day	Mean 5.4 ±1.20
Risk of Covid-19 exposure	35 (13.7)
The family lives in a high risk area	76 (30.2)
Have Covid-19-like symptoms	49 (19.1)
Have a relative or close acquaintance with Covid-19	8 (3.1)
No confidence about the Covid-19 policy of the college	171 (67.6)

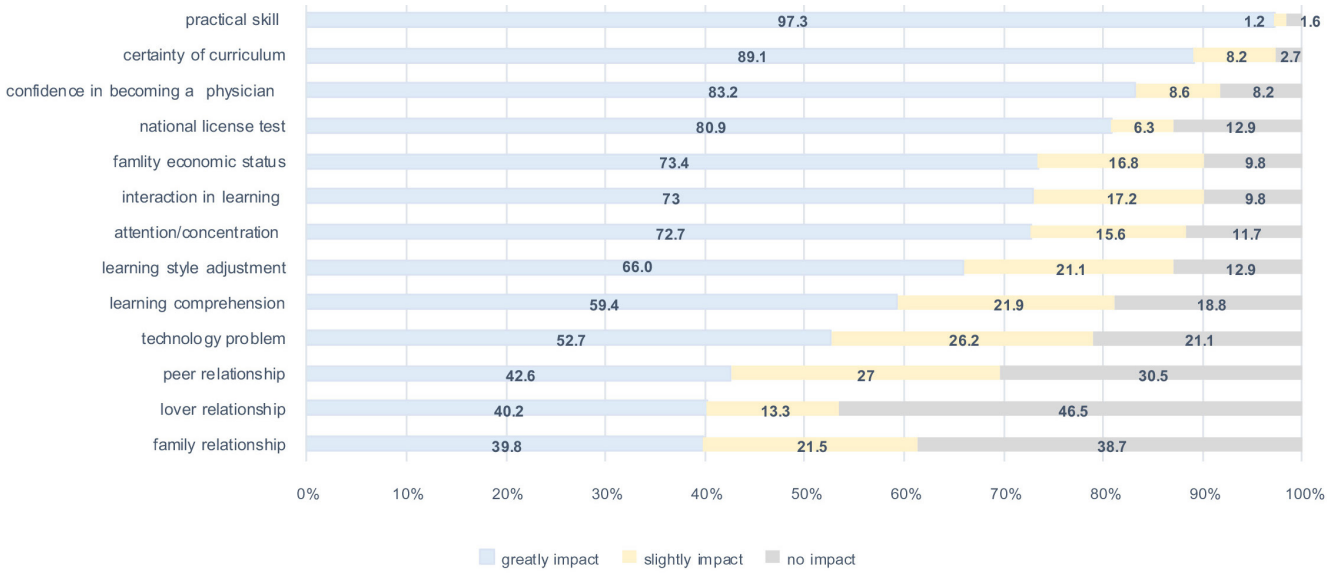

Figure 1. Impact of Covid-19 pandemic

For impact of the COVID-19 pandemic, medical students had been affected by both academic and personal issues; 97.3% reported a significant lack of practical skills, 89.1% were affected by unstable learning methods and promotion to the upper class, 83.2% had greatly decreased confidence in practicing medicine in the future and 73.4% had greatly affected family economic status (**Figure 1**).

Psychological impact on medical students

Using the DASS-21 form, 140 (54.7%) reported psychological impact. The second to sixth year medical students were affected by mental health issues at 70.7, 55.6, 44.2, 44.6 and 37.9%, respectively. The subscale and severity are shown in **Table 2**.

Table 2. Psychological impact among medical students

	MD 2 n (%)	MD 3 n (%)	MD 4 n (%)	MD 5 n (%)	MD 6 n (%)
Psychological impact (at least 1 symptom)	65 (70.7)	20 (55.6)	19 (44.2)	25 (44.6)	11 (37.9)
Depression					
normal	35 (38.0)	25 (69.4)	30 (69.8)	35 (62.5)	20 (69.0)
mild	20 (21.7)	3 (8.3)	3 (7.0)	3 (5.4)	2 (6.9)
moderate	25 (27.2)	4 (11.1)	5 (11.6)	10 (17.9)	7 (24.1)
severe	7 (7.6)	4 (11.1)	2 (4.7)	3 (5.4)	0 (0.0)
extremely severe	5 (5.4)	0 (0.0)	3 (7.0)	5 (8.9)	0 (0.0)
Anxiety					
normal	47 (51.1)	23 (63.9)	30 (69.8)	39 (69.6)	23 (79.3)
mild	15 (16.3)	6 (16.7)	6 (14.0)	8 (14.3)	2 (6.9)
moderate	17 (18.5)	3 (8.3)	2 (4.7)	3 (5.4)	3 (10.3)
severe	7 (7.6)	0 (0.0)	0 (0.0)	4 (7.1)	1 (3.4)
extremely severe	6 (6.5)	4 (11.1)	5 (11.6)	2 (3.6)	0 (0.0)
Stress					
normal	52 (56.5)	27 (75.0)	34 (79.1)	40 (71.4)	27 (93.1)
mild	9 (9.8)	2 (5.6)	0 (0.0)	7 (12.5)	1 (3.4)
moderate	19 (20.7)	3 (8.3)	5 (11.6)	4 (7.1)	1 (3.4)
severe	8 (8.7)	4 (11.1)	4 (9.3)	4 (7.1)	0 (0.0)
extremely severe	4 (4.3)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)

Clinical students (MD 4-6), Preclinical students (MD 2-3)

Fear of Illness and Virus Evaluation (FIVE)

Regarding the evaluation of all participants, a positive correlation was observed between FIVE scale scores and the psychological impact (DASS-21 scale) ($r=0.449$; $p<0.001$). Using the FIVE adult report form, the results showed that the fear about virus and illness subscale were as followed: 12.9% of students were always afraid of family members might get sick or die, and 11.3% were constantly afraid that they might do something to cause someone getting illness or infection. About the fear of social distancing subscale, 22.3% were unable to enjoy good things and 13.7% had a difficult time to do things they liked. About their behaviors related to illness and virus fear subscale, 57.4% wore masks over their faces or used other protective gear all the time and 38.3% used social media to continue connecting to their friends. About the impact of illness and virus fear subscale, 2.3% were afraid that an illness or virus infection would cause them to experience stress emotion and 1.2% were afraid that an illness or virus infection would involve the way of enjoying their lives **(Figures 2-5).**

Coping activities that were used to help with mental wellbeing

Common coping activities comprised the use of social media, video chats and exercise. Comparing coping strategies between preclinical and clinical students, a significant difference in using social media applications, consulting with friends/senior and joining activities/club of the college was observed (**Table 3**).

Table 3. Coping activities used among medical students

Coping activity	Total (%)	Preclinical students (%)	Clinical students (%)	*p-value*
Social media applications	241 (94.1)	125(97.6)	116(90.6)	0.017*
Video call/Chat	211 (82.4)	109(85.1)	102(79.6)	0.250
Exercises	207 (80.9)	107(83.1)	100(78.1)	0.266
Search for more information to be prepared and protect oneself	145 (56.6)	72(56.2)	73(57.0)	0.900
Consult with friends/seniors	144 (56.3)	84(65.6)	64(50.0)	0.002*
Join activities/clubs of the college	92 (35.9)	54(42.1)	38(29.6)	0.037*
Do meditation or study religious teachings	69 (27)	38(29.6)	31(24.2)	0.324
Consult with advisors	60 (23.4)	35(27.3)	25(19.5)	0.140
Use drugs, alcohol, and/or cigarettes	23 (9)	13(10.1)	10(7.8)	0.512
Consult a psychologist/ psychiatrist	20 (7.8)	9(7.0)	11(8.5)	0.641
Consult via online mental health/telephone	19 (7.4)	9(7.0)	10(7.8)	0.812
Others	40 (15.6)	25(19.5)	15(11.7)	0.085

*Significance at $p< 0.05$

Factors associated with the psychological impact

The results of ordinal multivariate analysis of factors associated with the psychological impact of medical cadets and medical students during the COVID-19 pandemic are presented in **Table 4**. Depression was associated with preclinical students, sleep problems and extreme deterioration in family economic status. Anxiety was associated with preclinical students, COVID-19-like symptoms, slight problems in adapting to a new learning method and extreme deterioration in family economic status. Stress was associated with preclinical students, COVID-19-like symptoms and no confidence in prevention or control of COVID-19 due to the college's policy.

Discussion

This study revealed that one half of students had psychological effects: depression, anxiety or stress. The findings on the effects on mental health were similar to those reported in other countries.[13, 20, 21] During unprecedented situations, improving medical students' mental health has become a higher priority to reduce psychological effects. Both males and females experienced similar effects on their mental health status;[8] thus, sex was not a determinant factor in developing psychological impact among medical students in Phramongkutklao College of Medicine. The results of this study did not agree with the findings reported from Australia and Turkey where being female had a significant psychological influence.[13, 22] Our study revealed that medical students studying in the preclinical year experienced increased depression, anxiety and stress during the Covid-19 pandemic. This was consistent with other studies reporting that preclinical medical students experienced greater psychological impact because of their lack of expertise in patient care and comprehension of the disease pandemic.[9, 23, 24] Because preclinical students were less resilient, they struggled more

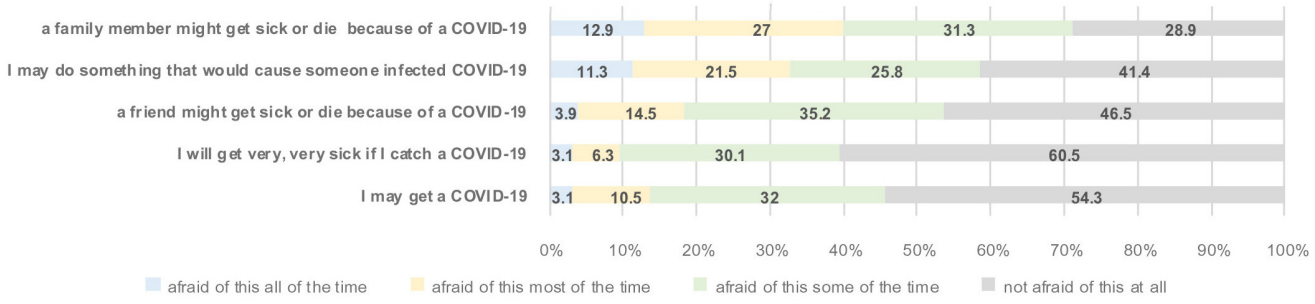

Figure 2. Fears about Contamination and Illness

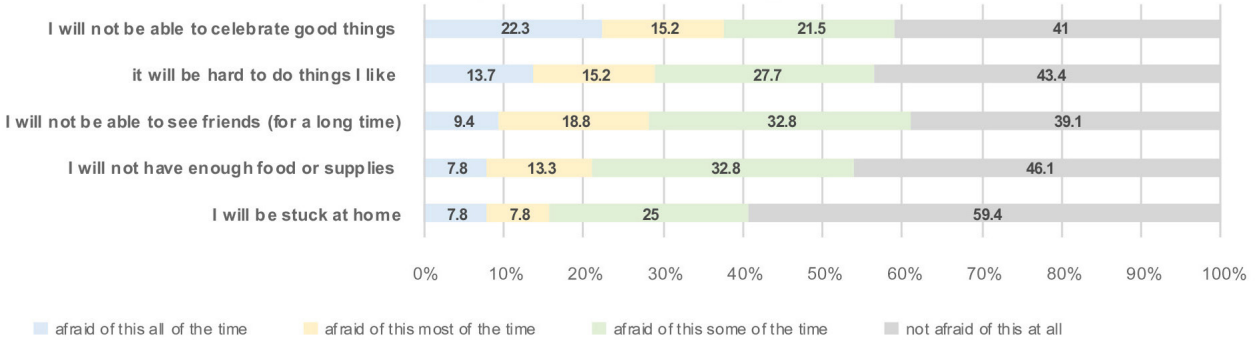

Figure 3. Fears about Social Distancing

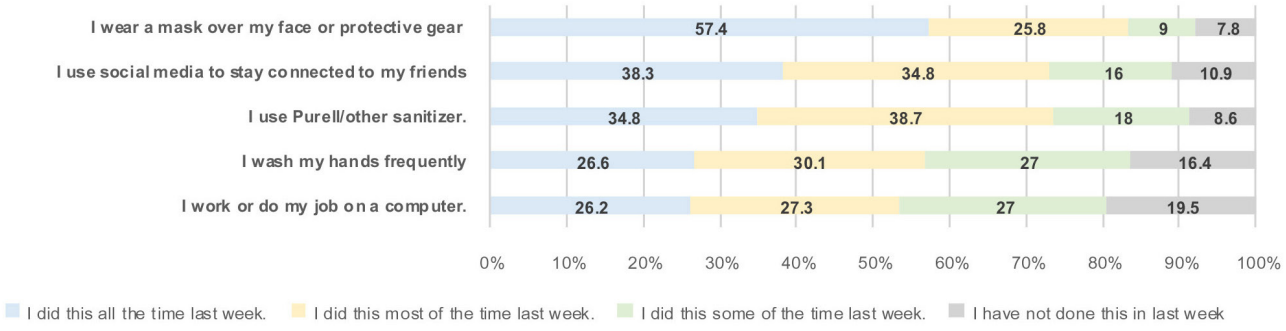

Figure 4. Behaviors Related to Illness and Virus Fears

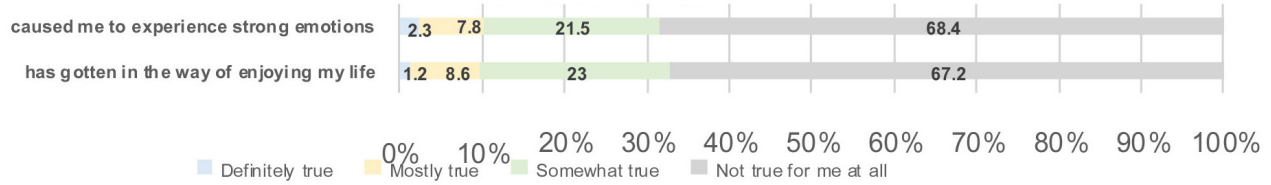

Figure 5. Impact of Illness and Virus Fears

Table 4. Factors associated with the psychological impact

	Depression			Anxiety			Stress		
	AOR	**95% CI**	***p*-value**	**AOR**	**95% CI**	***p*-value**	**AOR**	**95% CI**	***p*-value**
Class									
clinic	1			1			1		
preclinical	3.03	1.54-5.97	0.001*	3.20	1.52-6.72	0.002*	3.84	1.75-8.40	0.001*
Sleep problem									
No	1			1			1		
Yes	2.20	1.16-4.16	0.015*	1.40	0.70-2.80	0.345	1.32	0.65-2.68	0.445
Covid-19 like symptoms									
No	1			1			1		
Yes	1.52	0.68-3.43	0.309	2.93	1.26-6.83	0.012*	3.54	1.53-8.19	0.003*
Confidence in Covid-19 policy of PCM									
Yes	1			1			1		
No	1.41	0.74-2.70	0.298	1.93	0.95-3.93	0.070	2.3	1.06-4.98	0.035*
Problems with adaptation to a new learning method									
No problem	1			1			1		
Slight problem	1.6	0.46-5.54	0.461	6.11	1.54-24.24	0.010*	1.29	0.32-5.23	0.719
Significant problem	1.53	0.66-3.54	0.323	1.08	0.43-2.74	0.872	0.54	0.20-1.43	0.215
Family financial status									
No problem	1			1			1		
Slight problem	3.11	0.67-14.34	0.146	2.31	0.33-16.2	0.401	3.05	0.48-19.61	0.240
Great problem	7.27	1.81-29.29	0.005*	8.29	1.44-47.59	0.018*	4.47	0.84-23.76	0.079

AOR= adjusted odds ratio, *Significance at *p*-value < 0.05

with self-adaptation to new learning patterns than clinical students. [25-26] According to a 26-year retrospective study in Thailand, preclinical students used counseling services at a higher rate than those of clinical students, and second year medical students were the most likely to use mental health services.[27] Besides the stress of studying medicine in PCM, the stress of the military training was added, especially in preclinical classes that had more intense military training than those of clinical classes.[28] In our study, preclinical students were more likely than clinical students to use social media applications, consult with friends/seniors and participate in college activities/clubs. The suitable techniques to promote their mental wellbeing were online therapy or online extracurricular activities.

During the Covid-19 outbreak, medical students with Covid-19-like symptoms were more likely to experience mental health issues. [10,11] The fear of Covid-19 was positively correlated with anxiety among US college students.[29] In Vietnam, medical students who reported having greater fear of the COVID-19 scales had lower health literacy and mental health deterioration.[24] Similarly, our study indicated that students with Covid-19-

like symptoms experienced significantly higher levels of anxiety and stress. Furthermore, using Pearson's correlation analysis, a correlation between the fear of illness/virus evaluation and the psychological impact was observed.

To avoid putting themselves at risk of developing COVID-19, it became critical to remain healthy throughout this time. Moreover, medical students' sleep hygiene should not be disregarded, as sleep problems constitute physical and mental health hazards. [20-21] Jianping et al. reported that 33.2% of medical students reported poor sleep quality during the Covid-19 pandemic.[30] A meta-analysis showed a higher prevalence of sleep problems among healthcare professionals during the COVID-19 pandemic.[31] According to the SLEEP-50 questionnaire, a related study showed two thirds of medical students were at risk of at least one sleep disorder. Furthermore, all sleep disorders were associated with low academic performance. [32] In Mexico, depression symptoms among medical students were associated with academic stress and sleep problems.[33] Our findings also revealed that 40.6% of students experienced sleep issues, with sleep issues being associated with depression among medical students. Depression is linked to a functional decrease in serotoninergic neurotransmission as well as specific sleep disturbances, particularly insomnia. [34] However, specific details defining the sleep disorder were not clarified in this study. From this study, medical students obtained an average of 5.4 hours of sleep nightly. According to a study conducted at Srinakharinwirot University, sleeping for less than six hours resulted in poor academic performance as well as increased stress. [35]

The learning platform for medical students was shifted to online according to the social distancing policy. This study found that students who had difficulties adapting to a new learning technique experienced anxiety. The majority of students reported lacking practical skills and had low confidence in their abilities to practice medicine in the future. As a result, developing a curriculum to improve the medical educational process during the COVID-19 pandemic remains a top goal. Our findings revealed that two thirds of medical students lacked trust in the institution's policies which was significantly associated with stress. During the pandemic, educational institutions and hospitals must have robust and immediate safeguards in place to decrease the danger of infection while also mitigating stress and anxiety.[23] In Pakistan, 73.9% of medical students had little faith in their institutions' policies since more time had to be spent on epidemic prevention.[9] Thus, the curriculum had to be expanded to include emergency responses, i.e., disasters, outbreaks and other emergencies. [36] Aside from the effects on learning and the likelihood of infection with Covid-19, the effects on the family in terms of economic stability and harmful relationship within the family had a direct association with the effects on mental health.[10] Our findings revealed that medical students encountering a significant impact on their family's socioeconomic situation were more likely to experience depression and anxiety. During the epidemic, most medical students relied on social networking applications, video chats and exercise to unwind. When they experienced troubles, only 23% had to meet their advisors, and only 7.8% had to see psychiatrists or psychologists. Medical students have used a variety of techniques to cope with their COVID-19-induced mental health difficulties in reported studies. Spending time outside, having physical activities and exercise, using video calls plus social media applications as well as being mindfulness and meditation retreat were some of the tactics used to increase mental wellbeing.[37] Following the COVID-19 outbreak, physical activities and exercise have been proven to be particularly useful tools in aiding mental health of English medical students. [38]

Problem-adaptive coping and resilience training should be included in mental health treatment for medical students, especially at the preclinical level to improve the abilities needed for improved health care, sleep quality and mental health. Teaching and learning plans should include defined evaluation criteria. Furthermore, topic teachers should be available for online consultations to facilitate learning support. To enhance the impact of comprehensive mental health care, an online adviser, psychiatrist and psychologist should be available.

Because this study employed a cross-sectional survey of the Phramongkutklao College of Medicine, the results should be interpreted with caution when applied to other medical institutions. Self-reported data collection has a limitation in terms of data dependability and is susceptible to recall bias. As a result, a study using molecular biomarkers tests, such as salivary cortisol levels[39] and salivary amylase, is required.[40] Following up the mental health condition to evaluate any changes is also necessary. The lack of clarity regarding sleep problems, the severity of the underlying mental or medical diagnosis and health literacy may all be key factors in the psychological impact involving this study.

Conclusion

Medical students represent a vulnerable group that is prone to high levels of stress as a result of their studies, particularly during the COVID-19 epidemic, which could disrupt their education, family and other areas of life. During the COVID-19 epidemic, a high prevalence of psychological effects was observed among medical students. It remains critical to address the consequences of COVID-19 on medical students' mental health to provide psychological support and resilience training to promote mental wellbeing.

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FREQUENCIES OF PREDICTED MIA ANTIGEN AMONG SOUTHERN THAI BLOOD DONORS

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Abstract

Background: The Mi^a antigen (MNS7) of the MNS blood group system is clinically important in Asian populations. Anti-Mi^a has been implicated in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn in Thai populations. However, data of this antigen frequency among southern Thais remains unknown.

Objective: This study aimed to determine and predict Mi^a antigen frequencies among southern Thai blood donors and to estimate the risk of alloimmunization among Thais.

Methods: A cross-sectional study was conducted. Altogether, 400 southern and 500 central Thai blood samples were genotyped for *GYP(B-A-B)* and *GYP(A-B-A)* MNS hybrids using polymerase chain reaction with sequence-specific primer (PCR-SSP).

Results: Among them, 19 of 400 (4.45%), and 28 of 500 (9.33%) were positive with the set of GP.Hut, GP.HF, GP.Mur, GP.Hop, and GP.Bun. No GP.Vw phenotype was found among southern and central Thais. The predicted Mi(a+)frequency among southern Thais was significantly lower than among central and northern Thais ($p<0.05$). Its frequency was similar to Vietnamese, Taiwanese, and Southern Han Chinese populations ($p>0.05$) but significantly differed from Indonesian, Filipino, and Chinese (Guangzhou) populations ($p<0.05$). The risk of Mi^a alloimmunization among southern Thais was significantly lower than among both Thai groups ($p<0.05$).

Conclusion: This constitutes the first study to report Mi(a+) frequencies among southern Thais, supporting the estimation risk of alloimmunization and providing transfusion safety among Thai populations.

Keywords: Genotyping, MNS blood group system, Predicted Mi(a+) frequency, Southern Thais

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Introduction

The Miltenberger (Mi) subsystem was formally classified by Cleghorn in 1966.[1] The antigens carried on glycophorin A (GPA), and GPB and encoded by the glycophorin genes consisted of *GYPA, GYPB,* and *GYPE* genes of the MNS blood group system.[2, 3] The formation of *GYP(A-B-A)* and *GYP(B-A-B)* hybrid genes encode various GP hybrid molecules expressed on the red cell membranes, and these hybrid GPs display an implicit phenotype profile of Mi^a antigens currently established as MNS7. Mi^a is an antigen illustrated in 7 hybrid GPs consisting of GP.Vw, GP.Hut, GP.HF, GP.Mur, GP.Hop, GP.Bun, and GP.Kip.[4] These antigen frequencies are rare in Caucasians (0.012%), African Americans (0%) and Indian (0.2%) populations; however, higher frequencies are reported in Thai (9.7%) and Chinese (7.3%) populations.[2, 5-7] Anti-Mi^a has been implicated in hemolytic transfusion reactions (HTRs),[8, 9] hemolytic disease of the fetus and newborn (HDFN),[10, 11] and a case of hydrops fetalis.[12] Hence, including Mi(a+) cells in reagent red cells would noteworthy for antibody detection in Asian populations. Southern Thai Muslim populations are concentrated mainly in three provinces: Pattani, Yala and Narathiwat near the border with Malaysia, and have different ethnic origins compared with other regions of Thailand. Concerning a related study, the frequency of Mi^a antigen varies among Malaysian blood donors in three ethnic groups consisting of Malay (2.08%), Chinese (4.9%), and Indian (3.0%).[13] The prevalence of this antigen may involve alloantibody formation among patients regularly transfused in different ethnic groups. To date, the data of Mi^a antigen frequency among southern Thai blood donors remains unknown. This study aimed to determine and predict Mi^a antigen frequency among southern Thai blood donors to estimate the risk of alloimmunization in Thai populations.

Methods

This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand COE No. 013/2564). Informed consent was signed by all study participants. The study included EDTA-anticoagulated blood from 900 unrelated healthy Thai blood donors. In all, 400 and 500 samples were obtained from the Regional Blood Centre 12th Songkhla, Thai Red Cross Society, Songkhla, and the National Blood Centre, Thai Red Cross Society (NBC-TRC), Bangkok, Thailand. All 400 samples were from Thai-Muslim donors living in the three southern border provinces of Pattani, Yala and Narathiwat.

Genomic DNA was extracted from peripheral blood samples using the DNeasy Blood & Tissue Kit according to manufacturer instructions (QIAGEN GmbH, Valencia, CA, USA), then stored at -20ºC until used for genotyping. A PCR-SSP technique to detect two MNS hybrid GPs; *GYP(B-A-B)* and *GYP(A-B-A)* were performed using two sets of primers, according to a relatedstudy.[14, 15] Control DNA of known Mi(a+) positive phenotypes was from our in house collections and GP.Vw DNA controls were provided by Dr. Genghis H. Lopez, Research and Development Laboratory, Clinical Services and Research Division, Australian Red Cross Blood Service, Brisbane, Australia. In the PCR, 1 µL of genomic DNA (50 ng/µL) was amplified using 0.5 µL of 10 µM F2 primer

(5'-CCCTTTCTCAACTTCTCTTATATGC AGATAA-3') and 0.5 µL of 10 µM Rccgg primer (5'-GAGCAACTATTTAAAACTAAGAACA TACCGG-3') for the first set of *GYP(B-A-B)* detection. For the second set of *GYP(A-B-A)* detection, 0.5 µL of 10 µM F1 primer (5'-CAG-CATTTCTCTAAAGGCTAAATAAGAAGATG-TA-3') and 0.5 µL of 10 µM RIN primer (5'-CA TATGTGTCCCGTTTGTGCA-3'). Moreover, 0.5 µL of 6 µ MHGH-434-F primer (5'-TGC CTTCCCAACCATTCCCTT A-3') and 0.5 µL, 6 µM of HGH-434-R(5'-CCACTCACGGATT TCTGTTGTGTTTC-3') primer were included in both sets of *GYP(B-A-B)* and *GYP(A-B-A)* detections. PCR of each MNS hybrid GP detection was performed using 5 µL of 2X PCR reaction mixture (Green Hot Start PCR Master Mix, Biotechrabbit GmbH, Hennigsdorf, Germany), and 2 µL of distilled water added to a final volume of 10 µL.

PCR amplification of two sets was performed in a T100 Thermal cycler (Bio-Rad Laboratories, Inc., USA). For the first set of *GYP(B-A-B)*

detection, the cycling parameters for the PCR program consisted of 1 cycle at 95°C for 5 min, followed by 30 cycles at 95°C for 30 sec, 62°C for 40 sec 72°C for 30 sec, with a final extension at 72°C for 5 min. For the second set of *GYP(A-B-A)* detection, the cycling parameters for the PCR program consisted of 10 cycles at 95°C for 30 sec, followed by 10 cycles at 95°C for 10 sec, 64°C for 1 min, 30 cycles at 95°C for 10 sec, 61°C for 50 sec, 72°C for 30 sec, with a final extension at 72°C for 5 min. PCR products were electrophoresed at 100 V with a 1.5% agarose gel containing 10,000X fluorescent DNA gel stain (SYBR Safe DNA gel stain) using 1X TBE buffer. Products were visualized under a blue-light transilluminator.

Altogether, 900 samples of Thai blood donors including 400 and 500 from southern and central Thais were employed for *GYP (A-B-A)* and *GYP (B-A-B)* hybrid detections using PCR-SSP.

Statistical analysis

The frequencies of predicted Mi(a+) were calculated using the gene counting method. The differences in Mi(a+) frequencies between southern Thai and other populations were compared using a chi-square test of homogeneity. In addition, the risk estimation of Mi^a alloimmunization was obtained by multiplying the probability of having a predicted Mi(a-) phenotype frequency by the probability of having a predicted Mi(a+) phenotype frequency. All statistical analyses were conducted using SPSS, Version 16.0 (SPSS Inc., Chicago, IL, USA). A *p*-value less than 0.05 was considered statistically significant.

Results

The PCR-SSP results of MNS hybrid GP detections are shown in **Figure 1**. The *GYP(B-A-B)* hybrids of *GYP*Hut, GYP*Mur, GYP*Hop, GYP*Bun,* and *GYP*HF* were amplified with the first set of primers (the product size of 148 or 151 bp), whereas *GYP*Vw* of the *GYP(A-B-A)* hybrids was amplified using the second set of primers (the product size of 296 bp). The DNA controls were also tested using these two sets of primers, and the results agreed.

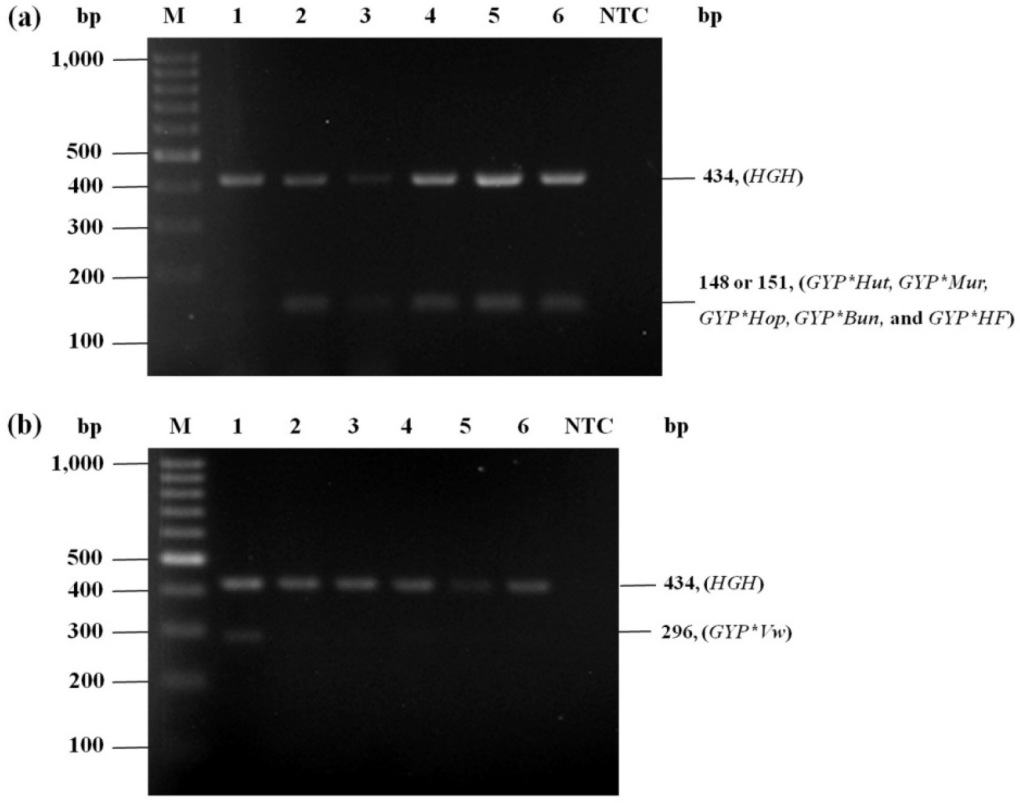

Figure 1. PCR-SSP results of MNS hybrid GP detections, (a) PCR products in Lanes 1-6 were amplified using the first set of primers, amplifying the fragments of the *GYP(B-A-B)* hybrids of *GYP*Hut, GYP*Mur, GYP*Hop, GYP*Bun,* and *GYP*HF*. (b) PCR products in Lanes 1-6 were amplified the second set of primers, amplifying the fragment of the *GYP(A-B-A)* hybrid of *GYP*Vw.* Lane NTC was the negative control (nontemplate control). Lane M: molecular weight 100-bp DNA Ladder.

Table 1. Occurrence of *GYP(B-A-B)* hybrids in Asian populations determined by PCR-based assays

Population	Number	*GYP(B-A-B)* hybrids		χ^2	*p*-value
		Mi(a+)	Mi(a-)		
Southern Thai (This study)	400	19	381		
Central Thai (This study)	500	51	449	9.202	0.002
Northern Thai [15]	300	67	233	49.183	< 0.001
Vietnamese [16]	160	10	150	0.524	0.469
Taiwanese[16]	167	7	160	0.084	0.772
Indonesian[16]	285	5	280	4.417	0.035
Filipino[16]	262	20	242	179.851	< 0.001
Southern Han Chinese[17]	3,104	201	2,903	1.792	0.181
Chinese (Guangzhou)[18]	528	51	477	7.864	0.005

Table 2. Estimations of risk for Mi^a alloimmunization in Thai populations

Population	Number	The frequencies of predicted Mi^a phenotype		Risk of Mi^a alloimmunization
		Negative	Positive	
Southern Thai	400	0.953	0.047	0.045
Central Thai	500	0.898	0.102	0.092*
Northern Thai [15]	300	0.777	0.223	0.173*

*p<0.05

Among 400 southern and 500 central Thai blood donors, 19 (4.45%), and 28 (9.33%) were positive only with the set of primers specific for GP.Hut, GP.HF, GP.Mur, GP.Hop, and GP.Bun. No GP.Vw phenotype was identified by PCR-SSP technique among southern and central Thais. Regarding the positive results of the MNS hybrid GPs by PCR-SSP, the predicted Mi(a+) frequency among southern Thais was significantly lower than that among central (p=0.002) and northern Thais (p<0.001).[15] The Mi(a+) frequency between southern Thais and other populations was compared. The frequencies were similar to Vietnamese, Taiwanese, and Southern Han Chinese populations (p>0.05).[16, 17] On the contrary, its frequency significantly differed from Indonesian, Filipino, and Chinese (Guangzhou) populations (p<0.05).[16,18], as shown in **Table 1**. In addition, the risk of Mi^a alloimmunization among southern Thais was significantly lower than those among central and northern Thais (p<0.05), as shown in **Table 2**.

Discussion

In Thailand, blood group allele frequencies have been studied among central, northern and southern Thais. The *DI*A* allele (predicted Di^a antigen) was significantly lower among southern Thais, while the *JK*01* allele (predicted Jk^a antigen) and *KEL*01* allele (predicted K antigen) were significantly higher among southern Thais than among central and northern Thais.[19] However, the Mi^a frequencies have not yet been reported in southern Thailand. This study constitutes the first to identify the predicted Mi^a frequencies in Thai Muslim populations, which have their own religion, culture and sustainable lifestyle.

Regarding the genotyping of MNS hybrid GP results using PCR-SSP among the four types; GP.Mur, GP.Hop, GP.Bun, and GP.HF of *GYP*

(B-A-B) hybrids, GP.Mur is commonly found in Asian and Australian populations.[4, 14, 16-18] Notably, GP.Vw of the *GYP(A-B-A)* hybrids was not found among southern and central Thais, similar to related studies in Thai and other Asian populations.[14, 16-18] The predicted Mi(a+) phenotype among southern Thais (4.8%) is closely related to Chinese-Malaysians (4.9%) but unrelated to Malay and Indian Malaysians (2.08% and 3.0%, respectively).[13] Even though the probability of finding the Mi(a+) phenotype among southern Thais is lower than among central and northern Thais, anti-E and anti-Mi^a are frequently found among southern Thai patients requiring repeated transfusions.[20, 21] Hence, the use of PCR-based assay to predict Mi^a antigen in blood donors and chronically transfused patients would be helpful to reduce alloimmunization risks and the above-mentioned adverse events. This study confirmed that it would pose no difficulty to find Mi^a antigen-negative donors for Thai patients with anti-Mi^a except among chronically transfused patients with multiple antibodies including anti-Mi^a. The possibility of finding compatible donor blood in complex cases caused by multiple alloantibodies may be more difficult concerning potential donors with a compatible blood type. Concerning patients requiring repeated transfusions such as thalassemia, cancer, and chronic renal diseases, performing Mi^a antigen typing is suggested before transfusion therapy and Mi^a-compatible donor (s) should be provided to patients. In concordance with the Clinical Practice Guidelines for diagnosis and management of thalassemia, the antigen typing of Rh (C, c, E, e) and MNS7 (Mi^a) is minimally required before the first transfusion.[22] This particular blood group antigen frequency could support not only determining genetic variation but also enhancing the characterization of Thai populations, especially in blood transfusion therapy.

Conclusion

This study is the first to report Mi (a+) frequencies among southern Thai blood donors. This finding is helpful to estimate the risk of alloimmunization and to provide transfusion safety in Thai populations.

Disclosures

The authors declare they have no conflicts of interest.

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PREVALENCE AND RISK FACTORS OF VITAMIN D INADEQUACY AMONG THAI ELDERLY PATIENTS WITH OSTEOPOROTIC HIP FRACTURE

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Abstract

Background: Vitamin D deficiency directly impacts bone biology, eventually resulting in elevated risk of fragility fracture. Despite its global abundance, data concerning its prevalence and risk factors among Thai patients with osteoporotic hip fractures remains lacking.

Objectives: This study aimed to evaluate the average level of serum vitamin D, prevalence of hypovitaminosis D and its risk factor among Thai elderly patients with fragility hip fractures.

Methods: A cross-sectional study was conducted among Thai patients with fragility hip fractures aged 60 years or older in a single center from April 2016-April 2020. The patients were divided according to serum 25-hydroxy vitamin D (25-(OH)D) levels. Demographic data were compared to identify risk factors of vitamin D inadequacy.

Results: Of 258 patients, 74.81% were females with mean age of 78.76 years. The average serum 25(OH)D level was 19.64 ng/mL. Prevalences of vitamin D inadequacy, vitamin D insufficiency and vitamin D deficiency were 86.05, 28.69 and 57.36%, respectively. When compared with the vitamin D sufficiency group, the vitamin D inadequacy group had a history of frequent falls, higher body mass index (BMI) as well as high parathyroid hormone (PTH) levels. Risk factors associated with vitamin D inadequacy were BMI >23 kg/m2 (AOR= 4.67, 95%CI=1.24-17.73), and two or more falls within a year (AOR= 3.96, 95%CI=1.38-11.33). Moreover, risk factors associated with vitamin D deficiency were being female (AOR= 2.87, 95%CI=1.06-7.78), BMI >23 kg/m (AOR=7.20, 95%CI =1.67-31.02), two or more falls within one year (AOR=7.32, 95%CI =2.17-24.69) and elevated PTH level (AOR= 3.38, 95%CI=1.17-0.74).

Conclusion: Most elderly patients with fragility hip fractures had hypovitaminosis D. Risk factors included high BMI, frequent falls for vitamin D inadequacy, being female and high PTH levels for vitamin D deficiency. Serum 25(OH)D assessment and appropriate supplement are recommended, especially for patients with fragility hip fractures and aforementioned risk factors.

Keywords: Vitamin D inadequacy, Vitamin D deficiency, Risk factor, Fragility hip fracture

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Introduction

Fragility hip fracture is a common osteoporotic fracture with significant morbidity, leading to loss of independence, mortality and financial burden.[1, 2] As the society ages, incidence of fragility fracture increases. The adjusted incidence rate of hip fractures in Thailand has increased more than 31% from 1997 to 2006.[3] Despite advances in medical care, one-year mortality after hip fracture has increased from 18% in 1999 to 21% in 2007.[4]

Vitamin D deficiency is common among the elderly, especially among housebound and geriatric patients.[5] It directly impacts bone biology, causing secondary hyperparathyroidism, high bone turnover, bone loss, mineralization defects and result in a decrease in bone mineral density, and ultimately, fragility fractures.[6] While vitamin D inadequacy is abundant, it remains one of the modifiable risk factors of fragility hip fractures where physicians can easily fix to lessen osteoporotic fracture risk. A related study in Thai population estimated the prevalence of vitamin D deficiency of 78% among patients with fragility hip fractures [7] corresponding to a 2014 study in Singapore showing an even higher number of 92%. [8]

Apart from the aforementioned investigations, data on vitamin D sufficiency among patients with osteoporotic fragility hip fractures remain limited, particularly in Asian populations.[9] Moreover, no research in Thailand has published risk factors of vitamin D inadequacy among patients with fragility hip fracture from osteoporosis. Therefore, in this study, we aimed to investigate the prevalence of vitamin D inadequacy and to identify risk factors associated with vitamin D inadequacy among Thai patients with fragility hip fractures.

Methods

Participants

Upon approval of the institutional ethics committee (COA No.89/2018), a cross-sectional study was conducted among patients admitted to the Police General Hospital, Thailand between April 1, 2016 and April 30, 2020. The inclusion criteria were patients aged 60 years or more with fragility hip fractures from a low energy trauma defined as a fall from standing height or less. The exclusion criteria were patients with pathologic fractures from skeletal tumor or malignancy, bone metastasis, metabolic bone diseases, long term use of oral steroid or other medical conditions that would affect bone quality, as well as patients whose serum 25-hydroxy vitamin D (25(OH)D) levels were unavailable for the study.

According to a study on hypovitaminosis D among patients with fragility hip fractures in Singapore (8), the percentage of vitamin D inadequacy was 92%. The proportion (p) of 0.92 was used to calculate, resulting in a required sample size of at least 114 for this study.

Using serum 25(OH)D level, patients were divided in three groups according to the Endocrine Society's definition. (10) Vitamin D sufficiency was defined as having serum 25(OH) D of 30 ng/mL or higher. Values of serum 25(OH) D below 30 ng/mL were defined as vitamin D inadequacy which was subdivided in vitamin D insufficiency with 25(OH)D level of 20 to less than 30 ng/mL and vitamin D deficiency with 25(OH)D level below 20 ng/mL. Demographic data were compared across groups to identify risk factors for each level of hypovitaminosis D.

Data acquisition

Patient demographic data and laboratory results were collected from the electronic database, including age, sex, body mass index (BMI), underlying diseases, previous fractures, pre-injury status, previous osteoporotic medications and any possible causes of secondary osteoporosis. Laboratory investigations included serum levels of 25(OH)D, parathyroid hormone (PTH), calcium, phosphate, albumin, creatinine and alkaline phosphatase including estimated glomerular filtration rate. Blood collection was performed within two days after admission. Serum 25(OH) D level was measured using electrochemiluminescence binding assay (ECLIA) on a Cobas e601 Analyzer (Roche Diagnostics Germany). All other laboratory investigations were performed at the central lab center, Police General Hospital using the same standardized machine and technique.

Statistical analysis

All statistical analysis was performed using STATA, Version 15 and statistical significance was set at $p<0.05$ with 95% confidence interval (95%CI). Chi-square test and Fisher's exact test were used to compare categorical variables. Student's t-test was applied for continuous data. Multivariate logistic regression analysis was also performed to assess independent risk factors associated with hypovitaminosis D.

Results

A total of 274 patients with hip fractures were admitted at the Police General Hospital during the study period. Twelve were excluded due to the lack of sufficient data of serum 25(OH) D levels. Another four were excluded because the fractures proved to have pathologic evidence. The remaining 258 patients were enrolled in this study. The majority of patients (74.81%) were females. The study population flow diagram is demonstrated in **Figure 1.** The mean age was 78.76 years and mean BMI was 21.52 kg/m^2. The mean serum 25(OH)D level was 19.64 ± 10.55 ng/mL.

The patients were divided in two groups according to their vitamin D status, 36 (13.95%) had sufficient 25(OH)D levels while the remaining 222 (86.05%) had vitamin D inadequacy. They were further classified in 74 (28.69%) with vitamin D insufficiency and 148 (57.36%) with vitamin D deficiency. The prevalence of each category is shown in **Figure 2**.

Of 193 women, 170 (88.09%) had vitamin D inadequacy. Fifty-two of 65 (80%) men had vitamin D inadequacy. A numerically higher percentage of women were found in the vitamin D inadequacy group, but without statistical significance. Comparing among age groups (60 to 69, 70 to 79 and ≥ 80 years old), no difference was observed in the proportion of vitamin D inadequacy to vitamin D sufficiency. The average BMI in the vitamin D inadequacy group was significantly greater than that of the vitamin D sufficiency group (21.73 ± 3.84 kg/m2 vs. 20.19 ± 2.72 kg/m2, p=0.022) with a significant tendency towards being overweight. Serum PTH level was significantly lower in the vitamin D sufficiency group (67.00 ± 43.09 pg/mL vs. 48.94 ± 30.31 pg/mL, p=0.012). Patients in the vitamin D inadequacy group fell more often than those observed in the vitamin D sufficiency group (two or more falls within one year 39.64% vs. 16.67%, p = 0.016). The characteristics of the patients comparing between the vitamin D sufficiency and inadequacy groups are shown in **Table 1**.

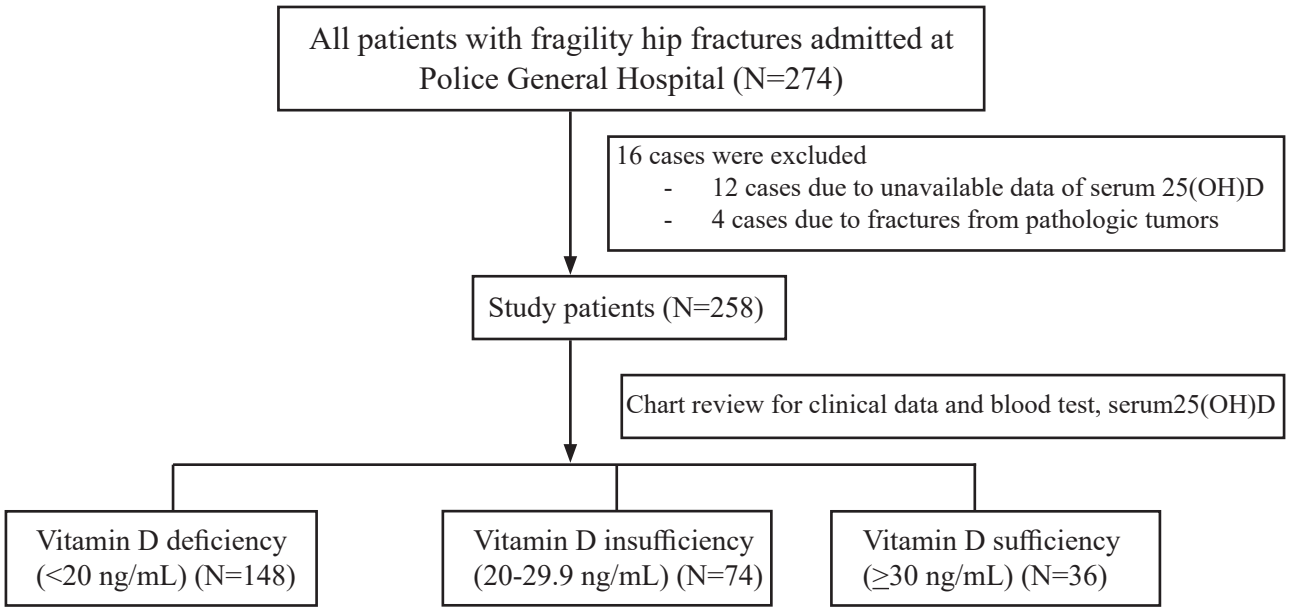

Figure 1. Flow diagram of study population

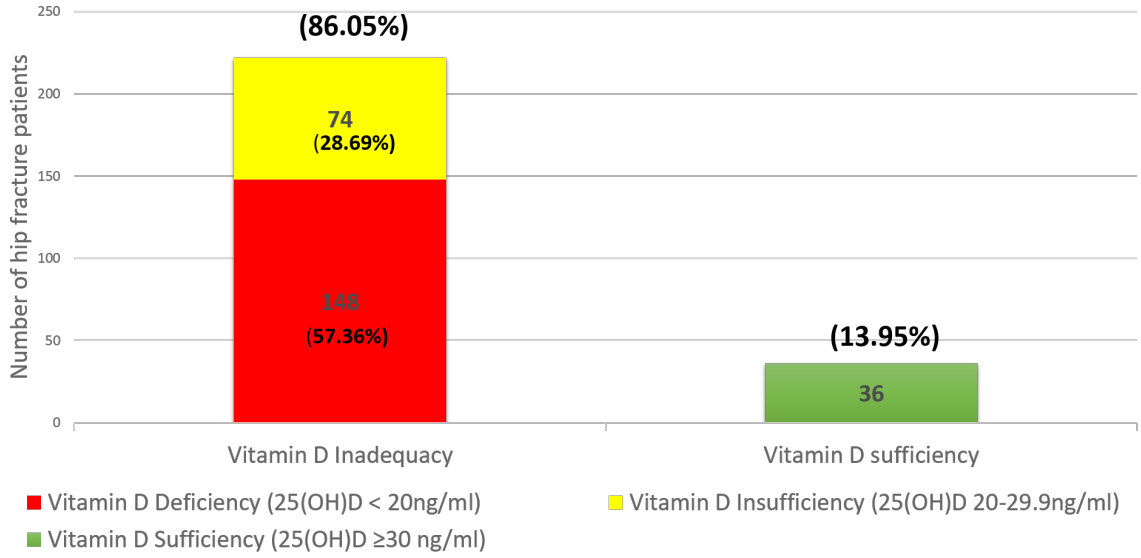

Figure 2. Prevalence of vitamin D sufficiency, vitamin D inadequacy (insufficiency and deficiency) among patients with fragility hip fractures
25(OH)D = 25-hydroxy vitamin D

Table 1. Characteristics of the cohort of patients with fragility hip fractures, patients with vitamin D inadequacy and vitamin D sufficiency. The variables were compared between vitamin D inadequacy and vitamin D sufficiency groups.

Characteristics	**Total** (n=258)	**Vitamin D inadequacy** (n=222)	**Vitamin D sufficiency** (n=36)	***p*-value**
Gender				
Female	193 (74.81%)	170 (76.58%)	23 (63.89%)	
Male	65 (25.19%)	52 (23.42%)	13 (36.11%)	0.104
Age (year)				
Mean ± SD	78.76 ± 8.86	79 ± 8.71	77.28 ± 9.71	0.281
60-69	36 (13.95%)	32 (14.41%)	4 (11.11%)	
70-79	93 (36.05%)	77 (34.68%)	16 (44.44%)	
≥80	129 (50%)	113 (50.90%)	16 (44.44%)	0.564
BMI (kg/m^2)				
Mean ± SD	21.52 ± 3.74	21.73 ± 3.84	20.19 ± 2.72	0.022*
<18.5	48 (18.61%)	39 (17.57%)	9 (25%)	0.040*
18.5-22.9	136 (52.71%)	113 (50.90%)	23 (63.89%)	
≥23	74 (28.68%)	70 (31.53%)	4 (11.11%)	
Underlying disease				
Hypertension	187 (72.48%)	165 (74.32%)	22 (61.11%)	0.100
Type 2 diabetes mellitus	99 (38.37%)	88 (39.64%)	11 (30.56%)	0.298
Previous fracture	28 (10.85%)	24 (10.81%)	4 (1.55%)	0.957
Pre-injury ambulation with gait aid	97 (37.60%)	83 (37.39%)	14 (38.89%)	0.863
Two or more falls within a year	94 (36.43%)	88 (39.64%)	6 (16.67%)	0.008*

Table 1. (continued)

Characteristics	Total (n=258)	Vitamin D inadequacy (n=222)	Vitamin D sufficiency (n=36)	*p*-value
Previous vitamin D supplement	32 (12.40%)	26 (11.71%)	6 (16.67%)	0.403
PTH level (pg/mL)	65.20 ± 42.01	67 ± 43.09	48.94 ± 30.31	0.016*
Albumin (g/dL)	3.83 ± 0.45	3.82 ± 0.46	3.91 ± 0.41	0.270
eGFR (ml/min/1.73 m^2)	66.44 ± 24.08	66.25 ± 24.74	67.60 ± 19.77	0.756

Values are presented as mean ± standard deviation or number (%)
BMI = body mass index; PTH = parathyroid hormone; eGFR = estimated glomerular filtration rate.
*$p < 0.05$ is statistically significant.

Table 2. Characteristics of the cohort of patients with fragility hip fractures, patients with vitamin D deficiency, and vitamin D sufficiency. The variables were compared between vitamin D deficiency and vitamin D sufficiency groups.

Characteristics	Total (n=184)	Vitamin D deficiency (n=148)	Vitamin D sufficiency (n=36)	*p*-value
Sex				
Female	145 (78.80%)	122 (82.43%)	23 (63.89%)	
Male	39 (21.20%)	26 (17.57%)	13 (36.11%)	0.015*
Age (year)				
Mean ± SD	78.30 ± 9.22	78.55 ± 9.12	77.28 ± 9.71	0.460
60-69	29 (15.76%)	25 (16.89%)	4 (11.11%)	
70-79	68 (36.96%)	52 (35.14%)	16 (44.44%)	
≥80	87 (47.28%)	71 (47.97%)	16 (44.44%)	0.555
BMI (kg/m^2)				
Mean ± SD	21.70 ± 3.98	22.07 ± 4.16	20.19 ± 2.72	0.011*
<18.5	33 (17.93%)	24 (16.22%)	9 (25%)	0.01*
18.5-22.9	94 (51.09%)	71 (47.97%)	23 (63.89%)	
≥23	57 (30.98%)	53 (35.81%)	4 (11.11%)	
Underlying disease				
Hypertension	132 (71.74%)	110 (74.32%)	22 (61.11%)	0.114
Type 2 diabetes mellitus	75 (40.76%)	64 (43.24%)	11 (30.56%)	0.165
Previous fracture	19 (10.33%)	15 (10.14%)	4 (11.11%)	0.863
Pre-injury ambulation with gait aid	77 (41.85%)	63 (42.57%)	14 (38.89%)	0.688
Two or more falls within a year	75 (40.76%)	69 (46.62%)	6 (16.67%)	0.001*
Previous vitamin D supplement	26 (14.13%)	20 (13.51%)	6 (16.67%)	0.626
PTH level (pg/mL)	67.77 ± 45.51	72.35 ± 47.46	48.94 ± 30.31	0.005*
Albumin (g/dL)	3.80 ± 0.46	3.77 ± 0.47	3.91 ± 0.41	0.103
eGFR (ml/min/1.73 m^2)	66.59 ± 25.17	66.35 ± 26.37	67.60 ± 19.77	0.790

BMI = body mass index; PTH = parathyroid hormone; eGFR = estimated glomerular filtration rate.
*$p < 0.05$ is statistically significant.

Table 2 demonstrates characteristics of patients with fragility hip fractures having vitamin D deficiency and vitamin D sufficiency. No difference was observed in the proportion of vitamin D deficiency to vitamin D deficiency among age group. The mean BMI was higher in the deficiency group (22.07 ± 4.16 kg/m^2 vs. 20.19 ± 2.72 kg/m^2, p=0.011). Patients reported two or more falls within one year in the vitamin D deficiency compared with the vitamin D sufficiency groups (46.62% vs. 16.67%, p=0.001). History of a previous fractures, previous injury ambulatory status, and previous vitamin D prescription did not differ between groups. The mean level of PTH in the vitamin D deficiency group was 72.35 pg/mL which was significantly higher than that found in the sufficiency group (48.94 pg/mL, p=0.005). Other laboratory results did not significantly differ.

Multivariate analysis showed that factors differentiating between vitamin D inadequacy and vitamin D sufficiency were higher BMI (23 kg/m2 or more) [adjusted odd ratio (AOR)= 4.67, 95% CI=1.24-17.63] and history of frequent falls (AOR=3.96, 95% CI=1.38-11.33), respectively. When analyzing the vitamin D deficiency group against the vitamin D sufficiency group, associated risks for vitamin D deficiency were being female (AOR= 2.87, 95%CI=1.06-7.78)), BMI ≥23 kg/m2 (AOR=7.20, 95% CI=1.67-31.02), two or more falls within one year (AOR= 7.32, 95%CI=2.17-24.69) and PTH levels more than 65 pg/mL (AOR= 3.38, 95%CI=1.17-9.74). **Table 3** shows multivariable analysis logistic regression of factors associated with vitamin D inadequacy and deficiency.

Table 3. Multivariable analysis of factors associated with Vitamin D inadequacy and vitamin D deficiency

Variable	Inadequacy vs Sufficiency			Deficiency vs Sufficiency		
	AOR	95% CI	*p*-value	AOR	95% CI	*p*-value
Age ≥ 80 years	1.45	(0.61-3.45)	0.395	1.14	(0.42-3.10)	0.800
Sex (female)	1.82	(0.78-4.24)	0.165	2.87	(1.06-7.78)	0.039*
BMI (kg/m^2)						
<18.5	1.00			1.00		
18.5-22.9	1.29	(0.50-3.34)	0.599	2.03	(0.67-6.13)	0.210
≥23	4.67	(1.24-17.63)	0.023*	7.20	(1.67-31.02)	0.008*
Fall ≥ 2 times	3.96	(1.38-11.33)	0.010*	7.32	(2.17-24.69)	0.001*
Hypertension	0.98	(0.40-2.42)	0.962	0.76	(0.27-2.14)	0.608
Diabetes	1.69	(0.70-4.11)	0.244	2.29	(0.87-6.06)	0.094
Previous fracture	0.51	(0.14-1.87)	0.311	0.45	(0.10-1.99)	0.294
Pre-injury status (without gait aids)	1.50	(0.66-3.41)	0.333	1.21	(0.48-3.07)	0.688
No Vitamin D supplement	2.23	(0.74-6.71)	0.154	2.85	(0.81-9.99)	0.101
PTH > 65 pg/mL	2.39	(0.92-6.19)	0.073	3.38	(1.17-9.74)	0.024*
Albumin < 3.4 g/dL	1.60	(0.52-4.86)	0.411	2.19	(0.65-7.43)	0.207
eGFR < 45	0.78	(0.24-2.57)	0.687	0.67	(0.19-2.39)	0.535

AOR =Adjusted odds ratio, CI = Confident Interval, BMI = body mass index, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate

*p < 0.05 is statistically significant.

Discussion

Vitamin D deficiency has many negative effects on bone quality including a defect in mineralization, leading to rickets and osteomalacia. It also leads to secondary hyperparathyroidism with concomitant increase in bone turnover, leading to osteoporosis. Moreover, it affects muscle strength and balances increasing the risk of fall.[11] Together with diminished bone quantity and quality, they lead to increased risk of fracture.

Our study showed a mean level of serum 25(OH)D of 19.64 ± 10.55 ng/mL among Thai elderly patients with fragility hip fractures. The result was similar to a related study among patients with osteoporotic hip fractures at Siriraj Hospital, Thailand[7] and in Singapore.[8] As expected, among these elderly patients, serum 25(OH)D level was much lower than an average level of 30.08 ng/mL among general Thai premenopausal women reported in 2009 [12] and 31.80 ng/mL among Thai adults with an average age of 40.3 years.[13] Considering the prevalence of vitamin D inadequacy, our study reported the prevalence of 86.05%, which was slightly higher than that of the study among patients with osteoporotic hip fracture at Siriraj Hospital, Thailand which was 78.4%. [7] Predictably, the figures were obviously higher than in a normal Thai population in which the rate of vitamin D inadequacy was found to be 50.9%. [13]

Aging has been proposed to affect vitamin D metabolism in many ways, magnifying the effect of vitamin D inadequacy among the elderly.[14] Aging skin has a lower concentration of 7-dehydrocholesterol, resulting in less conversion to inactive vitamin D3. Renal function also decreases with age, hindering the capability of the kidney to synthesize 25-hydroxylase required in the final step of vitamin D activation. The elderly are also at higher risk of being housebound and exposed to less sunlight. However, comparing among elderly themselves, age at 80 or above is not a predictor of vitamin D inadequacy.

In this study, the proportion of vitamin D inadequacy was about the same among women (88.09%) and men (80.0%). On the other hand, a related study among Thai healthy adults showed that vitamin D inadequacy was 3.1-fold more frequent among women than among men.[15] The prevalence of vitamin D inadequacy was only 13.9% among men, which was far lower than that of our study, indicating that patients with hip fractures were at higher risk of hypovitaminosis D, especially among men. However, the study was conducted among healthy adults with a mean age about 40 years old whose vitamin D metabolism would differ from the elderly.

The results of this study showed that BMI of 23 kg/m2 or more was associated with vitamin D inadequacy and deficiency. This relationship was consistent with another study which showed a significantly higher BMI in a vitamin D deficiency group, compared with vitamin D insufficiency and vitamin D sufficient groups. [16] Obesity-associated vitamin D insufficiency is likely to be related to decreased bioavailability of cholecalciferol synthesized from subcutaneous tissue when exposed to ultraviolet light, and ergocalciferol or cholecalciferol from dietary sources because of increased vitamin D deposition in body fat compartment. [17]

Loss of muscle strength affects functional ability and mobility putting an elderly individual at increased risk of falls and fractures. [18, 19] Vitamin D has direct impact on muscle strength by acting on vitamin D receptors present on muscle cells which becomes fewer with aging. [20] Hypovitaminosis D may sometimes present myopathy, which can be reversed with prescription of vitamin D.[21] A meta-analysis of eight randomized controlled trials recommended an optimal serum 25(OH)D level of 24 mg/mL (60 nmol/L) to achieve 23% reduced falls.[22] Similarly, supplemental high dose vitamin D could reduce all by 19%. Correspondingly, in our finding, patients with vitamin D inadequacy and vitamin D deficiency experienced more frequent falls than individuals with optimal vitamin D status.

Production of active vitamin D is under a tight control by serum calcium, phosphate and PTH. A decrease in serum 25(OH)D leads to a minute decrease in serum calcium.[6, 23] This in turns trigger a release of PTH, referred to as, "secondary

hyperparathyroidism" and a normalization of calcium level. As expected, the mean PTH level was higher in vitamin D deficiency compared with vitamin D sufficiency groups (72.35 ± 47.46 vs. 48.94 ± 30.31 pg/mL, p=0.005). However, serum calcium level was normal in all groups of participants.

Suboptimal vitamin D status is commonly observed among elderly people as the result of various risk factors and interacting physiologic changes in this population.[14] However, this condition is treatable and also preventable. Vitamin D and calcium supplementation in osteoporotic patients is also decidedly reasonable and cost-effective from an economic point of view.[24] Therefore, we highly recommend evaluating vitamin D level among patients with fragility hip fracture and supplementing with vitamin D according to their vitamin D status, especially among patients with risk factors of vitamin D inadequacy and deficiency.

This constitutes the first study to evaluate the risk factors of hypovitaminosis D among Thai patients with fragility hip fractures. Moreover, our study also showed a high prevalence of vitamin D inadequacy among Thai elderly men raising awareness of assessing vitamin D level and optimization not only among women but also men. However, several limitations were addressed in this study. First, the study constituted a cross-sectional design which could not directly explain causation. Secondly, the study was conducted at a single institution with limited representation as nationwide data. Furthermore, not all risk factors were included in the study, i.e., daily dietary consumption of calcium and vitamin D, exposure to sunlight, and dose of previous over-the-counter vitamin D supplementation due to inability to accurately assess such information and potential risk of confounders.

Conclusion

The majority of elderly patients with fragility hip fractures had suboptimal vitamin D status. Risk factors of vitamin D inadequacy included BMI >23 kg/m2, and two or more episodes of falls. Being female and high PTH levels were also associated with vitamin D deficiency. Serum 25(OH)D assessment as well as vitamin D supplement was recommended in this patient group, especially among those with risk factors including being female, presenting obesity, high risk for fallings and elevated PTH levels.

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Disclosure

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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APPLICATION OF ARTIFICIAL INTELLIGENCE TO ASSIST HIP FRACTURE DIAGNOSIS USING PLAIN RADIOGRAPHS

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Abstract

Background: Most hip fractures occur among elderly people. They are usually treated in the emergency room where orthopedic surgeons may not be readily available. The problem of delayed diagnosis and treatment results increase risks of further complications and mortality rate. Thus, applying artificial intelligence (AI) can assist physicians having limited experience to rapidly and confidently diagnose hip fractures using radiographs.

Objective: This study aimed to validate AI programs to assist diagnosing of hip fractures on plain radiographs.

Methods: This study employed a retrospective diagnostic study design. From 1 January 2015 to 31 December 2019, compiled ortho pelvis, anterior-posterior (AP) films from the diagnosis of hip fractures at Ananthamahidol Hospital were performed. The performance of the AI program was compared with one orthopedic surgeon who reviewed the same images. The accuracy, sensitivity and specificity of the diagnosis of hip fractures between the orthopedic surgeon and AI program were analyzed.

Results: In total, 217 patients were enrolled in this study. Of these, 56 (28.5%) were male and 161 (74.2%) female. Areas of hip fractures were as follow: intertrochanteric (108, 49.8%), femoral neck (102, 47.0%), subtrochanteric (6, 2.7%) and femoral head (1, 0.5%). The orthopedic surgeon and AI program revealed an accuracy of 93.59% (95%CI 90.8-95.73) vs. 81.24% (95% CI 77.17-84.85), sensitivity of 90.30% (95% CI 85.60-93.90) vs. 89.40% (95%CI 84.50-93.20) and specificity of 97.10% (95%CI 93.60-98.90) vs. 72.5% (95%CI 65.90-78.50), respectively.

Conclusion: Our results showed that the AI model (VGG16) showed a sensitivity of 89.40% vs. 90.30% obtained from the orthopedic surgeon. Thus, improvement in the sensitivity and specificity of AI software is further required. In the future, AI models have the potential as useful tools for emergent screening and evaluation of patients with hip fractures using plain radiographs, especially in the Emergency Department where orthopedic surgeons may not be readily available.

Keywords: Artificial intelligence, Hip fracture

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Introduction

The incidence of hip fractures is expected to increase due to rising elderly populations worldwide. In 2000, approximately 1.6 million hip fractures were reported [1] which is expected to increase to 4.5 to 6.3 million cases in 2050 according to the International Osteoporosis Foundation.[2] Hip fractures are a common problem impacting socioeconomic status. The cumulative mortality after one year of hip fractures among patients occurs 20 to 40%.[3, 6, 10] Thus, the mortality rate among patients with hip fractures greatly increases to 28.7% compared with that in the general population.[5] A systematic review of 229,851 patients with hip fractures from 36 countries from 2013 to 2017 revealed that mean overall one year mortality after hip fractures was 22.0%.[6] Risk factors that increased mortality rate included higher American Society of Anesthesiology (ASA) score. Odds ratio of 2.3 for every ASA point added, being male[4], age and type of hip fracture[7] were associated risk factors. Intertrochanteric fracture had a higher mortality rate than that of femoral neck fracture (17.40% vs. 9.83%). One year mortality after hip fractures among patients receiving nonoperative treatment was more than those receiving operative treatment.[8] Seung-Ju Kim, et al. also showed that one year mortality after hip fractures among patients receiving nonoperative treatment was 48.5% while among those receiving surgical treatments was 19.9%.[9]

The definition of delayed surgical treatment of hip fractures varied in several studies. The optimal time for surgical treatment is 48 hours[11-15] starting from admission to the time of operation. From 25 studies, 21% of patients undergoing surgery after 48 hours died within one year, while one year mortality of those undergoing operations within 48 hours was less than 20%. Factors of delayed surgical treatment included patients' medical conditions, i.e., using an anticoagulant drug, having unstable medical conditions etc. The problem of diagnosing hip fractures, especially that of occult hip fracture constituted a number of both false positive and false negative diagnoses found based on radiography findings alone. Additionally, the experience of physicians working in the Emergency Department proved very crucial. Poor sensitivity and specificity of radiographs of the proximal femur and pelvis among patients with pain or suspected trauma around these structures were recorded.[16] Dominquez et al. reported that 4.40% of patients who were suspicious of hip fractures at the Emergency Department received a subsequent diagnosis as having fractures. The incidence of occult hip fracture was 3 to 10%.[18, 19] Magnetic Resonance Imaging (MRI) and Computerized Tomography Scan (CT) are useful tools to help diagnose occult hip fractures. MRI, an investigation of choice to diagnose occult hip fractures, is highly accurate with 100% sensitivity and specificity. MRI can be helpful for patients to receive an operation within the optimal time.[20-22] CT scan [21-23] is also a second line of choice when results obtained from MRI were contraindicated or could not be obtained within 24 hours. However, MRI and CT scans are costly and require more time to prepare patients.

Artificial Intelligence (AI), a useful tool for assisting the diagnosis of hip fractures using plain radiographs can reduce delayed diagnosis resulting in delayed surgical treatment when orthopedic surgeons or radiologists may not be available at the Emergency Department. At present, in general hospitals, all radiographs are recorded to digital files that can be analyzed and interpreted using Picture Archiving Communication Systems (PACS). AI is a field of Computer Science that has a competence of helping diagnosis close to human performances especially deep learning and supervised learning for training AI. The input data provided specific results. After training the AI, it can predict results from all input datasets. AI can prove and continue to predict the results until obtaining the correct results. Urakawa et al.[24] detected fractures from radiographs using Convolutional Neural Networks (CNNs). Visual Geometry Group 16 layer (VGG16) is CNN-selected to detect intertrochanteric hip fractures compared with the performance to detect intertrochanteric hip fractures between VGG16 and orthopedic surgeons. The accuracy of VGG16 and orthopedic surgeons was 95.5% vs. 92.2%, sensitivity 93.3%

vs. 88.3% and specificity 97.4% vs. 96.8%. A similar study conducted by Cheng et al.[25] found that DCNN (Deep Convolutional Neural Network) achieved an accuracy of 91% sensitivity and 98% specificity. Recognition of hip fractures using DCCN could be performed with less than one hour of perceptual training.[26]

Most hip fractures occur among elderly people. They are usually brought to the Emergency Department where orthopedic surgeons may be unavailable at that time. To detect fractures using radiographs or occult hip fractures, the diagnostic problems of hip fractures could result from inexperienced physicians leading to delayed treatment, increasing both complications and mortality rate. Application of AI to assist young and inexperienced physicians to diagnose hip fractures using plain radiographs could help these physicians be confident with rapid diagnosis of hip fractures decreasing adverse events at the process of diagnosis.

Methods

Study population

The study was reviewed and approved by the Institutional Review Board of the Medical Department, Royal Thai Army (approval number S015h/63_EXP). A retrospective diagnostic study design was conducted from 1 January 2015 to 31 December 2019. Patients, undergoing both operative and nonoperative treatments, received a diagnosis of hip fracture and were admitted at Ananthamahidol Hospital.

The sample size was calculated from the prevalence of 17% osteoporosis and the sensitivity of AI to detect hip fractures conducted by Urakawa et al.[24] A total of 524 radiograph images of hip fractures and nonhip fractures were used in this study.

Definition

Hip fracture is a bone fracture from the edge of the femoral head to 5 cm below the lesser trochanter of the femur.

Criteria

The inclusion criteria included patients aged more than 50 years having a diagnosis of osteoporosis or hip fractures from accidents, i.e., slipping, falling or vehicle accidents. The exclusion criteria comprised patients having a diagnosis of bone tumor, periprosthetic hip fractures, nonfractured hip side without instruments, i.e., cement, screw, nail and prosthesis.

Compiled ortho pelvis and anterior-posterior (AP) films from the diagnosis of hip fractures were performed using 541 images which were divided to 120 images for AI training procedures and 421 images for test procedures. The performance of the AI program was compared with one orthopedic surgeon who reviewed the same images. The accuracy, sensitivity and specificity of the diagnosis of hip fractures between the orthopedic surgeon and AI program were analyzed.

Radiographs using deep learning techniques. the AI model comprised the VGG16, which is a public model and was used in this study. The VGG model was created in 2014 for which the model VGG16 has advantages of a design architecture with conv2D 3x3 pixels, 1 stride same padding and max pooling 2x2 pixels, 2 strides and hyperparameter. The VGG16 has 16 layers with a large network and hyperparameters encompassing about 138 million units.

Procedure

Preprocessing images

The 120 ortho pelvis radiograph images were provided for training procedures while 421 images were used for test procedures. Images were taken from digital film photographs and input to Picture Archiving Communication Systems (PACS) and all image data were recorded in the Digital Imaging and Communications in Medicine (DICOM). The DICOM was changed to JPG and adjusted by histogram equalization to brighten adjustment, reduce noise, and rotate and crop images as shown in **Figure 1.**

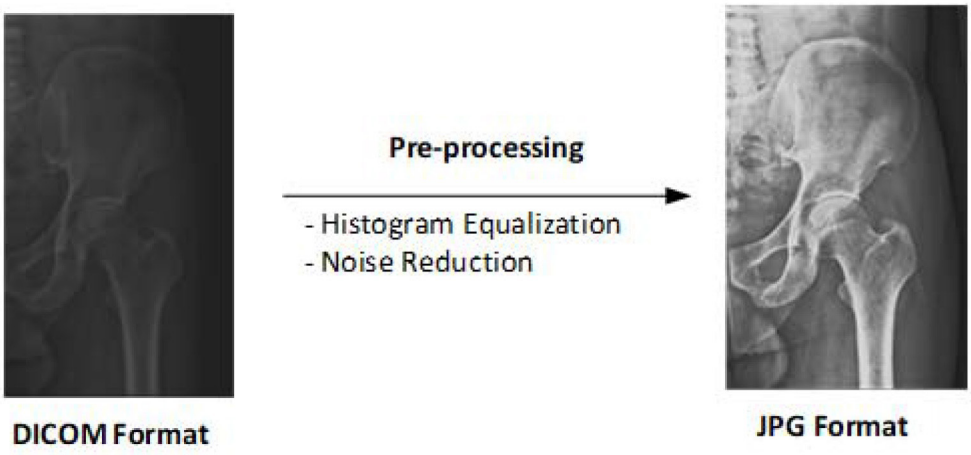

Figure 1. Preprocessing image

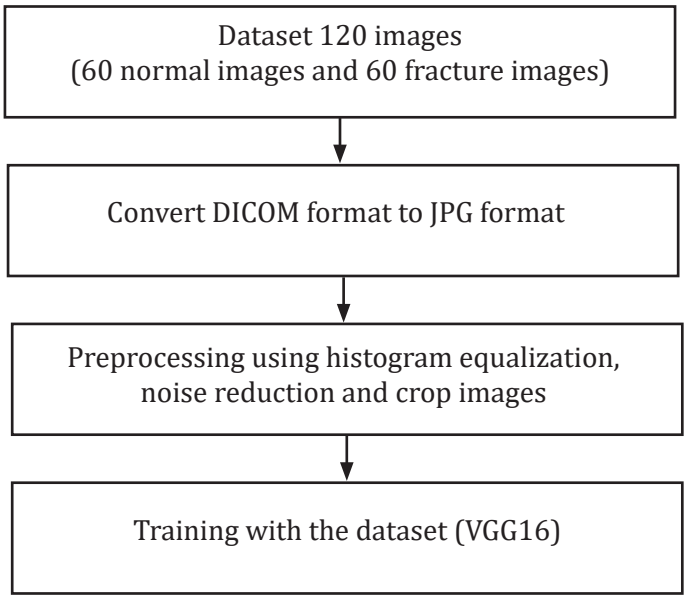

Figure 2. Training procedures

Training Procedures

Images for training using the VGG16 were classified in two groups: nonhip fracture and hip fracture. The size of each image was 200x200 pixels, then the images were input to the Image Data Generator by keras preprocessing that was used in the preprocessing process. The Image Data Generator adjusted the size, rotation and zoom of the images before training procedures. CPU Intel Core i5 Generation 11 and GPU NVIDIA RTX3060 were used for the training procedures involving VGG16 use 50 times as shown in **Figure 2**.

Test Procedures

The 421 Ortho Pelvis radiograph images were changed from DICOM to JPEG and adjusted using histogram equalization to reduce noise, rotate and crop images, then the preprocessing was repeated using the Image Data Generator I of VGG16. This process took less than 2 minutes to analyze the data of 421 images (**Figure 3**).

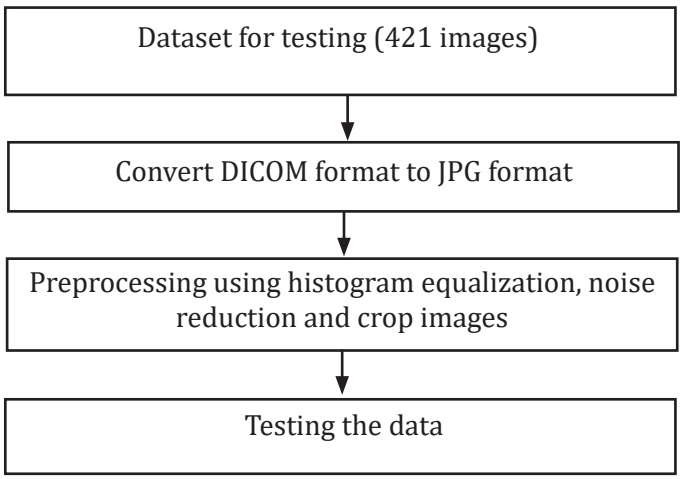

Figure 3. Training procedures

Table 1. Demographic data of enrolled patients

	number	%
Sex		
Male	56	25.8
Female	161	74.2
Age		
Mean± SD	76.89±10.6	
Median (min-max)	80 (50-93)	
Sideding		
Left	116	53.5
Right	101	46.5
Area		
Intertrochanteric fracture	108	49.8
Neck of femur fracture	102	47.0
Subtrochanteric fracture	6	2.7
Femeral head fracture	1	0.5

Table 2. Analysis of interpreted radiographics by AI

Gold Standard	Positive	Negative	Total
Abnormal	194	23	217
Normal	56	148	204
Total	250	171	421
		95%CI	
Sensitivity (%)	89.40	84.50	93.20
Specificity(%)	72.50	65.90	78.50

Table 2. (Continued)

Gold Standard	Positive	Negative	Total
Positive Predictive Value (%)	77.60	71.90	82.60
Negative Predictive Value (%)	86.50	80.50	91.30
Accuracy (%)	81.24	77.17	84.85

Table 3. Analysis of interpreted radiographics by the orthopedic surgeon

Gold standard	Positive	Negative	Total
Abnormal	196	21	217
Normal	6	198	204
Total	202	219	421
		95%CI	
Sensitivity (%)	90.30	85.60	93.90
Specificity(%)	97.10	93.70	98.90
Positive Predictive Value (%)	97.00	93.60	98.90
Negative Predictive Value (%)	90.40	85.70	94.00
Accuracy (%)	93.59	90.80	95.73

Results

In total, 217 patients were enrolled in this study. Of these, 56 (28.5%) were male and 161 (74.2%) female. Areas of hip fractures were as follow: intertrochanteric (108, 49.8%), femoral neck (102, 47.0%), subtrochanteric (6, 2.7%) and femoral head (1, 0.5%) as shown in **Table 1**. The orthopedic surgeon and AI program revealed accuracy of 93.59% (95%CI 90.8-95.73) vs. 81.24% (95% CI 77.17-84.85), sensitivity of 90.30% (95% CI 85.60-93.90) vs. 89.40% (95%CI 84.50-93.20), and specificity of 97.10% (95%CI 93.60-98.90) vs. 72.5% (95%CI 65.90-78.50), respectively (**Tables 2 and 3**).

Discussion

The AI model (VGG16) used in this study revealed an accuracy of 81.24% which was less than that reported by Takaaki et al. (95.5%).[24] However, the accuracy of the orthopedic surgeon was similar between the two studies (93.59% vs. 92.22%). The difference resulted from the quality of images such as shooting distances, brightness, clearance, noise and size of images. In this study, among 217 images of hip fractures, 21 (9.7%) were occult hip fractures of which the incidence of the occult hip fracture was 3 to10 %[18, 19]: this could confound the accuracy of AI. The accuracy of AI used in this study could not detect the occult hip fracture to the same degree as those of the orthopedic surgeon. Preprocessing of images is an important procedure resulting in the accuracy of AI. In addition, the number of images, which were used for training AI procedures could have affected the accuracy of AI. In this study, 120 images (60 images of hip and 60 images of nonhip fractures) were used for the step of training procedures. Other related studies [24, 25] could provide more images for this step than our study. Takaaki et al. [24] used 2,678 images for training procedures of AI images (1,408 images of hip and 1,270 images of nonhip fractures) while Cheng et al. [25] used 3,605 images for training procedures of AI images (1,975 images of hip and 1,630 images of nonhip fractures). Additionally, the area of hip fractures used in

the study of Takaaki et al. was specific at the intertrochanteric area of the femur while those in the study of Cheng et al. was specific at the neck and intertrochanteric area of the femur. Compared with our study, the areas of hip fractures starting from the head to the subtrochanteric area of the femur were used covering larger areas than those of related studies. Different areas had varieties of bone architectures and artifacts that reduced the accuracy of AI. Thus, one advantage of our study was being able to detect overall areas of the hip fractures.

The competence of AI for detecting hip fractures using plain radiographs was reliable as a screening tool to diagnose hip fracture because the sensitivity did not significantly differ from the performance of the orthopedic surgeon, 89.40% (95%CI= 84.50-93.20) vs. 90.30% (95% CI =85.60-93.90), respectively. Further studies to develop the competence of AI are required using more images for the AI training step as well as improving qualities of images in the pre-processing image. In the future, multihospital assessment of hip fractures would also be required to validate the developed AI model.

Conclusion

Our results showed that the AI model (VGG16) showed a sensitivity of 89.40% vs. 90.30% obtained from the orthopedic surgeon. However, improvement in the accuracy of AI software, both sensitivity and specificity, is further required. In the future, AI models have the potential to be a useful tool for emergent screening and evaluation of patients with hip fractures using plain radiographs, especially in the Emergency Department where orthopedic surgeons may not be readily available.

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CORRELATION BETWEEN CLINICAL AND PATHOLOGIC FEATURES OF DIABETIC NEPHROPATHY

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Background: Diabetic nephropathy is the most common cause of end stage renal disease in Thailand. Renal biopsy remains the gold standard investigation to diagnose and classify diabetic nephropathy.
Objectives: In this study, we aimed to evaluate the correlation between clinical parameters and renal pathology classification among patients with type 2 diabetic and nephropathy.
Methods: We conducted an observational study and enrolled 63 patients undergoing renal biopsy between 1 January 2014 and 31 December 2018. Pathologic classification established by the Renal Pathology Society was used to assess the severity of histologic lesions in diabetic nephropathy. Clinical parameters including age, sex, duration, presence of diabetic retinopathy, blood urea nitrogen, creatinine, urine protein creatinine ratio, fasting plasma glucose and hemoglobin A1C were collected.
Results: At the time of biopsy, mean age was 50.25±11.46 years. Median duration of diabetes mellitus was 10 years with interquartile range (IQR) 3.75-12.00 years, mean serum creatinine was 2.44 ± 1.31 mg/dL and estimated glomerular filtration rate was 22.41±12.16 mL/min/1.73 m^2. Based on the glomerular classification, 1 patient (1.6%) was in class I, 16 (25.3%) in class II, 25 (39.7%) in class III and 21 (33.3%) in class IV. Using multivariate analysis, class IV was associated with rising serum creatinine compared with class II [adjusted odds ratio (AOR)= 2.58; 95% CI= 1.13-5.89]. Patients with interstitial fibrosis and tubular atrophy (IFTA) <25%, 25-50% and >50% were observed in 10, 27 and 22 patients, respectively. Patients with IFTA >50% were significantly associated with duration of diabetes (OR=1.27; 95%CI=1.21-1.57), serum creatinine (OR=3.92; 95%CI=1.34-11.48) and urine protein (OR= 1.25; 95%CI=1.01-1.55) compared with patients with IFA<25%. Using multivariate analysis, only serum creatinine (AOR=3.48; 95%CI=1.23-12.65) was confirmed as independently correlated to IFTA >50% compared with IFTA <25%. A univariate analysis revealed no significant correlation between vascular indexes and renal function.
Conclusion: The results revealed that advanced glomerular lesions and high IFTA >50% correlated with impaired renal function in type 2 diabetic nephropathy.

Keywords: Diabetic nephropathy, Arteriolar hyalinosis, Interstitial fibrosis and tubular atrophy, Renal function

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Introduction

Almost 40% of patients with type 2 diabetes in a nationwide cross-sectional study in Thailand presented impaired glomerular filtration rate.[1] Diabetic nephropathy is the major cause of end stage renal disease worldwide and exhibits a higher risk of mortality, mostly from cardiovascular complications.[2] Renal complications reflect a complex pathophysiology, whereby various genetic and environmental factors determine susceptibility and progression to end stage renal disease.[3] Renal pathology and structure defect are detected even at the onset of diabetes mellitus and further development of renal changes in advanced stages of diabetic nephropathy.[4] Renal biopsy remains the gold standard investigation to diagnose and classify diabetic nephropathy. Patients with type 2 and advanced nodular glomerulosclerosis have structural-functional relationships similar to type 1, and tubulointerstitial and mesangial expansion correlates with renal function. [5]

The Research Committee of the Renal Pathology Society developed a consensus classification of diabetic nephropathy.The classification is based on glomerular, tubulointerstitial and vascular lesions. This classification showed good interobserver agreement, improved problematic definitions of diabetic glomerulopathy and was easy to use in clinical practice.[6] One study documented that renal pathologic diagnosis showed a good predictor for renal prognosis in type 2 diabetes, [7] but limited studies have yet elucidated the correlation between renal pathologic and clinical parameters according to this classification system.[8] In this study, we aimed to identify the correlation between evidence-based clinical factors and renal pathologic findings according to Tervaert's pathologic classification among patients with diabetic nephropathy.[6]

Methods

The study was approved by the Ethics Committee of the Institute Review Board, Royal Thai Army. We collected patients with biopsy-confirmed diabetic nephropathy from January 2014 to December 2018 at Phramongkutklao Hospital. Enrolment criteria consisted of patients with type 2 diabetes as defined by the American Diabetes Association criteria [9] and documented diabetic nephropathy were enrolled in the study. The sample size was calculated at 82 patients to reach statistical power of 80%with a type I error of 5.[10] Exclusion criteria included other glomerular diseases and inadequate tissue biopsy with less than eight glomeruli. The study was conducted under the provisions of the Declaration of Helsinki and the protocol was approved by the local ethics committee. Informed consent was obtained at the time of registry enrollment.

Baseline clinical and laboratory data were collected at the time of biopsy. Clinical data were recorded for each patient at the time of biopsy including age, sex, weight, duration of disease and diabetic retinopathy. Laboratory variables including fasting plasma glucose, hemoglobin A1C (HA1C), urine examination, serum creatinine and urine protein creatinine ratio, were measured at the time of renal biopsy.

All histologic diagnoses were sent to a single renal pathologist, unaware of patients' clinical data, to evaluate the biopsies according to Tervaert's pathologic classification.[6] The glomerular classification was as follows: class I, only glomerular basement membrane (GBM) thickening; class II, mesangial expansion; class III, nodular sclerosis and class IV, global glomerulosclerosis in more than 50% of glomeruli. Interstitial fibrosis and tubular atrophy (IFTA) scores were classified as follows: 0, absent; 1, less than 25; 2, 25 to 50% and 3, greater than 50% of the total area. Interstitial inflammation was scored as follows: 0, absent; 1, inflammation only in relation to IFTA and 2, inflammation in areas without IFTA. Arteriolar hyalinosis was scored as follows: 0, absent; 1, hyalinosis present in a minimum of one arteriole; and 2, more than one arteriole present in the total area. Arteriosclerosis was scored in the most severely affected artery as follows: 0, no intimal thickening; 1, intimal thickening less than the thickness of the tunica media and 2, intimal thickening greater than the thickness of the tunica media. For every patient, the histologic slides included hematoxylin and eosin, periodic acid–Schiff, Masson trichrome and periodic acid methenamine silver stains for light microscopy.

The study exclusively analyzed morphologic data; however, immunofluorescence (IgG, IgA, IgM, C3, C1q, fibrinogen, kappa and lambda light chains) was always performed to confirm the central diagnosis.

Statistical analysis

Descriptive data with mean ± standard deviation and median with interquartile range (IQR) were expressed on the histologic and clinical data collected at the time of biopsy. The Chi-square was used for categorical variables comparing renal histologic groups. The independent t-test, one-way analysis of variance, Mann-Whitney U and Kruskal-Wallis tests were used for continuous variables comparing renal histologic groups. Univariate and multivariate logistic regression analyses were performed to identify clinical factors associated with severity of renal pathologic changes in diabetic nephropathy. Statistical analysis was performed using SPSS Software (SPSS, Version 20, Chicago, IL, USA). All tests were two-sided with a significance level of 0.05.

Results

A total of 63 patients with biopsy-proven diabetic nephropathy were enrolled in the study. The patients were male (n=22, 34.9%), mean age at renal biopsy was 50.25±11.46 years and the median onset of type 2 diabetes preceding the time of renal biopsy was 10 years with IQR 3.75 to 12.00 years. The baseline renal profiles included urine protein creatinine ratio of 7.3 with IQR 4.7-11.3 g/gCr, BUN of 32.85±16.50 mg/dL, serum creatinine of 2.44±1.31 mg/dL and HA1C of 7.83±2.16%. The glomerular and IFTA classification, 33.3, 39.7, 25.3 and 1.6% of patients comprised classes IV, III, II and I, respectively and 34.9, 42.9 and 15.9% had IFTA <25%, 25 to 50% and >50, respectively. For one patient in glomerular class I, electron microscopy confirmed GBM thickening according to Tervaert's pathologic classification. The baseline clinical characteristics are summarized in **Table 1**.

Table 1. Characteristics of enrolled patients

Clinical characteristic	n = 63
Male	22 (34.9%)
Age± SD (years)	50.25±11.46
Median duration of DM (years)	10 (3.75 to 12.00)
Diabetic retinopathy	45 (71.4%)
BUN (mg/dL)	32.85±16.50
Creatinine (mg/dL)	2.44±1.31
Estimated glomerular filtration rate (mL/min/1.73 m^2)	22.41±12.16
Median urine protein creatinine ratio (g/g Creatinine)	7.3 (4.7 to 11.3)
Fasting plasma glucose (mg/dL)	165.6±82.1
Hemoglobin A1C (%)	7.83±2.16
Diabetic nephropathy	
Class I	1 (1.6%)
Class II	16 (25.4%)
Class III	25 (39.7%)
Class IV	21 (33.3%)
Interstitial fibrosis and tubular atrophy (IFTA) (N=59)	
< 25%	10 (16.9%)
25-50%	27 (45.7%)
> 50%	22 (37.2%)

Table 1. Characteristics of enrolled patients (Continued)

Clinical characteristic	n = 63
Interstitial inflammation (N=59)	
Absent	1 (1.6%)
Infiltration only relation to IFTA	57 (96.6%)
Infiltration in areas without IFTA	1 (1.6%)
Arteriolar hyalinosis (N=63)	
Absent	2 (3.2%)
At least one area of arteriolar hyalinosis	12 (19.0%)
More than one area of arteriolar hyalinosis	34 (54.0%)
Inadequate tissue diagnosis	15 (23.8%)
Presence of large vessels arteriosclerosis (n=63)	
No intimal thickening	6 (9.5%)
Intimal thickening less than thickness of media	13 (20.6%)
Intimal thickening greater than thickness of media	15 (23.8%)
Not present	29 (46.0%)

Data presents as mean±SD, median with IQR and percentage

Comparisons of pathologic findings among the groups according to glomerular lesions are shown in **Table 2**. Significant differences were found in BUN and serum creatinine between IFTA<25%, 25 to 50% and >50% (**Table 3**). No significant differences were found regarding age, serum creatinine, urinary protein excretion rate or clinical factors between patients with and without arteriolar hyalinosis (**Table 4**). A significant difference was found in age and HA1C between patients with and without large vessel arteriosclerosis (**Table 5**). Overall, no clinical findings differed among class II, III and IV groups, except serum creatinine was significantly higher in class IV (3.4 with IQR=2.0-4.7) than those in class III (1.80 with IQR=1.49-2.75) and class II (1.93 with IQR=1.47-2.75) groups (p=0.003). The initial step using univariate analysis for all clinical factors associated with the glomerular classification was analyzed. On univariate analysis, and compared with glomerular class II, glomerular class IV was found to be significantly associated with serum creatinine (OR=2.37; 95%CI=1.21-4.65). Univariate analysis indicated that duration of type 2 diabetes (OR=1.27; 95%CI=1.03-1.57), serum creatinine (OR= 3.92; 95%CI=1.34-11.48) and urine protein creatinine ratio (OR=1.25; 95%CI=1.01-1.55) were associated with severity of IFTA >50 compared with IFTA<25%. (**Table 6**). However, concerning univariate analysis, presence of large vessels arteriosclerosis was found to be insignificantly associated with any clinical findings. (**Table 6**). Using multivariate analysis, only serum creatinine [adjusted OR (AOR)=3.48; 5%CI=1.23 to 12.65] was confirmed as independently correlated with IFTA >50% compared with IFTA <25 after adjusting for age, duration of type 2 diabetes, serum creatinine and urine protein creatinine ratio (**Table 7**).

Discussion

Renal biopsy is considered the gold standard to evaluate histologic findings and assess severity of diabetic nephropathy lesions. Renal pathology could provide additional information concerning patient outcomes. However, the relationship between clinical parameters and renal histologic findings remain controversial regarding the definitions of Tervaert's pathologic classification and limiting its use in clinical practice. The main finding in our study confirmed that advance glomerular lesions in class IV and high tubulointerstitial fibrosis scores (>50) were independently related factors to impaired renal function among Thai patients with diabetic nephropathy.

Table 2. Clinical findings according to glomerular classification

	Class II (n=16)	Class III (n=25)	Class IV (n=21)	*p*-value
Age (years)	52.31±15.24	48.84±9.62	50.57±10.72	0.264
Gender, male, N (%)	6 (37.5)	8 (32.0)	8 (38.1)	0.894
Duration of DM (years)	10.0 (5.0-18.0)	7.0 (1.0 -10.5)	10.0 (5.2 -12.0)	0.256
BUN (mg/dL)	26.0 (17.2-35.6)	28.0 (21.5 -43.5)	40.0 (25.7 - 44.5)	0.099
Creatinine (mg/dL)	1.93 (1.47-2.75)	1.80 (1.49 -2.75)	3.4 (2.0-4.7)*	0.003
Urine protein creatinine ratio (g/gCr)	6.74 (4.32 -7.62)	7.26 (4.85-12.65)	8.4 (5.7 -14.0)	0.189
Fasting plasma glucose (mg/dL)	154.0 (112.0-221.5)	137.0 (115.0 -175.0)	158.0 (103.0-212.0)	0.901
Hemoglobin A1C (%	7.6 (7.0-8.3)	6.85 (6.4 -8.2)	7.3 (6.8-8.8)	0.490
Diabetic retinopathy, N (%)	9 (56.3)	20 (80.0)	16 (76.2)	0.143

Data presents as mean±SD, median with IQR and percentage. *$p < 0.05$ versus class II.

Table 3. Clinical findings according to interstitial fibrosis and tubular atrophy

	IFTA <25% (n=10)	IFTA 25-50% (n=27)	IFTA >50% (n=22)	*p*-value
Age (years)	54.10±11.63	50.63±12.03	47.95±10.14	0.688
Gender, male, N (%)	1 (10)	11 (40.7)	8 (36.4)	0.205
Duration of DM (years)	4.5 (0.9 -10.0)	10.0 (2.0-12.5)	10.0 (6.7-12.2)	0.118
BUN (mg/dL)	20.6 (11.5-37.2)	25.7 (20.5-40.0)*	39.4 (25.3-45.5)*	0.038
Creatinine (mg/dL)	1.28 (0.81-2.08)	1.80 (1.60-2.60)*	2.96 (2.07-4.66)*	0.001
Urine protein creatinine ratio (g/gCr)	5.60 (1.17-7.57)	7.30 (5.50-12.90)	8.96 (5.59-13.2)	0.093
Fasting plasma glucose (mg/dL)	173.5 (120.5-208.7)	145.0 (121.0-183.0)	127.5 (103.2-205.7)	0.367
Hemoglobin A1C (%	7.65 (6.77-9.80)	7.10 (5.95-8.07)	7.30 (6.67-7.97)	0.564
Diabetic retinopathy, N (%)	5 (50)	19 (70.4)	18 (81.8)	0.222

Data presents as mean±SD, median with IQR and percentage. *$p < 0.05$ versus IFTA<25%.

Table 4. Clinical findings according to arteriolar hyalinosis

	Absent arteriolar hyalinosis (n=2)	At least one area (n=12)	More than one area (n=34)	*p*-value
Age (years)	52.00±8.48	51.42±13.05	49.41±11.43	0.617
Gender, male, N (%)	1 (50.0)	4 (33.3)	11 (32.4)	1.000
Duration of DM (years)	4.0-7.0	10.0 (2.0-14.0)	7.5 (2.0-10.0)	0.376
BUN (mg/dL)	8.8-14.9	26.4 (20.2-35.5)	33.5 (23.0 -45.5)	0.271
Creatinine (mg/dL)	1.1-1.4	1.80 (1.19-2.78)	2.25 (1.67-3.34)	0.202
Urine protein creatinine ratio (g/gCr)	2.80-5.91	6.87 (5.35-12.19)	7.5 (5.42-12.92)	0.680
Fasting plasma glucose (mg/dL)	104.0 -146.0	175.0 (130.7- 210.2)	152.0 (113.7-201.2)	0.341
Hemoglobin A1C (%	6.9 -8.0	8.0 (5.6-11.0)	7.2 (6.4 -8.3)	0.663
Diabetic retinopathy, N (%)	1 (50.0)	6 (50.0)	25 (73.5)	0.410

Data presents as mean±SD, median with IQR and percentage. Compared between arteriolar hyalinosis at least one area and arteriolar hyalinosis more than one area

Table 5. Clinical findings according to large vessels arteriosclerosis

	No intimal thickening (n=6)	Intimal thickening less than thickness of media (n=13)	Intimal thickening greater than thickness of media (n=15)	*p*-value
Age (years)	42.33±11.69	51.54±12.34*	50.33±7.45*	0.027
Gender, male, N (%)	1 (16.7)	2 (15.4)	5 (33.3)	0.488
Duration of DM (years)	8.5 (6.2-12.0)	3.0 (1.0-12.2)	7.0 (3.0-10.0)	0.427
BUN (mg/dL)	40.0 (17.9 -55.3)	28.0 (20.2-39.4)	31.5 (24.0-47.0)	0.639
Creatinine (mg/dL)	2.68±1.61	1.90±0.88	2.68±1.65	0.401
Urine protein creatinine ratio (g/gCr)	8.90 (6.40-9.03)	9.35 (6.25-13.90)	7.10 (4.41-11.29)	0.437
Fasting plasma glucose (mg/dL)	174.5 (118.2-251.0)	162.0 (151.0-195.0)	136.0 (103.0-212.0)	0.685
Hemoglobin A1C (%	6.0 (5.4-7.2)	7.0 (6.8-8.1)	8.1 (7.6-9.8)*	0.012
Diabetic retinopathy, N (%)	4 (66.7)	6 (46.2)	11 (73.3)	0.605

Data presents as mean±SD, median with IQR and percentage. *$p < 0.05$ versus no intimal thickening

Table 6. Univariate analysis of clinical factors associated with severity of glomerular class, IFTA and arteriolar hyalinosis

	Class IV vs. class II (OR with 95%CI)	*p*-value	IFTA >50% vs IFTA <25% (OR with 95%CI)	*p*-value	Arteriolar hyalinosis more than one area vs. at least one area (OR with 95%CI)	*p*-value
Age (years)	0.99 (0.94 to 1.04)	0.676	0.95 (0.88 to 1.02)	0.144	0.99 (0.93 to 1.04)	0.609
Gender, male, N (%)	1.03 (0.27 to 3.92)	0.970	5.14 (0.55 to 48.37)	0.152	0.96 (0.24 to 3.87)	0.950
Duration of DM (years)	0.96 (0.86 to 1.07)	0.420	1.27 (1.03 to 1.57)*	0.027	0.93 (0.83 to 1.04)	0.207
BUN (mg/dL)	1.06 (0.99 to 1.12)	0.056	1.06 (0.99 to 1.12)	0.084	1.04 (0.98 to 1.07)	0.485
Creatinine (mg/dL)	2.37 (1.21 to 4.65)*	0.012	3.92 (1.34 to 11.48)*	0.013	2.06 (0.99 to 1.09)	0.154
Urine protein creatinine ratio (g/gCr)+	1.19 (0.99 to 1.43)	0.061	1.25 (1.01 to 1.55)*	0.040	0.99 (0.86 to 1.14)	0.900
Fasting plasma glucose (mg/dL)	0.99 (0.59 to 12.02)	0.318	0.99 (0.99 to 1.00)	0.165	0.99 (0.99 to 1.01)	0.666
Hemoglobin A1C (%)	1.00 (0.72 to 1.39)	0.993	0.81 (0.55 to 1.19)	0.291	0.92 (0.69 to 1.21)	0.538
Diabetic retinopathy, N (%)	2.67 (0.59 to 12.02)	0.202	4.80 (0.79 to 28.90)	0.087	2.38 (0.52 to 10.86)	0.263

*$p < 0.05$ versus control group

Table 7. Multivariate analysis of clinical factors associated with severity of glomerular class, IFTA and arteriolar hyalinosis

	Class IV vs. class II (OR with 95%CI)	Class IV vs. class II (Adjusted OR with 95%CI)[a]	IFTA >50% vs IFTA <25% (OR with 95% CI)	IFTA >50% vs IFTA <25% (Adjusted OR with 95% CI)[a]
Age (years)	0.99 (0.94-1.04)		0.95 (0.88-1.02)	
Duration of DM years)	0.96 (0.86-1.07)		1.27 (1.03-1.57)*	
BUN (mg/dL)	1.06 (0.99-1.12)		1.06 (0.99-1.12)	
Creatinine (mg/dL)	2.37 (1.21-4.65)*	2.58 (1.13-5.89)*	3.92 (1.34-11.48)*	3.48 (1.23-12.65)*
Urine protein creatinine ratio (g/gCr)	1.19 (0.99-1.43)		1.25 (1.01-1.55)*	

*$p < 0.05$ versus control group

[a] adjusted for age, duration of type 2 diabetes, serum creatinine and urine protein creatinine ratio

The impact of clinical renal involvement on long term prognosis in type 2 diabetes has been described in several studies. Our results demonstrated that serum creatinine levels in glomerulopathic class IV were significantly greater than those in other glomerular classes in type 2 diabetes. These results agreed with several cross-sectional studies demonstrating the relationship of renal histologic lesions with clinical features among patients with diabetes and nephropathy.[11, 12] Nevertheless, one study showed no significant differences in renal survival rates between glomerular classes.[8] However, advanced diabetic glomerulosclerosis in class IV exhibited the lowest estimated glomerular filtration rate and the worst five-year renal survival rate among patients with type 2 diabetes.[7] Several studies indicated an association between the severity of renal histological injury and renal prognosis.[13-15] Thus, quantitative scoring of glomerulosclerosis according to Tervaert's pathologic classification might be beneficial in determining worsening renal outcomes.

Nodular glomerulosclerosis reflected an advanced stage of nephropathy. Patients with type 2 diabetes and Kimmelstiel-Wilson nodules or at least glomerular class III according to Tervaert's pathologic classification revealed elevated serum creatinine compared with patients without nodular glomerulosclerosis.[16] Our results did not demonstrate significantly increased serum creatinine levels in glomerular class III, compared with glomerular class II. Similar to one study, the nodular glomerulosclerosis index did not correlate to renal function and proteinuria.[17] Nodular glomerulosclerosis might be a weaker predictor of renal progression than established indicators such as IFTA score. Moreover, several patients showed that the severity of interstitial lesions did not correlate with glomerular lesions [18] and the classification of nodular glomerulosclerotic lesions might have been influenced by sampling bias due to the number of glomeruli obtained by renal biopsy. Further studies are needed to determine the pathophysiologic change and clinical significance of nodular glomerulosclerosis in type 2 diabetes.

Abnormalities in tubulointerstitial lesions are important when assessing the outcome of patients with type 2 diabetes.[19-21] Tervaert's pathologic classification focuses on tubular atrophy and interstitial fibrosis in diabetic nephropathy. The majority of patients (87.8%) included in our study had IFTA >25%; this renal chronicity score related to poor renal function and high proteinuria. The findings were consistent with related prospective studies that identified a correlation between the degree of tubulo-interstitial injury with renal function [22] and interstitial lesions as a predictor for renal prognosis among patients with type 2 diabetes and overt proteinuria.[8] Early identification of ITFA among patients with type 2 diabetes could prompt more therapeutic interventions.

Arteriosclerosis manifests itself as a lesion of the intimal layer of the arterial wall and accumulation of plaque [23] and develops as a result of a multistep process ultimately leading to cardiovascular disease in type 2 diabetes. [24] Several studies have documented the development of atherosclerosis related to aging and chronic hyperglycemia. [25, 26] Our study also confirmed that a significant difference was found in aging and uncontrolled glycemic with renal vessel arteriosclerosis.

The main limitation encountered was the relatively small sample size. In addition, a single-center prospective study of clinical and histopathologic lesions among patients with type 2 diabetes, indicated our study may have been underpowered to detect meaningful histopathologic lesions and subjects might not be representative of all populations in type 2 diabetes. Second, only the cross-section study was evaluated. A further study of renal pathologic scores with main renal outcomes including double serum creatinine and long term renal replacement therapy is needed. Third, a high percentage of inadequate tissue diagnosis for vascular lesion and a limited sample regarding the interstitial inflammation were found in this study. Therefore, they could have resulted from assessing the correlation of vascular and interstitial pathological lesions and clinical findings. More clinical studies based on renal biopsy are needed to clarify the correlation between vascular lesions and tubulointerstitial inflammation in diabetic nephropathy.

Conclusion

In conclusion, advanced diabetic glomerulosclerosis (class IV) and high percentage of IFTA (>50%) according to Tervaert's pathologic classification were significantly associated with impaired renal function among patients with type 2 diabetes and overt proteinuria. These results indicated that the advanced glomerular and IFTA pathologic classification correlated with renal outcome in type 2 diabetic nephropathy.

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Disclosures

All authors report no conflicts of interest associated with the study.

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RADIOLOGIC CHARACTERISTICS OF COMPUTERIZED TOMOGRAPHY ATTENUATION IN RENAL CELL CARCINOMA

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Background: Renal cell carcinoma (RCC) is the most common kidney cancer in adults. Computed Tomography (CT) with contrast study is used to diagnose RCC. The enhancement in the nephrogenic phase more than 15 Hounsfield units (HU) is suspected of RCCs. However, this threshold HU shows 15-20% false positive results for RCCs.
Objectives: This study aimed to determine RCC enhancement in CT that was below the standard threshold and to analyze the attenuation range of RCCs in noncontrast CT.
Methods: Patients with pathological RCC and undergoing CT with contrast study were retrospectively reviewed. An average of attenuation values of three regions of interest (ROI) were measured in noncontrast and nephrogenic phases, by avoiding foci of calcification and peritumoral region. ROI values were calculated for enhancement and range of attenuation values in the noncontrast CT.
Results: A total of 152 pathologically RCCs were included in the study. Mean ± SD attenuation values were 32.54 ± 8.02 HU (range 13.3-57.23 HU) and 71.26 ± 33.1 HU (range 16.87-202.8 HU) for noncontrast and contrast CT, respectively. Thirty-one (20.4%) of RCCs did not reach 15 HU enhancement. Using multivariate analysis, significant differences among subtypes (p<0.001) and renal mass less than 7 cm (p<0.001) were observed. In noncontrast CT, using a range of 20-60 HU, 129 (84.9%) RCCs were entirely within this range. To improve the accuracy of RCC diagnosis, the combined use of both non-contrast attenuation group (<20 HU and >20 HU) and enhancement >15 HU could increase the accuracy to 96.7%.
Conclusion: One-fifth of RCCs did not reach the standard enhancement threshold that were mostly found in nonclear cell subtype. Especially, when the mass was larger than 7 cm or involved nonclear cell RCCs, the enhancement threshold >15 HU must be carefully used for diagnosis. Using a noncontrast phase regardless HU combined with enhancement >15 HU could improve the accuracy of RCC diagnosis.

Keywords: renal cell carcinoma, attenuation, Hounsfield unit, kidney, cancer

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Introduction

Renal cell carcinoma (RCC), the most common kidney cancer in adults, comprises approximately 90% of kidney cancers.[1] Incidence of RCC is 3.8 cases per 100,000 populations, while the incidence in Thailand is 1.6%.[2] Since 1971, incidence of renal mass has significantly increased due to increase use of Computed Tomography (CT) scan to diagnose other symptoms[3], particularly unenhanced CT has been increasingly used for kidney stones and CT colonography.[4]

CT scan with contrast study has been extensively recognized as a potential tool for diagnosis of RCC. Renal mass that is enhanced in nephrogenic phase on CT more than 15 Hounsfield units (HU) should be suspected of RCC.[5] Currently, many studies illustrated that an enhancement level above 15 HU indicated a false negative result for RCC diagnosis. A Canadian study found that around 17.2% of RCCs did not reach the 15 HU enhancement on CT studies.[6] Another study found that 15% of more than 15 HU enhancements in surgically resected renal masses were benign lesions.[7] Unenhanced CT has been increasingly used to evaluate other conditions, especially in patients who have chronic kidney diseases or allergy to contrast. Many studies concerning the noncontrast CT have suggested the range of HU more than 70 HU or less than 20 HU indicated benign lesion and required no further workup.[8-10]

This study aimed to determine whether RCC enhanced in CT is below the standard enhancement threshold and to analyze the HU range of RCC using the noncontrast CT where possible malignancy should be considered.

Methods

This retrospective descriptive study was approved by the Institutional Review Board of the Royal Thai Army Medical Department and acquisition of consent was waived (R041h/60). Using the Urology Department's database, Thai patients with renal cell carcinoma who were operated on, both radical and partial nephrectomy, at Phramongkutklao Hospital from January 2007 to December 2017 were enrolled in this study. The inclusion criteria included patients with pathologically proven RCCs with the size of renal lesion more than 1 cm. A related study has shown that renal lesions of less than 1 cm were too small to adequately characterize.[11] To reduce misunderstanding between each RCC and CT imaging, patients who had more than one mass in each kidney, polycystic kidney disease or von Hippel Lindau disease were excluded. The demographic data presenting symptoms and cell type of RCC were collected.

CT technique and interpretation

All patients were imaged with CT scanner (Brilliance CT 64, Phillips) after an intravenous bolus of 120 mL of contrast material with rate 3 mL/sec. Four phases of CT scan were acquired: unenhanced, corticomedullary, nephrographic and delayed phase. The CT studies were reviewed by a radiologist who was specialized in genitourinary imaging and was blinded to the final diagnosis. Renal masses were measured by placing similarly sized regions of interest (ROI), between 25 to 100 mm2 and avoiding areas of focal calcification and extreme periphery of the tumor to minimize effects from surrounding normal tissue. The mean HU were corrected in each ROI in the noncontrast and nephrogenic phase systematically as shown in **Figure 1.**

Statistical analysis

Mean HU in the noncontrast, contrast and enhancement were collected. Fisher's exact test was used to identify the affecting factor of the attenuation enhancement more and or less than 15 HU. Using logistic regression to analyze the factors that affected enhancement more than 15 HU, McNemar's test was used to see differentiation of sequence using HU between the noncontrast and enhancement in RCCs. Statistical analysis was performed using SPSS Software, Version 16.0 (SPSS Inc., Chicago, IL, USA).

A total of 152 patients with pathologically proven RCC included 37 women and 115 men, ages ranging from 31 to 89 years with mean age of 61 years. Of these, 66 patients (43.4%) had incidental findings including 130 (85%) clear cell RCC, 17 (11%) papillary RCC, 2 (1%) chromophobe RCC and 3 (2%) other types. The attenuation values, mean±SD, were 32.54±8.02 HU in the noncontrast CT, 71.26±33.10 HU in the contrast CT and 38.72±30.82 for the enhancement as shown in **Table 1**.

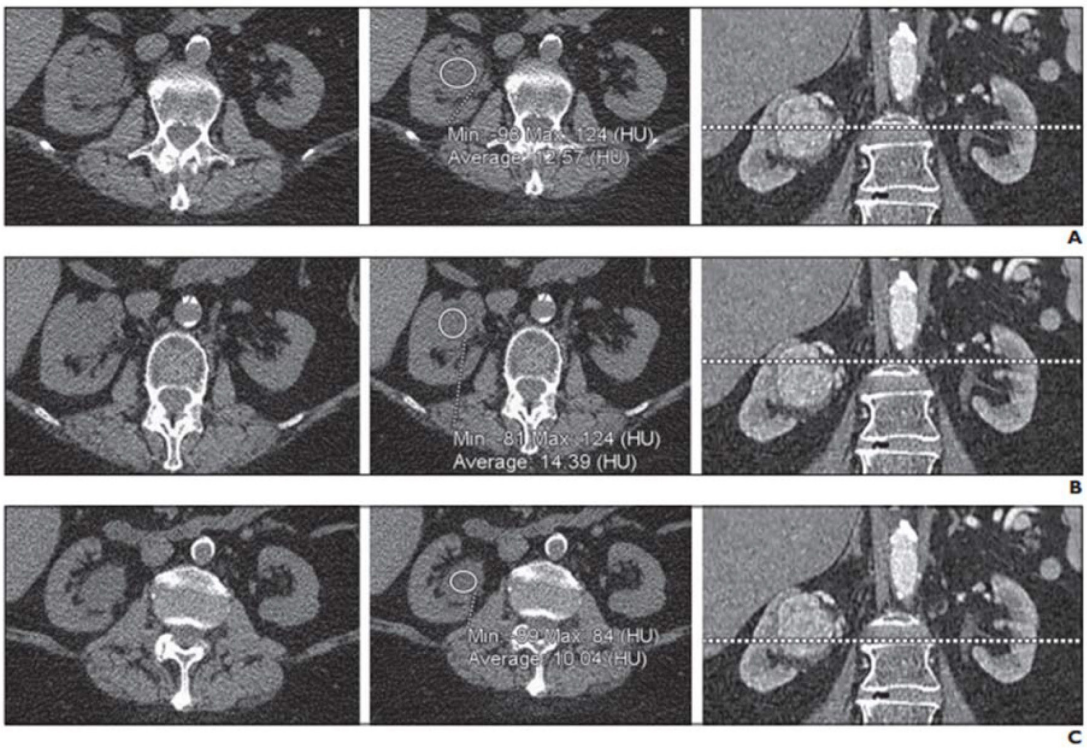

Figure 1. shows the regions of interest measurement (ROI) with separate renal mass in 3 parts by coronal image. **Picture A:** region of interest 1 is the mid part of renal mass. ROI was measured in Hounsfield attenuation in the noncontrast axial image. **Picture B:** region of interest 2 is midway between the middle and cranial margin. ROI was measured in Hounsfield attenuation in the noncontrast axial image. **Picture C:** region of interest 3 is midway between the middle and caudal margin. ROI was measured in Hounsfield attenuation in the noncontrast axial image. ROI was measured similar to the nephrogenic phase. Average attenuation of three measurements were recorded for each tumor.

Table 1. Clinical and pathological characteristics of patients

Characteristics		n = 152 (%)
Age (years)	Mean±SD	61.0±12.4
Gender	Male	115 (75.7)
	Female	37 (24.3)
Symptoms	Yes	86 (56.6)
	No	66 (43.4)
Histologic	Clear cell RCC	130 (85.5)
	Papillary RCC	17 (11.2)
	Chromophobe RCC	2 (1.3)
	Others	3 (2.0)
Location	Right	70 (46.0)
	Left	82 (54.0)
Size	<4 cm	35 (23.0)
	4 - 7 cm	38 (25.0)
	>7 cm	79 (52.0)

Table 1. Clinical and pathological characteristics of patients (Continued)

Characteristics		n = 152 (%)
Mass calcification	Yes	40 (26.3)
	No	112 (73.7)
Mass characteristic	Homogenous	40 (26.3)
	Heterogenous	112 (73.7)
Pattern	Endophytic	34 (22.4)
	Exophytic	118 (77.6)
Non-contrast HU	Mean±SD	32.54±8.02
	Median (min, max)	32.00 (13.30, 57.23)
Contrast HU	Mean±SD	71.26±33.10
	Median (Min, Max)	62.48 (16.87, 202.80)
Enhancement HU	Mean±SD	38.72±30.82
	Median (Min, Max)	32.12 (-3.20, 156.97)

*SD – standard deviation, RCC – renal cell carcinoma, HU – Hounsfield unit

We found that 31 RCCs did not reach the enhancement threshold at >15 HU. As a result, 20.4% of the RCCs could not be diagnosed because of no enhancement in the nephrogenic phase. Both cell type and size of tumor that affected enhancement were statistically significant (p <0.001). Using multiple logistic regression, enhancement of clear cells had 25 times greater than that of non-clear cells (AOR = 25.71, 95% CI=6.23-101.1). The size of tumor less than 7 cm also had 11 times greater enhancement. (AOR=11.52, 95% CI=2.846.6) after having adjusted with clear cell histologic subtype. (**Table 2**). Significant differences in proportion of different subtypes of RCC that did not reach the 15 HU enhancement threshold were observed, namely, 100% chromophobe RCC, 58.8% papillary RCC and 13.1% clear cell RCC (p<0.001). Using HU cut points in the noncontrast study, no significant differences between cell type groups were observed. (**Table 3**)

This study also analyzed the attenuation range of RCCs in the noncontrast CT. The minimum and maximum attenuations were 13.30 and 57.23 HU, respectively (32.54±8.02 HU). Minimum attenuation less than 20 HU was 23 (15%) of the tumor, and no tumor had a maximum attenuation greater than 60 HU. CT attenuation between 20 and 60 HU accounted for 84.9% of the RCCs. Accuracy of the standard 15 HU enhancement threshold was 79.6% while 20 to 60 HU in the noncontrast CT was 84.9%. The 20 to 60 HU attenuation gave more accuracy for RCC diagnosis than that of the standard contrast enhancement (**Table 4**).

DISCUSSION

Due to increased use of CT, more RCCs have been detected. Related studies have proposed where enhancement thresholds of 15 HU might lead to 17.2% misdiagnosis of RCCs especially in papillary RCC.[6] Therefore, this study aimed to evaluate the accuracy of the criteria set by enhancement of more than 15 HU of pathologically proven RCCs in Thai patients. The result was similar to the related study that 20.4% of RCCs did not reach 15 HU enhancement.

The classification of RCC is based on histopathology. Histological subtype is related to different tumor characteristics and cancer prognosis. Several studies found that attenuation values can differentiate RCC subtypes, especially in clear cells and other subtypes.[14] This study found that clear cell RCC appeared to be better enhanced than nonclear cell RCC. Due to high vascular supply and alveolar structure of histologic studies, the clear cell subtype showed higher enhancement of HU when compared with other subtypes.[15,16]

Table 2. Multiple logistic regression for factors of enhancement attenuation

	Enhancement <15 HU (%)	Enhancement ≥15HU (%)	*p*-value	Adjusted OR (95% CI)	*p*-value
Age (years) (mean±SD)	60.3±13.6	61.2±12.1	0.702		
Sex					
Male	21 (18.3)	94(81.7)	0.250		
Female	10 (27.1)	27 (72.9)			
Symptoms					
Yes	21 (24.5)	65 (75.5)	0.160		
No	10 (15.2)	56 (84.8)			
Histologic type					
Clear cell RCCs	17 (13.1)	113 (86.9)	**<0.001**	25.17 (6.23- 101.1)	**<0.001**
Non clear cell RCCs	14 (63.6)	8 (36.4)			
Location					
Right	17 (24.3)	53 (75.7)	0.296		
Left	14 (17.1)	68 (82.9)			
Size					
< 7 cm	6 (9.2)	67 (91.8)	**<0.001**	11.52 (2.8-46.6)	**0.001**
≥ 7 cm	25 (31.6)	54 (68.4)			
Size					
< 4 cm	4 (11.4)	31 (88.6)	0.133		
≥ 4 cm	27 (23.1)	90 (76.9)			
Mass Calcification					
No	21 (28.8)	91 (81.2)	0.701		
Yes	10 (25.0)	30 (75.0)			
Mass characteristic					
Homogenous	6 (15.0)	34 (85.0)	0.324		
Heterogenous	25 (23.1)	87 (76.9)			
Pattern					
Endophytic	3 (8.8)	31 (91.2)	0.057		
Exophytic	28 (23.7)	90 (76.3)			

*SD – standard deviation, RCCs – renal cell carcinoma, HU – Hounsfield unit

Table 3. Attenuation and enhancement of Hounsfield unit in subtype of renal cell carcinoma

	Cell type (%)				*p*-value*
	Clear cell RCC	Papillary RCC	Chromophobe RCC	Others	
Enhancement					**<0.001**
<15 HU	17 (13.1)	10 (58.8)	2 (100.0)	2 (66.7)	
>15 HU	113 (86.9)	7 (41.2)	0	1 (33.3)	
Non-contrast					0.457
<20 HU	21 (16.2)	1 (5.9)	0	1 (33.3)	
>20 HU	109 (83.8)	16 (94.1)	2 (100.0)	2 (66.7)	

*Fisher's exact test

Table 4. Accuracy of using attenuations between noncontrast and enhancement in RCC

	Enhancement>15(%)	Enhancement<15(%)	Total
Non-contrast>20	103 (67.8)	26 (17.1)	129 (84.9)
Non-contrast<20	18 (11.8)	5 (3.3)	23 (15.1)
Total	121(79.6)	31 (20.4)	152 (100.0)

McNemar's test=0.291

Other significant affecting factors enhancing threshold less than 15 HU was the tumor size. Tumor size less than 7 cm also had 11 times greater enhancement. Some studies have shown that pseudo-enhancement increases with smaller masses.[17, 18] Inversely, when the mass is larger than 7 cm or non-clear cell RCC, the use of enhancement threshold >15 HU must be carefully used for diagnosis.

The noncontrast CT has been used more frequently to diagnose disease in urologic and nonurologic conditions, in situations when patients have contraindication for contrast media. Incidental finding of renal mass is the most common extracolonic cancer identified in CT studies.[19] The role of HU in RCCs involving noncontrast CT studies has not been specified in other literature. However, several studies have shown that the mean attenuation of RCCs of noncontrast CT are within the range of 20 to 70.[20, 21] Homogenous renal mass with HU less than 20 HU or more than 70 HU can be considered benign without need for further investigation.[8-10, 22] Therefore, we used the lower range of attenuation between 20 to 60 HU in the noncontrast CT as a threshold to identify the length of pathologically proven RCC in Thai patients. Our study found that pathologically proven RCC in this range was 84.9%, similar to the study of Dustin Pooler et al.[13] Surprisingly, the results showed more accuracy than the standard enhancement (>15HU) in the nephrogenic phase at 79.6%. However, our study encountered limitations, not including the benign lesion of kidneys such as oncocytoma and angiomyolipoma in the study. As the result, we cannot conclude that the use of non-contrast with HU >20 was more accurate than enhancement in the nephrogenic phase.

Our study found that 15% of RCCs had an attenuation less than 20 HU. A related study showed that a few solid RCCs had attenuation like the water (-10 to 20 HU) on the noncontrast study, but all were clear cell subtypes with heterogeneous mass.[12] None of the RCCs had attenuation more than 60 HU which was similar to the results of the study by Jonisch et al.[9] We concluded that renal mass with an attenuation of 60 HU or higher of the noncontrast CT had a greater chance of representing a high attenuation cyst and not RCC. This study showed that most RCCs in this series could be measured at values between 20 and 60 HU with the noncontrast CT.

An unenhanced renal mass containing this range of attenuation should be carefully evaluated because of malignancy risk.

Accuracy of the standard 15 HU enhancement threshold and 20 to 60 HU in the non-contrast CT were 79.6% and 84.9%, respectively. Altogether 20 to 60 HU attenuation gave more accuracy for RCC diagnosis than the standard contrast enhancement. To improve the accuracy of RCC diagnosis, the combined use of both noncontrast attenuation group (<20 HU and >20 HU) and enhancement >15 HU could increase the accuracy to 96.7%.

In clinical practices, when malignancy from an imaging study is unsuspected (<20 HU or enhancement <15 HU), an active surveillance strategy was used to follow up the patient. As a result, tissue diagnosis from this group of patients could not be obtained. In this study, only one radiologist reviewed the CT scans. Thus, no inter-observers were used to examine the results of HU. Finally, our study only showed lower enhanced HU in nonclear cells compared with clear cell subtype of RCC. In future studies, comparison of HU among noncontrast, corticomedullary and nephrogenic phases could help differentiate subtypes of RCCs.

Conclusion

One fifth of RCCs did not reach the standard enhancement threshold (>15 HU). Clear cell RCCs and small renal mass (<7 cm) had more chance to receive enhancement of HU than those of the other groups. Inversely, when the mass was >7 cm or nonclear cell RCC, the use of enhancement threshold >15 HU must be carefully used for diagnosis. The renal lesion in the noncontrast CT for which attenuation ranged from 20 to 60 HU should be carefully evaluated because of malignancy risk. Using a noncontrast phase regardless HU combined with enhancement >15 HU could improve the accuracy of RCC diagnosis.

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Conflict of interest

The authors have no conflicts of interest to declare.

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CORRELATION BETWEEN RETICULOCYTE HEMOGLOBIN EQUIVALENT AND IRON STATUS IN PEDIATRIC CHRONIC KIDNEY DISEASE

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Abstract

Background: Anemia is a major complication of pediatric chronic kidney disease (CKD). Iron deficiency is one of the most common causes of anemia. Conventional markers of iron deficiency anemia, transferrin saturation (TSAT) and serum ferritin could be interfered with various factors. in CKD. Reticulocyte hemoglobin equivalent (Ret-He) is useful for assessing iron status among these patients.

Methods: A descriptive cross-sectional study enrolling children with CKD stage 3 and above was conducted between April and November 2021. Demographic information was also collected. Correlation of Ret-He, anemia indices and markers of iron status were analyzed.

Results: Among 50 participants, we found moderate positive correlations between Hb and Ret-He (r=0.518; p <0.001), Hct and Ret-He (r=0.403; p=0.004), and MCHC and Ret-He (r=0.667; p<0.001); a modest negative correlation between RDW and Ret-He (r=-0.616; p<0.001) and strong correlations between MCV and Ret-He (r=0.747; p<0.001) including MCH and Ret-He (r=0.865; p<0.001). No correlations between TSAT and Ret-He, serum ferritin and Ret-He, TSAT and Hb, or TSAT and Hct were observed. In addition, weak negative correlations between serum ferritin and Hb (r=-0.307; p=0.032) and between serum ferritin and Hct (r=-0.305; p=0.033) were detected. The median Ret-He was 28.42 ± 3.37 pg. Twenty-seven participants (54%) met the criteria for iron deficiency anemia (cut-off value <29 pg) of which 2 (4%) had absolute iron deficiency and 9 (18%) had functional iron deficiency defined by conventional markers.

Conclusion: Ret-He is a relevant marker of iron status among pediatric patients with CKD and correlates well with anemia indices which could help identify more patients with iron deficiency.

Keywords: Reticulocyte hemoglobin equivalent (Ret-He), Pediatric, Chronic kidney disease (CKD), Anemia, Iron deficiency

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Introduction

The worldwide prevalence of chronic kidney disease (CKD) is estimated to be 8 to 16% and continues to grow, driven by aging populations and increasing rates of obesity and type 2 diabetes mellitus.[1] Prevalence of 1.5 to 3.0 per 1,000,000 was found among children younger than 16 years old. The most common causes of CKD among children are urologic abnormalities (30-33%), glomerulopathies (25-27%), hereditary nephropathies (16%) and renal hypoplasia/ dysplasia (11%).[2] CKD affects children in many aspects such as growth, bone mineral density, hypertension, cardiovascular events and anemia. [3]

Anemia is one of the most common complications in pediatric CKD causing cognitive decline, decreased quality of life, cardiovascular risks, hospitalization and mortality. [4-7] Among adults, the National Kidney Foundation (NKF) has been working to improve patient outcomes by developing, disseminating and implementing the Dialysis Outcomes Quality Initiative (DOQI) Clinical Practice Guidelines (KDOQI), defining anemia as hemoglobin (Hb) <13.5 g/dL among males and <12 g/dL among females.[8] The most recent NKF-KDOQI clinical practice guidelines employed reference data from the National Health and Nutrition Examination Survey (NHANES) III to cite normative values among children and recommended initiating an evaluation for anemia when Hb levels fall below the age- and sex-specific 5th percentile value, although Hb differences by race are still not considered. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) have demonstrated that the prevalence of anemia among children was 73% at stage 3 CKD, 87% at stage 4 and higher than 93% at stage 5.[9] Anemia in CKD can be caused by erythropoietin deficiency, iron deficiency, increased hepcidin and iron-restricted erythropoiesis.[1, 3, 10, 11] Therefore, to evaluate iron status in CKD is important to manage properly.

Kidney Disease Improving Global Outcomes (KDIGO) recommended measuring serum ferritin and transferrin saturation (TSAT) to evaluate iron status and commencing oral iron or intravenous iron among patients undergoing hemodialysis (HD) when TSAT is ≤20% and ferritin is ≤100 ng/ml (≤100 μg/l).[12] However, interpreting serum ferritin and TSAT among patients with CKD as both measurements are confounded by inflammation and several other factors such as malnutrition, liver disease, and malignancy can give rise to high serum ferritin. TSAT is not actually measured but derived from measurement of serum iron and total iron binding capacity (TIBC). TIBC concentration in plasma is reduced by inflammation as it constitutes a negative acute-phase reactant. These implicate poor reliability of TSAT and serum ferritin in assessing iron status in CKD and end stage kidney disease (ESKD) exhibiting numerous confounding factors.[11, 13] Therefore, alternative measures were proposed to estimate available iron to incorporate in emerging erythrocytes. Reticulocyte hemoglobin content (CHr) is a measure using the Siemen blood count analyzer (Siemen ADVIA, Siemens Bayer Diagnostics, Tarrytown, NY, USA). As reticulocytes develop into erythrocytes within a few days, and hemoglobin production depends on available iron, the hemoglobin content of reticulocytes would represent short term changes in available iron for erythropoiesis. Another measure to assess iron bioavailability for emerging erythrocytes is reticulocyte hemoglobin equivalent (Ret-He), which is available using the Sysmex blood analyzers (Sysmex XN-series, Sysmex, Kobe, Japan). In this method, fluorescent markers were used to identify cellular RNA of red blood cell, reticulocytes and platelets. The reticulocyte hemoglobin equivalent (Ret-He) is calculated from a combination of fluorescence and forward scatter.[11] Both CHr and Ret-He are appropriate markers for assessing iron status among children with CKD as they measure a short half-life element that is dependent on availability of iron.

Among patients undergoing hemodialysis (HD), Brugnara et al. found that Ret-He is a reliable marker of cellular hemoglobin content and can be used to identify the presence of iron - deficient states. [14] Ret-He has been used as a marker of iron availability for erythropoiesis among adults on HD, although the cut-off values as well as specificity and sensitivity vary. [15]

Garzia et al. described an excellent diagnostic efficiency of Ret-He in evaluating patients needing iron support and demonstrated a strict correspondence between the classic CHr and the new Ret-He. [16] El-Halim et al. found that a statistically significant increase in Ret-He was observed after IV iron supplementation compared with baseline values. [17] The British Committee for Standards in Haematology guidelines for the laboratory diagnosis of functional iron deficiency 2013[18] and the National Institute for Health and Care Excellence (NICE) anemia guidelines 2015 update[19] recommended measuring CHr and Ret-He for diagnosis of iron deficiency. Analysis of CHr and Ret-He is performed on the same EDTA blood sample used for complete blood count; hence, this would require less blood sampling and reduce costs compared with traditional measures.[11] In CKD, absolute iron deficiency is likely to be present when TSAT is ≤20% and serum ferritin concentration is ≤100 ng/mL in predialysis and among patients on peritoneal dialysis (PD) or ≤200 ng/mL among patients undergoing hemodialysis. [20] Functional iron deficiency is usually characterized by TSAT ≤20% with elevated ferritin levels. [20]

Most studies of Ret-He were conducted among adults with CKD; however, studies in the pediatric population remain limited. Related studies of Ret-He among pediatric patients with CKD were conducted by Davidkova et al. [15] and Pinto et al. [21] In this study, we aimed to investigate the relationship between Ret-He and the currently-used traditional markers of iron and erythropoiesis among pediatric patients with CKD. We hypothesized that Ret-He could be a clinically useful tool to evaluate iron status in this population.

Methods

Study design and settings

A descriptive cross-sectional study of children with chronic kidney disease from stage 3 and above was conducted under the care of Phramongkutklao Hospital, Bangkok, Thailand. CKD classification was based on the KDIGO international guidelines.[22] This study was approved by the Ethics Committee of the Institutional Review Board, Royal Thai Army Medical Department; approval number R034h/64.

Participants

Between April and November 2021, children aged 6 months to 18 years with a diagnosis of CKD stages 3 to 5, as well as patients of post kidney transplant with impaired GFR (3T-5T) at the Division of Pediatric Nephrology, Phramongkutklao Hospital were enrolled in this study. Exclusion criteria included the presence of underlying diseases requiring regular blood transfusion, transfusion-dependent thalassemia, aplastic anemia, previous blood transfusion within three months, hemolytic anemia and chronic blood loss. Informed consent was obtained from all participants.

Data collection

Patients underwent history-taking, physical examination and laboratory tests. Data regarding age, sex, weight, height, causes of CKD, stage of CKD, mode of renal replacement therapy, history of kidney transplantation, thalassemia and hemoglobinopathy, erythropoietin use and iron supplement were collected. Anemia and iron status indices including Hb, hematocrit (Hct), serum iron, TIBC, serum ferritin, reticulocyte count, corrected reticulocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), c-reactive protein (CRP), serum albumin, serum creatinine (SCr), blood urea nitrogen (BUN) and Ret-He were measured. TSAT was calculated as TSAT = (serum iron divided by TIBC) × 100. [20] Glomerular filtration rate (eGFR) was calculated among all patients using Schwartz formula.[23]

Statistical analysis

Data were processed using SPSS Software, Version 21. Descriptive statistical analysis was performed; data with normal distribution was reported as mean ± standard deviation. Pearson's correlation coefficient was used to analyze relationships between Ret-He and Hb, Hct, serum iron, TIBC, serum ferritin, reticulocyte count, corrected reticulocyte count, MCV, MCHC,

MCH, RDW, CRP, SCr, BUN and TSAT, as well as correlations between TSAT and Hb, TSAT and Hct, serum ferritin and Hb, and serum ferritin and Hct. The prevalence of iron deficiency anemia (IDA) was calculated using the percentage of participants having Ret-He below 29 pg according to NICE anemia guidelines 2015 update.[19] Receiver operating characteristic (ROC) analysis was used to determine the performance of Ret-He in diagnosing iron deficiency. Results with p <0.05 were considered to be statistically significant.

Results

Patient characteristics

Fifty patients were enrolled in the study, and their characteristics are summarized in **Table 1**. The majority of participants were male (64%) with a mean age of 12.52 ± 5.18 years. The most

Table 1: Demographic and clinical characteristics of study population

Characteristics	N	%
Gender		
- Male	32	64
- Female	18	36
Age (yrs)		
- Mean ± SD	12.52 ± 5.18	
Weight (kg)		
- Mean ± SD	35.61 ± 20.07	
Height (cm)		
- Mean ± SD	130.71 ± 28.46	
Cause of CKD		
- CAKUT	17	34
- Glomerular disease	11	22
- Unknown	11	22
- Ischemia	3	6
- Other	8	16
CKD stage		
- 3	9	18
- 3T	16	32
- 4	8	16
- 4T	4	8
- 5	11	22
- 5T	2	4
Dialysis		
- Yes	12	24
- Hemodialysis	2	4
- Peritoneal dialysis	10	20
Transplantation		
- Yes	22	44

Table 1: Demographic and clinical characteristics of study population (Ext.)

Characteristics	N	%
Thalassemia		
- Hb E trait	3	6
- HbH disease	1	2
- No thalassemia	10	20
- Not tested	36	72
Erythropoietin		
- Receiving treatment	13	26
- Dose of erythropoietin (u/kg/week)	207.23 ± 142.74	
Iron supplement		
- Receiving treatment	26	52
- Dose of oral iron supplement (mg/kg/day)	3.09 ± 1.70	
- Route of administration		
o Oral	25	50
o IV	1	2

SD=standard deviation, yrs=years, kg=kilogram, cm=centimeter, CKD=chronic kidney disease, CAKUT= congenital anomalies of urinary tract and kidney, Hb= hemoglobin, u= unit, mg= milligram, IV= intravenous

common cause of CKD was CAKUT (34%). Most participants were in CKD stage 3T (32%). Twelve participants had undergone renal replacement therapy, two had hemodialysis, and ten had peritoneal dialysis. Twenty-two participants had undergone kidney transplantation (44%). Most patients were not tested for thalassemia (72%) and most were not receiving erythropoietin (74%). More than one-half of the patients received iron replacement therapy (52%), mostly via the oral route.

Anemia and iron status

Table 2 illustrated laboratory parameters of the participants. Mean Hb was 11.37 ± 2.12 g/dL; mean Hct was 35.24 ± 6.22 %.

Median Ret-He was 28.42 ± 3.37 pg. When applying a Ret-He threshold of 29 according to NICE anemia guidelines 2015 update [19], 27 participants (54%) met the criteria of IDA. On the other hand, when using conventional markers, two participants (4%) had absolute iron deficiency and nine (18%) had functional iron deficiency. One participant had TSAT <20% but lacked the data of serum ferritin. Thereby, a higher number of patients with iron deficiency could be identified with the use of Ret-He compared with conventional measures.

Linear regression analysis revealed moderate positive correlations between Hb and Ret-He (r=0.518; p <0.001) **(Figure 1)**, Hct and Ret-He (r=0.403; p=0.004) (**Figure 2)**, and MCHC and Ret-He (r=0.667; p<0.001) **(Figure 3)**. A modest negative correlation between RDW and Ret-He (r=-0.616; p<0.001) was depicted (**Figure 4)**. We also found strong correlations between MCV and Ret-He (r=0.747; p<0.001) **(Figure 5)** and between MCH and Ret-He (r=0.865; p<0.001) (**Figure 6)**. On the other hand, no correlations were found between TSAT and Ret-He (r=0.170; p=0.239) or serum ferritin and Ret-He (r=-0.021; p=0.887). Weak negative correlations between serum ferritin and Hb (r=-0.307; p=0.032), and serum ferritin and Hct (r=-0.305; p=0.033) were observed. However, no correlations between TSAT and Hb (r=-0.011; p=0.940) or TSAT and Hct (r=-0.030; p=0.838) were detected.

ROC analysis was performed to determine

Table 2. Laboratory parameters

Parameters	n	Mean ± SD
Hemoglobin (g/dL)	50	11.37 ± 2.12
Hematocrit (%)	50	35.24 ± 6.22
Serum iron (μg/dL)	50	78.50 ± 42.84
TIBC (μg/dL)	50	235.16 ± 54.35
Serum ferritin (ng/mL)	49	377.18 ± 347.59
Reticulocyte count (%)	50	1.79 ± 0.81
Correct reticulocyte count (%)	50	1.73 ± 0.75
TSAT (%)	50	33.93 ± 20.12
MCV (fl)	50	80.60 ± 8.08
MCHC (g/dL)	50	32.22 ± 1.39
MCH (pg)	50	26 ± 3.08
RDW (%)	50	14.54 ± 2.96
CRP (mg/L)	45	4.47 ± 8.19
Albumin (g/dL)	43	4.22 ± 0.69
SCr (mg/dL)	50	3.00 ± 2.84
eGFR (mL/min/1.73m^2)	50	31.74 ± 18.54
BUN (mg/dL)	50	36.07 ± 24.06
Ret-He (pg)	50	28.42 ± 3.37
- Ret-He below 29 pg		27 (54%)

TIBC = total iron binding capacity, TSAT = transferrin saturation, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, RDW = red cell distribution width, CRP = c-reactive protein, SCr =serum creatinine, eGFR= estimated glomerular filtration rate, BUN = blood urea nitrogen, Ret-He = reticulocyte hemoglobin equivalent

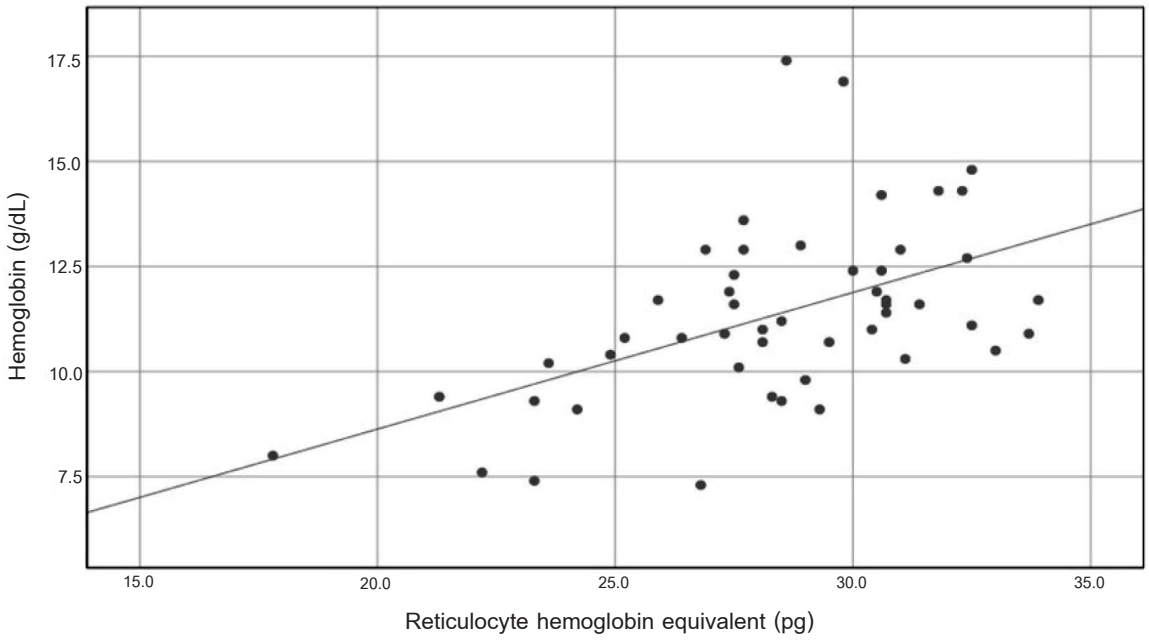

Figure 1. Correlation between hemoglobin and Ret-He

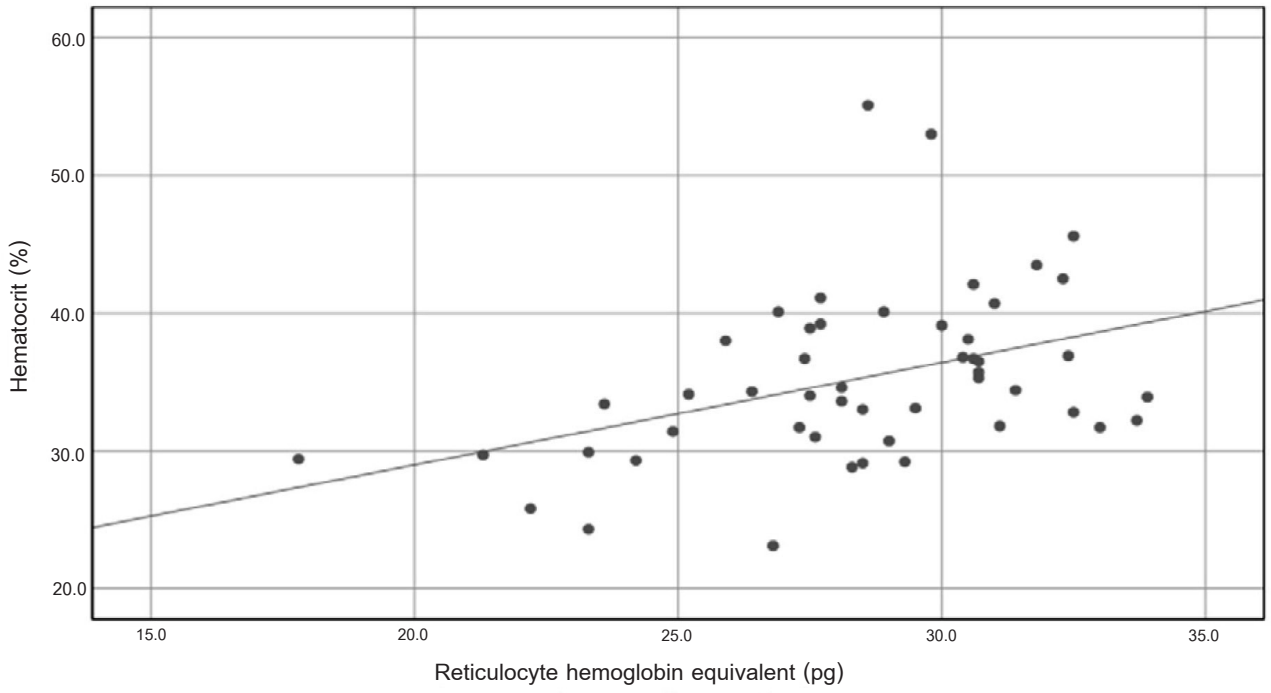

Figure 2. Correlation between hematocrit and Ret-He

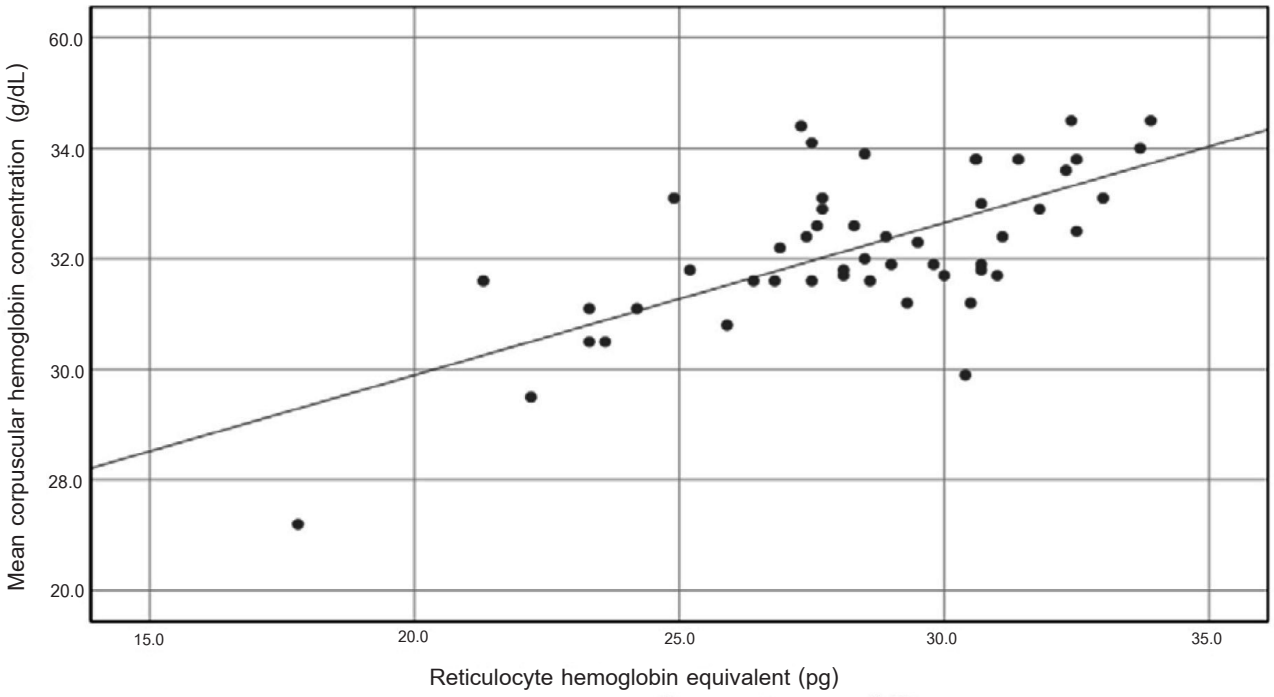

Figure 3. Correlation between mean corpuscular hemoglobin concentration and Ret-He

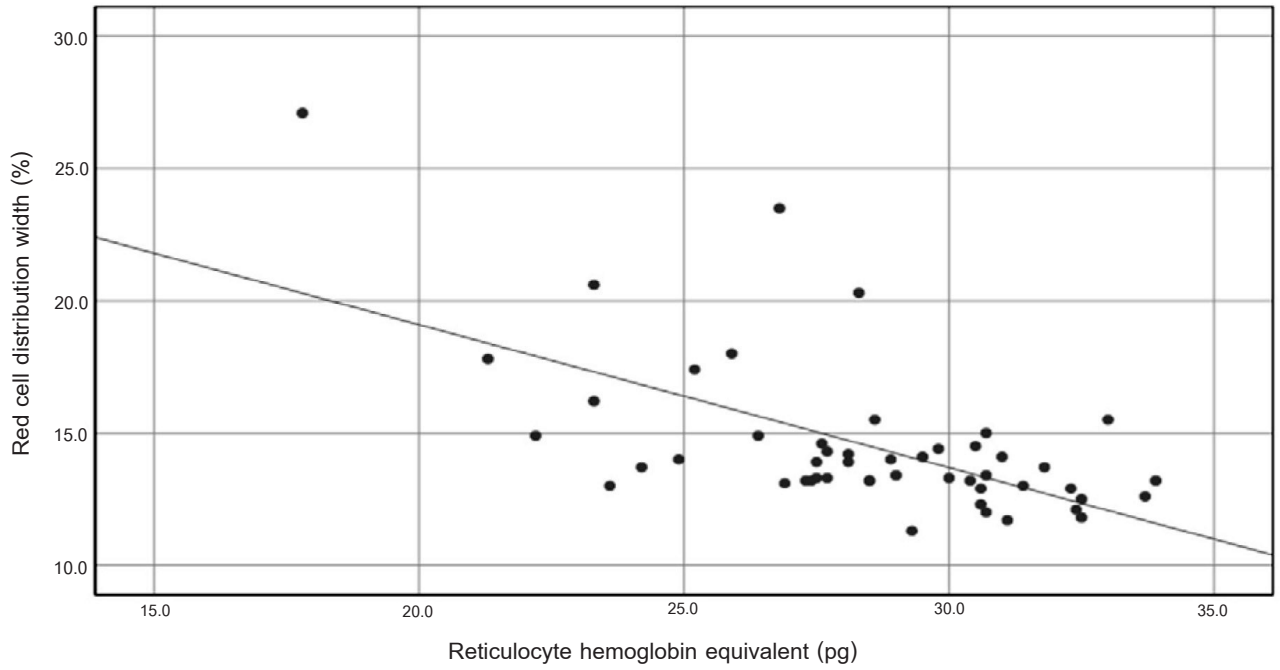

Figure 4. Correlation between red cell distribution width and Ret-He

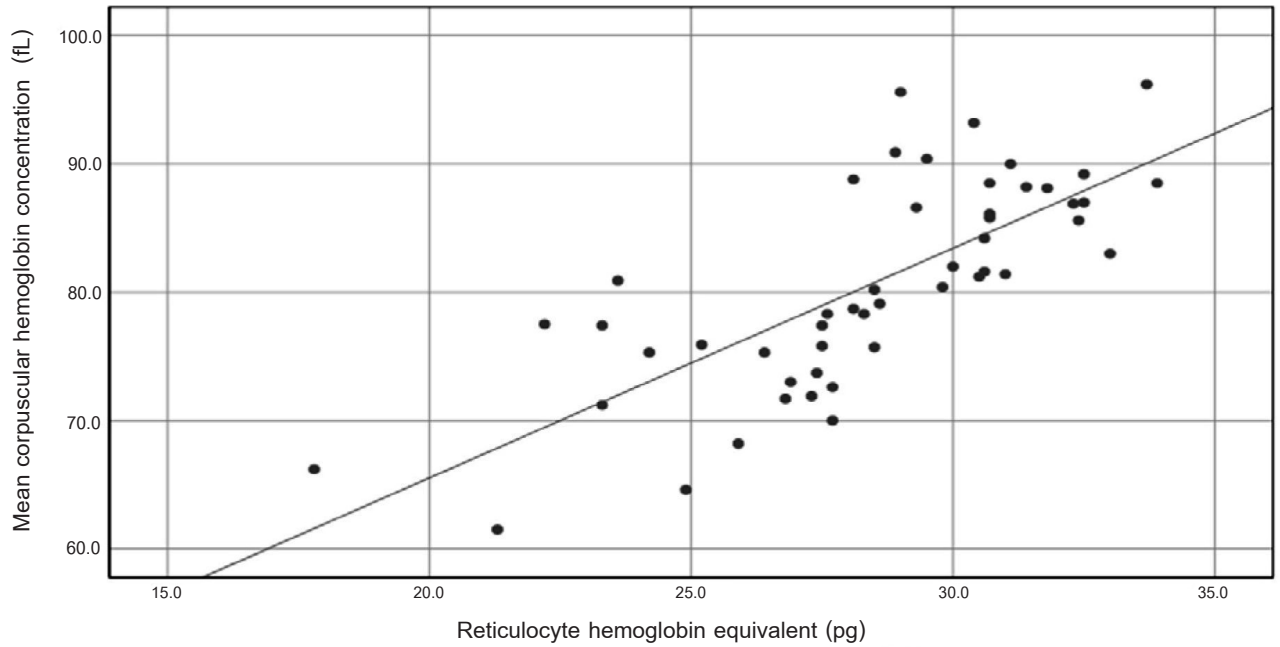

Figure 5. Correlation between mean corpuscular volume and Ret-He

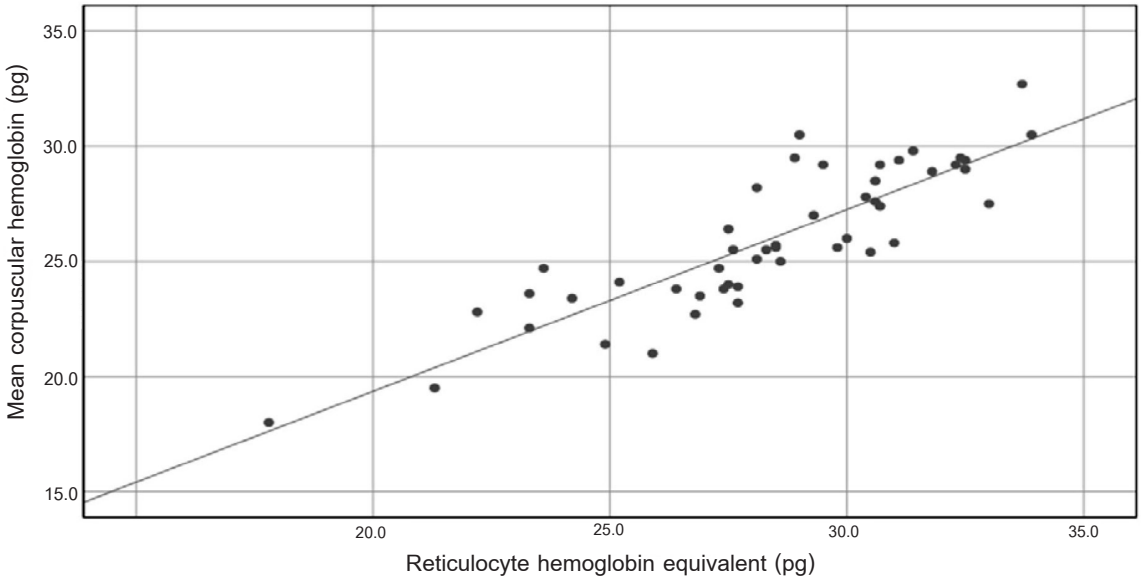

Figure 6. Correlation between mean corpuscular hemoglobin and Ret-He

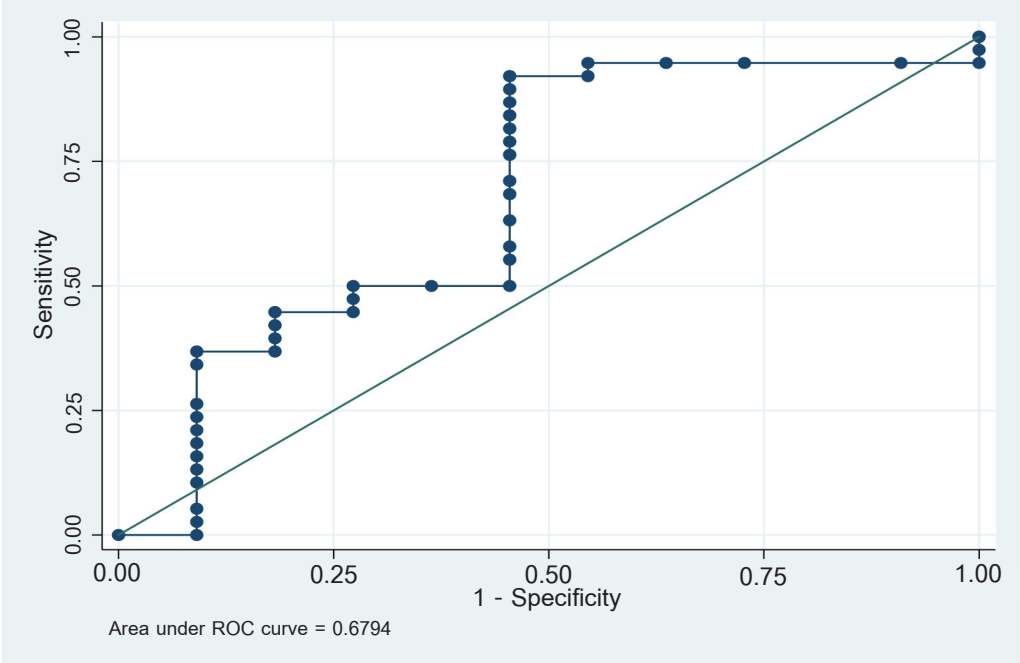

Figure 7. ROC curve of reticulocyte hemoglobin equivalent (Ret-He) to detect absolute and functional iron deficiency

the diagnostic performance of Ret-He against traditional diagnostic markers of iron deficiency, both functional and absolute iron deficiency. The area under the curve (AUC) was 0.68 (**Figure 7**). The ROC analysis demonstrated that a cut-off value of Ret-He was ≤25.9 pg detected iron deficiency with 92.11% sensitivity and 54.44% specificity.

Discussion

Anemia is an important problem among children with CKD. Lower glomerular filtration rates (GFR) are associated with lower levels of hemoglobin and become more pronounced when GFR falls below 60 mL/min per 1.73 m^2 among adults. [24] This study investigated the use of Ret-He as an indicator of iron deficiency in pediatric CKD, particularly in the low clearance group, namely CKD 3 and above. We identified

a higher prevalence of iron deficiency anemia (54%) based on Ret-He criteria compared with those of 4 and 18% for absolute and functional iron deficiency, respectively, diagnosed using traditional markers, namely, serum ferritin and TSAT. The result was comparable with that of the related study by Pinto et al. among children on hemodialysis, reporting the incidence of absolute iron deficiency being 13% when using traditional markers and 44% when using Ret-he with a cut-off point 29 pg.[21]

Before correcting anemia, accurate measurement of iron status is required to avoid unnecessary supplement. The widely-used markers in CKD are TSAT and serum ferritin, although they are known to have limited reliability in assessing iron availability among patients with CKD. Our present study demonstrated that Ret-He had moderate positive correlations with Hb (r=0.518; p <0.001), Hct (r=0.403; p=0.004) and MCHC (r=0.667; p<0.001), which was consistent with related studies. Davikova et al. conducted a study among children on chronic dialysis, exhibiting a modest positive correlation between Ret-He and Hb (r=0.22, p<0.001).[15] A study among children on hemodialysis from Pinto et al. also found a weak positive correlation between Hb and Ret-He (r=0.35, p<0.001).[21] Moreover, a moderate negative correlation between RDW and Ret-He (r=-0.616; p<0.001) and strong correlations between Ret-Hb and MCV (r=0.747; p<0.001) and MCH (r=0.865; p<0.001) were demonstrated. Therefore, this could imply that Ret-He had strong correlations with RBC indices.

Davikova et al. showed a modest relationship between Ret-He and TSAT (r=0.34, p<0001) and a poor correlation between Ret-He and ferritin (r=0.09, p=0.04).[15] However, Pinto et al. reported a significant positive correlation between TSAT and Ret-He (r=0.52, p<0.001).[21] In adult studies, Rovani et al. observed significant correlations between Ret-He and TSAT (r=0.416, p=0.019) among adult patients with CKD.[25] Dalimunthe et al. also found significant correlations between Ret-He and serum ferritin (r = 0.499, p< 0.0001) and TSAT (r = 0.592, p<0.0001) among adult patients on hemodialysis.[26] We were unable to find correlations between Ret-He and TSAT (r=0.170; p=0.239) or serum ferritin (r=-0.021; p=0.887); this could have been due to the small sample size.

Our study was concordant with the study from Pinto et al.[21], in which neither study found correlations between TSAT and Hb (r=-0.011; p=0.940), or Hct (r=-0.030; p=0.838, in contrast to the study from Davidkova et al. that demonstrated a weak positive correlation between Ret-He and Hb (r=0.12, p=0.007).[15] This could have resulted from inflammation that confounded the measurements. In the context of systemic inflammation, reduction in TIBC leads to a higher level of TSAT independent on the patient's iron status. Therefore, inflammation is implicated in the poor reliability of TSAT as a measure of iron status in CKD. [11]

Weak negative correlations between serum ferritin and Hb (r=-0.307; p=0.032), and Hct (r=-0.305; p=0.033) were demonstrated in this study, which was consistent with findings from related studies.[15,21] Ferritin is an intracellular iron storage protein, and its concentration is influenced by several factors, such as intracellular iron stores. Serum ferritin is also an inflammatory marker and plays a key role in diagnosing systemic inflammatory processes such as macrophage activation syndrome[11] and also affects the patient's nutritional status, as observed among patients on adult hemodialysis.[27] Given various confounding influences, the use of ferritin as a marker of iron status among patients with kidney disease has been questioned.

From ROC analysis, we found that a cut-off value of Ret-He was ≤ 25.9 pg with 92.11% sensitivity and 54.44% specificity. The cut-off value was lower than that of the study of Davikova et al., conducted among children undergoing chronic dialysis demonstrating a cut-off value of 28.9 pg to detect absolute iron deficiency anemia with 90% sensitivity and 75% specificity, and a cut-off value of 27.7 pg to detect functional iron deficiency anemia with 55% sensitivity and 83% specificity.[15] In addition, our cut-off value was lower than the standard guidelines, and and the NICE anemia guidelines 2015 update, recommending

using Ret-He below 29 pg in chronic kidney disease in diagnosing iron deficiency. [19] These could be due to the interference of baseline hemoglobinopathies among our patients due to the high prevalence of thalassemia in Thai populations. [28]

The major limitation encountered in this study was the small sample size due to the COVID-19 pandemic. Most patients avoided traveling and preferred to have blood work done at nearby hospitals, affecting the Ret-He investigation. We did not achieve the calculated sample size of 62 that could have affected the results of this study. Another challenge was that we could not check thalassemia among all participants. In Southeast Asia, hemoglobinopathies such as α-thalassemia, β-thalassemia, hemoglobin (Hb) E and Hb Constant Spring (CS) are highly prevalent.[28] Therefore, interpreting Ret-He in this population should be done with caution. Kadegasem et al. conducted a school-based study among Thai children to determine Ret-He in various groups: Ret-He of patients in the thalassemia trait group (26.7 ± 2.4 pg), iron deficiency group (29.0 ± 2.9 pg), iron deficiency anemia group (25.4 ± 2.7 pg), iron deficiency+ thalassemia trait group (26.6 ± 2.8 pg), and iron deficiency anemia + thalassemia trait group (24.6 ± 2.3 pg) were significantly lower than those in the normal control group (30.8 ± 1.7 pg; p<0.001, 0.01, 0.006, 0.002 and < 0.001, respectively). [29] Chaipokam et al. conducted a study among adults over 18 years of age having MCV <80 fl and treated at King Chulalongkorn Memorial Hospital, Thailand. They found that CHr among patients with IDA and thalassemia disease were significantly less than those among patients with anemia of inflammation and thalassemia trait (p<0.001), who in turn had lower CHr compared with normal controls (p<0.001).[30] This emphasized that hemoglobinopathies could interfere with Ret-He level, especially in Thai populations which are known to be highly prevalent with thalassemia.

In this study, we were able to display a moderate positive correlation between Hb and Ret-He among pediatric patients with CKD. This could give confidence to the use of Ret-He in evaluating iron status that would not be inferior to conventional markers. In fact, we could possibly diagnose a greater number of patients and provide prompt treatment. Furthermore, additional advantages included less blood sampling and reduced costs by 78% when compared with conventional measures.

Conclusion

Ret-He had significant moderate correlations with Hb, Hct and MCHC, a moderate negative correlation with RDW and strong positive correlations with MCV and MCH among pediatric patients with CKD. Thus, Ret-He is a relevant marker to assess iron status among pediatric patients with CKD and correlates well with anemia indices. Ret-He could identify more patients with iron deficiency than those of conventional markers. Ret-He value ≤25.9 pg had 92.11% sensitivity and 54.44% specificity in pediatric chronic kidney disease. Further studies with a larger sample size are still needed.

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Disclosure: The authors declare they have no conflict of interest.

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PULMONARY TUBERCULOSIS MORTALITY AND ITS RISK FACTORS AMONG PATIENTS WITH TYPE 2 DIABETES AND PULMONARY TUBERCULOSIS IN FOUR COMMUNITY HOSPITALS, CENTRAL THAILAND

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Abstract

Background: Tuberculosis (TB), a communicable disease, is currently a significant health problem in Thailand. Type 2 diabetes (T2D) is an indicator of poor TB outcomes; however, data according to specific antihyperglycemic use and tuberculosis outcomes in community hospital settings in Thailand remain limited. We aimed to determine TB mortality as well as explore the demographic and clinical risk factors among patients with pulmonary TB and underlying T2D.

Methods: A retrospective cohort study was conducted between January 1, 2013, and December 31, 2020, to determine tuberculosis mortality and its risk factors among patients with T2D and pulmonary TB visiting three community hospitals, in central Thailand. T2D and pulmonary TB were determined according to the International Classification of Diseases, Tenth Revision codes presented in medical records. TB mortality data were reviewed and retrieved from the tuberculosis treatment cards. Patients were classified as "dead" when they died before completing treatment regardless of the causes. Multivariable cox proportional regression analysis was performed to obtain the adjusted hazard ratios (AHR) and 95% confidence interval (CI) of factors related to TB mortality.

Results: A total of 133 patients with T2D and pulmonary TB were enrolled in the present study; 74 (55.6%) participants were males. At baseline, the average age of participants was 57.29±12.51 years. During the study period, the TB mortality rate was 15.74 (95% CI 8.13-27.50) deaths per 100 person-years. The independent risk factors for TB mortality included age ≥70 years (AHR 5.45, 95% CI; 1.36-21.84), use of insulin (AHR 4.62, 95% CI; 1.11-19.21), and positive sputum test result at 1st follow-up (AHR 16.10, 95% CI; 2.10-123.40).

Conclusion: TB mortality among patients with T2D should be emphasized. Insulin use may be a proxy indicator for poor glycemic control associated with mortality. Additionally, elderly patients should be closely observed for successful treatment as well as monitoring for any adverse events.

Keywords: Tuberculosis, Mortality, Diabetes, Community Hospital, Thailand
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Introduction

Tuberculosis (TB) is a communicable disease, and currently, a significant health problem in Thailand, securing one of the top ten causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS).[1] The World Health Organization (WHO) announced TB as an emergency health problem worldwide, in which Thailand was included in one of the 22 high TB burden countries in 2016.[1] Type 2 diabetes (T2D) is one of the most common noncommunicable diseases, developed in more than 10% of the Thai population, aged 30 to 60 years old.[2, 3] Patients with poorly controlled diabetes develop complications such as diabetic retinopathy, diabetic nephropathy, coronary vascular diseases, and chronic wound ulcers.[2, 4–6] Furthermore, poorly controlled diabetes was also found to be associated with poor innate and adaptive immune response and worsened TB outcomes.[7–10] Several studies reported that diabetes was associated with TB treatment outcome failure and increased morbidity and mortality, especially in pulmonary TB.[11–13]

Several systematic reviews demonstrated an increased mortality rate and TB relapse among patients with TB and underlying diabetes compared with patients with only TB.[10] Patients with poorly controlled diabetes were also found to develop TB more easily compared with a well-controlled diabetic group.[10] The prevalence of TB was also significantly higher among patients with T2D, and a recent meta-analysis found that the prevalence of T2D among patients with TB was about 21% in South Asian countries, where the prevalence is quite diverse.[9] We are interested in studying patients with pulmonary TB and underlying T2D due to its controversies, potential for diabetic treatment, and improved pulmonary tuberculosis outcomes. Although T2D is an indicator of poor TB outcomes, data according to specific antihyperglycemic and TB outcomes remain limited, especially in community hospital settings in Thailand. Rising socioeconomic status as well as lowering rural area socioeconomic status causes relatively inadequate health literacy, difficulty in accessing health care services, and a complicated specialist consultation system.[14] The persistence of TB in this setting presents a challenge for controlling tuberculosis.[15,16] Therefore, in the present study, we aimed to determine TB mortality and explore demographics and clinical risk factors among patients with T2D and pulmonary TB in community hospitals.

Methods

Study design and subjects

A retrospective cohort study was conducted between January 1, 2013, and December 31, 2020, to determine TB mortality and its risk factors among patients with T2D and pulmonary TB attending Thai community hospitals. In Thailand, the number of hospitals under the Ministry of Public Health (MoPH) totaled 833, including 33 regional, 83 general, and 717 community hospitals, of which 86% were community hospitals.[17] In the present study, we enrolled three community hospitals (F2 level; 60 to 90 beds) located in Lopburi Province and one community hospital (M2 level; 120 beds) in Chachoengsao Province. Eligible participants comprised patients with T2D receiving a diagnosis of pulmonary TB, aged at least 15 years, attending Sanam Chai Khet Hospital, Chachoengsao Province and Pattananikom Hospital, Ta Luang Hospital, and Ta Wung Hospital in Lopburi Province. A total of 133 individuals with T2D and pulmonary TB were enrolled in the present study.

Data collection

After obtaining permission from the directors of the enrolled hospitals, we retrieved data from medical records. Baseline characteristics of participants were collected including sex, age, comorbidities, smoking status, and alcohol consumption. We also collected TB diagnosis, treatment, and outcome information including a history of previous TB, sputum AFB examination, and chest x-ray. In addition, fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and antihyperglycemic medication use were collected at the start of treatment. Pulmonary TB and T2D were determined according to the International Classification of Diseases, Tenth Revision (ICD-

10) codes A15.0 to A19.9, and sputum AFB positive test results with underlying diabetes diagnosed by ICD10 codes E10.0 to E15.0 presented in the medical records. In addition, mortality data were reviewed and retrieved from the tuberculosis treatment card. Patients were classified as 'dead' when they died before completing treatment regardless of causes.[18,19]

Statistical analysis

Data were analyzed using StataCorp 2021, *Stata Statistical Software: Release 17*, College Station, TX: StataCorp LLC. We analyzed baseline characteristics using descriptive statistics. Continuous data were presented as mean and standard deviation (SD), while categorical data were presented as a percentage. We calculated the person-time of observation for each participant as the duration between the participant's baseline data and the date at which death, complete treatment, or loss-to-follow up took place, whichever occurred first[20]. We calculated the TB mortality rate with 95% confidence intervals (CI) per 100 person-years of observation. The Kaplan–Meier estimator was used to describe survival patterns, and we computed the log-rank test to compare survival across age groups. Validity of the proportional-hazards assumption was assessed; then Cox proportional hazard regression analysis was used to investigate risk factors for TB death. The magnitude of associations, including unadjusted and adjusted hazard ratios (HR) with 95% confidence intervals were presented. A two-sided *p*-value less than 0.05 was considered statistically significant.

Ethics consideration

The study was reviewed and approved by the Institutional Review Board, RTA Medical Department (M002h/64_Exp) in compliance with international guidelines including the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use-Good Clinical Practice (ICH-GCP). Due to using secondary data, a waiver of documentation of informed consent was used, and the waiver for informed consent was granted by the Institutional Review Board, RTA Medical Department.

Results

Baseline characteristics

Baseline characteristics are presented in **Table 1**. A total of 133 patients with T2D and diagnosed pulmonary TB were followed up for a mean of 209.2 days, ranging from 11 to 675 days. Of these, 67 (50.4%) participants came from the M2 level hospital. In all, 74 (55.6%) were male. The average age of participants was 57.3±12.5 years. In terms of comorbidities, the participants had HT, and CKD accounting for 27.1%, and 6.0%, respectively. The average fasting plasma glucose of participants at baseline was 203.2 ±112.6 mg/dL while the mean HbA1c was 9.7 ±3.0%. In all, 35 (26.3%) participants used insulin, while 89 (66.9%) individuals used metformin.

Table 1 Baseline characteristics of participants (N=133)

Characteristics	n (%)
Sex	
Male	74 (55.6)
Female	59 (44.4)
Age (years)	
mean±SD	57.3±12.5
median (max-min)	58 (26-85)
<70	110 (82.7)

Table 1 Baseline characteristics of participants (N=133) (Cont.)

Characteristics	n (%)
≥70	23 (17.3)
Hospital level	
Middle level (M2; 120 beds)	67 (50.4)
First level (F1; 60-90 beds)	66 (49.6)
HIV positive	2 (1.5)
Chronic obstructive pulmonary disease	4 (3.0)
Chronic kidney disease	8 (6.0)
Hypertension	36 (27.1)
Current smoker	46 (34.6)
Current alcohol use	36 (27.1)
Fasting plasma glucose (mg/dL)	
mean±SD	203.2±112.6
median (min-max)	162.5 (82-655)
80-130	32 (24.1)
>130	101 (75.94)
HbA1c (%)	
mean±SD	9.7±3.0
median (min-max)	8.9 (4.9-21.3)
<7	17 (18.7)
≥7	74 (81.3)
Metformin use	89 (66.9)
Pioglitazone use	4 (3.0)
Glipizide use	51 (38.3)
Insulin use	35 (26.3)
History of the previous TB	19 (14.3)
Clinical manifestation at diagnosis	
Typical	121 (91.0)
Atypical	12 (9.0)
Sputum grading at diagnosis	
≤2+	108 (81.2)
>2+	25 (18.8)
Chest x-ray at diagnosis	
One or no lung lesion	12 (9.0)
Multiple lung lesion	121 (91.0)

Table 1 Baseline characteristics of participants (N=133) (Cont.)

Characteristics	n (%)
1st Follow-up sputum	
Negative	104 (78.2)
Positive	29 (21.8)
Direct observational therapy	74 (61.2)
Treatment regimen	
Standard	98 (73.7)
Extended	32 (24.1)
Multidrug	3 (2.3)

SD; standard deviation

Pulmonary TB mortality rate among patients with T2D

A total of 12 (9.0%) participants died from pulmonary TB, representing an incidence rate of 15.7 (95% CI 8.1 to 27.5) per 100 person-years. The mortality rates among males and females were 14.1 (95% CI 5.0 to 30.6) per 100 person-years and 17.9 (95% CI 6.6 to 39.0) per 100 person-years, respectively. **Figures 1 to 4** show the Kaplan–Meier survival curves of pulmonary TB death overall, by age group, insulin use, and 1st follow-up sputum, respectively.

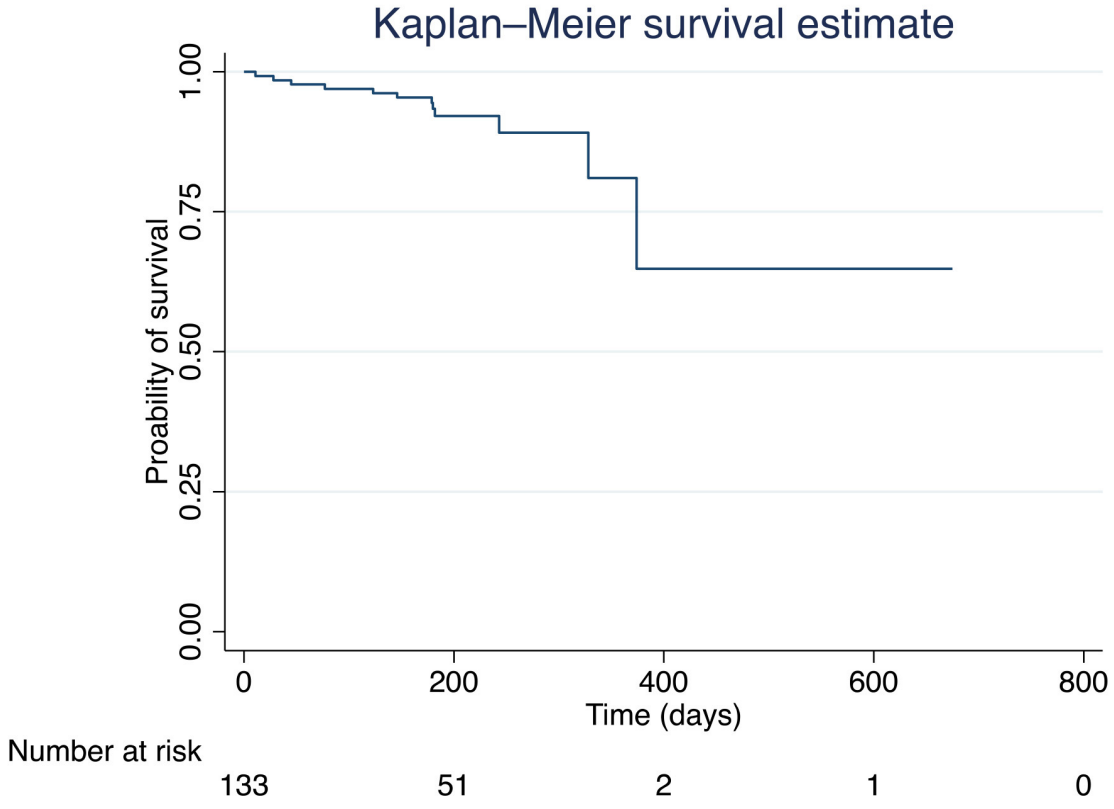

Figure 1. Kaplan–Meier survival curve of pulmonary TB mortality

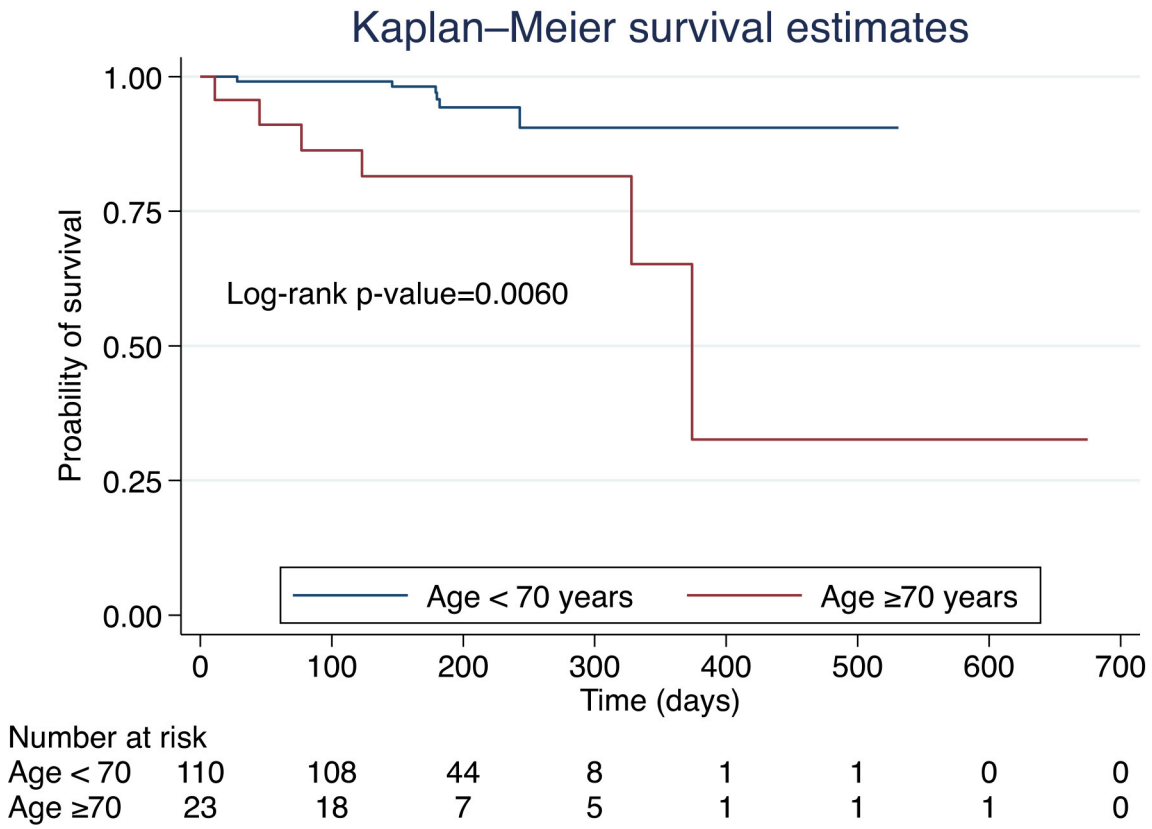

Figure 2. Kaplan–Meier survival curve of pulmonary TB mortality by age group

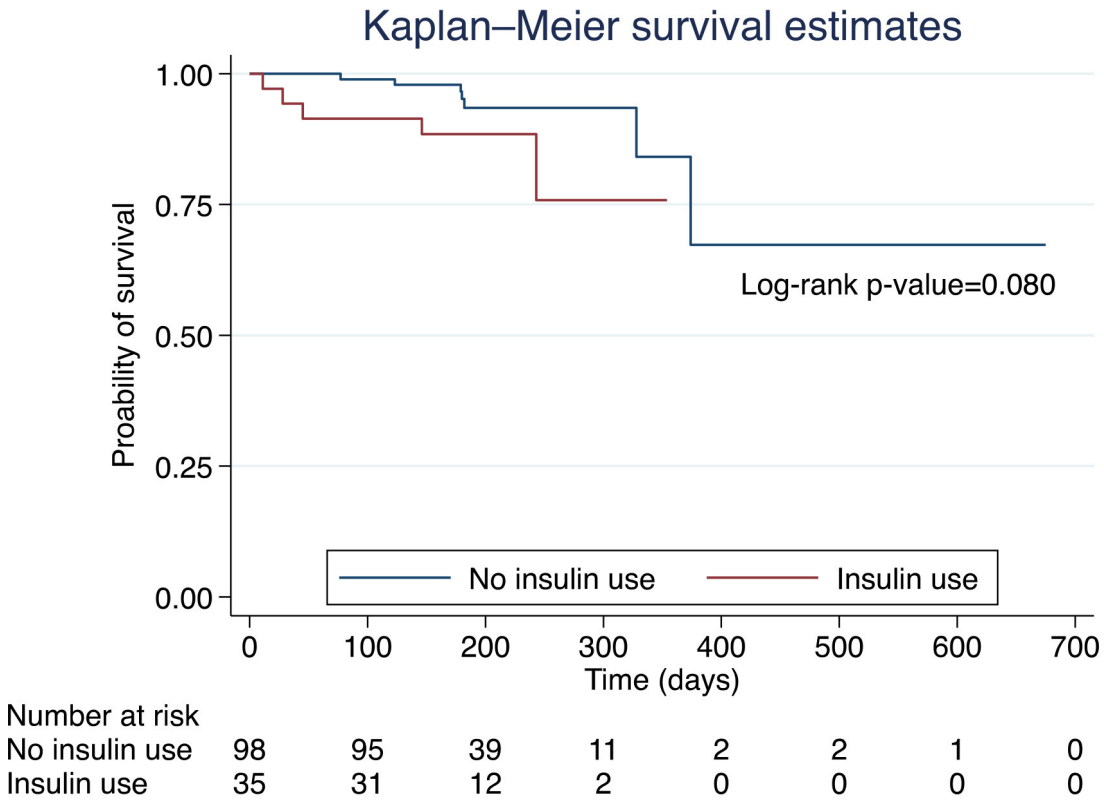

Figure 3. Kaplan–Meier survival curve of pulmonary TB mortality by insulin use

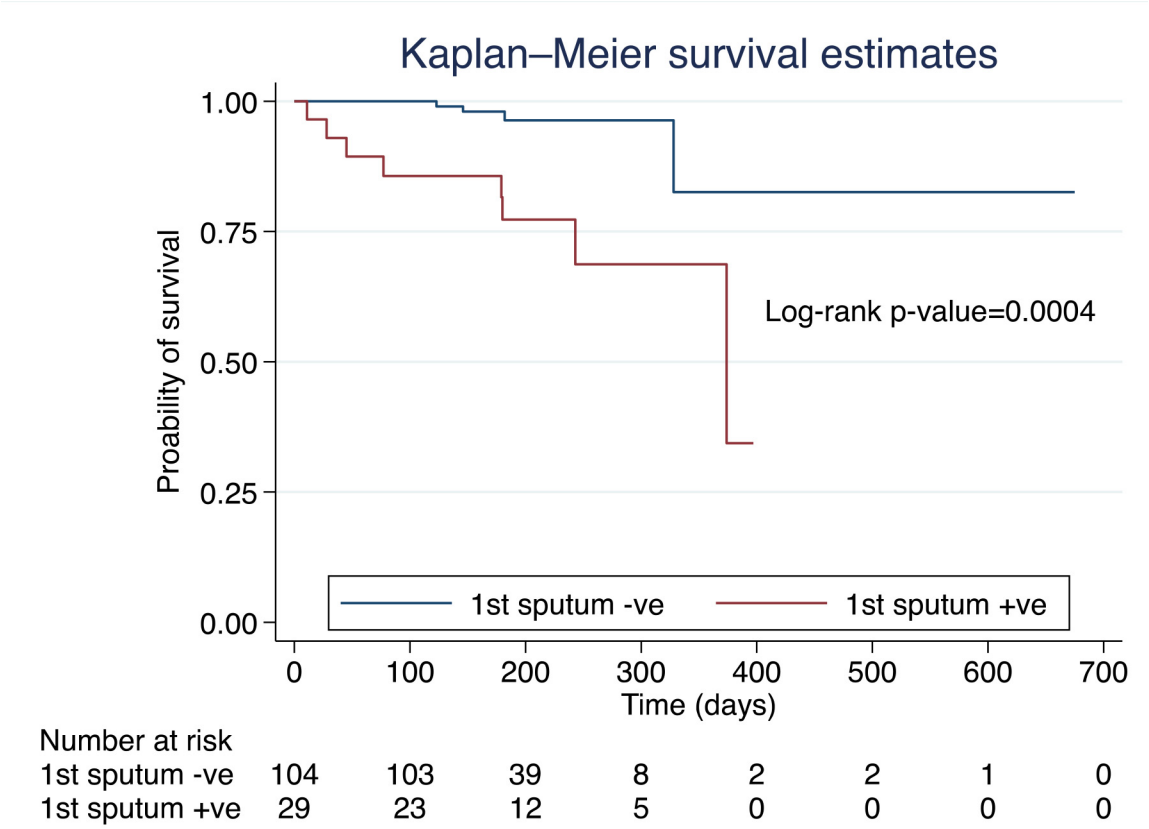

Figure 4. Kaplan–Meier survival curve of pulmonary TB mortality by 1st follow-up sputum AFB

Risk factors for pulmonary TB mortality among patients with T2D

Univariate and multivariate cox regression analysis of risk factors for pulmonary TB mortality are presented in **Tables 2 and 3**. After mutually adjusting for demographics and clinical characteristics, the mortality rate was higher for participants aged ≥70 years (HR 5.45; 95% CI 1.36-21.84), using insulin (HR 4.62; 95% CI 1.11-19.21) and having sputum AFP positive test results at first follow-up (HR 16.10; 95% CI 2.10-123.40).

Table 2. Univariable analysis of the association between demographics and clinical factors and TB mortality among patients with T2D

Factors	No. of death	Person-days of observation	Mortality rate / 100 person-years	Unadjusted Hazard Ratio	95% CI	*p*-value
Total	12	27823	15.74			
Sex						
Male	6	15584	14.06	1		
Female	6	12239	17.91	1.14	0.37-3.57	0.817
Age (years)						
<70	6	23079	9.50	1		
≥70	6	4744	46.20	4.59	1.42-14.79	0.011
Hospital level						
Middle level (M2; 120 beds)	3	13491	8.12	1		
First level (F1; 60-90 beds)	9	14332	22.94	2.38	0.63-9.07	0.203

Table 2. Univariable analysis of the association between demographics and clinical factors and TB mortality among patients with T2D (Cont.)

Factors	No. of death	Person-days of observation	Mortality rate / 100 person-years	Unadjusted Hazard Ratio	95% CI	*p*-value
HIV positive						
No	12	417	15.99	1		
Yes	0	27406	0	N/A	N/A	N/A
Chronic obstructive pulmonary disease						
No	12	26917	16.28	1		
Yes	0	906	0	N/A	N/A	N/A
Chronic kidney disease						
No	10	1739	14.00	1		
Yes	2	26084	42.01	2.47	0.53-11.58	0.250
Hypertension						
No	7	19945	12.82	1		
Yes	5	7878	23.18	1.56	0.52-5.17	0.830
Current smoker						
No	9	17690	18.58	1		
Yes	3	10133	10.81	0.57	0.15-2.11	0.401
Current alcohol use						
No	9	19874	16.54	1		
Yes	3	7949	13.78	0.85	0.23-3.14	0.806
Fasting plasma glucose (mg/dL)						
80-130	5	6456	28.29	1		
>130	7	19090	11.48	0.43	0.13-1.41	0.163
HbA1c (%)						
<7	0	3831	0			
≥7	6	15017	14.59	N/A	N/A	N/A
Metformin use						
No	6	8702	25.18	1		
Yes	6	19121	11.46	0.41	0.13-1.28	0.125
Pioglitazone use						
No	11	27064	14.85	1		
Yes	1	759	48.12	3.33	0.41-26.75	0.258
Glipizide use						
No	8	16639	17.56	1		
Yes	4	11184	13.06	0.68	0.20-2.27	0.533
Insulin use						
No	7	21259	12.03	1		
Yes	5	6564	27.82	2.80	0.84-9.29	0.093
History of the previous TB						
No	8	23567	12.40	1		
Yes	4	4256	34.33	2.63	0.77-8.94	0.122

Table 2. Univariable analysis of the association between demographics and clinical factors and TB mortality among patients with T2D (Cont.)

Factors	No. of death	Person-days of observation	Mortality rate / 100 person-years	Unadjusted Hazard Ratio	95% CI	*p*-value
Clinical manifestation at diagnosis						
Typical	12	24938	17.58	1		
Atypical	0	2885		N/A	N/A	N/A
Sputum grading at diagnosis						
≤2+	9	22325	14.72	1		
>2+	3	5498	19.93	1.31	0.35-4.85	0.684
Chest x-ray at diagnosis						
One or no lung lesion	1	2668	13.69	1		
Multiple lung lesion	11	25155	15.97	1.06	0.13-8.35	0.959
1st Follow-up sputum						
Negative	4	22225	6.57	1		
Positive	8	5598	52.2	7	2.09-23.45	0.002
Direct observational therapy						
No	9	15557	21.13	1		
Yes	3	10156	10.79	0.32	0.09-1.10	0.071
Drug regimen						
Standard	9	17594	20.34	1		
Extended	1	8990	4.06	0.12	0.01-1.07	0.057
Multidrug	1	1239	29.48	0.47	0.04-5.01	0.530

Table 3. Multivariable analysis of the association between demographics and clinical factors and the TB mortality among patients with T2D

Factors	Adjusted Hazard Ratio	95% CI	*p*-value
Sex			
Male	1		
Female	0.91	0.24-3.52	0.897
Age (years)			
<70	1		
≥70	5.45	1.36-21.84	0.017
Hospital level			
Middle level (M2; 120 beds)	1		
First level (F1; 60-90 beds)	0.38	0.05-3.06	0.364
Chronic kidney disease			
No	1		
Yes	0.14	0.08-2.57	0.144

Table 3. Multivariable analysis of the association between demographics and clinical factors and the TB mortality among patients with T2D (Cont.)

Factors	Adjusted Hazard Ratio	95% CI	*p*-value
Insulin use			
No			
Yes	4.62	1.11-19.21	0.035
History of the previous TB			
No			
Yes	6.18	0.85-44.77	0.072
1st Follow-up sputum			
Negative			
Positive	16.1	2.10-123.4	0.007
Sputum grading at diagnosis			
≤2+			
>2+	0.38	0.11-1.76	0.245
Direct observational therapy			
No			
Yes	0.44	0.11-1.76	0.245

Discussion

This study demonstrated pulmonary TB mortality among patients with T2D attending community hospitals in central Thailand. In addition, we found that demographics and clinical characteristics were associated with pulmonary TB death. The present study reported that pulmonary TB mortality was 9.0%, comparable to a related study in Texas, USA (10.3%).[20] Compared with another study in California, USA (13.1%),[21] the pulmonary TB mortality in the present study was relatively low.

Our finding reported that the pulmonary TB mortality rate among participants aged ≥70 years was higher than that among those aged <70 years which was compatible with recent evidence from Japan indicating that patients aged ≥75 years with pulmonary TB experienced increased mortality related to TB during treatment.[22] However, another report in Thailand presented that older patients were not associated with unsuccessful pulmonary TB outcomes including death, default, treatment failure, and transfer due to multidrug-resistant TB.[23]

We found that individuals with first follow-up sputum AFB positive had a higher mortality rate when compared with those with first follow-up sputum AFB negative. This observation was likely due to the well-documented positive relationship between unsuccessful pulmonary TB treatment and previously treated TB.[23–25] This finding may explain that previous treatment outcomes could be used to predict the development of drug-resistant TB.[24]

In terms of antihyperglycemic medication use, we observed that metformin use was also found as a protective factor for TB outcomes in several studies.[27–29] Metformin was found to inhibit intracellular growth of mycobacteria by inducing mitochondrial reactive oxygen species and facilitating phagolysosome fusion. Metformin can also modify the function of different biological pathways, such as down-regulating type I IFN pathways interfering with IFN-γ-mediated activation of macrophages, which are known to be associated with active TB improving the outcome of anti-TB therapy among patients with TB-infected DM.[29–32] However, the findings were not significant in our study possibly due to the small sample size.

Our finding reported that the pulmonary TB mortality rate among patients with T2D using

insulin was higher than that among those not using it. According to Thai Clinical Practice Guidelines of Diabetes 2017, insulin therapy would be initiated when fasting plasma glucose exceeds 300 mg/dL or HbA1c exceeds 11% with hyperglycemic symptoms or are currently treated with oral antihyperglycemic drugs.[2] In other words, insulin therapy could be an indicator of poor glycemic control leading to macrovascular and microvascular complications as well as increased mortality.[4, 5, 33] Insulin initiation tends to be delayed and irreversible complications can already be present by the time it starts especially when specialists are unavailable, in rural areas. Therefore, insulin use may also function as a marker for advanced disease and could affect hospitalization infection risk as well as outcomes.[34, 35] A global overview with a particular focus on the situation in Asian countries with high TB-DM burden also showed that patients with TB-DM with tight HbA1C control of lower than 7% had better outcomes regarding TB treatment[12] and an extensive systematic review and meta-analysis also showed that diabetes was related to increased mortality rate among patients with TB compared with those with TB without diabetes.[9] Our finding suggested that improving glycemic control should be encouraged among patients with T2D and pulmonary TB; additionally, individuals using insulin should be closely monitored for treatment and further complications.

Our study encountered several limitations. Firstly, according to a retrospective cohort study, some variables were collected very broadly. For example, we did not have detailed data on the number of alcoholic beverages consumed daily. Similarly, we did not have details of the smoking history, such as the current number of cigarettes smoked daily. Secondly, the present study comprised a small sample size; thus, the association between the well-known risk factors such as HIV co-infection and multiple lung lesions could not be presented.[9, 10] Third, the analysis did not include unmeasured confounders such as body mass index, anti-TB drug allergy, and diabetic complications, including macrovascular and microvascular complications. Another limitation was missing data, such as HbA1c, leading to the difficulty of including HbA1C in the analysis. Finally, the result of our study may not be generalized to the whole country but may reflect the situation of patients with T2D receiving a diagnosis of pulmonary TB in a community hospital setting in Thailand.

Conclusion

Mortality among patients with TB-DM should be emphasized. Insulin use may be a proxy indicator for poor glycemic control which was associated with mortality in this study population. Additionally, elderly patients should be closely observed for successful treatment as well as monitoring for any adverse events.

Acknowledgments

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Conflict of interest

The authors declare they have no conflict of interest.

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PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND OTHER STAPHYLOCOCCAL NASAL CARRIAGES AMONG HEALTH-CARE WORKERS, PHRAMONGKUTKLAO HOSPITAL

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a group of *S. aureus* strains containing the SCC*mec* gene causing beta-lactam antibiotic resistance. MRSA is common in healthcare settings and can cause serious problems.

Objective: The study aimed to investigate the prevalence of MRSA nasal colonization among privates of the Medical Private Company, Phramongkutklao Hospital, including antibiotic susceptibility pattern of *S. aureus* isolates and risk factors of *S. aureus* nasal carriage.

Methods: Nasal swabs were obtained from the anterior nares of 170 privates. Staphylococcal isolates were identified using a catalase test, tube coagulase test and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). MRSA detection was screened using cefoxitin disk diffusion and confirmed using the *mec*A gene detection and SCC*mec* typing. Antibiotic susceptibility patterns of *S. aureus* were examined using the disk diffusion method. A questionnaire was collected from the subjects to determine risk factors for *S. aureus* nasal carriage.

Results: Of 170 subjects, 157 (92.35%) revealed staphylococcal positive, yielding 161 staphylococcal isolates. The prevalence of MRSA, methicillin-resistant *Staphylococcus epidermidis* (MRSE), and methicillin-susceptible *Staphylococcus aureus* (MSSA) nasal carriage was 0.59, 1.18 and 8.82%, respectively. The MRSA isolate carried *mecA* revealing SCC*mec* type II. The MSSA isolates indicated low resistance to tetracycline (13.3%), whereas the MRSA isolate resisted ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin and tetracycline. Using multiple logistic regression analysis, a significant risk factor for *S. aureus* nasal carriage was utensil sharing (adjusted odds ratio=4.41; 95% CI=1.33-14.61).

Conclusion: Healthcare-associated MRSA existed among privates of the Medical Private Company. An associated risk factor for acquiring *S. aureus* was utensil sharing which could be used to help improve prevention and control management among privates.

Keywords: MRSA, MALDI-TOF MS, *Staphylococcus epidermidis*, MSSA, *mecA* gene, SCC*mec*

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Introduction

Staphylococci are gram-positive bacteria in the genus *Staphylococcus,* under the bacterial family Staphylococcaceae.[1] Fifty-nine species of staphylococci are widespread in nature.[2] Their significant natural habitats are mammals' skin and mucous membranes, including humans.[3] Staphylococcal species generally associated with human infections are *S. aureus*, *S. epidermidis* and *S. saprophyticus*.[4] Others may also be related to human diseases such as *S. haemolyticus*, *S. hominis*, *S. simulans* and *S. warneri*.[5] *S. aureus,* one of the most important bacterial pathogens among humans, causes various diseases ranging from mild skin and soft tissue infections (SSTIs) to severe life-threatening diseases such as complicated SSTIs, osteomyelitis, bacteremia, infective endocarditis and toxic shock syndrome.[6] Among humans, it commonly inhabits the nasal cavity and skin surface.[7] Most strains of *S. aureus* have developed antibiotic resistance, creating a serious problem for treatments, especially those that resist methicillin (methicillin-resistant *Staphylococcus aureus* or MRSA).[8]

Since the first MRSA report in 1961, MRSA has been one of the essential causative pathogens of healthcare-associated infections (HAIs) affecting patients and healthcare systems worldwide.[9, 10] MRSA infections are capable of causing severe infections such as osteomyelitis, endocarditis, pneumonia and sepsis, all of which could ultimately lead to mortality.[11] MRSA is multidrug-resistant (MDR), not only resists beta-lactams such as penicillin and cephalosporins but also resists nonbeta-lactam antibiotics such as macrolides, lincosamides, quinolones, tetracyclines and aminoglycosides.[12] According to a recent study, MRSA was susceptible to vancomycin (100%), mupirocin and rifampicin (99.2%), followed by chloramphenicol (82.3%) and gentamicin (76%).[13] Thus, vancomycin injection is the first drug of choice for MRSA infections; mupirocin nebulization is a widely used treatment for MRSA nasal colonization.

The methicillin-resistance mechanism is the production of an altered penicillin-binding protein (PBP) from PBP-2 turning into PBP-2a, and decreasing affinity to betalactams. The *mec*A gene encodes this protein on a mobile genetic element named Staphylococcal Cassette Chromosome *mec* (SCC*mec*).[14] To date, 13 different SCC*mec* types (I-XIII) have been identified.[15] In Thailand, most SCC*mec* were IIIA and IIA types.[16] On the other hand, other staphylococci can also carry this SCC*mec* and have the property of being methicillin-resistance such as methicillin-resistant *S. epidermidis* (MRSE), which is a kind of methicillin-resistant coagulase-negative staphylococci (MRCoNS). For this reason, MSSA can become MRSA upon obtaining the SCC*mec* from MRSA or MRCoNS.[17]

Hospital-acquired MRSA (HA-MRSA) is generally defined as those that develop infections within 48 hours of discharge from a hospital, clinic or healthcare facility. Community-acquired MRSA (CA-MRSA) among healthy individuals stems from those who have not been hospitalized or had a medical procedure within the past year.[18] CA-MRSA typically causes skin infections, and 40 to 90% of CA-MRSA strains are accompanied by an exotoxin named Panton-Valentine leukocidin (PVL).[19] Regarding the molecular aspect,

HA-MRSA and CA-MRSA are distinguished by *mecA* types. The HA-MRSA usually presents *mecA* gene types I, II or III,[15] while the CA-MRSA regularly shows *mecA* gene types IV or V.[20] MRSA has been found in Thailand for more than 40 years.[21] Occasionally, MRSA outbreaks are incident, especially in hospitals.[22] MRSA possesses increased; risk factors enhancing MRSA prevalence are antibiotic use, prolonged hospitalization, intravascular intervention and hospitalization in an intensive care unit.[23] MRSA is usually spread by skin-to-skin contact. At-risk populations of MSRA carriers include groups such as dormitory dwellers, healthcare workers, military privates or conscripts, prisoners and those living in crowded conditions. Among healthcare workers (HCWs), the prevalence of MRSA nasal colonization has been estimated to be 4.6% in non-outbreak settings in Europe and the United States.[24] In Thailand, the prevalence of MRSA in HCWs was 1% to 7.36%.[25, 26] This MRSA colonization is potentially transmitted from HCWs to patients. It consequently causes serious problems with a significant concern of MRSA carriage among HCWs. The incidence of MSRA isolates from clinical specimens of Phramongkutklao Hospital has been increasing, including coagulase-negative staphylococci and *Escherichia coli*. Moreover, vancomycin-intermediate resistance *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) have been reported in Thailand.[27,28] Routinely, MRSA screening among physicians and nurses of Phramongkutklao Hospital also revealed the continuous incidence of MRSA. However, an interesting group for screening MRSA comprised privates of the Medical Private Company, a group of HCWs working in the hospital. Thus, this study aimed to investigate the prevalence of MRSA nasal carriage, determine antibiotic resistance patterns in *S. aureus* isolates, and determine risk factors of *S. aureus* nasal carriages among privates of the Medical Private Company, Phramongkutklao Hospital.

Methods

Study design

A cross-sectional study was conducted to evaluate the prevalence of MRSA and *S. aureus* nasal carriage among privates of the Medical Private Company, Phramongkutklao Hospital. The study was approved by the Institutional Review Board (IRB), Royal Thai Army Medical Department (approval no. RF08_2555). Informed consent was obtained before collecting specimens. The study included 170 privates; the number of subjects was based on sample size calculation at a 95% reliability level using Yamane's formula.[29]

Sample collection

This study was conducted in the Medical Private Company of Phramongkutklao Hospital in 2015. The details of this study were informed to the subjects. A nasal swab was applied to collect specimens with a rotating technique neatly in both anterior nares of the subjects. All swabs were transported within Amies's transport media and then streaked on MRSA chromogenic agar and mannitol salt with egg-yolk agar, incubated at 35°C as a screening culture method. The bacterial growth was examined after 24 to 48 hours.

Isolation and identification

For all bacterial isolated colonies, macroscopic and microscopic examination using gram stain was performed for preliminary identification. All gram-positive cocci were confirmed by catalase test, and then all staphylococci were examined using the tube coagulase test. Furthermore, species were identified using the matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). For staphylococci isolates, MRSA was detected using cefoxitin disk diffusion. Therefore, this study could also recover MRSE and MSSA as an outgrowth.

Molecular characterization for MRSA

The MRSA isolates were detected for the *mecA* gene using the Polymerase Chain Reaction (PCR). The isolates were inoculated in tryptic soy broth (TSB) at 35°C overnight. The culture was diluted

in 0.5 McFarland standards (approximately 1.5 x 10^8 CFU/mL) and extracted for DNA using the Pure-gene Yeast/Bact. Kit B (Qiagen, Germany). To detect the *mecA* gene, specific primers were used to amplify the 310-bp of the *mecA* gene according to a method described by McClure,[30] and SCC*mec* typing was determined by amplifying the *mec* and *ccr* gene complex.[31]

Antibiotic susceptibility pattern

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, all MRSA and MSSA isolates were tested for antibiotic susceptibility patterns, including cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fosfomycin, fusidic acid, gentamicin, linezolid, tetracycline and trimethoprim/sulfamethoxazole using the disk diffusion method of Kirby-Bauer according to CLSI guidelines.[32] *S. aureus* (ATCC29213) was the control strain used for the susceptibility test.

Questionnaire for risk factors

A questionnaire was provided to collect data from the subjects. Demographic data were collected, including age, sex, hometown and occupation, before entering the military draft. Behavioral data included handwashing habits with and without soap, bathing, utensil sharing, nose-picking, smoking and a history of alcohol consumption. Health data included a history of skin infection, previous hospitalization, underlying disease, antibiotic use within the last two months, and history of surgery. Finally, operations while working in the hospital included touching patients, touching medical equipment, glove-wearing and mask-wearing.

Statistical analysis

The *S. aureus* positive samples were statistically analyzed using the data of independent factors possibly contributing to *S. aureus* nasal carriage. The crude odds ratio (OR) was determined using bivariate analysis with 95% confidence intervals (CI). All *p*-values were two-sided, with a $p < 0.05$ considered a significant correlation. The statistically significant factors were analyzed for adjusted odds ratio (AOR) using Cochran's and Mantel-Haenszel's multiple logistic regression analysis. All analyses were performed using IBM SPSS, Version 22.0.

Results

Prevalence of MRSA, MSSA, MRSE and other staphylococci

A total of 185 isolates were recovered from 170 samples, of which 157 were staphylococci (92.35%), including 161 staphylococcal isolates. Of 157 samples, 27 were coagulase-positive staphylococci (CoPS) (15.88%), and 131 were coagulase-negative staphylococci (CoNS) (77.06%). Of 27 CoPS, 16 were *S. aureus* (9.41%), and 11 were for *S. intermedius* (6.47%). Of 16 samples of *S. aureus*, one was MRSA (0.59%), and 15 were MSSA (8.82%). Of 131 CoNS, 103 were *S. epidermidis* (60.59%), and 32 were other CoNS (18.82%). Of 103 samples of *S. epidermidis*, 2 were MRSE (1.18%), and 101 were methicillin-sensitive *S. epidermidis* (MSSE) (59.41%). The prevalence of MRSA, MSSA, and MRSE nasal carriage were 0.59, 8.82, and 1.18%, respectively (**Figure 1**).

Isolation of bacterial strain

Of 185 bacterial isolates, 12 species were confirmed using the MALDI-TOF. The predominant one was *S. epidermidis* (55.68%), followed by *Corynebacterium* sp. (8.65%), *S. aureus* (8.65%), *S. hominis* (8.11%), *S. intermedius* (5.95%), *S. warneri* (3.24%), *Micrococcus* sp. (2.70%) and *S. saccharolyticus* (2.16%). Others were *Corynebacterium accolens*, *S. capitis*, *S. caprae*, and *S.haemolyticus,* at 1.08% each (**Table 1).**

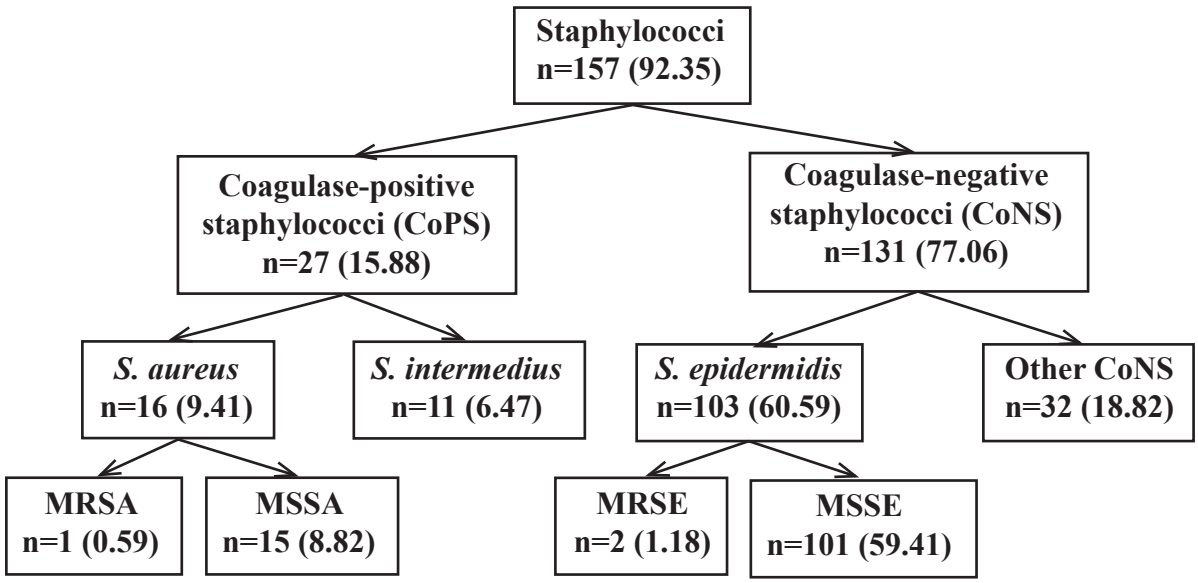

Figure 1. Prevalence of staphylococci nasal carriage among 170 privates of the Medical Private Company, Phramongkutklao Hospital

Table 1. Bacterial species were identified among 185 bacterial isolates from privates of the Medical Private Company, Phramongkutklao Hospital

Bacterial species	Number of isolates	%
Gram-positive cocci		
Staphylococci		
CoPS		
S. aureus	16	8.65
S. intermedius	11	5.95
CoNS		
S. epidermidis	103	55.68
S. hominis	15	8.11
S. warneri	6	3.24
S. saccharolyticus	4	2.16
S. capitis	2	1.08
S. caprae	2	1.08
S. haemolyticus	2	1.08
Micrococci		
Micrococcus sp.	5	2.70
Gram-positive bacilli		
Corynebacterium sp.	16	8.65
Corynebacterium accolens	2	1.08

Antibiotic susceptibility pattern for S. aureus

The cefoxitin disk diffusion test for the 16 isolates of *S. aureus* revealed that only one isolate was MRSA. The MRSA isolate showed resistance to ciprofloxacin, clindamycin, erythromycin, gentamicin and tetracycline. On the other hand, the isolate was susceptible to chloramphenicol, fosfomycin, fusidic acid, linezolid and trimethoprim/sulfamethoxazole. In addition, the MSSA isolates were 13.33% resistant to tetracycline. Nevertheless, the isolates were 100% susceptible to chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fosfomycin, fusidic acid, gentamicin, linezolid, cefoxitin and trimethoprim/sulfamethoxazole.

MRSA genotyping

Of 185 isolates, only one isolate was suspected of MRSA by phenotypic investigation. The existence of the *mecA* and *ccrA2* genes in an MRSA isolate was observed. The SCC*mec* typing was SCC*mec* type II, as shown in **Table 2**.

Risk factors associated with S. aureus nasal carriage

A total of 170 subjects were privates, male, aged 20 to 23 years, and residing together in dormitories in a military camp of the Medical Private Company, Phramongkutklao Hospital. Using bivariate analysis, utensil sharing (OR: 3.92; 95% CI=1.21-12.72) and antibiotic use within the last two months (OR: 4.85; 95% CI=1.12-21.00) were significantly associated with *S. aureus* nasal carriage as shown in **Table 3**. However, using multiple logistic regression analysis, utensil sharing was independently associated with *S. aureus* nasal carriage (AOR= 4.41; 95% CI=1.33-14.61)

Table 2. PCR amplification of the *mecA* and *ccr* genes for SCC*mec* type using the MRSA isolate

Gene amplification	PCR	Result
mecA (310 bp)	Positive	Methicillin-resistant isolate
ccrA1 (415 bp)	Negative	
ccrA2 (937 bp)	Positive	Type II of SCC*mec*
ccrA3 (518 bp)	Negative	

Table 3. Bivariate analysis and multiple logistic regression analysis for possible factors associated with *S. aureus* nasal carriage

Factor	*S. aureus* nasal carriage (%)		Bivariate analysis		Multiple logistic regression analysis	
	positive (n=16)	negative (n=154)	OR (95%CI)	*p*-value	AOR (95%CI)	*p*-value
Personal behavior						
Handwashing	14 (87.50)	147 (95.45)	3.00 (0.57-15.85)	0.1958		
Handwashing with soap	15 (93.75)	145 (94.16)	1.07 (0.13-9.07)	0.9477		
Bathing habit at least twice/day	15 (93.75)	146 (94.81)	1.22 (0.14-10.40)	0.8578		
Utensil sharing	5 (31.25)	16 (10.39)	3.92 (1.21-12.72)	0.0229*	4.41 (1.33-14.61)	0.016*
Nose-picking	14 (87.50)	115 (74.68)	1.02 (0.31-3.34)	0.2667		
Smoking	13 (81.25)	105 (68.18)	2.02 (0.55-7.42)	0.2885		
Alcohol consumption	12 (75.00)	109 (70.78)	1.24 (0.38-4.05)	0.7232		

Table 3. Bivariate analysis and multiple logistic regression analysis for possible factors associated with *S. aureus* nasal carriage (Cont.)

Factor	*S. aureus* nasal carriage (%)		Bivariate analysis		Multiple logistic regression analysis	
	positive (n=16)	negative (n=154)	OR (95%CI)	*p*-value	AOR (95%CI)	*p*-value
Health information						
History of skin infection	3 (18.75)	17 (11.04)	1.86 (0.48-7.19)	0.3687		
Previous hospitalization	1 (6.25)	7 (4.55)	1.40 (0.16-12.16)	0.7603		
Underlying disease	1 (6.25)	13 (8.44)	0.72 (0.09-5.92)	0.7624		
Antibiotic use within the last two months	3 (18.75)	7 (4.55)	4.85 (1.12-21.00)	0.0349*	3.077 (0.85-18.07)	0.079
History of surgery	0 (0.00)	13 (8.44)	0.32 (0.02-5.60)	0.7840		
Operation in hospital						
Touching patients	1 (6.25)	12 (7.79)	0.79 (0.10-6.50)	0.8255		
Touching medical equipment	2 (12.50)	13 (8.44)	1.55 (0.32-7.57)	0.5886		
Glove-wearing	4 (25.00)	37 (24.03)	0.95 (0.29-3.12)	0.9309		
Mask-wearing	11 (68.75)	107 (69.48)	1.03 (0.34-3.14)	0.9519		

*Significantly different (*p*-value <0.05), OR: crude odds ratio, AOR: adjusted odds ratio, CI: confident interval

Discussion

This study showed a low prevalence (0.59%) of MRSA nasal carriage among privates working in the hospital, which was less than other studies. In this study, MRSA genotyping was performed using only the cefoxitin-resistant *S. aureus* isolates, which might have revealed less MRSA prevalence. In addition, PCR amplification of all *S. aureus* isolates would be very helpful in determining the true prevalence of MRSA nasal carriage. However, in this study, PCR was used only for bacterial genotyping. Many reports showed a high MRSA prevalence, ranging from 0.67 to 36.06% among healthcare workers. Most healthcare workers could have acquired MRSA after contact with MRSA-positive patients. [33, 34] In Thailand, related studies showed that the prevalence of MRSA nasal carriage was 1% among medical sciences students in the north [25] and 7.36% among medical students in the central region.[26] As mentioned, a wide range of prevalence could be due to different sensitivities of methods used for the study. For this reason, a study not recovering MRSA did not imply that MRSA would be absent. [35]

In this study, the risk factor constituted utensil sharing, similar to a related study in which risk factors for MRSA and *S. aureus* were personal hygiene, especially sharing items between household individuals.[36] These results support that this MRSA isolate was likely to be CAMRSA. However, the MRSA-positive subject confirmed the *mecA* DNA fragment[37] and SCC*mec* typing.[38] The result revealed type II of SCC*mec* at 937 bp of the *ccrA2* gene. Therefore, this MRSA isolate might be HA-MRSA which commonly carries SCC*mec* type II [18]. However, whole genome sequencing of the MRSA isolate should be further performed to distinguish between HA-MRSA and CA-MRSA.

For MSSA, our result was similar to related studies indicating 15.0 and 20.25%, respectively, the MSSA nasal colonization among medical science students and medical students.[25, 26] These data emphasized that the establishment of MSSA could be generally seen as nasal colonization among healthcare workers. Our result

showed 13.3% tetracycline-resistant MSSA isolates, differing from that of medical science students (23.3%).[39] MSSA from nasal colonization among medical science students resisted penicillin, clindamycin and erythromycin at 88.4, 39.5, and 37.2%, respectively, revealing higher resistance than our results. [39]

In this study, management of the private identified as the nasal carriage of MRSA was treated with the proper antibiotic. Methicillin-resistant horizontal transmission hypotheses suggested that MSSA obtained *mec*A DNA fragments transferred from MRSE or MRCoNS into itself and then became MRSA.[40] Our study also showed various species of CoNS indicating the potential for exchanging the *mecA* gene among staphylococcal species.

Conclusion

The study confirmed the existence of MRSA in a group of privates working as HCWs in a hospital. A risk factor of healthcare-associated MRSA was utensil sharing, likely to occur when they resided in the same accommodation. Therefore, infection control measures in hospitals should be realized for the transmission of MRSA, even from HCWs. However, MRSA screening would be more reliable when using molecular techniques for confirmation.

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DIFFERENTIATION BETWEEN ALLERGIC AND NON-ALLERGIC RHINITIS IN CHILDREN WITH CHRONIC RHINITIS, ALLERGY CLINIC, PHRAMONGKUTKLAO HOSPITAL

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ABSTRACT

Background: Chronic rhinitis in children is a common problem. Investigation to diagnose allergic rhinitis (AR) using a skin prick test for aeroallergens requires a specialist doctor, which is not simply performed in primary health care facilities. Therefore, diagnosing patients with AR and non-allergic rhinitis (NAR) is based on clinical symptoms essential for treatment planning. This study compared clinical symptoms between AR and NAR, comorbidities, disease severity, and common aeroallergen sensitization.

Methods: A retrospective descriptive study was conducted among participants aged between 2-18 years with chronic rhinitis who were treated at the Department of Allergy and Immunology, Division of Pediatrics, Phramongkutklao Hospital, between 2014 and 2018. The medical records were reviewed on clinical symptoms, allergic test results, environmental data, and the severity according to Allergic Rhinitis and its Impact on Asthma (ARIA) classification. If the patient tested positive for aeroallergen, the allergist diagnosed AR. NAR is characterized by the same symptoms but with a negative skin prick test.

Results: Three hundred and seven participants were included. Among these patients, 226 (73.6 %) were categorized as AR, and 81 (26.4%) were NAR. The AR group had a higher percentage of males than the NAR group. Nasal pruritus and ocular symptoms were more commonly found in AR than in NAR. Regarding comorbidities, both groups had similar snoring, sinusitis, asthma, and atopic dermatitis. The most common aeroallergens among AR patients were *Dermatophagoides pteronyssinus* (82.7%), *Dermatophagoides farinae* (81.4%), followed by American cockroaches (38.1%), and German cockroaches (37.6%). Cat owners were associated with cat sensitization in AR patients (OR =2.77; 95% CI = 1.27-5.88).

Conclusions: In this study, the proportion of AR was higher than NAR. Nasal pruritus, ocular symptoms, or both strongly supported AR. The most common aeroallergen sensitization was house dust mites, followed by cockroaches. Initial treatment with antihistamine and other drugs can improve the severity of the disease.

Keywords: Chronic rhinitis, Allergic rhinitis, Non-allergic rhinitis, Thai children, Aeroallergen

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Introduction

Any inflammatory illness of the nasal mucosa is referred to as rhinitis. The development of one or more symptoms: rhinorrhea, sneezing, nasal itching, and nasal blockage, is clinically characterized as rhinitis.[1] Chronic rhinitis in children is a common problem in pediatric practice. The results of ISAAC Phase Three in Thai children revealed a significant increase in rhinitis symptoms in Bangkok and Chiang Mai.[2] Due to the prolonged period of this disease, it can affect daily activities such as academic performance, ability to work, and quality of life, leading to several indirect costs. Although allergic rhinitis (AR) is the most often recognized cause of chronic rhinitis in children, an alternative cause unrelated to allergic or infectious agents is known as non-allergic rhinitis (NAR). AR is caused by immunologic sensitization to aeroallergens resulting in the synthesis of specific IgE, which causes inflammatory processes leading to nasal symptoms. NAR is a chronic condition of the nasal mucosa with no evidence of allergic sensitization through skin prick tests (SPT) or specific IgE for aeroallergens.

A study of 660 children (aged 1 to 18 years) with chronic rhinitis in Singapore showed that AR was identified in 75.9 percent of cases, and NAR accounted for 24.1 percent of the total.[3] Previous research has shown that NAR patients would acquire more symptoms such as nasal obstruction and postnasal drip[4]; however, distinguishing between AR and NAR is less defined in children. Allergists will do further testing to diagnose allergic rhinitis using a skin prick test or specific IgE; however, it is difficult to carry out in primary care settings. As a result, diagnosing allergic rhinitis and non-allergic rhinitis by clinical symptoms before making treatment selections is essential. Gender disparities in the prevalence of atopic diseases are observed in many epidemiological studies. However, little is known about whether sex is more common among children and adolescents with rhinitis. A recent meta-analysis found sex-related differences in rhinitis prevalence, switching from a male to a female predominance around puberty. The male predominance from childhood seemed to persist in adolescence only in Asia[5]. This study aimed to describe the clinical profile of AR and NAR among children with chronic rhinitis diagnosis who visited a tertiary hospital's allergy clinics and had a skin prick test result. Furthermore, to evaluate the clinical difference and general characteristics between AR and NAR, the association between skin prick test results and area of residence, as well as how direct exposure as a pet owner contributes to the development of pet sensitization in allergic rhinitis patients.

Methods

The study protocol was approved by the Institutional Review Board, Royal Thai Army Medical Department (R045h/62). Data were collected retrospectively from patients aged 2-18 years diagnosed with chronic rhinitis between January 2014 to December 2018 at the Allergy and Immunology Clinic, Department of Pediatrics, Phramongkutklao Hospital. Diagnosis of chronic rhinitis was defined when patients had two or more of the following symptoms: rhinorrhea, nasal congestion, sneezing, or nasal itching that were present on most days for ≥ 4 weeks in the past year. Those who previously used some medications that could induce rhinitis symptoms were also excluded from the study. Patients with confirmed sinonasal disorders (such as a nasal

polyp, sinusitis, or nasal septum deviation that seriously impaired nasal cavity airflow) or pregnancy were excluded. All patients with neither contraindication for SPT nor active skin diseases were indicated for aeroallergen skin testing. SPT using stainless steel lancet was performed on all patients. Standard aeroallergen extracts including house dust mite [*Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df)], American cockroaches, German cockroaches, cat dander, dog epithelia, *Alternaria* spp., *Cladosporium* spp., mixed *Aspergillus* spp., *Curvularia* spp., Bermuda grass, Johnson grass, careless weeds, and acacia as well as positive and negative controls (histamine and saline, respectively) (ALK-Abello Pharmaceuticals Inc., Canada) were evaluated. An immediate reaction (wheal and erythema) was read after 15 minutes. The result of SPT was considered positive when the allergen wheal size was 3 mm or larger than the negative control. The allergists diagnosed a patient with AR if the test was positive for an aeroallergen. The same set of symptoms defined NAR but with a negative SPT.

The number of patients who participated in this study and underwent an aeroallergen SPT is shown in **Figure 1.** When the patients visited the Allergy Clinic, a symptom-based questionnaire adapted from ARIA guidelines was used to inquire about their symptoms and documented in the medical records. Age, sex, age at onset, family history of any atopic diseases, living area, and environmental data (presence of household pets and smoking) were collected. Patients' charts were reviewed for rhinitis symptoms (rhinorrhea, nasal congestion, nasal and eye itching or sneezing) and physician-diagnosed comorbidities; the severity of rhinitis according to ARIA classification and SPT result were recorded.

Statistical analysis

Continuous variables were summarized using mean and standard deviation, and categorical variables were summarized using counts and percentages. The association between allergic rhinitis status and baseline characteristics, including demographic data, clinical features, and comorbidities, were analyzed based on the distribution and expected values of data. Continuous data were assessed using Student's t-test, and categorical data were evaluated using the chi-square test and Fisher's exact test. Bivariate logistic regression was performed to estimate the association between aeroallergen sensitization profiles and living areas (Bangkok vs. other provinces). The association between pet sensitization test results and history of pet exposure was also explored using multivariable logistic regression adjusted by type of pet

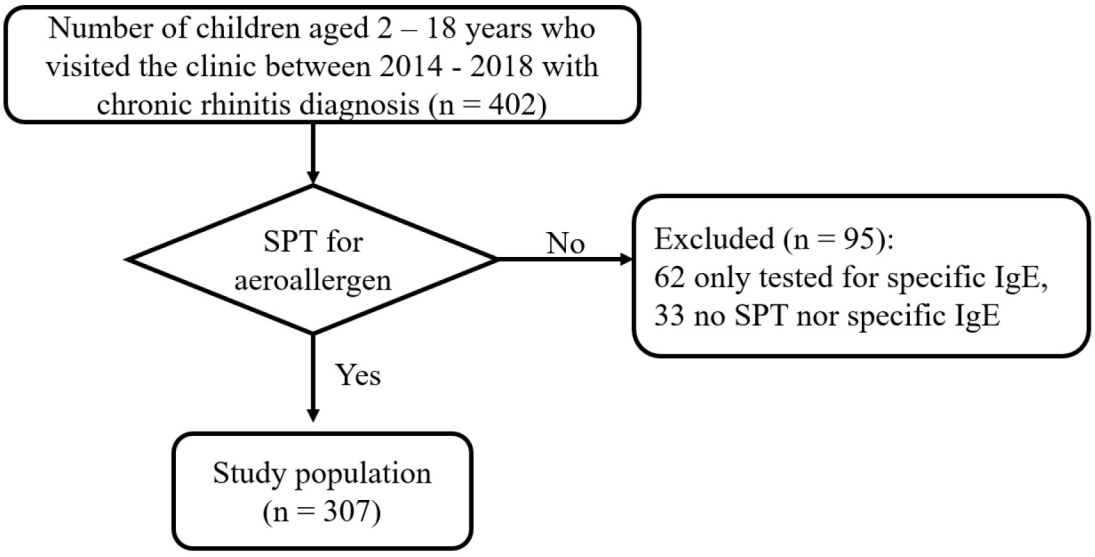


Figure 1. Number of patients who were assessed for chronic rhinitis and underwent skin prick test

exposure (cat, dog, others), age (years) at first diagnosis, and sex of patients. We assumed that age and sex were potential confounders between pet sensitization and history of pet exposure since age and sex are associated with allergy[5-7] and potentially related to pet exposure history. All analyses were performed using R version 4.0.2 software (R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: http://www.R-project.org/).

Results

Characteristics of children with rhinitis

During the study, 402 children with rhinitis were assessed for symptoms of chronic rhinitis in the Pediatric Allergy Clinic, Phramongkutklao Hospital. Three hundred and seven patients had undergone SPT for aeroallergen. Among these patients with SPT results, 226 (73.6 %) were classified as AR, and 81 (26.4%) were NAR. Most of them were living in Bangkok (69.7%). The mean age of onset for patients was not different between AR and NAR; however, the mean age at first diagnosis for patients with AR was older than NAR (mean ages 7.53 vs. 6.39 years, p=0.012 (**Table 1**). Most patients in the AR group were male (65.0%), which differed from the NAR group in that males and females were equal. Most patients had no other medical conditions (72.6%), although we observed that 8% had attention deficit hyperactivity disorder (ADHD) before they were diagnosed with AR or NAR. Patients with NAR were more likely to clean the house every week than those with AR (p=0.005) (**Table 1**); however, there was not enough evidence to conclude the differences in the history of allergic disease in the family, passive smoking, presence of household pets, and rhinitis severity between AR and NAR patients.

Table 1. Baseline characteristics of allergic rhinitis and non-allergic rhinitis patients in the pediatric allergy clinic, Phramongkutklao Hospital, 2014–2018

Characteristics	Total (n = 307)	NAR (n = 81)	AR (n = 226)	*p*-value
Age of onset (years)				
Mean (SD)	5.36 (3.16)	5.06 (3.47)	5.47 (3.04)	0.308 [a]
Age at first diagnosis (years)				
Mean (SD)	7.23 (3.50)	6.39 (3.27)	7.53 (3.54)	0.012 [a]
Sex, n (%)				
Male	186 (60.6)	39 (48.1)	147 (65.0)	0.011 [b]
Family history of atopic diseases, n (%)				
No family history	122 (39.7)	35 (43.2)	87 (38.5)	
Allergic rhinitis	153 (49.8)	40 (49.4)	113 (50.0)	0.793 [c]
Asthma	21 (6.8)	4 (4.9)	17 (7.5)	
Others †	11 (3.6)	2 (2.5)	9 (4.0)	
Passive smoking, n (%) ‡	67 (22.2)	12 (15.2)	55 (24.7)	0.113 [b]
Household pets, n (%) ‡	92 (30.4)	20 (25.0)	72 (32.3)	0.283 [b]
Frequency of house cleaning, n (%)				
Every one week	169 (58.1)	55 (73.3)	114 (52.8)	0.005 [b]
Every two weeks	85 (29.2)	16 (21.3)	69 (31.9)	
Longer than two weeks	37 (12.7)	4 (5.3)	33 (15.3)	

Table 1. Baseline characteristics of allergic rhinitis and non-allergic rhinitis patients in the pediatric allergy clinic, Phramongkutklao Hospital, 2014–2018 (Cont.)

Characteristics	Total (n = 307)	NAR (n = 81)	AR (n = 226)	*p*-value
Severity of disease at baseline, n (%)				
Mild intermittent	20 (6.5)	8 (9.9)	12 (5.3)	0.059 [b]
Mild persistent	195 (63.5)	57 (70.4)	138 (61.3)	
Moderate to severe intermittent	2 (0.6)	1 (1.2)	1 (0.4)	
Moderate to severe persistent	90 (29.3)	16 (19.8)	74 (32.9)	
Living area, n (%)				
Bangkok	214 (69.7)	60 (74.1)	154 (68.1)	0.392 [b]

† Other family history included: atopic dermatitis, food allergy, chronic urticaria

‡ Missing passive smoking (n=5); missing household pet (n=4)

Statistical tests for *p*-value: a) t-test, b) chi-square, c) Fisher's exact

Clinical characteristics and comorbid disease

Table 2 shows the comparison of nasal symptoms between the two groups. The most prevalent symptom in both NAR and AR was rhinorrhea, followed by nasal congestion. The AR group had a higher proportion of nasal itching (52.2% vs 37.0%; $p = 0.027$) and ocular symptoms (50.9% vs 28.4%; $p = 0.027$) than NAR group. The proportions of rhinorrhea, nasal congestion, and sneezing were not different between the NAR and AR groups. Snoring and acute sinusitis were the most common comorbidities; nevertheless, comorbidities including snoring, acute sinusitis, asthma, and atopic dermatitis seemed to be similar between the two groups.

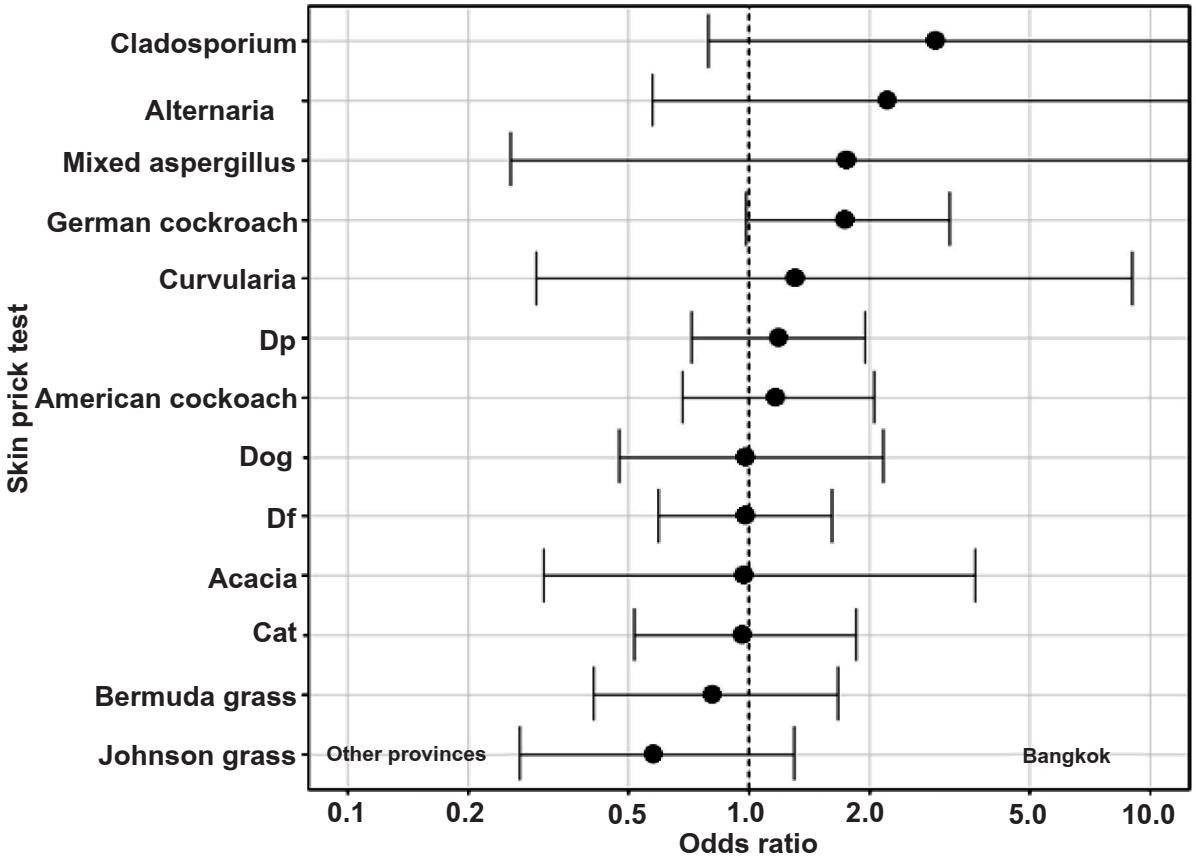
Table 2. Clinical symptoms and comorbidities between allergic rhinitis and non-allergic rhinitis patients in the pediatric allergy clinic, Phramongkutklao Hospital, 2014–2018

Clinical symptoms and comorbidities	NAR (n = 81)	AR (n = 226)	Chi-square *p*-value
Clinical symptoms, n (%)			
Rhinorrhea	70 (86.4)	193 (85.4)	0.968
Nasal congestion	57 (70.4)	160 (70.8)	1.000
Sneezing	39 (48.1)	125 (55.3)	0.328
Nasal itching	30 (37.0)	118 (52.2)	0.027
Ocular symptoms	23 (28.4)	115 (50.9)	0.001
Comorbidities, n (%)			
Snoring	19 (23.5)	50 (22.1)	0.927
Acute sinusitis	18 (22.2)	39 (17.3)	0.412
Asthma	11 (13.6)	41 (18.1)	0.443
Atopic dermatitis	2 (2.5)	22 (9.7)	0.065

NAR = non-allergic rhinitis, AR = allergic rhinitis

Aeroallergen sensitization profile in children with allergic rhinitis

The number of patients who lived in Bangkok was 214 (69.7%). **Figure 1** shows the prevalence of aeroallergen sensitization (based on SPT results) classified by patients who lived in Bangkok and other parts of Thailand. During the 4-year study period, Dp and Df were the most common aeroallergens sensitization (82.7% and 81.4%, respectively), followed by American cockroaches (38.1%), German cockroaches (37.6%), cat (24.3%), Bermuda grass (18.1%), dogs (15.9%), Johnson grass (12.8%), *Cladosporium* spp. (6.6%), acacia (5.8%), *Alternaria* spp. (5.3%), careless weed (3.5%), *Curvularia* spp. (3.5%), and mixed *Aspergillus* spp. (2.2%). Patients who lived outside of Bangkok seemed to have a higher chance of Bermuda grass sensitization (OR = 1.22; 95% CI = 0.61-2.46) than those who lived in Bangkok, similar to Johnson grass sensitization (OR = 1.71; 95% CI = 0.78-3.75), although their 95% CI were compatible with the null. On the other hand, patients who lived in Bangkok appear to have higher odds of aeroallergen sensitization profiles than those who lived outside Bangkok, for instance, *Cladosporium* (OR = 2.94; 95% CI = 0.79-19.07), *Alternaria* (OR = 2.23; 95% CI = 0.57-14.7), Mixed *Aspergillus* spp. (OR = 1.75; 95% CI = 0.25-34.53), German cockroaches (OR = 1.74; 95% CI = 0.99-3.17) and *Curvularia* spp. (OR = 1.31; 95% CI = 0.30-9.07) (**Figure 2**).



Dp = *Dermatophagoides pteronyssinus*, Df = *Dermatophagoides farinae*; could not determine odds ratio and 95% confidence interval for Careless weed due to sparse data.

Figure 2. Odd ratios and 95% confidence intervals for associations between aeroallergen sensitization profiles and area of living (Bangkok vs. other provinces)

Table 3. Association between household pets and pet allergen sensitizations among allergic rhinitis patients in the pediatric allergy clinic, Phramongkutklao Hospital, 2014–2018

	Skin prick test positive n (%)	Skin prick test negative n (%)	Adjusted OR†	95% CI†	*p*-value
Cat sensitization	55	252			
Cat ownership	14 (25.5)	25 (9.9)	2.77	1.27 - 5.88	0.009
Dog ownership	10 (18.2)	44 (17.5)	1.01	0.44 - 2.14	0.975
Dog sensitization	36	271			
Cat ownership	5 (13.9)	34 (12.5)	1.15	0.37 - 3.06	0.787
Dog ownership	9 (25.0)	45 (16.6)	1.63	0.67- 3.67	0.254

† OR = adjusted odds ratio, CI = confidence interval

Multivariable model logistic regression models included: type of pet ownership (cat, dog, others), age (years) at first diagnosis, and sex of patients

History of pet exposure in household and pet allergen sensitization

In this study, 30.4% of allergic rhinitis patients had pets in their houses. The proportion of cat sensitization (55/307=17.9%) was higher than dog sensitization (36/307=11.7%). The association between household pets and pet sensitization is shown in **Table 3**. After adjusting for type of pet exposure, age at first diagnosis, and sex of patients, the odds of sensitization to cats among rhinitis patients who were cat owners were 3.11 times that of those who did not own a cat. Using this statistical model, a plausible range of values for the odds ratio was 1.46 to 6.43 (95% CI=1.46-6.43).

Discussion

This study described the clinical characteristics of children with chronic rhinitis attending Phramongkutklao Hospital. The patients were divided into two groups: AR and NAR. The results revealed that children with AR were more common than NAR, similar to the findings of Vichyanond et al. [8], Visitsunthorn et al. [9], and Lee et al. [10] while differing from Westman et al. [11] and Lee et al.[12] However, our study could have a higher prevalence of AR than the general population because the patients were recruited from a pediatric specialist clinic. Since the symptoms of NAR were more severe than those of AR[13], patients with NAR went to see the doctor earlier. As a result, patients with NAR had a significantly lower age at first diagnosis than those with AR. Boys were shown to be more likely than girls to have AR in the children's age group, comparable to adulthood with chronic rhinitis, when men were also less likely to have NAR than females.[14] There were sex differences in the prevalence of AR over the life span, with boys having a greater frequency than girls during childhood, followed by an equal distribution in adolescence. [14]

Nasal itching and ocular symptoms were more frequent in AR than in NAR, similar to the study by Vichyanond et al. [8]; however, rhinorrhea, nasal congestion, and sneezing between the two groups were not different. Our study found that snoring was the most common comorbidity in children with AR and NAR. Snoring in these groups of patients might be due to adenoid hypertrophy or nasal congestion, affecting sleep quality. Patients with nasal congestion were more likely to have moderate to severe obstructive sleep apnea than allergic patients without nasal congestion.[15] Unfortunately, our questionnaires might be insufficient to assess sleep disorders,

and objective testing such as polysomnography should have been used to evaluate sleep disturbance accurately in the following study. Similar asthma prevalence in children with allergic and nonallergic rhinitis was consistent with previous studies.[16] Asthma was common comorbidity in children and adults with allergic rhinitis. Treatment for AR could reduce asthma symptoms; therefore, the clinician should look for other comorbidities and proper management if a patient presents with either AR or asthma symptoms.

House dust mites were shown to be the most frequent aeroallergen sensitization from studies in children with AR who lived in Bangkok[9] and Chiangmai Province[17], accounting for 80% of cases, similar to our study. House dust mite sensitization is relatively high because Thailand is located in a tropical environment. The temperature is generally warm and humid, favorable for mite proliferation. American cockroaches (*P. americana*) and German cockroaches (*B. germanica*) are common cockroach species found in people's homes, and their allergens can induce atopic diseases, particularly allergic rhinitis. In 2004, Tungtrongchitr et al. found that P. americana was the predominant cockroach species in the house of people who lived in Bangkok, followed by *B.germanica.*[18] We found more prevalence of German cockroach sensitization than American cockroach sensitization in children who lived in Bangkok, which differed from previous studies in Thailand.[9, 17] Furthermore, in this study, approximately 35% of children with AR in Bangkok were only sensitized to the German cockroach. A higher prevalence of German cockroach sensitization might be due to the increasing number of this type of cockroaches in Bangkok. We know that exposure to pet allergens is a significant risk factor for developing allergic sensitization and respiratory allergic diseases such as allergic rhinitis or allergic asthma. The presence of a pet at home is commonly regarded as the most significant risk factor for allergic sensitization. In previous studies, the association between cat ownership and sensitization to cats has remained questionable[19]; however, dog ownership was preventative for sensitization to dogs.[20] Our findings showed that cat ownership was strongly associated with cat sensitization in children with allergic rhinitis, opposite to dog ownership, which was not associated with dog sensitization. The timing of exposure, duration of the pets exposure, level of pet allergen exposure, or genetic risk seem to modify or induce pet sensitization.[21]

A strength of this study was the use of symptom-based questionnaires to collect medical data and SPT for aeroallergens, which were performed in most patients with chronic rhinitis to classify them into AR and NAR. However, a retrospective review was a significant limitation of this study; this data collection could not be as complete as in a prospective study. Patients with local allergic rhinitis (LAR) would have been indistinguishable in this study because NAR was differentiated from AR based only on rhinitis symptoms and the absence of negative SPT results. A systematic review of studies subjected to nasal allergen provocation tests (NAPT) has recently demonstrated local allergen reactivity in 16.1% of children previously considered NAR. [22] It is essential to use NAPT to evaluate rhinitis to identify LAR patients. The development of systemic atopy is not a common situation in LAR individuals. Nevertheless, LAR worsens rapidly with progressive rhinitis severity and impairment in quality of life. Therefore, a more accurate diagnostic test to define the type of chronic rhinitis is warranted to optimize patient management.

Conclusion

This study describes clinical characteristics between AR and NAR among children with chronic rhinitis. The proportion of AR was higher than NAR. The most common aeroallergen sensitization was house dust mites, followed by cockroaches. This information could benefit children who visit primary care for initial management and appropriate common allergen avoidance. Nasal pruritus or ocular symptoms strongly supported AR, of which additional allergy testing in those children may be required.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Acknowledgments

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DIAGNOSIS OF IRON DEFICIENCY ANEMIA IN THAI FEMALE ADOLESCENTS USING RETICULOCYTE HEMOGLOBIN EQUIVALENT

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Abstract

Background: Female adolescents aged 10 to 19 years are at remarkable risk of iron deficiency anemia (IDA). Reticulocyte hemoglobin equivalent (Ret-He) is an initial indicator of iron incorporation in red blood cells (RBCs) hemoglobin and reflects the iron functional availability in the RBCs.

Objective: This study aimed to assess the diagnostic performance of Ret-He to identify IDA and determine a specific cut-off value for Thai female adolescents.

Methods: Blood samples of 191 Thai female adolescents, ages ranging from 12 to 18 years, were included. Patients underwent complete blood count, reticulocyte count, Ret-He, serum iron (SI), total iron-binding capacity (TIBC), and transferrin saturation (TSAT). The correlation of Ret-He with other parameters and the diagnostic performance to identify IDA were evaluated.

Results: Among 191 patients, 89 and 102 were defined as IDA and non-IDA groups. Ret-He value in the IDA group was significantly lower than that in the non-IDA group (p<0.001). Strong positive correlations were observed between Ret-He and RBC indices and SI and TSAT (p<0.001). A Ret-He value of ≤27.0 pg could distinguish IDA from non-IDA with a sensitivity of 91.2% and a specificity of 100.0% (area under the curve, AUC of 0.99, 95% CI: 0.98-0.99; p<0.001).

Conclusion: This study confirmed that Ret-He is a cost-effective parameter representing an advantage over other traditional iron markers. A specific Ret-He cut-off value of ≤27.0 pg is suitable for distinguishing IDA from non-IDA with excellent diagnostic performance among Thai female adolescents.

Keywords: Iron deficiency anemia, Reticulocyte, Hemoglobin, Reticulocyte hemoglobin equivalent, Thai female adolescents

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Introduction

Iron deficiency anemia (IDA) is the most common cause of disorders of nutritional anemia, especially among reproductive-age women and people with low iron intake. Untreated iron deficiency (ID) can affect growth and development, especially in children.[1,2] Because of rapid growth, menstrual blood loss, and inadequate iron intake, female adolescents aged 10 to 19 years are at remarkable risk of iron ID.[3] Patients with IDA may experience effects on cognitive function, audiovisual reaction time, physical capacity, and work performance. In clinical practice, symptoms improve promptly with iron supplementation.[3,4]

Laboratory testing consisting of hemoglobin (Hb) and mean corpuscular volume (MCV) is used to screen for anemia owing to its widespread availability and ease of interpretation. According to the high prevalence of thalassemia in Thailand and Southeast Asian countries, further testing such as iron study, Hb typing, and molecular studies to identify mutations of globin genes are required to differentiate between IDA and thalassemia. [5,6] Conventional biomarkers such as serum ferritin (SF), serum iron (SI), total iron-binding capacity (TIBC), and transferrin saturation (TSAT) are used to define the iron status of clinically anemic individuals. However, those biomarkers are influenced by several factors of diurnal variation and dietary intake. [7,8]

Reticulocyte hemoglobin equivalent (Ret-He) is a reticulocyte-derived parameter available on Sysmex-XN series analyzers. It constitutes an initial indicator of iron incorporation in red blood cells (RBCs) Hb and reflects the iron functional availability in the RBCs.[9] Related studies have demonstrated the applications of Ret-He in IDA and various clinical settings.[10-16] Notably, Ret-He provided speedy results to indicate iron status and was not affected by other chronic diseases. Therefore, Ret-He is convenient in the diagnosis and follow-up treatment of IDA among infants, pregnant women, and patients with chronic renal failure.[17-19]

Recently, the diagnostic performance using Ret-He cut-off values to identify IDA and non-IDA among various Thai populations has been reported.[20-22] However, the information on Ret-He levels and a specific cut-off value among Thai female adolescents is currently unavailable. This study aimed to assess the diagnostic performance of Ret-He to identify IDA and determine the cut-off value for Thai female adolescent populations.

Methods

This study was approved by the Committee of the Institutional Review Board, Royal Thai Army Medical Department, Bangkok, Thailand (IRBRTA 984/2564), and the Human Research Ethics Committee of Thammasat University (HREC-TUSc) Pathumthani, Thailand (COE No. 018/2564).

The cross-sectional study enrolled female adolescents, ages ranging from 12 to 18 years attending the Division of Hematology/Oncology, Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand, from September 2021 to February 2022, were recruited. The sample size calculation was based on a single proportion formula, this study was based on the prevalence of anemia in Thai female adolescents of 25.2%,[23] with a confidence interval of 95% and a margin of error of 6.5%. The calculated sample size of 191 participants was sufficient to meet the study objective. The subjects were categorized into two groups; IDA and non-IDA. Anemia was defined as a Hb concentration <12.0 g/dL.[24] IDA was defined as serum iron (SI) <50.0 mg/dL and/or TSAT <16.0%.[25] Informed consent forms were signed by the subjects' parents. Ethylene-diaminetetraacetic acid (EDTA) and clotted blood samples were collected and analyzed at the Laboratory of Hematology, Department of Pathology, Phramongkutklao Hospital, to perform laboratory tests. The laboratory has received International Organization for Standardization (ISO) 15189:2012 and ISO 15190:2003 certifications.

Complete blood count (CBC), reticulocyte count, and Ret-He were analyzed using a Sysmex XN-9000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Serum from clotted blood samples was analyzed for iron markers, including SI and unsaturated iron-binding capacity

(UIBC) using COBAS INTEGRA Iron Gen.2 reagent (Roche Diagnostics GmbH, Germany) and COBAS INTEGRA UIBC reagent and analyzed by a Cobas 8000 series c502 Chemistry Analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland). TIBC was calculated as a sum of SI and UIBC. In addition, a transferrin saturation (TSAT) was calculated using the formula; TSAT (%) = (SI/TIBC) × 100. All blood samples were assessed within two hours after collecting blood. In addition, quality control samples were run daily to ensure adequate functionality of the analyzers.

Statistical analysis

The IBM SPSS software for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism, Version 9 (GraphPad Software, CA, USA) were used for statistical analysis. Demographic characteristics were analyzed using descriptive statistics, and continuous variables were presented as mean and standard deviation (SD). The comparison between the study groups was carried out using the Student's *t*-test. The correlation (r) between the Ret-He and other blood parameters was evaluated using Pearson's correlation coefficient with a 95% confidence interval (95% CI). The diagnostic performance of RET-He to detect IDA was determined, including sensitivity, specificity, predictive values, and the area under the receiver operating characteristic (ROC) curve (AUC), and compared with the reference assays, SI and TSAT. A $p<0.05$ was considered statistically significant.

Results

The study population included 191 female participants, 89 IDA, and 102 non-IDA patients. No difference was found between the two groups regarding age and body mass index (BMI). The RBC parameters and iron biomarkers included in this study are summarized in **Table 1**. All red blood cell indices, including Hb, Hct, and RBC indices, except RDW, in the IDA group, were significantly lower than those of the non-IDA group ($p<0.001$). For iron biomarkers, lower SI and TSAT and higher TIBC were observed in the IDA group compared with those of the non-IDA group ($p<0.001$). There is no significant difference in the reticulocyte count between the two groups ($p>0.05$). However, Ret-He was significantly lower than that in the non-IDA group ($p<0.001$) (**Figure 1**).

In the IDA group, the baseline Ret-He level was positively correlated with baseline Hb level (r=0.72, $p<0.001$), MCV level (r=0.84, $p<0.001$), MCH level (r=0.88, $p<0.001$) and MCHC level (r=0.45, $p<0.001$) (**Figure 2**). In addition, the baseline Ret-He level was correlated with SI level (r=074, $p<0.001$) and TSAT (r=0.71, $p<0.001$).

The reference iron biomarkers to define IDA, SI <50.0 mg/dL, and TSAT <16.0% and the ROC analysis among female adolescents with Hb levels of less than 12.0 g/dL is shown in **Figure 3**. The sensitivity and specificity of those biomarkers were calculated (**Table 2**). By ROC analysis, the optimal Ret-He cut-off for IDA detection was generated using the best combination of sensitivity and specificity. The ROC curve revealed the area under the curve of 0.99 (95% CI: 0.98-0.99; $p<0.001$) at cut-off ≤27.0 pg (**Figure 3**), at which IDA was distinguished with a sensitivity of 91.2%, a specificity of 100.0% specificity, a positive predictive value (PPV) of 92.0%, and a negative predictive value (NPV) of 100.0% (**Table 2**).

Table 1. Demographics and clinical findings of the patients

Variable	IDA (n= 89)	Non-IDA (n=102)	*p*-value
Age (years)	14.83±2.10	15.20±2.32	0.258
BMI (kg/m^2)	22.46±5.70	21.74±5.50	0.373
RBC (×10^6/L)	4.51±0.85	4.84±2.70	0.262
Hb (g/dL)	9.35±1.94	12.60±1.77	<0.001
HCT (%)	30.71±5.53	38.53±5.30	<0.001
MCV (fL)	68.94±8.33	84.71±5.98	<0.001
MCH (pg)	21.11±3.59	27.72±2.08	<0.001
MCHC (g/dL)	30.36±2.18	32.32±3.11	<0.001
RDW (%)	19.23±4.75	16.11±18.60	0.125
Reticulocyte (%)	1.48±2.43	2.06±2.71	0.213
Ret-He (pg)	21.03±4.19	30.40±3.52	<0.001
SI (µg/dL)	22.86±10.49	78.87±28.95	<0.001
TIBC (µg/mL)	300.24±113.92	78.73±28.84	<0.001
TSAT (%)	8.50±4.16	29.10±12.42	<0.001

Abbreviations: BMI, body mass index; Hb, hemoglobin; HCT, hematocrit; IDA, iron deficiency anemia; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Ret-He, reticulocyte hemoglobin equivalent; SI, serum iron; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

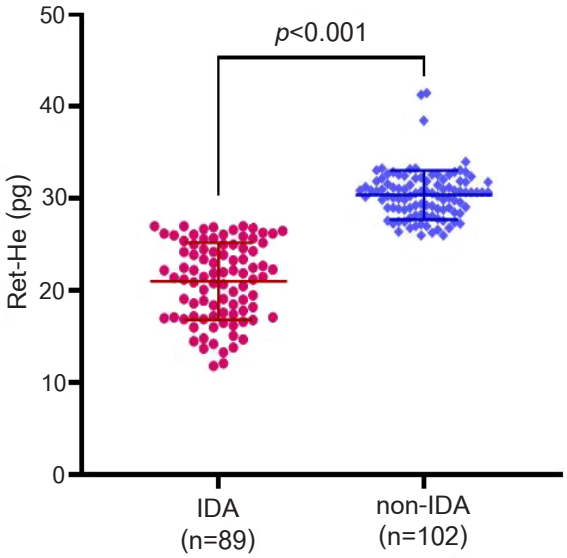

Figure 1. Differences in the Ret-He level among IDA and non-IDA patients

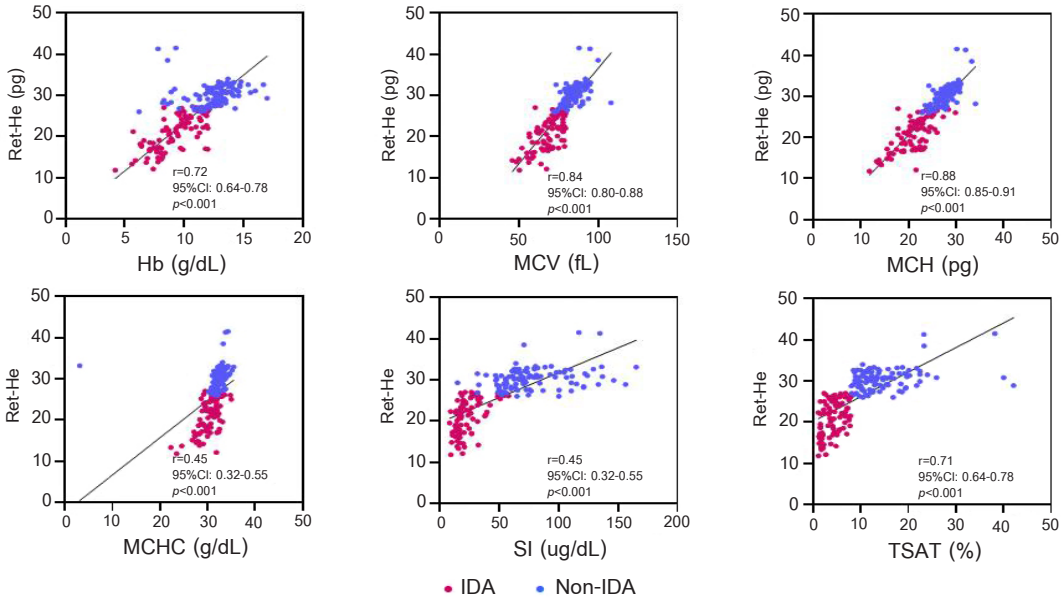

Figure 2. Relationship of Ret-He and Hb, MCV, MCH, MCHC, SI, and TSAT

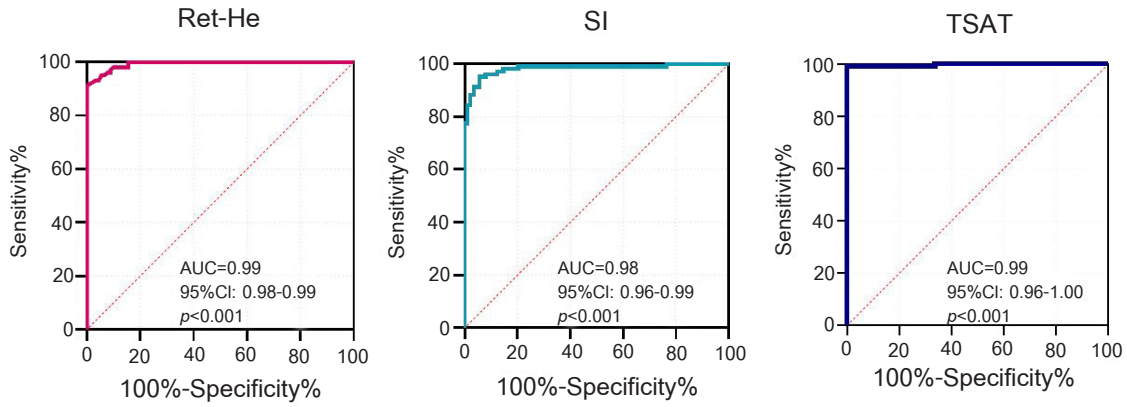

Figure 3. Receiver operating characteristic (ROC) curve analyses of Ret-He, SI, and TSAT to determine IDA among female adolescents

Table 2. Diagnostic performance of Ret-He, SI and TSAT to determine IDA

Parameter	Cut-off	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Ret-He (pg)	≤27.0	0.99	91.18	100.00	92.00	100.00
SI (mg/dL)	<50.0	0.98	91.18	96.63	85.00	96.00
TSAT (%)	<16.0	0.99	99.18	100.00	98.87	100.00

Abbreviations; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Ret-He, reticulocyte hemoglobin equivalent; SI, serum iron; TSAT, transferrin saturation.

Discussion

Measurement of Ret-He has been implemented to detect changes in iron status. This marker is beneficial in monitoring the response to both iron-replacement therapy and bone marrow response after starting treatment.[26] Related studies revealed that Ret-He constitutes a capable parameter for detecting the early stages of IDA. The reference intervals for Ret-He have already been determined in different patient populations, ranging from 29.8 pg to 38.2 pg.[27] The proposed 31.2 pg cut-off value of Ret-He was used to differentiate IDA from non-IDA patients.[28] In Thailand, the differences in Ret-He among school-aged children were evaluated, and the Ret-He cut-off ≤27.0 pg was suggested to identify IDA in a thalassemia-prevalent area.[21] Additionally, the diagnostic performance of the Ret-He has been implemented in different Thai patient populations, and the optimal cut-off >30.0 pg could signify a non-IDA state.[22] Because the Ret-He cut-off value varies in study populations, the specific cut-off value should be determined before clinical applications.

A total of 191 female adolescents were included in this study. According to their Hb levels, SI, and TSAT, they were divided into two groups, IDA and non-IDA. Ret-He was significantly lower in the IDA group compared with that of the non-IDA group. A positive correlation between Ret-He and CBC parameters, including Hb levels, MCV, MCH, and MCHC found in the target population, was concordant with related studies.[21,22,29,30] Notably, Ret-He exhibited a strong positive correlation with MCV and MCH, corresponding to the Hb of the young RBCs entirely released from the bone marrow. Hence, Ret-He provides real-time information on the functional availability of iron for effective erythropoiesis and changes in iron status earlier than the Hb content of mature RBCs. In addition, a strong positive correlation between Ret-He and SI and TSAT was also observed.

Concerning Ret-He diagnostic performance assessment among Thai female adolescents (12 to 18 years), a Ret-He value of 27.0 pg and below could distinguish IDA from non-IDA with a sensitivity of 91.2% and a specificity of 100.0%. This result was supported by a recent study in which a Ret-He cut-off value of 27.0 pg was found to predict IDA with a sensitivity of 91.7% and a specificity of 81.0% among school-aged Thai children.[21] In addition, the diagnostic performance of Ret-He was comparable to SI and TSAT. Therefore, Ret-He constitutes a practical parameter to diagnose IDA. Presently, the advanced hematology analyzer provides information on reticulocyte (RET) and Ret-He, which allows clinicians to determine the quality and quantity of the young RBCs fraction. The test is fast, inexpensive, and practical to perform within two hours after collecting blood.

Even though SI and SF levels and TSAT were routinely used to assess IDA, in this study, only SI levels and TSAT were used as reference methods because SF levels can alter by other non-physiological changes, such as inflammatory disorders and infections.[31] The disadvantages of using SI and TSAT included increased patient expenses and required additional blood specimens. Further, the limitation of this study involved using the population at a single center in a high thalassemia-prevalent area. Unfortunately, hemoglobin typing and molecular studies were not performed among all subjects. Also, only 12-18-year-old females were included. Hence, the results cannot be generalized to other populations. A larger population study is recommended. Our results clearly showed that Ret-He constitutes a helpful marker in diagnosing IDA and non-IDA among female adolescents. Further multi-center studies in different geographical regions are suggested.

Conclusion

Ret-He constitutes a parameter included in reticulocyte testing using automated analyzers. Its use is cost-effective and does not require more sample collection, illustrating an advantage over other conventional biomarkers. This study was the first to use a Ret-He cut-off value of ≤27.0 pg to distinguish IDA from non-IDA with excellent diagnostic sensitivity and specificity among Thai female adolescents.

Disclosures

The authors declared they have no conflicts of interest.

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EFFECT OF AGOMELATINE AND SERTRALINE ON PATIENTS WITH MAJOR DEPRESSIVE DISORDERS AND CHRONIC KIDNEY DISEASE: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Background: Depression is highly prevalent and is well known to affect patients with chronic kidney disease (CKD). Agomelatine exerts psychotropic effects upon mood and anxious states. There is limited data on agomelatine treatment among patients with CKD.

Methods: Patients with CKD stage 3-5 with DSM-5-defined major depressive disorder (MDD) were randomly assigned to receive 25 mg/day of agomelatine or sertraline 50 mg/day for eight weeks at Phramongkutklao Hospital. Hamilton Depression Rating Scale (HDRS) score and concerning adverse events were measured at baseline and the end of the study. Efficacy assessment compared the improvements in clinical response and remission between the agomelatine and placebo groups.

Results: Of 53 enrolled patients, 27 were assigned to the agomelatine group and 26 to the sertraline group. The mean age was 64.8±13.4 years. Baseline characteristics were comparable across treatment groups. After eight weeks, agomelatine-treated showed reductions in HDRS score from baseline (-15.6 with 95% CI -18.6 to -12.5). A significant difference was observed in the reduced HDRS scores between agomelatine and sertraline groups (-12.4; 95% CI -18.4 to -6.5). Over the 6-week treatment period, clinical response (55.0 vs. 9.0%, p <0.001) and remission (45.0 vs. 17.4%, p =0.049) improved significantly more with agomelatine than with sertraline. Both agomelatine and sertraline were well-tolerated during the treatment period.

Conclusion: Agomelatine showed superior antidepressant efficacy over sertraline in treating CKD patients with depression after eight weeks, with a good tolerability profile.

Keywords: Major depressive disorders, Chronic kidney disease, Depression, Agomelatine, Sertraline

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Introduction

Quality of life and functional health are influenced by physical, cognitive, and emotional factors among patients with chronic kidney disease (CKD).[1] Major depressive disorder (MDD) is a common disorder, and its prevalence is 3-4 times more among patients with CKD than in other chronic diseases.[2] MDD is associated with an increased risk of adverse clinical outcomes, including rapid decline in renal function, dialysis therapy initiation, death, or hospitalization among patients with CKD.[3-5] Potential mechanisms of depression might differ in CKD versus normal populations, and intervention regarding the type and dose of antidepressant medications might vary between patients with and without renal impairment. Future studies should examine interventions to prevent and treat depression in CKD populations.

Antidepressant drugs are effective in the general population, but pharmacokinetics might vary among patients with renal impairment. The evidence on the effectiveness of antidepressants among patients with CKD is insufficient, and further clinical trials are greatly needed.[6] Current guidelines suggest selective serotonin reuptake inhibitors to treat depression among patients undergoing dialysis, but evidence regarding these medications for these patients is sparse and inconclusive.[7] Agomelatine is a potent agonist of melatonin receptors (MT1 and MT2) with 5-HT2C antagonist properties and is effective in treating depression. Agomelatine is well-tolerated, exhibits few serious side effects, and may provide a useful alternative antidepressant drug among patients with CKD.[8] One randomized controlled trial indicated that agomelatine had significantly better efficacy in treating depressive and anxiety symptoms among patients with CKD with low and transient adverse events.[9] Presently, no studies have yet been conducted to compare the efficacy and acceptability of agomelatine versus sertraline in treating depressive symptoms among patients with CKD. The initial study aimed to demonstrate the efficacy and safety of agomelatine versus sertraline in treating MDD among patients with CKD.[10]

Methods

Study designs

This study protocol was reviewed and approved by the Institutional Review Board, the Royal Thai Army Medical Department, Bangkok, Thailand, approval number (IRB Number R011h/62). The study was registered on the Thai Clinical Trials Registry (TCTR20200319005) on 19 March 2020. The study constituted a randomized controlled trial comparing the efficacy of agomelatine and sertraline treatment among MDD patients with CKD. The study was conducted among MDD patients with CKD treated at Phramongkutklao Hospital between 1 June 2019 and 28 February 2020, with all subjects selected using inclusion criteria. The study was conducted per good clinical practice guidelines and the principles of the Declaration of Helsinki. All subjects provided their informed consent before they were enrolled.

Subjects

Psychiatrists evaluated patients for MDD using the Diagnostic and Statistical Manual of Mental Disorders (DSM-5).[11] The inclusion criteria included age 18 years or older, MDD, Thai Hamilton Depression Rating Scale (HDRS) ≥8[12], nondialysis CKD stages 3-5, and stable glycemic and blood pressure control at least three months before enrolling. The exclusion criteria comprised severe depression or suicidal ideation, other psychiatric conditions, active infections, advanced liver disease, advanced malignancy, renal transplant recipient, history of hypersensitivity to agomelatine or sertraline, and use of antidepressants or psychotherapy before randomization.

Patients were randomized in a 1:1 ratio using blocks of four randomizations based on a computerized random-number generator and allocation concealment, then divided into two groups, as shown in **Figure 1**. Assuming similar standard deviations (SD) from a previous study of the efficacy of agomelatine on depressive symptoms in patients with major depressive disorder, [13] a t-test comparison using a 2-sided α of .05 was estimated to be able to detect an effect size of 0.5 with 80% power in a sample of 70 participants (35 per group). One group consisted of 27 patients

treated with oral agomelatine 25 mg/day before bedtime. In contrast, the other group consisted of 26 patients treated with oral sertraline 50 mg/day before bedtime for eight weeks. All subjects typically continued their normal daily activities during both treatments and were instructed to adhere to the disease throughout the study.

Data collection

Data relating to demographics, medical and psychiatric history, and CKD treatment were reviewed from medical records before and after the study. At baseline and weeks 4 and 8, the depressive symptoms of patients were assessed using HDRS and Patient Health Questionnaire-9 (PHQ-9) score.[14] The HDRS is used as a clinician-administered depression assessment scale, and the HDRS contains 17 items about symptoms of depression experienced over the past week. The response was defined as a 50% reduction in HDRS scores, and remission was described as an HDRS score ≤7. At baseline and week 8, the World Health Organization Quality of Life-BREF (WHOQOL-BREF) was also used to assess four domains of quality of life (QOL): physical health, psychological health, social relationships, and environment.[15] Signs related to drug reactions and medication compliance were closely monitored every four weeks. Drug tolerability was defined as the percentage of complete medical prescriptions during the study. All subjects underwent routine laboratory blood tests at baseline and eight weeks at the end of the study.

Clinical outcomes

The primary outcome of the present study was a change in the HDRS score. The secondary outcome measures were the response rate, remission rate, and WHOQOL-BREF score. Adverse events that were or were not considered related to treatment were monitored every four weeks. The patients were systematically questioned about their experiences concerning adverse events during the previous four weeks.

Statistical analysis

Using the intention-to-treat principle, participants were analyzed in the groups to which they were randomized. Data analysis was performed using SPSS 19.0. Descriptive statistics were used to summarize demographics and baseline characteristics, including percentages, averages, and SD in the case of normally distributed continuous data. The Chi-square or Fisher's exact test was used for discrete or categorical variables. Paired-sample t-tests were used for continuous variables. Differences in HDRS and WHOQOL-BREF between the two groups were established by independent t-test and Mann-Whitney U test and presented using the relative risk of 95% confidence intervals. All results were considered significant when p was <0.05.

Results

From the screening, of a total of 986 patients with CKD in the outpatient clinic, 931 were ineligible. The main reasons for ineligibility were current antidepressant medication or psychologic therapy, medical and other psychiatric problems, and a decline to consent. A total of 53 patients with a mean age of 64.8±13.4 years and male (41%) were eligible according to the entry criteria in **Figure 1**. In all, 27 patients were assigned to the agomelatine group and 26 to the sertraline group. Forty-three (81%) patients completed the trial: 20 (74%) in the agomelatine group and 23 (88%) in the sertraline group. Characteristics of the study population are shown in **Table 1**. The baseline and laboratory characteristics were not different in both groups. Over 80% had at least one comorbidity, with type 2 diabetes and hypertension being the most common. Of the 53 patients, 27.9% had CKD stage 3, 16.2%, stage 4; and 55.8%, stage 5. The mean (SD) baseline score on the HDRS of 25.3 (5.0) in the agomelatine group and 22.5 (4.4) in the sertraline group were included in the study.

Efficacy Outcomes

Over eight weeks, the HDRS score was significantly decreased at -15.6 (95% CI -18.6 to -12.5) from baseline in the agomelatine group (p<0.05) but was not significantly changed from baseline in the sertraline group (-3.1; 95% CI -8.4 to 2.1). A significant difference was observed in the reduced HDRS scores between agomelatine and sertraline groups. (-12.4; 95% CI -18.4 to -6.5) (**Figure 2**).

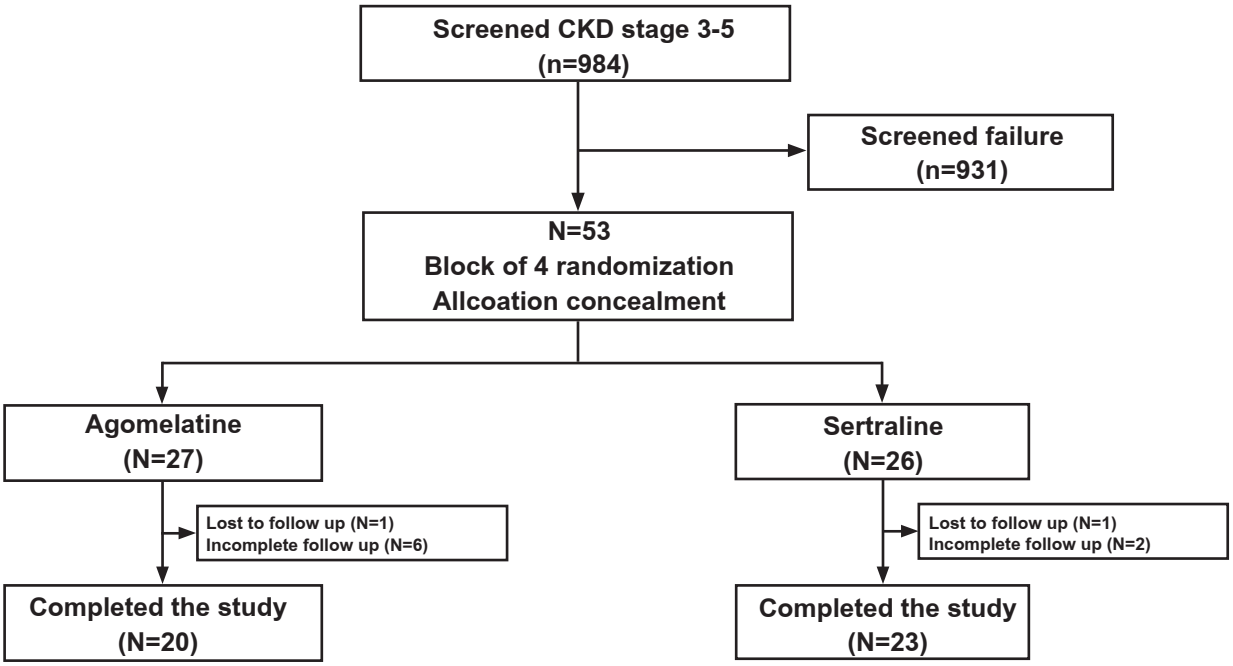

Figure 1. Flow chart of study

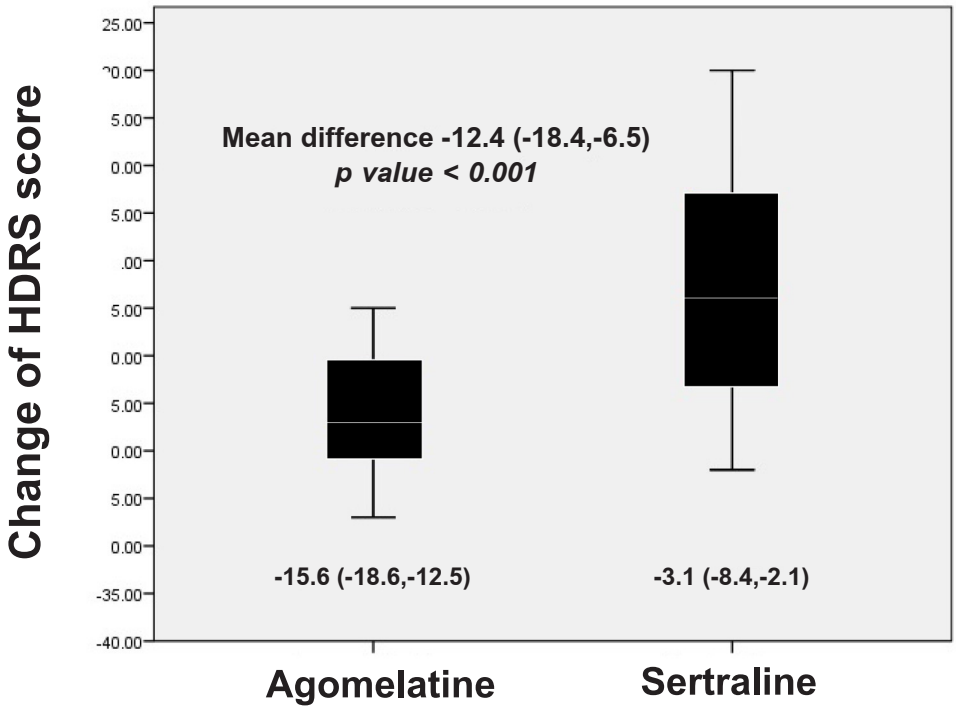

Figure 2. Mean change of HDRS after eight weeks of treatment. Significant between-group differences were noted in the mean change of HDRS (p <0.001).

Table 1. Baseline characteristics of patients

Characteristics	Agomelatine (N=20)	Sertraline (N=23)	*p*-value
Age (years±SD)	64.7±13.6	64.9±14.1	0.944
Male, N (%)	9 (45)	9 (39)	0.697
Etiology of kidney disease, (N (%)			
Type 2 diabetes	17 (85)	10 (43.5)	0.050
Hypertension	1 (5)	7 (30.4)	0.050
Chronic glomerulonephritis	2 (10)	2 (8.7)	1.000
Others	0 (0)	4 (17.4)	0.111
HDRS score	25.3±5.0	22.5±4.4	0.059
PHQ-9 score	14.4±5.7	12±4.8	0.124
WHOQOL-BREF score	72.5±12.4	70.7±9.9	0.607
Hemoglobin (g/dL)	10.2±2.1	10.7±1.5	0.354
BUN (mg/dL)	41.9±26.3	36.8±14.8	0.447
Serum creatinine (mg/dL)	5.2±3.4	4.3±2.6	0.388
Estimated GFR (mL/min/1.73 m^2)	18.9±17.6	19.7±15.5	0.679
Sodium (mEq/L)	138.8±2.5	138.7±2.9	0.914
Potassium (mEq/L)	4.4±0.7	4.4±0.7	0.862
Chloride (mEq/L)	99.9±3.9	100.3±5.3	0.775
Bicarbonate (mEq/L)	24.6±2.9	23.6±3.4	0.284
AST (U/L)	18.3±6.1	23.6±12.9	0.172
ALT (U/L)	12.7±7.9	18.9±10.7	0.098
Albumin (g/dL)	3.8±0.5	3.7±0.8	0.593
HemoglobinA1C (%)	7.4±1.8	7.1±2.1	0.655

Data presented as mean ± SD or number with a percentage. GFR; glomerular filtration rate, HDRS; Hamilton Depression Rating Scale, PHQ-9; Patient Health Questionnaire-9 (PHQ-9), WHOQOL-BREF; World Health Organization Quality of Life-BREF

Similarly, the proportion with remission, defined as a 50% reduction in HDRS score, was 55% in the agomelatine group and 9% in the sertraline group ($p<0.001$) (**Figure 3**). The proportion of patients with a treatment response defined as an HDRS score ≤7 was 45% in the agomelatine group and 17.4% in the sertraline group ($p=0.049$) (**Figure 3**). No significant difference was found in change in patient-reported overall health on the WHOQOL-BREF between groups ($p=1.640$), and no differences were observed in quality-of-life components from baseline to the end of the study (**Figure 4**). The percentage of medication adherence ascertained by pill count was 95% in the agomelatine group and 43.5% in the sertraline group ($p<0.001$) (**Figure 4**). At the end of the study, no statistically significant differences were noted between the two groups in body weight, hemoglobinA1C, hemoglobin, and estimated glomerular filtration rate.

Adverse events

Nausea or vomiting was the most commonly reported at 60.9% in the sertraline group compared with 0% in the agomelatine group ($p<0.001$), and headaches related to treatment were 21.7% vs. 0%, respectively ($p=0.051$; **Table 2**). Finally, no deaths or drug-related serious adverse events were reported in both groups. These results indicated that agomelatine was well-tolerated in the study.

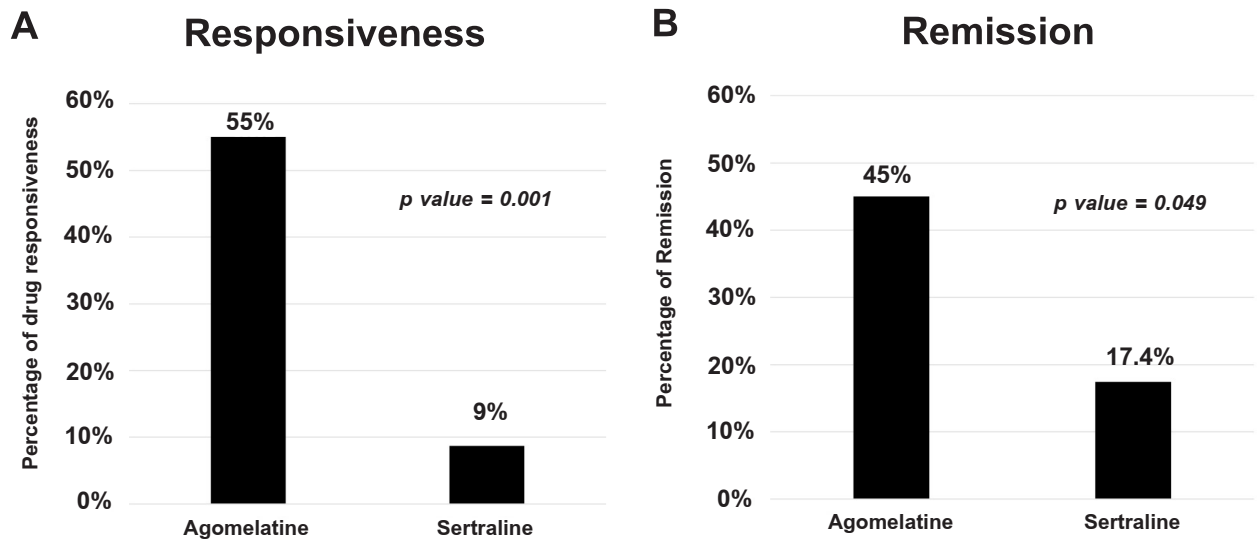

Figure 3. Response and remission rate after eight weeks of treatment. Significant between-group differences were noted in response and remission rates (p<0.001).

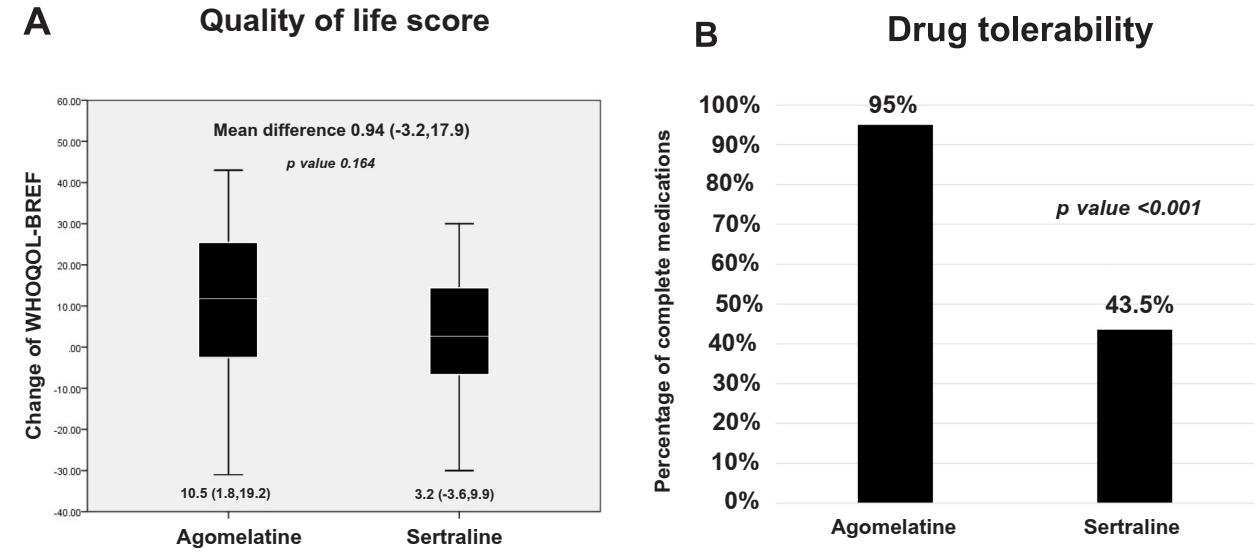

Figure 4. Mean change of WHOQOL-BREF and percentage of drug tolerability after eight weeks of treatment. Significant between-group differences were noted in drug tolerability (p<0.001).

Table 2. Adverse events reported in patients

Adverse effects, N (%)	Agomelatine (n=20)	Sertraline (n=23)	*p*-value
Nausea and vomiting	0 (0)	14 (60.9)	0.001
Headache	0 (0)	5 (21.7)	0.051
Dizziness	1 (5)	4 (17.4)	0.351
Dry mouth	0 (0)	5 (21.7)	0.051
Sleepiness	5 (25)	2 (8.7)	0.222
Fatigue	0 (0)	2 (8.7)	0.491
Anxiety	2 (10)	3 (13)	1.000
Increased appetite	1 (5)	1 (4.3)	1.000
Insomnia	1 (5)	1 (4.3)	1.000
Irritability	0 (0)	2 (8.7)	0.491
Agitation	0 (0)	3 (13)	0.236
Decreased libido	1 (5)	2 (8.7)	1.000
Erectile dysfunction	0 (0)	1 (4.3%)	1.000

Data are presented as the number with a percentage.

Discussion

The present study constituted a randomized controlled trial of the effect of agomelatine on depression score and drug tolerability among patients with advanced CKD. Treatment with agomelatine improved depressive symptoms and medication adherence among patients with CKD at stages 3-5 and decreased adverse effects compared with sertraline treatment. The risk of serious adverse events was not higher among patients receiving agomelatine vs. sertraline, but those treated with agomelatine experienced a significantly lower incidence of gastrointestinal adverse effects. This constitutes the first randomized controlled trial to provide evidence concerning MDD treatment with agomelatine among patients with advanced CKD compared with standard therapy.

Depression is common among patients with CKD but is often unrecognized, and few studies have investigated effective methods for treating depression among patients with CKD.[16] Additionally, data on the benefits and risks of antidepressants in this setting remain limited. Generally, patients with MDD are initially treated with antidepressant monotherapy, often serotonin reuptake inhibitors or sertraline. In randomized control trials, treatment with sertraline did not significantly improve depressive symptoms among patients with CKD.[17,18] A higher withdrawal rate was observed in the sertraline group due to gastrointestinal adverse events. Patients with CKD were at a higher risk for antidepressant adverse events due to the potential accumulation of toxic metabolites, increased risk of drug-drug interactions, and already prone to uremic symptoms such as nausea and vomiting. Initial studies found that agomelatine had advantages over standard antidepressants in treating depressive symptoms and glycemic control among patients with type 2 diabetes.[19,20] Additionally, clinical data supported that agomelatine exhibited good antidepressant efficacy, favorable tolerability profile, and fewer cases of discontinuation syndrome.[21-23] The findings indicated that agomelatine might be a promising agent in treating depression among patients with CKD.

One study demonstrated that agomelatine had significantly better efficacy in treating depressive and anxiety symptoms among patients with CKD than paroxetine, but the. Still, remission rates were nonsignificantly higher in the agomelatine group.[9] Our study also supported that agomelatine yielded better efficacy in treating depressive symptoms than sertraline. It might be mainly due to the following reasons: patients at

stages 3-5 CKD versus patients at stage 2-4 CKD and agomelatine compared with sertraline versus agomelatine compared with paroxetine. However, results in a related randomized control trial among patients without CKD also followed higher response rates for agomelatine than sertraline.[24] A published pooled analysis of head-to-head studies also confirmed that a significant reduction of depressive symptoms and better response rates were observed with agomelatine than with other antidepressants.[25]

Approximately 75-90% of patients completed the study. Adherence to the medication assessed by pill counts seems adequate in the agomelatine treatment group at 95%. Overall, the findings in this study were much higher than in other antidepressant trials involving patients with CKD, comparable with large trials among participants without CKD. A multicenter observational study also observed good adherence to agomelatine treatment in clinical practice.[26] This satisfactory adherence could be explained by the low relapse rate among patients receiving agomelatine.[27]

Several limitations were associated with the present study, particularly the small sample size. Recruitment was complex and constrained by the exclusion of a high number of patients already receiving treatment for depression and that declined to participate. A short-term study of sertraline improved depressive symptoms among patients with CKD. However, this study could not assess long-term efficacy and safety effects among patients with CKD. No proof was evident that the decreasing HDRS score with antidepressive agents would have long-term effects on clinical endpoints. Additional research is needed to confirm the results and determine long-term clinical outcomes. Finally, the study was an open-label randomized controlled design. The study's strength stemmed from measuring subjective scores, including HDRS and WHOQOL-BREF.

Conclusions

The present study indicated that treatment with agomelatine compared with sertraline for eight weeks significantly improved depressive symptoms among nondialysis patients with CKD. These findings supported the use of agomelatine to treat MDD among patients with CKD. Future long-term clinical studies are needed further to explore agomelatine's efficacy and safety in CKD populations.

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Conflict of interest

The authors declare that they have no competing interests.

Availability of data and materials

Data supporting this study are available upon request. All authors contributed substantially to the conception and design of the study, the acquisition of data, the analysis and interpretation of data, drafting of the article or critical revision for important intellectual content, and final approval of the submitted version.

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DETERMINING STRESS AND ASSOCIATED FACTORS IN A RURAL COMMUNITY DURING COVID-19 PANDEMIC USING THE COVID STRESS SCALE

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Abstract

Background: The COVID-19 pandemic has affected people worldwide, both physically and mentally. Stress is one of the burdens being faced, especially in the working class. Therefore, this study aimed to explore and compare associated stress factors during the COVID-19 pandemic lockdown among adults in a rural community in Thailand using the COVID stress scale.

Methods: This cross-sectional study was conducted from December 2021 to March 2022 in Chachoengsao Province. It included adults aged 20 to 60 years old. The questionnaire included demographic data and the Thai COVID stress scale (T-CSS) version. The data were collected using face-to-face interviews. Associated factors of stress were assessed using linear regression.

Results: Data were compared with their counterparts, illiteracy (adjusted β=18.4, 95% CI 5.9-30.1) and agriculturists (adjusted β=13.2, 95% CI 3.1-23.4). At the same time, age 51-60 (adjusted β=-11.1, 95% CI 3.9-27.3) and vaccination with ≥3 doses of COVID-19 vaccine (adjusted β=-8.9, 95% CI -16.4 to -1.5) were associated with decreased stress level.

Discussion: Illiteracy and agriculturists were associated with higher stress scores. COVID-19 vaccination doses might affect stress levels due to the efficacy of preventing infection and severe illness. Older people had less stress due to better experience in stress management. Limitations included that T-CSS cannot determine the cut-off point of stress and nonstress in the population due to multiple factors. However, it might be possible to imply that outlier scores from a normal distribution are likely to be most stressful during the COVD-19 pandemic.

Keywords: Stress, COVID-19, COVID-stress scale, Rural

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Introduction

Currently, the incidence of coronavirus 2019 (COVID-19), which the World Health Organization (WHO) has declared the epidemic of the new coronavirus to be a "pandemic" after the outbreak, has spread to various countries and territories around the world.[1]

In Thailand, the pandemic started in January 2020. As of April 2022, there have been five significant pandemic waves with varying virus strains.[2, 3] Distribution of COVID-19 vaccines was not ready until February 2021 with two major vaccines, CoronaVac and Oxford-Astra Zeneca COVID-19.[4, 5] However, CoronaVac's weak efficacy against the Delta strain shifted the mRNA and viral vector vaccine regimens by October 2021.[5] Therefore, by the beginning of the Omicron outbreak in late 2021 to early 2022, people, especially vulnerable groups, were encouraged to take booster doses due to its effect in preventing severe COVID-19.[6]

The pandemic resulted in problems involving many aspects, including health and the economy. The situation generated impacts including problems in self-isolation at home, follow-up visits to patients with underlying medical conditions and the lack of opportunities for patients with other diseases to receive treatment due to the COVID-19 pandemic.[7-11] Stress might have accumulated from lifestyle changes, altered working patterns, social distancing, lack of daily supplies and consumption supplies, and being informed about the COVID-19 outbreak and lockdowns.[10-13]

These health, economic and social problems affected all groups, families and institutions up to the national level, as well as the mentality of people in the country, especially the working class. Adults in the working class were most likely to be affected by outbreak situations and lockdowns during the COVID-19 pandemic, which can be stressful events.[14]

Previous studies have reported depression and stress in rural areas before the pandemic. [15-17] However, vulnerabilities due to inadequate logistic supplies and infrastructures, poor socioeconomic status, insufficient healthcare coverage and lower support combined with the pandemic situation, might have affected stress among people living in rural areas. Therefore, this study aimed to determine the associated factors of stress during the COVID-19 pandemic among adults in a rural community in Thailand.

Methods

Study design and subjects

A cross-sectional study was carried out to address the associated stress factors during the COVID-19 pandemic among participants residing in Baan Nayao, Chachoengsao, Thailand, from December 2021 to March 2022. Individuals eligible for this study were adults aged 20 to 60 years old and living in Baan Nayao, Chachoengsao, Thailand, during the study. The study focused on working-age people due to the possible impacts of COVID-19 on work and economic status. Participants were excluded if they had difficulties answering the questionnaire, such as people with visual or auditory disabilities and those with psychiatric conditions. Subjects were randomly selected.

This study was approved by the Institutional Review Board of the Royal Thai Army Medical Department. The approval number was M027q/64. Consent was appropriately obtained from all participants.

Baan Nayao is a rural community in Chachoengsao Province in eastern Thailand, located 145 km east of the capital city of Bangkok. The total population is approximately 4200, comprising 1152 households in this area. Of these, 85% are agriculturists. An aging population pyramid represents the population structure.[15]

Questionnaire and data collection

The questionnaire of this quantitative study included two parts, namely, demographic data and the 5-Likert Thai version of the COVID stress scale (T-CSS).[18] The COVID stress scale was translated and modified to Thai. The translators of this questionnaire comprised two linguistic experts. The translation process included translating the questionnaire to Thai by the first translator; the second translator re-translated the Thai questionnaire back to English. The content of

the original English language questionnaire and re-translated English questionnaire were compared. The corrections on discrepancies between the two English versions were made on the Thai questionnaire. Finally, the content of the Thai questionnaire was examined by three psychiatrists from the Department of Psychiatry, Phramongkutklao Hospital.

Demographic data included age, sex, marital status, educational level, tobacco and alcohol use, health issues and comorbidities, living status and COVID-19 vaccine reception. Apart from the original five parts of the COVID stress scale, T-CSS comprised six parts by adding COVID fear of contamination. The six parts included COVID 1) danger, 2) socioeconomic consequences, 3) xenophobia, 4) fear of contamination, 5) traumatic stress, and 6) compulsive checking. Items in each aspect were rated on a 5-point scale ranging from 0 (not at all) to 4 (extremely). Three experts confirmed the validity. Item Objective Congruence (IOC) was more than 0.5. All parts and overall reliability were examined using Cronbach's alpha coefficient, which had values greater than 0.8. Results were then generated to stress scores according to the COVID stress scale.

The questionnaire was carried out using face-to-face interviews, and data were collected on paper and online using Google Forms. Age, sex and marital status were collected from the individual's identity card. In addition, educational level, tobacco and alcohol use, health issues and comorbidities and living status were interviewed. COVID-19 vaccination history was collected via the Moh Prompt application, which stores each person's online vaccination card. Responses from participants were held on Google Sheets.

Operational definition

This study used smoking and alcohol consumption definitions from The Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO). CDC defines smoking as the following: a current smoker can be defined as an adult who has smoked 100 cigarettes in his/her lifetime and who currently smokes cigarettes. A former smoker was an adult who had smoked at least 100 cigarettes in his/her life time but quit smoking at the time of the interview.[19] WHO defined alcohol consumption as the followings: a former drinker is an adult (15+ years) in a given population who did not consume alcohol in the last 12 months but did previously. A current drinker comprises those having consumed a drink containing alcohol in the previous 12 months.[20]

Statistical analysis

STATA 17.0 (Stata Corporation, College Station, TX, USA) was used for statistical analysis. Regression diagnosis found linear relationships between the predictors and the outcome and homogeneity of variance. General characteristics were calculated using descriptive statistics. Associated factors of increased stress score were assessed using linear regression with univariate analysis. Statistically significant factors, p <0.20 and previously significant in other studies, were eligible for multivariate analysis. The final model included sex, age, education level, occupation, alcohol consumption, smoking, diabetes mellitus and COVID-19 vaccine doses. We used the β-coefficient and 95% confidential interval to represent the relationship between variables and outcomes. Statistical significance was considered at $p \leq 0.05$ at a 95% confidential interval.

Results

Demographic data

Participants' demographic data are shown in **Table 1**. The average age of participants was 47.8 ± 0.7 years. Most participants were female (61.0%), married and living with a partner (77.5%), educated to at least a primary level (56.6%), agriculturist (38.7%), had no comorbidities (47.2%), living with family or partner (89.2%), non-smokers (76.3%), non-drinkers (57.5%), vaccinated with two doses of COVID-19 vaccine (80.5%), and received CoronaVac (1st dose) and Oxford-AstraZeneca COVID-19 vaccine (2nd dose) vaccination regimen (52.8%).

Table 1. Demographic data among adults in Baan Nayao Village, Chachoengsao, 2022

Characteristic	n	%
Sex		
Female	260	61.0
Male	166	39.0
Age (year)		
Mean S.D.	47.77 ± 0.65	
≤30	56	13.2
31-40	65	15.3
41-50	104	24.4
51-60	201	47.1
Marital status		
Married/living with a partner	330	77.5
Single	55	12.9
Widowed/Divorce/Separated	41	9.6
Education level		
Illiterate	36	8.5
Primary school	241	56.6
Secondary school and higher	149	35.0
Occupation		
Agriculturist	165	38.7
Business owner	135	31.7
Employee	65	15.3
Unemployed	50	11.7
Government officer	11	2.6
Comorbid illnesses		
Hypertension	84	19.7
Dyslipidemia	57	13.4
Diabetes mellitus	44	10.3
Cardiovascular disease	16	3.8
Chronic kidney disease	10	2.4
Asthma	6	1.4
Emphysema	4	0.9
Cancer	4	0.9
No comorbidities	201	47.2
Living status		
Living with family/partner	380	89.2
Living alone	46	10.8
Smoking		
Non-smoker	325	76.3
Ex-smoker	40	9.4
Current smoker	61	14.3
Alcohol consumption		
Nondrinker	245	57.5
Former drinker	78	17.3
Current drinker	103	24.2

Table 1. Demographic data among adults in Baan Nayao Village, Chachoengsao, 2022 (Cont.)

Characteristic	n	%
COVID-19 Vaccine dose		
0 dose	12	2.8
1 dose	13	3.1
2 doses	343	80.5
3 doses	58	13.6
COVID-19 Vaccine regimen		
Unvaccinated	12	2.8
Incomplete vaccination[A]	13	3.1
SV+SV	13	3.1
SV+AZ	225	52.8
SP+PF	4	0.9
AZ+AZ	35	8.2
SP+SP	3	0.7
SP+AZ	13	3.1
PF+PF	3	0.7
AZ+PF	33	7.8
MDN+MDN	13	3.1
SV+SV+AZ	4	0.9
SV+SV+PF	7	1.6
SV+SV+MDN	6	1.4
SV+AZ+PF	22	5.2
SV+AZ+MDN	3	0.7
SV+AZ+AZ	10	2.4
AZ+AZ+PF	10	2.4

SV = CoronaVac; SP = Sinopharm BIBP COVID-19 vaccine; AZ = Oxford-AstraZeneca COVID-19 vaccine; PF = Pfizer–BioNTech COVID-19 vaccine; MDN = Moderna COVID-19 vaccine
[A] An incomplete vaccination was for individuals receiving only one of any provided

COVID-stress scale

The mean overall score and standard deviation for the COVID-stress scale were 53.8±31.8. The mean score and standard deviation in each domain included COVID danger (10.5±6.6), COVID socioeconomic consequences (5.9±6.9), COVID xenophobia (12.5±8.6), COVID fear of contamination (12.5±6.6), COVID traumatic stress (3.9±6.1), and COVID compulsive checking (8.7±6.3). As shown in **Figure 1**, the histogram of the overall mean score was right-skewed.

Associated factors of increased and decreased stress score

Associated factors for increased stress during the COVID-19 pandemic were illiteracy (adjusted β=18.4, 95% CI 5.9 to 30.1) and agriculturists (adjusted β=13.2, 95% CI 3.1 to 23.4) compared to their counterparts. At the same time, age 51 to 60 (adjusted β=-11.1, 95% CI 3.9 to 27.3) and vaccination with ≥3 doses of COVID-19 vaccine (adjusted β=-8.9, 95% CI -16.4 to -1.5) were associated with decreased stress level. Results are summarized in **Table 2**.

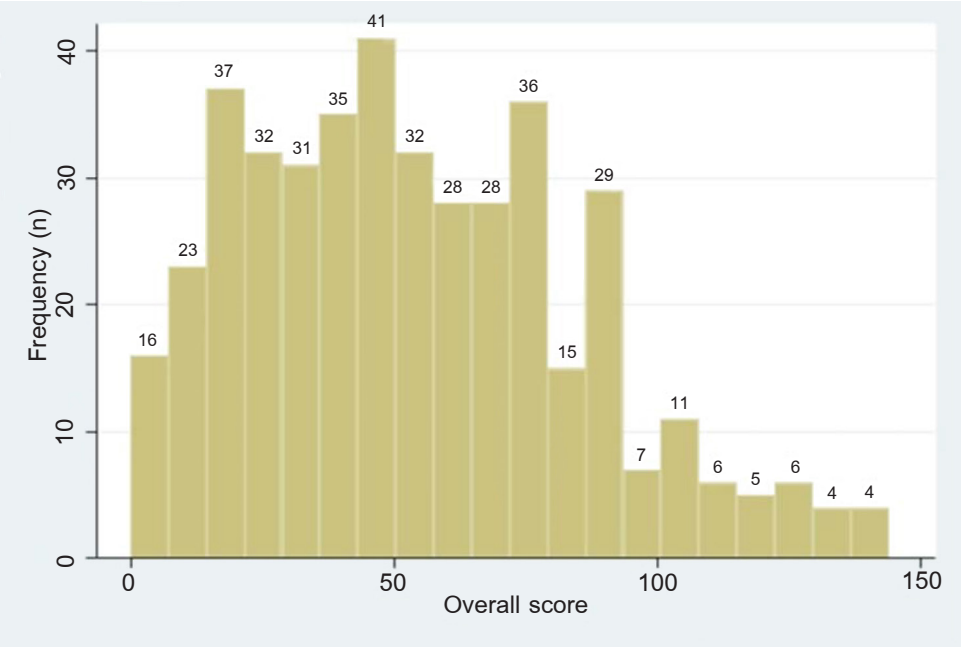

Figure 1. The overall mean score of T-CSS

Table 2. Univariate and multivariate analysis identified the factors that independently affect the T-CSS in Baan Nayao, Chachoengsao, Thailand, 2022

	Univariate analysis		Multivariate analysis	
Characteristic	**Crude β (95% CI)**	***p*-value**	**Adjusted β (95% CI)**	***p*-value**
Sex				
Male (as reference)				
Female	7.8(1.6-14.0)	0.013	6.9(-0.6-14.3)	0.070
Age (year)				
≤30 (as reference)				
31-40	-9.6(-21.5-2.4)	0.116	-10.1(-22.0-1.8)	0.095
41-50	-4.6(-15.3-6.1)	0.401	-9.3(-20.7-2.0)	0.107
51-60	-5.4(-15.1-4.3)	0.275	-11.1(-22.2- -0.3)	0.049*
Marital status				
Single (as reference)				
Married/living with a partner	-4.8(-13.8-4.3)	0.305		
Widowed/Divorce/Separated	6.8(-6.1-19.6)	0.301		
Education level				
Secondary school and higher (as reference)				
Primary school	-0.8(-7.2-5.6)	0.801	-1.2(-8.9-6.3)	0.761
Illiterate	21.2(9.8-32.7)	<0.001	18.4(5.9-30.8)	0.004*
Occupation				
Unemployed (as reference)				
Agriculturist	12.2(2.2-22.2)	0.017	13.2(3.1-23.4)	0.011*
Business owner	-0.7(-10.9-9.5)	0.895	-1.3(-11.5-9.0)	0.808
Employee	9.6(-2.0-21.2)	0.104	9.6(-2.1-21.3)	0.109
Government officer	6.0(-14.7-26.5)	0.569	5.7(-15.4-26.8)	0.596

Table 2. Univariate and multivariate analysis identified the factors that independently affect the T-CSS in Baan Nayao, Chachoengsao, Thailand, 2022 (Cont.)

	Univariate analysis		Multivariate analysis	
Characteristic	**Crude β (95% CI)**	***p*-value**	**Adjusted β (95% CI)**	***p*-value**
Alcohol consumption				
Nondrinker (as reference)				
Former drinker	-7.4(-15.5-0.7)	0.074	-6.1(-14.4-2.2)	0.151
Current drinker	-6.4(-13.7-0.9)	0.086	-3.3(-11.2-4.7)	0.418
Smoking				
Non-smoker (as reference)				
Former smoker	-1.2(-11.7-9.2)	0.817	3.0(-8.4-14.4)	0.607
Current smoker	-9.0(-17.7- -0.2)	0.044	-3.5(-13.8-6.8)	0.504
Living status				
Living with family/partner (as reference)				
Living alone	-0.5(-10.3-9.3)	0.920		
Comorbid illnesses				
Diabetes mellitus				
No (as reference)				
Yes	-7.3(-17.2-2.7)	0.152	-7.4(-17.5-2.8)	0.153
Dyslipidemia				
No (as reference)				
Yes	-2.3(-11.2-6.6)	0.613		
Hypertension				
No (as reference)				
Yes	0.6(-7.0-8.2)	0.879		
Cardiovascular disease				
No (as reference)				
Yes	4.0(-12.0-19.9)	0.626		
Chronic kidney disease				
No (as reference)				
Yes	-11.4(-31.4-8.6)	0.264		
Respiratory disease				
No (as reference)				
Yes	-10.1(-32.5-12.2)	0.373		
Cancer				
No (as reference)				
Yes	-12.2(-43.6-19.3)	0.447		
COVID-19 vaccine doses				
Unvaccinated (as reference)				
2 doses of vaccination	-3.4(-14.1-7.3)	0.533	-4.4(-14.8-6.1)	0.413
≥ 3 doses of vaccination	-9.6(-17.2- -2.0)	0.013	-8.9(-16.4- -1.5)	0.019*

* Statistically significant at $p<0.05$

Discussion

The study identified the associated stress factors during the COVID-19 pandemic lockdown among adults in a rural community in Thailand. Our results showed that the factors associated with stress during the COVID-19 lockdown were illiteracy, working as a farmer and vaccination with COVID-19 vaccine regimens other than CoronaVac (1st dose) and Oxford-AstraZeneca COVID-19 vaccine (2nd dose).

Related studies have addressed varying populations' stress, anxiety and depression during the COVID-19 pandemic.[21-25] Related studies were conducted in China and in Paraguay, using the Depression, Anxiety and Stress Scale (DASS-21) to classify depression, anxiety and stress levels as normal, mild, moderate, severe and highly severe.[21, 25] Stress levels are affected by multiple factors, such as varying severity of situations and measurements.[21, 25] This issue was already considered using the original COVID stress scale as the cut-off point was difficult to determine and the mean score on the scales changed throughout the COVID-19 pandemic.[18, 26] As a result, the cut-off points of 'stress' and 'non-stress' were not absolute and should be interpreted along with the response of a given population to the pandemic at a given time.[26] The higher the score, the higher the stress an individual may exhibit. According to this information, this study collected the total scores of the studied population as continuous data and stratified them on the histogram; the chart was right-skewed. As those with a score over 100 points were outliers from a normal distribution, we assumed they were more likely to be affected by stress than those in the normal distribution.

According to the COVID-stress scale, increased stress levels were strongly associated with illiteracy. Related studies suggest that people with lower educational levels are more likely to experience stress and anxiety.[21, 27, 28] However, other studies showed that during the COVID-19 pandemic, people with higher literacy were more affordable with expenses, had a healthier diet, performed more physical activities, could better use health resources obtained on the Internet, and had better adjustment capabilities.[29, 30] This study also hypothesized that due to uncertainty of pandemic development and public health measures, people with higher literacy could better perceive news from reliable resources than those with lower literacy.

The agriculturist occupation was found to have a relationship with elevated stress levels during the COVID-19 pandemic lockdown. The reason might be that farmers were more pessimistic about income loss.[31] However, farmers also tended to be affected by many factors, including problems in marketing goods, problems in transportation and a lack of financial support from the government.[32]

Increasing age was associated with less pandemic-related stress. A related study also reported a similar result.[33] Gerontologic theories suggest that older people cope with stress better than younger people.[34] The related study hypothesized that the already lonely nature of older people might make them feel less socially isolated than younger individuals who may have experienced a sudden reduction in their social contact due to social distancing measures.[33] Our study also hypothesized that older people were less likely to be affected by the loss of employment during the COVID-19 pandemic than younger, working-age adults; thus, resulting in less stress.

This study discovered that three and more doses of the COVID-19 vaccine influenced lowering stress during the COVID-10 pandemic. Most of the 'booster' dose of COVID-19 in Thailand was the mRNA vaccine. According to related studies comparing the efficacy of vaccine regimens, other vaccine regimens, including mRNA vaccines as second or booster doses, enhance immunogenicity very well.[6, 35, 36] Therefore, it could be possible that people receiving booster doses had lower stress levels during the COVID-19 pandemic because of participants' confidence in vaccine efficiency against infection and severe illness.

The study encountered several significant limitations. First, the questionnaire of this study did not include diagnostic assessments such as DSM-V or ICD-10, which would have helped

evaluate the prevalence of stress. Second, 13 interviewers were involved during data collection. The questionnaire results could be influenced differently because each interviewer had different verbal and nonverbal communication skills. Third, enrolled participants had limited age group variety, with 47.2% of participants in the age group 51 to 60 years. The study was conducted during the Omicron outbreak, two years after the first COVID-19 outbreak. Stress levels could be lower than in the previous waves since the Omicron outbreak in Thailand was less severe, and people might be able to adapt to a new normal lifestyle. Finally, this study's generalizability and external validity might be limited due to the small number of subjects, and being conducted in a single area might not represent the whole country.

The strength of this study was that the COVID stress scale was translated to Thai and tested for validity and reliability. It constituted the first to be used in the epidemiology field. In further studies, T-CSS might be able to be used as a brief measurement of COVID-19-related stress if compared with a standard stress scale or structured diagnostic assessment. The COVID stress scale is recommended to be used in future studies. Total scores should be collected as continuous data and stratified for distribution to determine the stress level of the studied population due to the lack of absolute cut-off point of 'stress' and 'nonstress' because of pandemic situation dynamics.

The COVID-19 pandemic is seemingly a long-run even though the outbreak's status will be shifted from pandemic to endemic in the future. Therefore, health education, financial support and social support for rural communities should be implemented to prevent mental illnesses in this vulnerable group. In addition, government priorities should alleviate burdens on people during the pandemic, such as control of prices of customer products, inexpensive and simple accessibility to healthcare services, adequate distribution of vaccines and clear public communication.

Conclusion

this study showed associated factors of stress levels during the COVID-19 pandemic in a rural community in Thailand. According to the COVID-stress scale, the stress level was significantly affected by age, illiteracy, farmer occupation, and COVID-19 vaccine doses. Overall mean stress score showed some outlying individuals with high-stress scores, which could be associated with other mental illnesses such as depression and anxiety. For healthcare practitioners and organizations, T-CSS can serve to aid in identifying individuals at risk for adverse emotional events during the COVID-19 pandemic and can be used as a brief measure of COVID-related stress.

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PROPOSAL FOR A MANDATORY HEALTHCARE PROGRAM FOR SURROGATES IN SURROGACY ARRANGEMENTS

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Abstract

Background: Surrogacy is controversial. The issue of surrogates' exposure to legal, ethical, health and social risks has been globally debated, and most literature explores policies and regulations that could be crafted or reconsidered to protect surrogates from such hazards. However, a discussion is lacking on the need for surrogacy healthcare programs (SHPs), although surrogacy has been possible for over four decades.

Objectives: This paper aimed to examine the existence (or lack) of SHPs and identify healthcare program needs to protect surrogates from risks in building surrogacy arrangements.

Methods: An interdisciplinary, nonsystematic literature review and media content analysis were conducted. Medline (Ovid) and PubMed were searched for articles published between 2012 and 2022. In addition, three search engines, Google, Bing and Yahoo, were used to identify high-profile and landmark cases to supplement the literature review.

Results: Seventy-eight articles were retrieved, but only 2 were reviewed. Using the 3 search engines, 53 cases were identified; however, 42 were duplicates, and only 11 were analyzed. The results suggested that SHPs do not exist. High-profile and landmark cases demonstrate a need to educate prospective surrogates.

Conclusion: SHPs should be compulsory for prospective surrogates to promote their well-being, and proposals for such programs should be further studied and implemented in healthcare policies.

Keywords: Surrogacy, Surrogacy arrangement, Surrogates, Education, Educational program, Healthcare

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Introduction

Surrogacy arrangements have been conducted since the 1980s; however, the possibility of creating surrogacy healthcare programs (SHPs) has never been thoroughly examined.[1] Surrogates, who commit to carrying a baby for intended parents, comprise a vulnerable population sector; they are at risk of exploitation, and their voices are often overridden in forming surrogacy arrangements.[2] Due to the lack of SHPs, surrogates may misunderstand their rights or not comprehend the potential risks involved in the treatment process. Consequently, surrogates are exposed to greater than normal legal, ethical, health and social risks.[3,4]

Several studies have investigated surrogates' health-related risks and advocated to protect their health at policy and regulation levels. For example, in some countries, such as the US, where commercial surrogacy is legal, firm policies and regulations (P&R) are in place to protect surrogates. Surrogates must receive legal advice and counseling and be provided their fully informed consent before conception.[5] In Australia, where altruistic surrogacy is allowed, strong policies are in place to protect the surrogate's health and optimize perinatal outcomes, such as a single embryo transfer (SET) policy for gestational surrogacy to prevent multiple pregnancies.[6-8] However, in countries where P&R are not established, laying the groundwork for surrogates' understanding of health-related facts and promoting their well-being is necessary. Education is one strategy for implementing health promotion programs for the target population.[9] Health education offers learning experiences on related health topics, including health benefits and threats. One broad purpose of health education is to increase individuals' knowledge and ensure their well-being by addressing and supporting P&R.[10] Health education builds individual health literacy and empowers patients. This paper aims to review the existence (or lack) of SHPs and to identify gaps in education for further development.

Methods

An interdisciplinary, nonsystematic literature review and media content analysis were conducted (**Figure 1**). Journal and original research articles and high profile and landmark cases published in English between 2012 and 2022 were identified. In addition, the author queried key search terms on Medline (Ovid) and PubMed, including surrogates, surrogate mothers, surrogacy, gestational surrogates, educational programs and healthcare programs.

The review centered on articles that discussed surrogacy education or SHPs. The literature was restricted to: (1) articles about the bioethical components of surrogacy, or arguments for/against it, that did not mention educational or healthcare programs and (2) studies on surrogacy arrangements and outcomes.

To supplement information found in the literature, the review also included sources from media such as news, reports, government agency websites and surrogacy agency websites. Three search engines (Google, Bing and Yahoo), which are considered the top three search engines in the world (updated in 2022),[11] were searched to further identify high profile and landmark cases. The media search was performed in January 2022 using the following phrases: high profile surrogacy cases and landmark surrogacy cases. Examining both the peer-reviewed literature and media content allowed the author to identify gaps in knowledge about healthcare program needs.

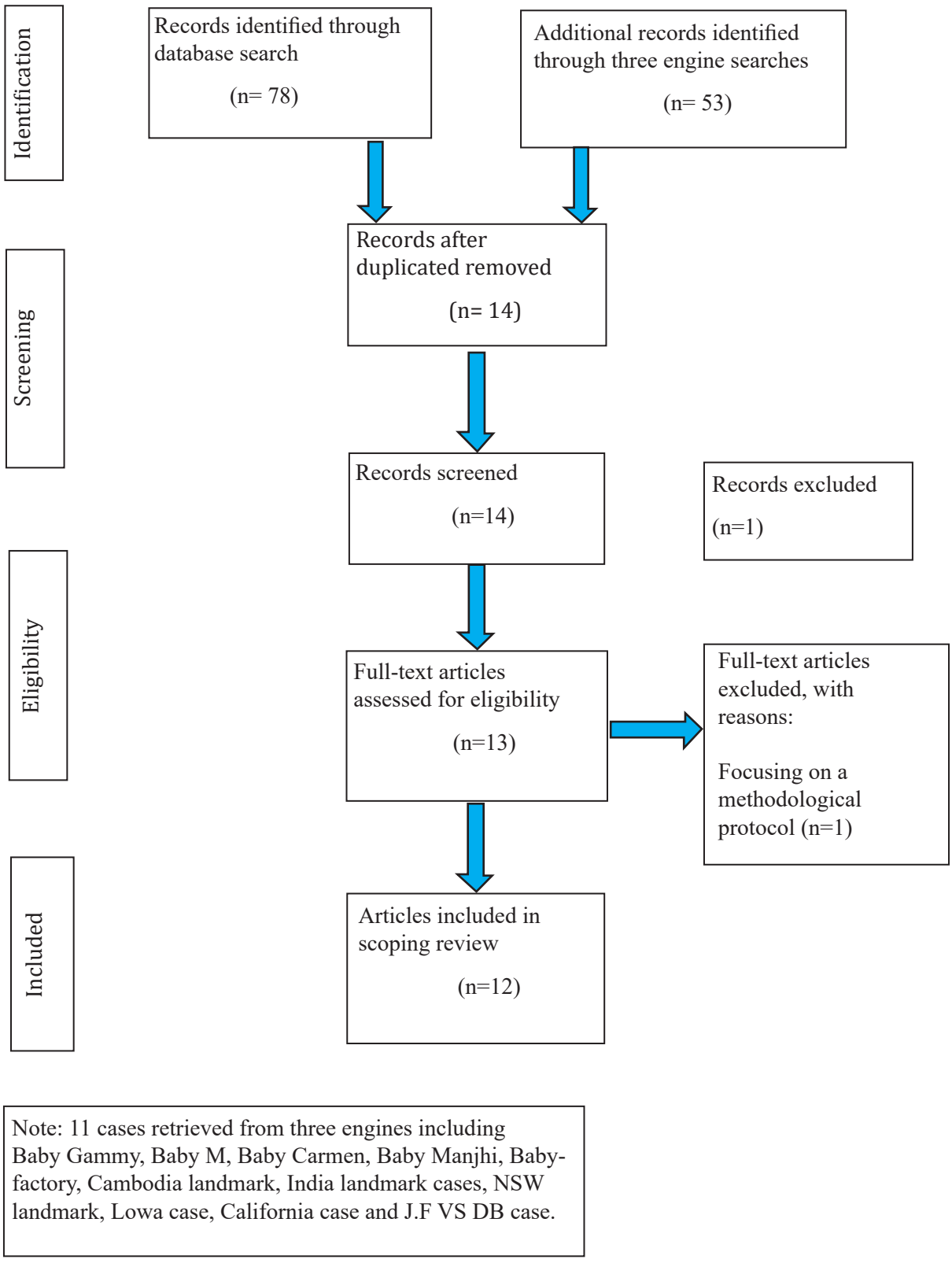

Figure 1: Search results

Results

Results from search strategies

The Medline (Ovid) search produced three articles, one of which was reviewed. The search of PubMed generated 75 articles, of which only one met the criteria for review, but it comprised a duplicate of the article identified through Medline (Ovid). The search Google led to 34 cases, Bing produced 9 cases and Yahoo generated 10 cases. The surrogacy cases identified by Bing and Yahoo were duplicated in Google. (**Figure 1.**)

The review identified one article about a healthcare program and 11 that covered high profile and landmark surrogacy cases. The findings suggested that SHPs were not practically implemented at policy and regulation levels. However, many studies and government agencies highlighted the importance of education to protect surrogates from health-related risks. Some surrogacy agency businesses[12, 13] provide education to surrogates through consultations before they enter a surrogacy arrangement; however, this could indicate a conflict of interest. Importantly, SHPs should be emphasized in surrogacy P&R. Countries' health departments and services could play a vital role in delivering educational programs to prospective surrogates to raise their awareness of risks, as well as to deepen their knowledge of surrogacy, identify their beliefs and opinions and enhance their self-efficacy and personal power.

Based on the above, two major themes emerged from the review:(1) building health literacy and (2) autonomy and self-knowledge.

Building health literacy

Health literacy is critical when using a health-related service and understanding the information needed to make appropriate decisions. Health literacy has been put forward as the pathway to education and health outcomes. In addition, education level is associated with the ability to comprehend health information, affecting one's capacity to maintain or improve one's health.[14] Hence, it would be unsurprising that people with low education may have poor health outcomes.[14]

In surrogacy, several high profile cases suggested that surrogates typically have low income and uneducated status.[15,16] Reflecting on education level, surrogates have less knowledge of surrogacy and the health risks involved, including the physical dangers resulting from complications of embryo transfer, pregnancy and delivery modes, as well as psychological, psychosocial and legal risks. For example, Tanderup and colleagues[17] found that none of the 14 surrogates they investigated could explain or understand the complications of multiple embryo transfer (MET) and multiple births. An interview by Attawet and peers[18] also supported the notion that surrogates are at risk during the surrogacy process:

> *I had three cycle attempts for embryo implantation. In each cycle, I received a triple embryo transfer. I became pregnant in the third embryo transfer cycle. At week 8 of my gestation, I had a regular check-up, and the doctor could not detect the baby's heartbeat. I was informed that I had to undergo a D&C [dilatation and curettage] procedure. The procedure was really painful, and I took a while to recover. I received less payment because I could not get through the pregnancy and delivery process. I received only 30,000 THB [approximately USD 960]. This was not worthwhile, and I swore I would not be a surrogate again. (p. 5)*

From this perspective, less health literacy knowledge could have been a barrier to the surrogate's ability to access or understand the health information affecting her regarding the health-related risks she might encounter. Research [19] suggests that people with limited health literacy are less likely to ask clinicians questions or to seek further information. Although education level is associated with the pathway of health literacy and health outcomes, healthcare systems should take proactive steps to promote people's well-being. Providing appropriately targeted health education programs using universal health literacy precautions is a clear strategy to improve people's understanding of health information, regardless of their literacy level or education.[20]

Autonomy and self-knowledge

The surrogacy context often involves informed information and consent. In surrogacy practice, the philosophy of autonomy has been revisited many times and its application among surrogates is ethically debated. Inadequately informed consent coercion, and dependence on decision-making often happen in surrogacy arrangements, especially in commercial surrogacy.[2, 17] Referring to philosophical autonomy, consent can be obtained through three standard approaches, namely, (1) the subjective standard: what an individual would need to know and understand for informed decision-making; (2) the reasonable patient standard: the information needed for patients to decide on their treatment and (3) the reasonable physician standard: a clinician's explanation of the procedure's nature to aid a patient's decision.[22] One of these approaches requires an assessment of the patient's understanding of the treatment procedure, including benefits and risks.[21] Therefore, the exercise of autonomy in the surrogacy process is needed for the surrogate's full knowledge and understanding of the treatment.

The healthcare provider must provide clear information to support patients' self-determination in their treatment. This depends on drawing the patient's attention to the fact that her self-knowledge is connected to her use of autonomy and could effectively support self-decision-making in the treatment. Thus, building surrogates' health literacy can bridge the gap between their autonomy and self-decision-making.

Discussion

The content analysis of the themes revealed a lack of health promotion for surrogates and SHPs. This issue should be brought forward and established in the healthcare system in the same way as other available programs for health promotion. In addition, government agencies should offer mandatory SHPs at the policy and regulation levels.

Although many theories exist concerning applying pedagogy in education, this paper does not discuss the form or approach that the education of surrogates should take. Instead, the author argues for the urgent need for SHPs. To date, no healthcare programs have been available to educate surrogates to help them better understand surrogacy arrangements, treatment and potential risks. In addition, although information about surrogacy is available on the Internet, some information might not be reliable.[22] The most accessible information comes from the recruiting agencies offering surrogacy services to intended parents.[22] Such information mainly focuses on the benefits of becoming a surrogate, but information about risks is largely lacking.[22] Some government websites—such as that of Australia's Victorian Assisted Reproductive Treatment Authority (VARTA)(23)—offer reliable information. However, they might not be easily accessible or be of limited use to surrogates whose first language differs from the website.

To ensure optimal use of the surrogate's autonomy and to avoid the influence of interested parties in the surrogate's decisions, the risks involved in the surrogacy process should be proposed in an educational context as discussed below.

1. Health risks, such as MET, multiple pregnancies, and cesarean section (C-section)

In one study, almost 80% of surrogates were likely to receive MET, resulting in 30% of the women birthing multiple children.[26] MET is an incentive for surrogates who accept to carry multiple pregnancies.[15, 16] Hence, before accepting the incentive model, surrogates should fully understand the risks of multiple pregnancies for themselves and their babies. Such surrogacy education could also help to formulate P&R in the future to protect surrogates from the risk of MET and possible exploitation by the surrogacy industry.

C-sections should also be included in the program. C-sections are commonly forced on surrogates to suit intended parents who are foreigners and must fly back to their countries on a preferred date.[24] Therefore, educating surrogates on the pros and cons of C-sections is necessary for them to make informed choices about their bodies and to discuss the matter with healthcare professionals. Further, this would help surrogates in their preparation and awareness of self-care.

2. The risk of legal aspects when surrogates move across borders

As is well known, surrogates frequently move across borders in response to efforts by the commercial surrogacy industry to avoid having to comply with local surrogacy laws. Unfortunately, this has increased surrogates' exposure to legal risk in the country of the transfer. For instance, after Cambodia banned commercial surrogacy in 2016, 33 surrogates were imprisoned in that country in a high-profile case and charged with human trafficking[25]. Therefore, before entering (commercial) surrogacy, in the context of education, potential surrogates should be aware of the legal risks involved in protecting their rights. This education would benefit surrogates and possess the advantage of framing international regulations to prevent cross-border surrogacy.

3. Understanding the context of psychosocial risks

Taking on the role of surrogate motherhood is problematic. Parental attachment and responsibility are likely to arise and cause complications in a surrogate's obligations. Although surrogates can address their psychological well-being regarding baby attachment when coping with relinquishment. [26-28] There have been cases of surrogates bonding with the baby. For example, Baby Carmen was born to a Thai surrogate who changed her mind about delivering the baby to the intended gay parents; however, the intended parents later won the case as the surrogate failed to return to court.[29] Although the surrogate had claimed to be a victim of human trafficking, the fact was that she wished to keep the baby in Thailand, and the true reason for her wish was not known. The issue of baby attachment and relinquishment often becomes delicate and has been well discussed in the literature;[26, 30] surrogates should be educated on this point to help them prepare mentally.

The scandal of Baby Gammy, who was left with his Thai surrogate mother when his intended Australian parents abandoned him, is another example of parent attachment and responsibility. In this case, the surrogate willingly accepted responsibility for Baby Gammy as one of her children after he was left in Thailand. The facts later clarified that at some point, the surrogate grew very attached Baby Gammy and his female twin, and she decided to keep Baby Gammy.[31, 32] As such, what would happen if a baby were left with a surrogate who was not physically or financially capable of raising the baby? Who would be responsible for this matter? Would the surrogate's pregnancy become a stigma?

Accordingly, education on managing psychosocial problems should be provided to raise the awareness of surrogates. These issues demonstrate the complexity of surrogacy treatment and highlight many matters that could go wrong that need to be addressed to develop regulations to cover all parties.

4. Understanding the context of surrogacy contracts and the signers' rights

The surrogacy contract is one of the most important documents in the surrogacy process. Before starting the surrogacy process, surrogates must fully understand and accept the conditions for their obligations.[33] While potential surrogates in the US receive mandatory legal counseling before deciding, in Southeast Asian countries, such as India and Thailand, no such process to supports surrogates.[34, 35] Reportedly, the rights of surrogates in India and Thailand have often been taken away. Indian surrogates had to reside in restricted areas provided by the surrogacy agency or fertility clinic.[2] Likewise, according to news reports, Cambodian surrogates had to live in a hostel provided for the duration of their pregnancy.[25] The worst part of this process was that the surrogates had to stay away from their families, which could cause psychological harm. In addition, they did not have access to insurance, postnatal counseling or other services.[34] Apart from the legal and ethical matters involved, surrogates should seriously consider other risks, such as health, financial and emotional hazards, along with potential irregularities in the legal contract. Yet, suppose surrogates are unable to understand a comprehensive contract. In that case, they could be misled and remain unaware of the risks, with the outcome that they fail to speak up for their rights and unintentionally accept the risks by signing the contract. In the

context of not receiving legal counseling, an educational program is critical to support surrogates' understanding of the contract. Not only do educational programs need to address this issue, but international policies or regulations should frame it so as to protect surrogates' rights.

This proposal for an educational program is a recommendation from the standpoint of public health to improve surrogates' well-being. This paper initially proposed an SHP to address problems with surrogacy at the individual and interpersonal levels. Evidence exists that surrogates have limited knowledge and understanding of the risks involved in surrogacy treatment.[2, 17] Many strategies are available to build surrogates' knowledge, but this paper proposes education in the form of a compulsory SHP before starting the process. However, this paper only reviewed knowledge and issues arising from high-profile cases without critically examining surrogates' education level. To move toward a more comprehensive health perspective, one recommendation is that research or surveys seeking support from the public and healthcare organizations for SHPs be included as an operable part of planning.

Conclusion

To protect surrogates' health and advocate for them, all governments should adopt SHPs in their P&R. This paper suggests that SHPs be made compulsory for women who want to become surrogates before starting the process. A complete understanding of surrogacy treatment and potential risks can ensure the optimal use of surrogates' autonomy in making decisions and enhance their perspectives on health.

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