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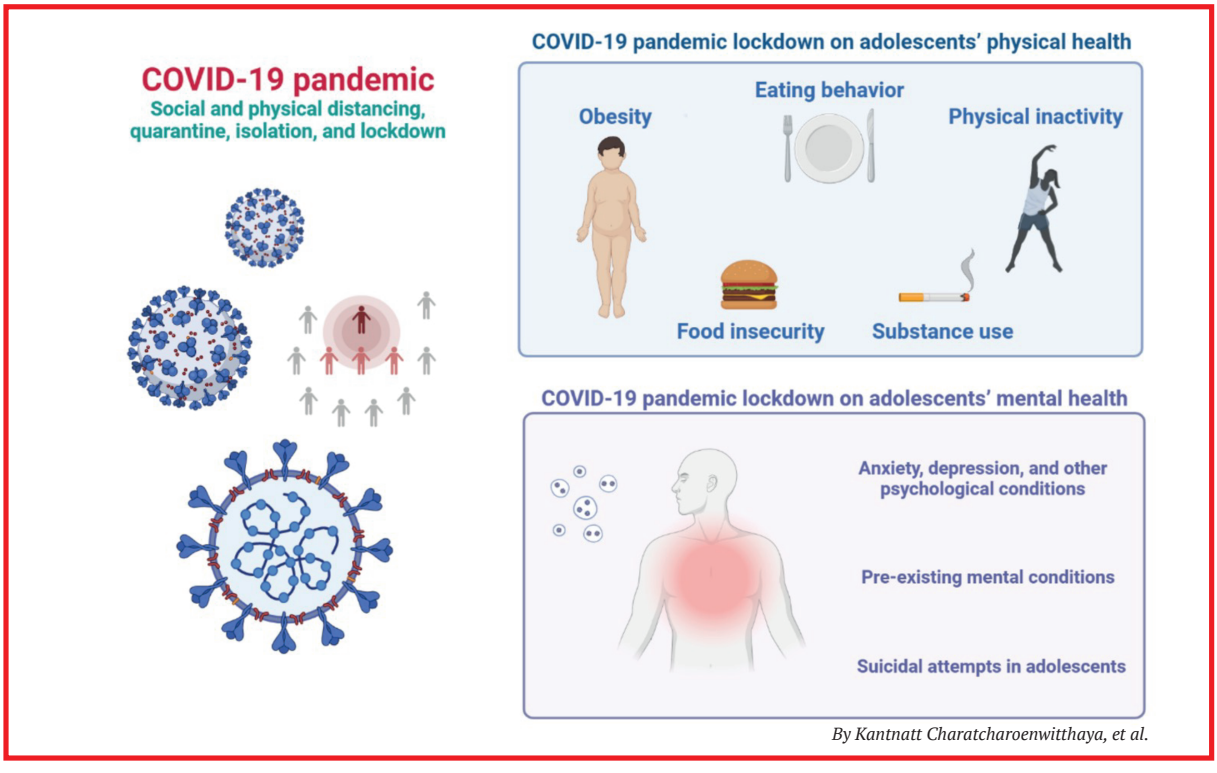

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Proinflammatory Cytokines Associated with *Inv*A and *Pag*C Genes of *Salmonella typhi* Isolated from Patients Undergoing Cholecystectomy

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ABSTRACT

Objective: Cholecystectomy is the surgical removal of the gallbladder. The gallbladder is often colonized by *Salmonella* during typhoid fever. The association between cytokine and virulence genes of *Salmonella* is not well known. The current study aims to identify the association between proinflammatory cytokines, TNF-α and IFN-γ, and two virulence genes, *Inv*A and *Pag*C, extracted from *S. typhi* isolates obtained from patients undergoing cholecystectomy.

Materials and Methods: One hundred and fifty clinical specimens, including gallbladder tissues and blood, were collected from patients undergoing cholecystectomy at AL-Sadder Medical City and AL-Furat General Hospital/ Iraq from December 2019 to September 2020. A Monoplex PCR technique was used to detect *Inv*A and *Pag*C, and an enzyme-linked immunosorbent assay was used to measure serum levels of TNF-α and IFNγ.

Results: From a total of 66 *S. typhi* isolates, the prevalence of *Inv*A and *Pag*C was (84.8% and 69.6%) respectively. Even after adjusting for potential confounding factors, serum levels of TNF-α and INF-γ were statistically significantly higher in *Pag*C detected in *S. typhi* isolates compared to *Pag*C not detected (p-value<0.001), but there was no significant difference between *Inv*A detected in *S. typhi* isolates and *Inv*A not detected (p-value > 0.05).

Conclusion: The virulence genes of *S. typhi*, especially *Pag*C, may be considered a potent inducer of proinflammatory cytokine secretion. Further studies are needed to present the patient's additional clinical course according to cytokine level.

Keywords: Typhoid; cytokine; TNF-α; IFN-γ; *Inv*A; *Pag*C (Siriraj Med J 2022; 74: 828-835)

INTRODUCTION

Cholecystectomy operations have been developed from open to laparoscopic surgery, which helps in reducing postoperative pain, returning faster to daily activities, and shortening the recovery period.[1,2] It remains the most effective treatment option for *Salmonella enterica serovar typhi (S. typhi)* carriers with gallstones.[3] *S. typhi* is a human-restricted pathogen that causes typhoid fever, a disease transmitted primarily through the fecal-oral route. Typhoid fever is considered to be one of the most important and under-reported diseases in the developing world. Based on the most recent global estimates, 9.9 and 24.2 million incident cases and 75,000–208,000 typhoid-related deaths occur annually.[4] In Asia, the incidence was more than 100 cases per 10^5 people per year, mostly among travelers; large outbreaks of *S. typhi* that are multidrug resistant have been observed in both urban and rural areas where access to sanitary facilities, food, and water are limited.[5] In Iraq, Mousa et al. estimated the incidence of typhoid fever to be 34.2 cases/10^5 individuals in 2012.[6]

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Typhoid fever is associated with a wide range of clinical symptoms from asymptomatic to severe, including fever, headache, malaise, and perforation of the ileum as complications of ileal ulceration. When some people are exposed to *S. typhi*, they become carriers, as they keep bacteria in the gallbladder that are responsible for storing and excreting bile from the liver to the small intestine.[7] Abdominal ultrasound screening showed an ulcerative inflammatory reaction with fluid accumulation and an obvious thickening of the gallbladder in more than 59% of patients with typhoid fever, which may be these features in response to a local infection.[8]

With the advent of molecular biology, including DNA fingerprinting and plasmid profiling, many vivo studies investigate gene variations of *S. typhi* that help bacterial organisms to adhere and survive in different environmental niches, as strains that lack the invasion machinery have drastically reduced capacity to colonize, spread[9] and/or drive inflammation[10] in mouse models. To our knowledge, live attenuated *S. typhi* strains delivered orally to humans or mice infected with *S. typhimurium*, which causes an illness similar to typhoid, have both been extensively used to study immune responses. These investigations showed a paradoxical phenomenon whereby the excessive synthesis of tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) by T cells reduces the protective immune response to *S. typhi* antigens.[11] The current study aims to identify the association between proinflammatory cytokines, TNF-α and IFN-γ, and two virulence genes, *Inv*A and *Pag*C, extracted from *S. typhi* isolates obtained from patients undergoing cholecystectomy.

MATERIALS AND METHODS

Following the acceptance of the Nursing Faculty's Ethical Review Board at the University of Kufa in Iraq (N: 10/05 on 02/September/2019) for our proposal, a case-control study was conducted in AL-Sadder Medical City and AL-Furat General Hospital/Iraq, from December 2019 to September 2020.

Subjects

One hundred and fifty patients undergoing either laparoscopic or open cholecystectomy were included as the study group. The patients were admitted to the hospital before 24 hours of surgery for preoperative investigations and assessment. Before surgery, no antimicrobial substances were prescribed while 3rd generation cephalosporins were given intra-operatively. Thirty individuals were included as the control group without suffering from acute fever or calcular cholecystitis. All subjects gave their written informed consent before enrollment.

Sample collection

A five mL blood sample was obtained from each participants by venous puncture and placed in a plain tube. After a blood clot, centrifugation at (3 x 10^3 rpm) for 20 minutes was used to separate the serum. All sera were stored at -20°C and had not been thawed before being analyzed. Gallbladder tissues taken from each patient after surgery were used for culture and immediately frozen at -20°C for DNA extraction.

Isolation and Identification of *S. typhi*

Sections of the gallbladder's tissue were injected into a Stewart transport medium (Hampshire, UK). In a sterile mortar, (1g) of gallbladder tissue was crushed and pestled in (1ml) saline, then it was cultured on selenite cystine medium (Hampshire, UK) and incubated for 24 hrs. at 37°C. For identification of individual colonies, they were subcultured on MacConkey agar, Salmonella Shigella agar, and Xylose Lysine Deoxycholate agar, as well as gram staining, IMViC test, triple sugar iron test, motility, lysine, and urease decarboxylase activities, were performed. The final identification was detected using an automated VITEK-2 compact system (BioMérieux –France). After these identification tests, we obtained 66 isolates of *S. typhi* out of 150 collected clinical specimens, and the remaining samples (84) were excluded from the current study.

By using the slide agglutination method, *Salmonella* antisera were performed for serotype and to determine corresponding antigens of *S. typhi* (somatic, flagellar, and virulence (Vi)) in the healthy control group according to the product's instructions.

Molecular Study

Genomic deoxyribonucleic acid (DNA) was extracted by using a DNA extraction kit (promega, USA), and a Monoplex PCR technique was performed to determine *Inv*A and *Pag*C. The forward and reverse primers used in this study for the InvA were 5'-ACA GTG CTC GTT TAC GAC CTG AAT-3' and 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3', resulting in a 260-bp product.[12] *Pag*C forward and reverse primers were 5'-CGC CTT TTC CGT GGG GTA TGC-3' and 5'-GAA GCC GTT TAT TTT TGT AGA GGA GAT GTT-3', respectively, yielding a 454-bp product.[13]

For PCR reaction, a 20µL tube was used, which contained 5 µl of master mix (Taq DNA polymerase, Tris-HCL pH9, dNTPs, 30 mM KCL,1.5 mM MgCl2, and Track dye from Bioneer company-Korea), 5µl DNA template, 2.5µl form each forward and reverse primer, and 5µl deionized water. PCR programming conditions for *Inv*A were 35 cycles of initial denaturation at 94°C

for 2 min, denaturation at 95°C for 1 min, annealing at 58°C for 1 min, elongation at 75°C for 1 min, and final elongation at 72°C for 10 min. PCR programming conditions for *Pag*C were 25 cycles of initial denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, annealing at 66.5°C for 30 s, elongation at 72°C for 2 min, and final elongation at 72°C for 10 min.

Proinflammatory cytokines

TNF-α was measured in serum samples using an enzyme-linked immunosorbent assay Kit (ab46087, UAS). IFNγ was measured in serum samples using an enzyme-linked immunosorbent assay Kit (ab174443, UAS). According to each standard curve and the dilution factor, the TNF-α and IFNγ concentrations in each plate were calculated.

Statistical analyses

A descriptive analysis of the qualitative variables was carried out, presenting the frequencies, median and interquartile range. A statistically significant difference was assessed by Chi- square test (X^2), student's t test, and Kruskal-Wallis test. For post hoc comparisons, the Dunnett C technique was applied. A general linear model analysis of covariance was conducted for adjusting for potential confounding factors. The statistical tests were executed using the GraphPad Prism version 8 software. Statistical significance was determined at a p-value < 0.05.

RESULTS

Demographic data of *S. typhi*- infected patients:

Demographic and clincal data of *S. typhi*-infected patients are summarized in Table 1.

The *S. typhi*-infected patients included 41 females and 25 males, with a mean age of 49.7±10.3 years (range, 32-65 years). The healthy individuals included 17 females and 13 males with a mean age of 50.6±8.7 years (range, 36-63 years). The clinical features of *S. typhi*-infected patients were 59 cases of pain, 51 cases of anorexia, 43 cases of nausea, 44 cases of vomiting, and 57 cases of fever, while the study did not find any of these features in healthy individuals.

For smoking and drinking alcohol, we found 27 cases of smoking and only two cases of drinking alcohol in patients, while there were six smokers and no drinkers in the control group. For other diseases, there were 15 patients who had other diseases and were taking different drugs. Furthermore, total white blood cells (WBCs) count were higher in patients than in the control group. As shown in Table 1, with the exception of age, gender, and drinking alcohol, the *S. typhi*-infected patients and control group significantly differed in demographic and clinical features (p-value <0.05).

Monoplex PCR Detection of Virulence Genes of *S. typhi* Isolates:

From a total of 66 *S. typhi* isolates, the prevalence of virulence genes was 56 (84.8%) for *Inv*A and 46 (69.6%) for *Pag*C (Fig 1).

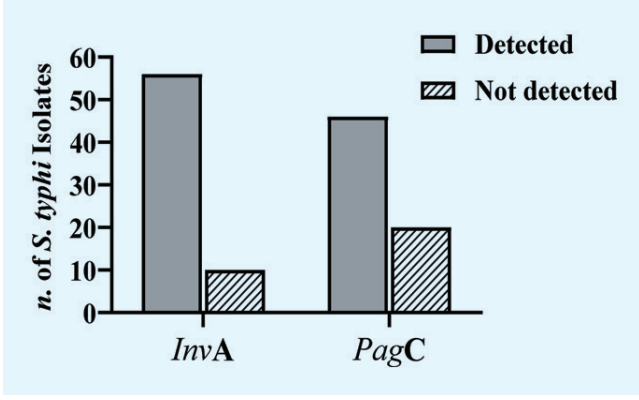

Fig 1. Prevalence of *Inv*A and *Pag*C, in *S. typhi* isolates which were collected from patients undergoing cholecystectomy by monoplex PCR assay.

By agarose gel electrophoresis, *Inv*A and *Pag*C were demonstrated in Fig 2A & 2B, respectively.

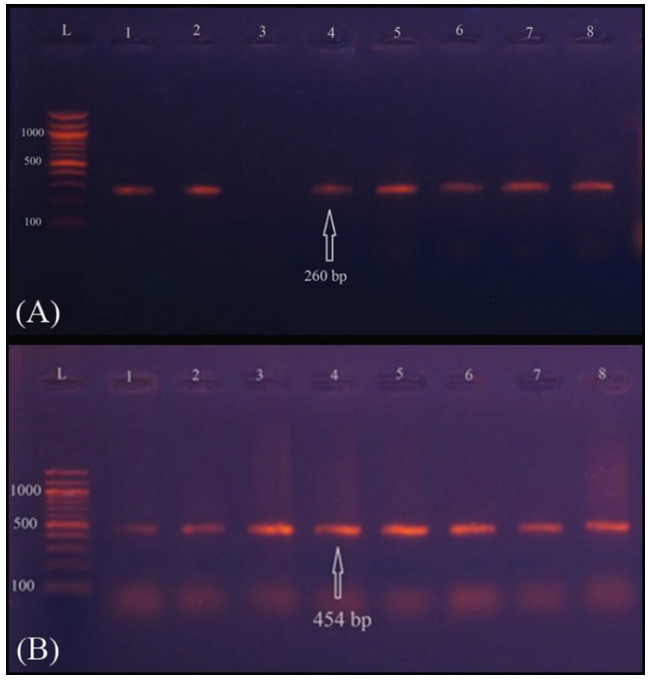

Fig 2. Ethidium bromide-stained agarose gel showing monoplex PCR for detection of the (A) *Inv*A and (B) *Pag*C of *S. typhi*. For both genes, lane 1: molecular weight marker and lanes 1 to 8: amplification products.

TABLE 1. Demographic and clinical data of participants (*S. typhi*-infected patients and healthy control).

	***S. typhi*- infected Patients n=66 Mean ± SD (rang)**		**Healthy Control n=30 Mean ± SD (rang)**		**Statistics**	***P* value**
Age groups (Years)	49.7±10.3 (32 - 65)		50.6±8.7 (36 - 63)		t = 0.415, df = 94	0.678
Total WBCs (x 10^3/µL)	7.3±1.71 (7 - 10)		5.2±1.32 (5 - 8.7)		t = 5.961, df = 94	<0.001**
	n	%	n	%		
Gender						
Male	25	37.88%	13	43.33%	X^2 = 0.257	0.612
Female	41	62.12%	17	56.67%	df = 1	
Abdominal Pain (RUQ tenderness)						
Yes	59	89.39%	-	-	X^2 = 69.58	<0.001**
No	7	10.61%	30	100%	df = 1	
Anorexia						
Yes	51	77.27%	-	-	X^2 = 49.45	<0.001**
No	15	22.73%	30	100%	df = 1	
Nausea						
Yes	43	65.15%	-	-	X^2 = 35.40	<0.001**
No	23	34.85%	30	100%	df = 1	
Vomiting						
Yes	44	66.67%	-	-	X^2 = 36.92	<0.001**
No	22	33.33%	30	100%	df = 1	
Fever						
< 38	9	13.64%	30	100%	X^2 = 4.514	0.034*
>= 38	57	86.36%	-	-	df = 1	
Smoking						
Yes	27	40.91%	6	20%	X^2 = 3.997	0.046*
No	39	59.09%	24	80%	df = 1	
Alcohol drink						
Yes	2	3.03%	-	-	X^2 = 0.928	0.566
No	64	96.97%	30	100%	df = 1	
Drugs						
Yes	15	22.73%	-	-	X^2 = 8.081	0.004**
No	51	77.72%	30	100%	df = 1	
Other Diseases						
Yes	15	22.73%	-	-	X^2 = 8.081	0.004**
No	51	77.72%	30	100%	df = 1	

Abbreviations: RUQ= Right upper quadrant; WBCs=White blood cells
*= significant; **= high significant

Proinflammatory cytokines findings

A general linear model analysis was conducted with age and total WBCs count as continuous predictors; gender, abdominal pain, anorexia, nausea, vomiting, fever, smoking, drinking alcohol, drugs, and other diseases as categorical predictors; and pro-inflammatory cytokines as dependent variables. Following their adjustment as covariates, the serum levels of TNF-α was statistically significantly different in *S. typhi*-infected patients and healthy control groups (X^2=50.46, df=2, *p*-value <0.001) (Fig 3).

Moreover, Fig 4 shows that, like TNF-α, the serum level of INF-γ was statistically significantly different in *S. typhi*-infected patients and healthy control group (X^2=46.99, df=2, *p*-value <0.001).

According to *Inv*A gene detection, the post hoc comparsion revealed that the serum level of TNF-α was statistically significantly higher in *Inv*A detected in *S. typhi* isolates (143.1, (97.5-149.8)) compared to healthy control (85,1 (79.9-91.4)) (X^2=36.14, df=2, *p*-value <0.001), and it was statistically significantly higher in *Inv*A not detected (143.4, (136.4-148.1)) compared to healthy control (X^2=42.0, df=2, *p*-value <0.001), but there was no significant difference between *Inv*A detected and *Inv*A not detected (X^2=-5.88, df=2, *p*-value >0.05) (Fig 3).

Like TNF-α, the post hoc comparsion of *Inv*A revealed that the serum level of INF-γ were statistically significantly higher in *Inv*A detected in *S. typhi* isolates (21.2, (13.7-22.5)) compared to healthy control (11.6 (9.9-14.6)) (X^2=27.9, df=2, *p*-value <0.001), and it was

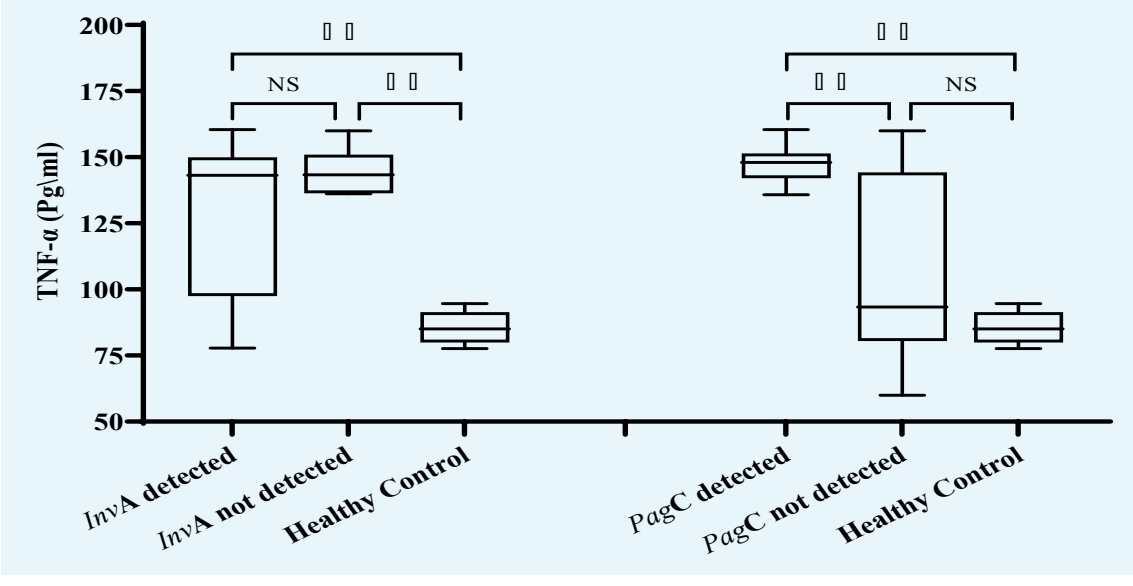

Fig 3. Boxplot of serum TNF-α levels in *S. typhi*-infected patients based on detection of virulence genes, *Inv*A and *Pag*C, and healthy control,**p*-value < 0.001.

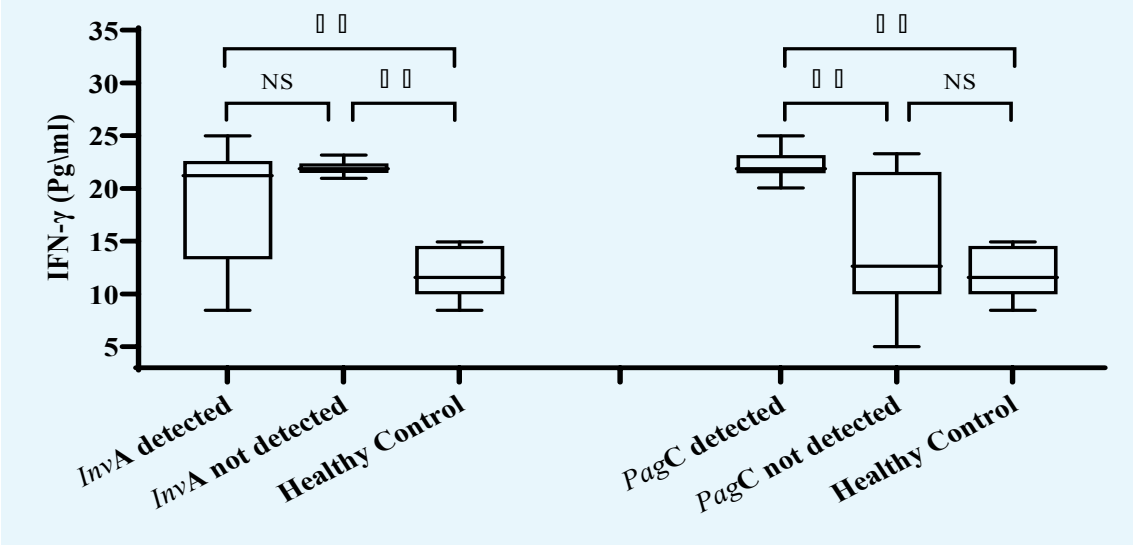

Fig 4. Boxplot of serum INF-γ levels in *S. typhi*-infected patients based on detection of virulence genes, *Inv*A and *Pag*C, and healthy control, **p*-value < 0.001.

statistically significantly higher in *Inv*A not detected (21.9, (21.6-22.1)) compared to healthy control (11.6 (9.9-14.6)) (X^2=41.2, df=2, *p*-value <0.001), but there was no significant difference between *Inv*A detected and *Inv*A not detected (X^2=-13.24, df=2, *p*-value >0.05) (Fig 4).

According to *Pag*C gene detection, the post hoc comparsion revealed that the serum level of TNF-α was statistically significantly higher in *Pag*C detected in *S. typhi* isolates (148.1, (142.1-151.2)) compared to *Pag*C not detected (93.4, (80.4-144.2)) (X^2=31.75, df=2, *p*-value <0.001), and it was statistically significantly higher in *Pag*C detected compared to healthy control (85,1 (79.9-91.4)) (X^2=48.78, df=2, *p*-value <0.001), but there was no significant difference between *Pag*C not detected and healthy control with (X^2=17.03, df=2, *p*-value >0.05) (Fig 3). Like TNF-α, the post hoc comparsion of *Pag*C revealed that the serum level of INF-γ was statistically significantly higher in *Pag*C detected in *S. typhi* isolates (21.8, (21.5-23.2)) compared to *Pag*C not detected (12.6, (9.9-21.5)) (X^2=31.78, df=2, *p*-value <0.001), and it was statistically significantly higher in *Pag*C detected compared to healthy control (11.6 (9.9-14.6)) (X^2=46.3, df=2, *p*-value <0.001), but there was no significant difference between *Pag*C not detected and healthy control (X^2=14.5, df=2, *p*-value >0.05) (Fig 4).

DISCUSSION

This study detect two virulence genes, *Inv*A and *Pag*C, in *S. typhi* isolates which were collected from patients undergoing cholecystectomy. Out of 66 *S. typhi* isolates, the findings of monoplex PCR of studied virulence genes were 56 (84.8%) *Inv*A detected, and 46 (69.6%) *Pag*C detected. A previous study showed that *S. typhi* isolates possess several genes for pathogenicity, such as *Inv*A, *Pag*C, *Spa*N, *Org*A, *Prg*H, *Inv*J, which give specific virulence traits and may deviate from the typical pattern of *S. typhi*, because they may be gained by horizontal transfer from another organism.[14]

In most strains of *S. typhi* isolated from humans and animals, the *Inv*A has been reported to be very important for pathogenicity as this gene encodes a protein responsible for adhesion and invasion of *S. typhi* into epithelial cells of the infected host.[15] In addition to the above virulence gene, the *Pag*C is also very important for bacterial adhesion, toxin transfer, and surviving with infected host cells through stimulating bacteria to form a vesicle called outer membrane vesicles (OMVs), spherical membranous compounds that are secreted from the surfaces of virtually all gram-negative microorganisms. High expression of *Pag*C accelerates *S. typhi*-OMVs formation.[16] In consistency with the rseults of our study, previous studies have observed a high prevalence of *Inv*A and *Pag*C in *S. typhi*.[17,18] On the basis of the results of this study, these two virulence genes, *Inv*A and *Pag*C, could be very useful genes for the fast determination of *Salmonella* pathogen.

Furthermore, despite *S. typhi* infection, our findings show that TNF-α release was significantly higher in *Pag*C detected in *S. typhi* isolates than in *Pag*C not detected, and INF-γ release was also significantly higher, but there was no significant difference between *Inv*A detected in *S. typhi* isolates and *Inv*A not detected, indicating that this phenomenon extends to other cytokine molecules. These findings could be attributed to strong defense responses, such as immune cell proliferation and excretion of T helper 1 (Th1) cytokines after expression of *Pag*C. This supports the findings of two studies from Iraq and Indonesia.[19,20] Following in vitro incubation of the peripheral blood mononuclear cells (PBMCs) with isolated flagella from *S. typhi*, Wyant et al.[21] have demonstrated a decrease in the number of cells expressing the lipopolysaccharide (LPS) receptor CD14. According to the same research group,[22] PBMCs that have been coincubated with flagella of *S. typhi* have a diminished response to mitogens and antigens. Patients with typhoid fever and cholecystitis show alterations in the total number of circulating white blood cells (WBCs).[23,24] However, the majority of participants in the study described here had total WBCs that were within the normal range at the time of enrollment. It remains to be proven whether this process manifests in vivo during typhoid fever and is the cause of the reported reduction in proinflammatory cytokine release. The serum of typhoid fever patients may, however, include soluble molecules that prevent or decrease *Pag*C-induced cytokine release, as has been observed for LPS-induced TNF-α release in mycobacterial infections.[25] LPS-induced IL-1beta and TNF-α production were inhibited by IL-6 and IL-1 receptor antagonist in vitro, and these two cytokines can both be increased in the blood of individuals with typhoid fever.[26]

TNF-α is a proinflammatory cytokine that is released when bacterial engulfment by macrophages occurs. Infected macrophages become *S. typhi* antigen presenters to Th1, which secretes IFN- γ and interleukin-12. IFN-γ stimulates many effector activities of mononuclear phagocytes, such as monocytes and macrophages, in order to kill intracellular pathogens.[27] A study discovered that incubating peripheral blood mononuclear cells for four days with *S. typhi* flagella raises TNF-α to 2000 pg/ml.[28] Depending on *Salmonella* flagellum expression by fliC gene, the ability of *salmonella* to enhance production of

TNF-α and IFN-γ by human monocytes was shown.[29] So, the LPS is not the only component of gram-negative microorganisms that enhances cytokine production, but also on the bacteria's genes. According to the results of this study, infection by *Pag*C -involved *S. typhi* is more harmful than infection by *Pag*C -not involved *S. typhi*, because excessive production of inflammatory cytokines, such as TNF-α, and the subsequent release additional inflammatory mediators might lead to decreased blood pressure, shock, and even death.[30] The present findings have limiting factors; the sample size was small to draw any firm conclusions on the relationship between cytokines and virulence genes, and due to a deficiency of laboratory supplies, we were unable to complete all analyses with other virulence genes.

CONCLUSION

The virulence genes of *S. typhi*, especially *Pag*C, may be considered a potent inducer of systemic inflammatory cytokine secretion. Further studies are needed to present the patient's additional clinical course according to cytokine level.

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Conflict of interest: none

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Impact of the COVID-19 Pandemic on Tertiary-care University Dermatology Outpatient Clinic and Dermatology Procedures

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ABSTRACT

Objective: This study aimed to determine the changes in patient characteristics, disease prevalence, and dermatology procedures during the COVID-19 pandemic.

Materials and Methods: We retrospectively reviewed the demographic data, diagnoses, and dermatology procedures of the patients who visited an outpatient dermatology clinic between March 1, 2018, and December 31, 2021. Changes in the number of patients, the patterns of diagnosis, and the dermatology procedures were analyzed.

Results: The number of patients decreased at the start of each of the 4 COVID-19 waves that occurred during the study period. Older adults increased their dermatology visits during the pandemic. The skin disease with the highest proportional increase in the frequency of visits was cutaneous T-cell lymphoma. The proportion of noncosmetic procedures significantly increased, in contrast to a decrease in the proportion of cosmetic procedures.

Conclusion: The COVID-19 pandemic significantly affected outpatient dermatology by changing the setting and frequency of patient visits to the outpatient clinic, the pattern of the diseases, and the dermatology procedures.

Keywords: COVID-19; Dermatology procedure; Dermatology; Outpatient department (Siriraj Med J 2022; 74: 836-843)

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a respiratory tract infection caused by Severe Acute Respiratory Syndrome Coronavirus-2. First reported in Wuhan, China, in late 2019,[1] it rapidly spread globally and was declared a pandemic by the World Health Organization in March 2020.[2,3] The first patient in Thailand was reported on January 12, 2020. An outbreak occurred in March 2020 due to several clusters in Bangkok's boxing venues and nightclubs, and the disease began spreading throughout Thailand.[4,5] To control the infection, the Thai government implemented a public health emergency decree on March 26, 2020.[5]

The COVID-19 pandemic has dramatically affected personal lifestyles, governing and political systems, and national economies. It has also greatly impacted healthcare systems through the need to control the disease while allocating finite medical resources to COVID-19 and non-COVID-19-related illnesses.[6-8] Access to medical services sometimes became limited, leading to appointment cancellations, and face-to-face visits have been adversely affected by social distancing and stay-at-home policies introduced by governments and businesses.[9] The various COVID-19 restriction policies have also disrupted the provision of dermatology services.[2,9] Skin diseases are usually nonfatal and involve few emergency conditions.

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Therefore, we anticipated that the COVID-19 pandemic would result in a decline in outpatient visits or a shift to teledermatology, especially with mild cases of skin disease. Previous European studies demonstrated a significant decline in patient visits to dermatology outpatient departments (OPD) and an increase in patients missing appointments during the pandemic.[7,9,10] There were also effects on the prevalence of certain skin diseases[11-13] and the performance of minor versus major dermatology procedures.[14,15] We aimed to evaluate the changes in patient characteristics, disease prevalence, and dermatology procedures during the COVID-19 pandemic in Thailand, which has different medical settings and legislation from Europe.

MATERIALS AND METHODS

Study design and participants

This single-center study was conducted at the Department of Dermatology, Faculty of Medicine, Siriraj Hospital, Mahidol University. The hospital is the largest tertiary care referral hospital in Thailand. Before this research began, the Siriraj Institutional Review Board approved its protocol (COA no. Si 571/2021). We retrospectively reviewed the electronic registration database to identify patients visiting the dermatology OPD between March 1, 2018, and December 31, 2021. All consecutive patients in the study period were selected for the analysis. Details were retrieved of the number of patients, their demographic data (age and sex), skin diagnoses (per the International Classification of Diseases [ICD]-10), and dermatology procedures (per the ICD-9 system). The number of COVID-19 cases in Thailand was obtained from Thailand's Ministry of Public Health website.[16] The primary outcome of the study is the changes in the proportion of visit from common diseases in dermatology outpatient unit before and during COVID-19 era. The secondary outcome is the changes in patients' demographic data, dermatological procedures before and during COVID-19 era.

Sample size consideration

The sample size was calculated with an equation for detecting the difference of two independent proportions. From the literature review, contact dermatitis is common diseases during COVID-19 pandemic; therefore, the number of visits for contact dermatitis was used for sample size calculation. Using reported frequency of contact dermatitis of 5.8% in 1,165 and 9.9% in 717 outpatient dermatology visits before and during COVID-19 pandemic, respectively[11], alpha error of 5%, the desired power of 90% and a predetermined allocation ratio of 1:1, the estimated sample size is 1,215 before COVID-19 era and 751 visits during COVID-19 era.

Data collection

The proportions of patients, diagnoses, and procedures from the pandemic period (March 2020-December 2021) were compared with the corresponding values for the preceding (March 2018-February 2020). The changes in the monthly number of patients and procedures were evaluated, and collinearity with the number of new COVID-19 cases in Thailand was assessed.

Statistical analysis

Data were analyzed using SPSS version 18 (SPSS Inc. Released 2009. PASW Statistics for Windows. Version 18.0. Chicago: SPSS Inc.). The proportion of visit from diseases and procedures before and during the COVID-19 pandemic were compared using either the Chi-squared or Fisher's exact test. A probability (*P*) value of less than 0.05 was considered statistically significant. Continuous data were reported using mean or median. Categorical data were reported using frequency and percentage.

RESULTS

Between March 2018 and December 2021, 200 368 patients visited the Siriraj dermatology OPD. The average number of patients during the pre-pandemic period-4925 patients/month-declined by approximately 30% to 3423 patients/month during the pandemic. Women patients predominated, with a female-to-male ratio of 2:1; however, the degree of female preponderance significantly decreased during the COVID-19 period. The most common patient age group in both the pre-pandemic and pandemic periods was 18- to 44-year-olds (40.7% and 40.1%, respectively). The proportion of older patients (> 65 years) significantly increased during the pandemic (Table 1).

The relationships between the number of new COVID-19 cases in Thailand and the number of patients and dermatology procedures conducted at the dermatology OPD are illustrated in the Figure. Although patient-case and dermatology-procedure numbers declined significantly in every wave of COVID-19, they returned to near normal approximately 1 month after each COVID-19 peak. Changes in the number of dermatology procedures performed during the pandemic corresponded with the variations in the number of OPD cases.

Table 2 classifies skin diseases according to ICD-10 and details percentage changes in the frequency of OPD visits for treatment before and during the pandemic. The diseases are presented in 3 groups: significantly increased visit frequency, significantly decreased visit frequency, and unchanged visit frequency. In terms of the degree of frequency, the top-3 skin diseases before and during the pandemic were identical: unspecified dermatitis,

TABLE 1. Demographic data of patients visiting Siriraj Hospital's dermatology outpatient department

Demographic data	Before COVID-19 pandemic (n = 118 209)		During COVID-19 pandemic (n = 82 159)	
	n	%	n	%
Sex				
Female	80 264	67.9	54 214	66.0
Male	37 945	32.1	27 945	34.0
Age group (years)				
< 18	3579	3.0	1819	2.2
18–44	48 070	40.7	32 979	40.1
45–64	40 009	33.8	26 962	32.8
> 65	26 551	22.5	20 399	24.8

Abbreviation: COVID-19, coronavirus disease 2019

TABLE 2. Distribution of diagnosed skin diseases before and during COVID-19 pandemic

Diagnosis (ICD-10)	Number of visits				Percentage change
	Before COVID-19 pandemic (n = 158 154)		During COVID-19 pandemic (n = 153 386)		
	n	%	n	%	
Diseases with significantly increased visit frequency					
CTCL, unspecified (C84.8)	25	0.02	68	0.04	180.46
Kaposi sarcoma (C46.0)	9	0.01	22	0.01	152.04
SPTCL (C86.3)	7	0	17	0.01	150.41
Other bullous disorders (L13)	99	0.06	191	0.12	98.93
Mycosis fungoides (C84.0)	1754	1.11	2985	1.95	75.47
Pemphigus (L10)	911	0.58	1499	0.98	69.66
Pemphigoid (L12)	821	0.52	1255	0.82	57.61
Infection due to other mycobacteria (A31)	225	0.14	314	0.2	43.89
Statis dermatitis (L83.1)	576	0.36	754	0.49	34.97
Drug reaction (L27)	835	0.53	1023	0.67	26.32
Herpes zoster (B02)	2129	1.35	2533	1.65	22.67
Atopic dermatitis (L20)	2596	1.64	3082	2.01	22.41
Other non-scarring hair loss (Telogen effluvium) (L65)	1097	0.69	1296	0.84	21.81
Exfoliative dermatitis (L26)	302	0.19	349	0.23	19.16
Urticaria (L50)	7363	4.66	8267	5.39	15.77
Psoriasis (L40)	13 211	8.35	14 821	9.66	15.67
Actinic keratosis (L57.0)	805	0.51	879	0.57	12.59
Nail disorders (dystrophy) (L60)	1842	1.16	2009	1.31	12.46
Androgenic alopecia (L64)	9695	6.13	10 457	6.82	11.21
Seborrheic dermatitis (L21)	6401	4.05	6894	4.49	11.05
Xerosis cutis and xerotic eczema (L85.3)	5385	3.4	5740	3.74	9.91
Dermatophytosis (B35; except 35.1)	2286	1.45	2425	1.58	9.38
Unspecified dermatitis (L30)	19 765	12.5	20 461	13.34	6.74

TABLE 2. Distribution of diagnosed skin diseases before and during COVID-19 pandemic (Continued)

Diagnosis (ICD-10)	Number of visits				Percentage change
	Before COVID-19 pandemic (n = 158 154)		During COVID-19 pandemic (n = 153 386)		
	n	%	n	%	
Diseases with significantly decreased visit frequency					
Mastocytosis (Q82.2)	70	0.04	22	0.01	-67.59
Sweet syndrome (L982)	47	0.03	18	0.01	-60.51
Leprosy (A30)	209	0.13	87	0.06	-57.08
Hemangiomas (D18.0)	265	0.17	118	0.08	-54.09
Varicella (B01)	139	0.09	63	0.04	-53.27
Panniculitis (M793)	123	0.08	84	0.05	-29.58
Anogenital herpes (A60)	1066	0.67	733	0.48	-29.1
Melasma (L81.1)	6774	4.28	4779	3.12	-27.26
Acne (L70)	11 335	7.17	8208	5.35	-25.34
Seborrheic keratosis (L82)	3743	2.37	2742	1.79	-24.47
Candidiasis (B37)	2024	1.28	1538	1	-21.65
Epidermal cyst (L720)	1132	0.72	892	0.58	-18.75
Unspecified contact dermatitis (L25)	391	0.25	319	0.21	-15.88
Vitiligo (L80)	13 227	8.36	10 792	7.04	-15.87
Syphilis (A51-53)	716	0.45	586	0.38	-15.61
Pityriasis versicolor (B36.0)	400	0.25	329	0.21	-15.19
Alopecia areata (L63)	5146	3.25	4349	2.84	-12.86
Cicatricial alopecia (L66)	967	0.61	819	0.53	-12.67
Lichen simplex chronicus and prurigo (L28)	4485	2.84	3824	2.49	-12.09
Other disorders of pigmentation (L81; except 81.1)	5543	3.5	4783	3.12	-11.03
Tinea ungiuum (B35.1)	3331	2.11	2887	1.88	-10.64
Irritant contact dermatitis (L24)	1333	0.84	1156	0.75	-10.58
BCC, SCC (C44)	1288	0.81	1128	0.74	-9.7
Viral wart (B07)	3051	1.93	2789	1.82	-5.75
Diseases without a significant change in visit frequency					
Malignant melanoma (C43)	40	0.03	28	0.02	-27.82
Scleroderma (M34)	374	0.24	321	0.21	-11.5
Scabies (B86)	120	0.08	104	0.07	-10.64
Lichen planus (L43)	480	0.3	421	0.27	-9.57
Erythema multiforme (SJS/TEN) (L51)	126	0.08	116	0.08	-5.07
Anogenital wart (A630)	207	0.13	193	0.13	-3.87
Herpes simplex infections (B00)	826	0.52	787	0.51	-1.76
Pityriasis rosea (L42)	179	0.11	171	0.11	-1.5
Lupus erythematosus (L93)	1367	0.86	1341	0.87	1.15
Vasculitis (L95)	2105	1.33	2084	1.36	2.08
Bacterial skin/mucosa diseases (L00-L08)	3416	2.16	3408	2.22	2.87
Allergic contact dermatitis (L23)	1887	1.19	1887	1.23	3.11
Dermatomyositis or inflammatory (M33)	118	0.07	118	0.08	3.11
Melanocytic nevus (D22)	1569	0.99	1588	1.04	4.36
Cutaneous tuberculosis (A18.4)	19	0.01	20	0.01	8.54
Pediculosis and phthriasis (B85)	21	0.01	23	0.01	12.93
Rosacea (L71)	356	0.23	390	0.25	12.96

* A *P* value < 0.05 indicates statistical significance (chi-squared test).

Abbreviations: BCC, basal cell carcinoma; COVID-19, coronavirus disease 2019; CTCL cutaneous T-cell lymphoma; ICD, International Classification of Diseases; SCC, squamous cell carcinoma; SJS, Stevens–Johnson syndrome; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; TEN, toxic epidermal necrolysis

vitiligo, and psoriasis. The skin disease with the highest percentage increase in frequency was cutaneous T-cell lymphoma, followed by Kaposi sarcoma, subcutaneous panniculitis-like T-cell lymphoma, other bullous disorders, and mycosis fungoides. Conversely, the diseases with the highest reductions in visit frequency were mastocytosis, sweet syndrome, leprosy, hemangioma, and varicella.

Regarding dermatology procedures, there was a marked decrease in the number of procedures conducted at the OPD following the COVID-19 outbreak. Overall, there was a 37% decline, from a mean of 2211 to 1382 procedures/month (Table 3). However, the fall was not uniform across all procedures. The proportion of noncosmetic procedures significantly rose (85.27% to 86.67%), whereas the proportion of cosmetic procedures fell (14.73% to 13.33%). The noncosmetic procedure with the highest frequency increase was radical excision of skin lesions, while the melanocyte-keratinocyte transplantation procedure demonstrated the largest frequency decline. Regarding cosmetic procedures, platelet-rich plasma injections saw the greatest frequency increase, and the use of iontophoresis fell the most.

DISCUSSION

This study demonstrated the influences of the COVID-19 pandemic on the number of patient visits and procedures performed at a dermatology OPD. The total number of patients visiting the OPD decreased,

TABLE 3. Number of dermatology procedures before and during COVID-19 pandemic

Procedure (ICD-9)	Number of procedures				Percentage change
	Before COVID-19 pandemic (n = 53 075)		During COVID-19 pandemic (n = 33 161)		
	n	%	n	%	
Noncosmetic procedures	*45 257*	*85.27*	*28 740*	*86.67*	*1.64*
Radical excision of skin lesion (864)	2	0.00	20	0.06	1500.52
Nail extraction (8623)	29	0.05	48	0.14	164.91
Wound care (dressing and wound debridement) (8622, 9357)	1739	3.28	1283	3.87	18.08
Biopsy (8611)	2514	4.74	1853	5.59	17.97
Ultraviolet light therapy (9982)	20 072	37.82	14 356	43.29	14.47
Aspiration, incision, and drainage (8601, 8604)*	249	0.47	162	0.49	4.13
Intralesional injection (steroid, 5-FU, MTX) (9923, 9925)	8037	15.14	4747	14.32	-5.47
Ablative treatment (863)	6169	11.62	3461	10.44	-10.21
Chemosurgery of skin (8624)	6311	11.89	2761	8.33	-29.98
Debridement of nail, nail bed, or nail fold (8627)*	92	0.17	40	0.12	-30.41
Sclerotherapy (3992)*	27	0.05	8	0.02	-52.58
Melanocyte-keratinocyte transplantation procedure (8660)	16	0.03	1	0.00	-90.00
Cosmetic procedures	*7818*	*14.73*	*4421*	*13.33*	*-9.49*
Platelet-rich plasma injection (9907)	80	0.15	153	0.46	206.10
Botulinum toxin, filler injection (including complication management) and injection or tattooing of skin lesion or defect (8602, 9929,9957)	1535	2.89	1188	3.58	23.87
Laser (8625)	4845	9.13	2684	8.09	-11.34
Hair transplantation (0863, 8664)*	91	0.17	49	0.15	-13.82
Comedone extraction (8603)	1173	2.21	345	1.04	-52.93
Iontophoresis (9927)	94	0.18	2	0.01	-96.59

***, Not statistically significant**

Abbreviations: 5-FU, 5-fluorouracil; COVID-19, coronavirus disease 2019; ICD, International Classification of Diseases; MTX, methotrexate

consistent with reports from other countries.[10,17] In addition, another study showed a more pronounced loss of patients from a hospital's dermatology OPD than its other departments.[18] The explanation could be that, given most dermatology conditions' nonfatal and nonemergency nature, the risk of acquiring a COVID-19 infection outweighed the perceived necessity for an OPD visit.[10,18] The Thai government's COVID-19 prevention policies also included interregional travel restrictions. The policies impeded or prevented patients in rural areas from accessing face-to-face medical consultations at tertiary healthcare centers in other provinces. Moreover, international surveys revealed that over 50% of dermatologists had been reassigned from dermatology departments to fields more directly related to COVID-19 to mitigate staff shortages resulting from the pandemic. However, the relocations of dermatologists created staff shortfalls in dermatology OPDs.[9,19] For a limited period, Thailand's COVID-19 prevention policies also placed severe restrictions on the conduct of cosmetic procedures throughout many areas of Thailand, including Bangkok, where our hospital is situated.

The COVID-19 pandemic also influenced the age and sex distribution of patients visiting the dermatology OPD. The proportion of older adults became significantly higher during the pandemic, which accords with another study.[5,12] However, the majority of patients at our dermatology clinic were teenagers and adults, who could be asymptomatic carriers of COVID-19. Therefore, to minimize the risk of COVID-19 transmission, nonemergency dermatology visits should be temporarily suspended or serviced through alternative methods, such as teledermatology.[20,21] Moreover, the number of female patients significantly decreased during the pandemic. This dissimilarity might reflect different levels of awareness among men and women of the hazards of COVID-19.

Certain skin conditions saw an increase in the frequency of visits during the pandemic. This was particularly the case with diseases with high severity or urgent situations, such as cutaneous T-cell lymphoma (ICD-10; C84.8, C86.3, C84) and vesiculobullous diseases (ICD-10; L10-13). Among the most commonly diagnosed diseases, the frequency of dermatitis, psoriasis, and urticaria significantly increased during the pandemic. For urticaria and psoriasis, the increase in prevalence might have been related to COVID-19-associated stress and anxiety, which would have precipitated these diseases.[12,22,23] Psychological stress affects or exacerbates many skin disorders, including psoriasis and urticaria but also atopic dermatitis, seborrheic dermatitis, vesiculobullous disorder (pemphigus, pemphigoid), and viral infections (herpes zoster).[24-26] Our results also revealed an increase in the frequency of these diseases after the pandemic, consistent with previous studies.[5,13] Moreover, the increased frequency of urticaria and dermatitis might have been related to mass vaccination programs against COVID-19. Urticaria is the most common cutaneous adverse reaction (34.44%) associated with COVID-19 vaccine administration, followed by eczematous skin reaction (6.95%).[27] Eczema levels might also rise due to the extended mask wearing and increased attention to hand hygiene occurring during the COVID-19 era.[10-13] Reactivation of varicella-zoster virus has been reported in association with COVID-19 infections because of weakened immune systems and the administration of different COVID-19 vaccines.[28,29] There are also many literature reports of acute telogen effluvium after recovery from COVID-19 infection.[30] Our findings showed an increased telogen effluvium frequency, supporting previous observations.

Particular skin conditions showed significantly lower visit frequencies. The decreases were possibly due to the nature of the diseases (not emergent, gradual onset, or rare). Alternatively, the skin conditions might have mainly had cosmetic issues, so hospital visits could be suspended. Vitiligo and acne were among the most commonly diagnosed skin diseases that had significantly decreased visit frequencies during the pandemic. Moreover, patients might have felt more comfortable seeking treatment for these conditions at primary or secondary healthcare facilities than at a crowded tertiary healthcare center, where they would have a higher risk of contracting COVID-19. Teledermatology is another treatment channel that had a significant uptake during the pandemic. Commonly occurring ambulatory dermatoses like dermatitis and acne were reported to be more amenable to being assessed and managed via teledermatology.[31] Furthermore, in Thailand, patients can easily buy medications for skin conditions over the counter without a prescription. Our study also found that the frequency of sexually transmitted diseases (STDs) such as syphilis and anogenital herpes infection significantly decreased during the pandemic. Previous studies in several countries reported lower incidences of STDs during the pandemic.[32-34] Possible factors contributing to the apparent fall in STD-case numbers are the introduction of strict social distancing measures (which reduced sexual risk behaviors) and a decline in STD screening services and resources for controlling STDs.[33,34]

In our study, the number of overall dermatology procedures decreased, and this occurred for both noncosmetic and cosmetic procedures. Similarly, there was a reduction

Fig 1. Changes in the number of patients and dermatology procedures during the COVID-19 pandemic
Abbreviations: COVID-19, coronavirus disease 2019; OPD, outpatient department

in scheduled and performed procedures in Italy after its lockdown, resulting from appointment cancellations by patients or the development of COVID-19 infections.[15] During the COVID-19 period, health authorities were obliged to prioritize medical activities and decrease nonurgent visits because healthcare systems had rapidly become overwhelmed. Most dermatology activities were suspended. However, life-saving or time-dependent dermatology procedures (such as radical excisions of skin lesions, biopsies, and resections of malignant lesions) continued to be performed at high levels. Conversely, the melanocyte-keratinocyte transplantation procedure for vitiligo was the least performed procedure, possibly because vitiligo is a prolonged condition. The present study found a proportional increase in noncosmetic procedures, with a corresponding fall in the number of cosmetic procedures. This change in proportions resulted from the hospital's protocol of limiting nonurgent procedures at our center, such as laser and hair transplantation. Among cosmetic procedures, the number of platelet-rich plasma injections had the highest increase, given that they were a substitute for hair transplantation surgery. However, the number of botulinum toxin and filler procedures significantly rose during the pandemic. The increasing levels of virtual socializing might have led to dissatisfaction of individuals with their onscreen appearance, resulting in a heightened interest in some cosmetic procedures.[35] A similar situation was observed with the demand for facial plastic surgery procedures.[36]

There are some limitations to the study. First, since the data was collected from a single tertiary center, it may lack generalizability on the national scale or in other settings such as primary or secondary care. Second, due to the study's retrospective nature, some data relating to patients' decisions to avoid OPD visits may not have been captured.

In conclusion, the COVID-19 pandemic affected outpatient dermatology by changing the setting and numbers of patients visiting the OPD, the pattern of the diseases, and the dermatology procedures. The study presents Thailand's COVID-19 circumstances that affected our dermatology practices.

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Are Technical Skills Assessed Using Medical Knowledge Associated with Non-technical Skill Knowledge in Anaesthesia Resident Training?

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ABSTRACT

Objective: We examined the association between midterm examinations and anaesthetists' non-technical skills (ANTS) knowledge using multiple choice questions (MCQs) for anaesthesia training.

Materials and Methods: A prospective cross-sectional study was implemented based on two cohort studies conducted in November 2017 and November 2019 at the university hospital in southern Thailand. Each cohort consisted of MCQs, short answer questions (SAQs), objective structured clinical examinations (OSCEs), and MCQs after ANTS simulation workshops during the midterm examinations. The main exposure variable was the midterm examination (MCQs/ SAQs/ OSCEs) whereas the ANTS MCQs were the outcome. The potential predictors were the residents' role, sex, PGY (1-3), and competency. Associations between midterm examinations and ANTS MCQ scores were analysed using Pearson's correlation coefficients (r) and multivariate linear regression analysis, and presented as beta coefficient (β) and 95% confidence limit (CL).

Results: Forty-eight anaesthesia residents were recruited for the study. After adjusting for PGY, knowledge score, and attitude evaluated by the staff, OSCE was found to be significantly associated with the pretest ANTS scores (β [95% CL]= 1.02 (0.06, 1.98)) and MCQ scores were significantly associated with the posttest ANTS scores (β [95% CL]= 0.14 (0.04, 0.24)). SAQ scores had negative associations with the pretest (β [95% CL]=-0.11 (-0.21, -0.01)) and posttest (β [95% CL]=-0.16 (-0.27, -0.05)) ANTS scores. It was found that there was a significant relationship between midterm examinations and posttest ANTS scores (r=0.52).

Conclusion: Technical skills using OSCE and midterm MCQ examination scores were associated with non-technical skill knowledge in anaesthesia training.

Keywords: Technical skill; anaesthesia's non-technical skill knowledge; multiple choice questions; short answer questions; objective structured clinical examination (Siriraj Med J 2022; 74: 844-856)

INTRODUCTION

Anaesthetists' non-technical skills (ANTS) are a crucial component of anaesthesia resident training because they emphasise communication skills and the ability to work in a multidisciplinary team during anaesthesia practise.[1] It is also part of the anaesthesia training requirement in conjunction with the technical skills required to answer multiple choice questions (MCQs), short answer questions (SAQs), objective structured clinical examinations (OSCEs), and oral examinations. Because it is a behavioural marker tool, at least two evaluators are required to assess residents' behaviour during the

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simulation workshop to accurately evaluate performances related to 15 elements of ANTS.[2] Some predictors – such as the number of evaluators, residents' skill level range, and the observational process – can affect the reliability of the evaluators.[3] Other predictors associated with higher non-technical skills were trainees' experience, recent training in the life support protocols,[4] and higher technical skills.[5,6] Most studies have demonstrated the correlation between technical and non-technical skills in simulation workshops.[5,6] To date, no studies have reported other evaluated tools besides the behaviour maker tool to examine the association between non-technical and technical skills in anaesthesia. Not all institutes like ours might be able to evaluate ANTS behaviour in all residents in one time period workshop; therefore, through midterm examinations, we applied the ANTS assessment by using the debrief session and evaluating ANTS knowledge to ensure ANTS comprehension and understanding. Moreover, we examined whether the technical skills assessed using medical knowledge during midterm examinations were associated with ANTS knowledge, based on the MCQ scores of residents who either participated in or observed the ANTS simulation workshop.

MATERIALS AND METHODS

This prospective observational study was conducted after receiving approval from the Ethics Committee, Faculty of Medicine, Prince of Songkla University on 1 October 2019 (REC 62-265-8-1). We recruited anaesthesia residents from different post-graduate years (PGY-1, PGY-2, and PGY-3) who attended a simulation-based ANTS training workshop at the simulation centre, Faculty of Medicine, Prince of Songkla University, in November 2017 and November 2019. Since the workshop was a part of the anaesthesia training programme, written informed consent from the participants was waived by the Ethics Committee.

Standard operating procedure

Midterm examinations held annually in the month of November and consist of MCQs, SAQs, and OSCEs (Fig 1). Residents' performance in terms of knowledge, skill, and attitude are simultaneously evaluated by the anaesthesia staff. The simulation ANTS workshop has been held biennially since 2015. All residents were informed of the schedule of the workshop one month in advance. The three case scenarios were based on the dominant ANTS category themes. Meanwhile, situation awareness (SA), teamwork and communication (TW), task management (TM), and decision making (DM), were developed by three different anaesthesia staff members for each theme. For 2019, the scenario 'Missing tooth' for the TW theme, the scenario 'Anaesthesia emergency service' for the TM theme, and the scenario 'Decision making in on call resident' for the DM theme, were created by SS, MO, and NP, respectively. All the residents (PGY-1, PGY-2, PGY-3) received the ANTS material one week before the workshop. The manikins and workstations were prepared and checked to ensure readiness one day before the workshop. On the day of the workshop, the residents received a ten-minute briefing on overall concepts of the ANTS by MO and completed a pretest consisting of 20 MCQs (Appendix 1). Each scenario consisted of a 30-minute simulation workshop and a 30-minute debriefing session. One or two residents from PGY-1, PGY-2, or PGY-3 were randomly selected to participate in the scenario theme (Fig 1), while the remaining residents observed the sessions through a monitor in the observer room. Each debriefing session consisted of de-rolling and applying the ANTS, and was facilitated by the anaesthesia staff who created the respective scenarios. After finishing the workshop, all residents completed the posttest (Appendix 1). An anaesthesia staff member (MO) discussed the MCQ answers with the residents after the posttest.

Operational definition

Scenario participant was defined as a first-, second- or third-year resident who participated as a team leader or team member in the scenario. Observer was defined as a first-, second- or third-year resident who observed the session from the observation room. Technical skills in anaesthesia training were represented by MCQ, SAQ, and OSCE scores during the same period of the simulation ANTS training (midterm examinations in November 2017 and November 2019).

Outcome of the study and outcome measurement

The primary outcome of the study was the posttest MCQ score after the ANTS workshop. The secondary outcome was the pretest MCQ score before the ANTS workshop. The pretest and posttest MCQs consisted of the same 20-item questions. The reliability of these pretest and posttest MCQ items were 0.95 and 0.98, respectively, as assessed by two anaesthesiologists (NP and SS). The MCQ consisted of 80% (16/20 items) application taxonomy-related questions and 20% (4/20) recalled taxonomy-related questions, with the ratio of ANTS content being 25% in SA (5/20 items), 25% in TM (5/20 items), 30% in TW (6/20 items), and 20% in DM (4/20 items).[7]

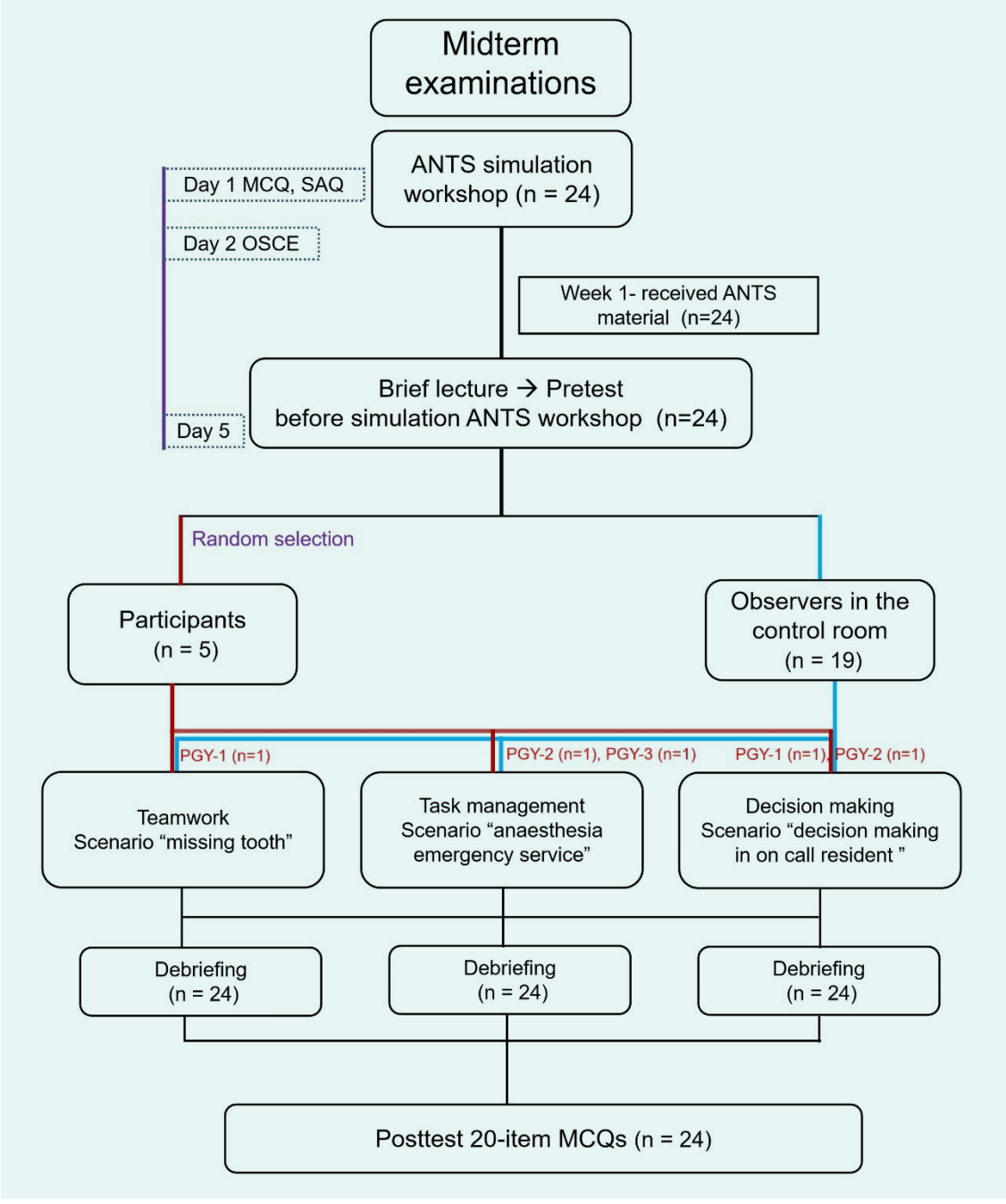

Fig 1. Midterm examinations of anaesthesia training.
Note: PGY: post-graduate year, ANTS: anaesthetists' non-technical skills, MCQ: multiple-choice question, SAQ: short answer question, OSCE: objective structured clinical examination.

Predictors and potential confounders

The main exposure variable was the midterm examination, which comprised 180 MCQs, 12 SAQs, and 24 OSCEs (Appendix 2). The average score of the anaesthesia residents' competency for each year in terms of knowledge, skills, and attitude was also evaluated by all anaesthesia staffs before the examination (Appendix 2). Potential predictors were the residents' role in the scenario (participant/observer), sex, PGY (1-3), and percentage of self-study prior to attending the workshop.

The step of statistical analysis

First, the basic comparison test to compare the residents' characteristics and the midterm examination scores as well as MCQ ANTS scores among PGY (1-3) were performed. Continuous variables were presented using the median and interquartile range for non-normally distributed data and using mean and standard deviation (SD) for normally distributed data. Categorical variables were presented using frequency and percentage, and were compared using Fisher's exact test or Pearson's

APPENDIX 1

MCQ ANTS pretest and posttest

1. You are an anesthesiologist on call and handed over a case of hypotension. The inotrope was given but the hypotension persisted. You did not notice that the line of inotrope was clamped. Which ANTS category is related to this event?
    A. Task management
    B. Teamwork & communication
    C. Situation awareness
    D. Decision-making
2. You are an anesthesiologist and a team leader of a fire situation in OR. You need to discuss with the surgeon, a chairman of the surgery department, to rapidly manage a patient. The surgeon has refused to abandon surgery until he has completed the operation. Which key element would you deal with in this situation?
    A. Providing & maintaining standards
    B. Co-ordinating activities with team
    C. Using authority & assertiveness
    D. Recognising & understanding
3. According to question 2, which ANTS category was referred to?
    A. Task management
    B. Teamwork & communication
    C. Situation awareness
    D. Decision-making
4. You are a third-year resident working in the emergency room. There is a pregnancy case with fetal distress; however, all 3 emergency rooms are occupied. Which ANTS category applies in this situation?
    A. Task management
    B. Teamwork & communication
    C. Situation awareness
    D. Decision making
5. From question 4, you managed the pregnancy case with fetal distress by taking the first available elective room. To which key element does this situation referred?
    A. Identifying & utilizing resources
    B. Co-ordinating activities with the team
    C. Reconising & understanding
    D. Balancing risks & selecting options
6. What is the key element of situation awareness?
    A. Providing & maintaining standards
    B. Assessing capabilities
    C. Recognising & understanding
    D. Identifying options
7. What is the key element of decision making?
    A. Planning & preparing
    B. Supporting others
    C. Anticipating
    D. Re-evaluating

APPENDIX 1 (Continued)

8. Which is FALSE regarding the key element of teamwork & communication?
   A. Identifying & utilizing resources
   B. Co-ordinating activities with the team
   C. Exchanging information
   D. Using authority & assertiveness
9. Failure of which ANTS category could lead to communication failure between two colleagues?
   A. Task management
   B. Teamwork & communication
   C. Situation awareness
   D. Decision making
10. What tool is the most important for the simulation of Anaesthetists' Non-Technical skills (ANTS) training?
   A. Simulation center
   B. Standardized patient
   C. Real-life case scenario
   D. Feedback or debriefing
11. You handed over the details, except for the information of morphine allergy, of a case of total abdominal hysterectomy with bilateral salpingo-oophorectomy to your colleague who was on call. This mistake could refer to which ANTS category(ies)?
   A. Teamwork & communication
   B. Teamwork & communication and task management
   C. Teamwork & communication and situation awareness
   D. Teamwork & communication and decision making
12. After placing an epidural catheter for painless labor at the labor room, you noticed that there was no basic monitoring. You managed this by informing an anesthetist nurse to palpate a radial pulse and a nurse aid to urgently get a monitor from the OR. What ANTS category are you concerned with the most?
   A. Task management
   B. Teamwork & communication
   C. Situation awareness
   D. Decision making
13. The anesthesiologist in charge did not have any plan for postoperative pain control for patient receiving total abdominal hysterectomy with bilateral salpingo-oophorectomy. The patient developed severe pain at the PACU. This error did not refer to which ANTS category?
   A. Situation awareness
   B. Teamwork & communication
   C. Task management
   D. Decision making
14. A surgeon would like to perform surgery in a case of immediate post-cardiac arrest in the OR. As the anesthesiologist in charge, you managed to discuss with him regarding the risk of performing surgery. Which ANTS category best reflects your action?
   A. Situation awareness
   B. Teamwork & communication
   C. Task management
   D. Decision-making

APPENDIX 1 (Continued)

15. You are an anesthesia resident who administered the wrong type of packed red cell to a patient with massive bleeding. Before you transfused the packed red cell, you checked only the patient's name without checking the hospital number nor cross-checking with an anesthetist nurse. This mistake refers to which ANTS category?
    A. Teamwork & communication
    B. Decision making
    C. Situation awareness
    D. Task Management
16. The mistake from question 15 was relevant to which key element of that particular ANTS category?
    A. Balancing risks & selecting options
    B. Providing & maintaining standards
    C. Co-ordinating activities with the team
    D. Anticipating
17. You were a team leader of advanced cardiac life support (ACLS) resuscitation. You noticed that your team member could not perform chest compression effectively, so you managed to exchange a chest compressor with a mask ventilation member. Your decision correlates with which key element of ANTS?
    A. Co-ordinating activities with the team
    B. Exchanging information
    C. Using authority & assertiveness
    D. Assessing capabilities
18. You are giving anesthesia to a patient with a history of difficult airway. After the patient loses consciousness, you cannot ventilate him, so you call a senior anesthesiologist staff to stand by in the room. Your action could refer to which ANTS category(ies)?
    A. Situation awareness
    B. Situation awareness and teamwork & communication
    C. Situation awareness and task management
    D. Situation awareness and decision making
19. An anesthesia resident who was on call was about to intubate a non-difficult airway patient when he overheard a CPR code. The resident arranged for an anesthetist nurse to do the intubation instead. The resident then informed the anesthesiologist staff by phone while he ran to the CPR scene. His action was relevant to which key element?
    A. Prioritising
    B. Recognising & understanding
    C. Co-ordinating activities with the team
    D. Balancing risks & selecting options
20. As an anesthesiologist staff, you informed an anesthetist nurse to administer intravenous succinylcholine to a child. You suspected the child might develop a laryngospasm since there was no reading of oxygen saturation on the monitor. After you checked the oxygen saturation probe, you found that it was not connected to the child's finger. This error was relevant to which ANTS category?
    A. Task management
    B. Teamwork & communication
    C. Situation awareness
    D. Decision making

APPENDIX 2

Combination of midterm examination score of resident training and resident's competency evaluated by staff in 2017 and 2019

PGY	MCQ (100)	SAQ (100)	OSCE (10)	Skill (10)	Attitude (10)	Knowledge (10)
1	58.33	59.17	5.73	7.74	6.28	7.79
1	59.44	59.63	6.75	7.9	6.34	10
1	46.67	41.11	6.24	7.3	6.14	9.69
1	64.44	50.46	5.55	7.5	6.38	7.83
1	61.67	53.52	6.19	7.76	6.26	10
1	56.11	52.59	6	7.74	6.26	9.79
1	54.44	49.63	5.78	7.6	6.26	8.2
1	62.78	50.93	7.46	7.84	6.08	9.48
1	93.75	83	7.75	7.86	7.15	7.55
1	90.28	67.5	8.48	7.53	7.04	6.97
1	91.67	68	8.31	8.13	7.24	7.98
1	75	70	7.4	8.16	7.06	8.13
1	100	63	8.33	7.95	7.15	7.59
1	77.78	68	7.04	7.5	7.11	7.06
1	89.58	70	6.1	7.76	7.15	7.46
1	90.97	77.5	7.52	7.61	7.13	7.46
2	83.89	84.14	7.26	8.66	6.5	9.06
2	64.44	60.93	5.8	7.86	6.36	10
2	78.89	61.79	7.46	8.1	6.26	10
2	59.44	57.43	6.29	8.26	6.5	9.44
2	70.56	75	5.86	8.1	6.36	10
2	54.44	54.43	5.25	7.34	6.02	8.72
2	73.33	64.57	6.2	8.2	6.22	9.06
2	63.34	64.72	6.93	7.71	7.15	7.32
2	78.83	65	7.81	8.55	7.3	8.29
2	72.35	66.39	8.44	8	7.06	7.67
2	64.47	69.17	6.52	7.32	6.93	7.15
2	76.8	65	7	7.5	7.11	7.06
2	74.61	68.11	7.95	8.17	6.39	8.1
2	67.85	60.11	7.07	8.24	6.4	7.85
2	67.57	62.89	7.8	8.08	7.17	7.81
2	63.34	61.11	7.44	8.03	7.18	7.5
3	58.26	48.04	5.37	8.04	6.2	10
3	66.09	55.18	6.24	8.26	6.48	9.76
3	62.61	70.27	5.41	8.1	5.76	9.18
3	57.39	52.68	5.34	8.4	6.22	10
3	70.43	65.36	6.13	8	6.34	8.51
3	76.52	80.36	6.86	8.3	6.16	10
3	64.35	45	5.32	8.5	6.56	10
3	62.61	61.94	7.28	7.97	6.4	8.11
3	63.96	66.67	8.19	8.78	7.34	8.94
3	60	55.39	7.18	8.47	7.3	7.98
3	63.06	58.67	7.87	7.76	6.07	7.5
3	62.61	63	8.01	8.76	7.21	8.86
3	63.29	67.78	7.32	8.65	7.35	8.86
3	60	61.67	6.59	7.61	6.25	7.02
3	62.16	69.72	6.65	7.84	7.17	7.76
3	60	64.39	7.1	8.28	7.29	7.96

Abbreviation: PGY: post-graduate year

chi-square test. Continuous variables were compared using Kruskal–Wallis tests and analysis of variance, as appropriate. Second, to determine the degree of correlation between midterm examinations and MCQ ANTS scores, Pearson's correlation coefficients (r) were used. Third, the r used among the main exposures and outcomes in the second step was considered a univariate analysis, and therefore, a directed acyclic graph (DAG) was used to select the potential confounders that could affect the main exposures and the outcomes into the multivariate model. Then, the DAGitty software version 3.0. was applied. Potential confounding variables including resident competencies (knowledge, skills, and attitude) suggested by the DAG were then selected for a multivariate linear regression model and were retained irrespective of their statistical significance.[8,9] The outcomes were tested to ensure that they fit the residual of linear distribution assumption. The association between the posttest/pretest MCQ scores and midterm examinations was presented as an adjusted beta coefficients with 95% confidence limits (CL) and considered significant if the F-test p values were <0.05. Finally, the effect modification between the potential predictors and midterm examinations on the outcomes were evaluated for each final model.

Sample size calculation

The sample size was calculated based on the correlation between midterm MCQ scores and posttest ANTS scores. A correlation coefficient (r) of 0.4, which represented a medium level of correlation, was used to calculate the required sample size of 47 patients under a significance level of 0.05. Since a total pool of 24 residents were available from PGY-1 to PGY-3, we used data from two cohorts – collected in 2017 and in 2019 – to include in this study project.

RESULTS

Forty-eight anaesthesia residents were recruited from among the two cohorts of ANTS training in 2017 and 2019, including 16 PGY-1, 16 PGY-2, and 16 PGY-3 residents. Table 1 compares the characteristics of the various three-year anaesthesia residents, which reveals no significant differences. Table 2 compares the ANTS pretest/posttest scores, midterm examination scores, and anaesthesia residents' competency as evaluated by the staff. There were no significant differences between the ANTS pretest/posttest scores and midterm examination scores among groups; however, the skill competency increased by year of resident training (P<0.001).

Table 3 shows the correlation between midterm examination scores, residents' competency as evaluated by staff, and MCQ ANTS scores. The correlation between the midterm examination scores and pretest/posttest ANTS scores was low (r<0.3). SAQ scores had a negative correlation with posttest ANTS scores but were not significantly different (r=-0.24, P=0.10).

Analysis of pretest MCQ score

Nine variables (sex, PGY, self-study, knowledge score, skill competency, attitude, MCQ score, SAQ score, and OSCE) as suggested by a previous literature review were related to ANTS scores.[4,5,7] There was an effect

TABLE 1. Comparison of characteristics of anaesthesia residents.

	Post-graduate year			
Characteristic	**1 (n=16)**	**2 (n=16)**	**3 (n=16)**	***P* value**
Sex (M/F)	4/12	5/11	4/12	1.00
Role				
Observer	13 (81.2)	12 (75)	8 (50)	0.131
Participant	3 (18.8)	4 (25.0)	8 (50.0)	
Percentage of self-study				
0-25	6 (37.5)	6 (37.5)	3 (18.8)	
>25-50	7 (43.8)	8 (50.0)	5 (31.2)	0.198
> 50	3 (18.8)	2 (12.5)	8 (50)	

*Numbers in the table represent frequency (%) unless stated otherwise.

TABLE 2. Comparison of ANTS pretest and posttest scores, midterm examination scores, and anaesthesia residents' competency as evaluated by staff.

Test	Overall (n=48)	Post-graduate year			*P* value
		1 (n=16)	2 (n=16)	3 (n=16)	
ANTS Pretest*	12.6 (2.2)	12.1 (2.2)	12.8 (1.7)	12.8 (2.6)	0.622
ANTS Posttest*	14.3 (2.8)	13.7 (2.3)	15.2 (2.4)	14 (3.5)	0.284
MCQ†	64.4 (60.8, 74.8)	69.7 (59.2, 90.5)	67.8 (63.9, 74.0)	62.6 (60.0, 64.1)	0.092
SAQ*	62.7 (9.3)	61.5 (11.3)	65.1 (7.2)	61.6 (9.0)	0.499
OSCE*	6.8 (1.0)	6.9 (1.0)	6.9 (0.9)	6.7 (1.0)	0.708
Knowledge score†	8.2 (7.7, 9.6)	7.9 (7.5, 9.5)	8.3 (7.7, 9.2)	8.9 (8, 9.8)	0.373
Skill competency*	8.0 (0.38)	7.7 (0.2)	8.0 (0.4)	8.2 (0.4)	<0.001
Attitude†	6.5 (6.3, 7.2)	6.7 (6.3, 7.1)	6.5 (6.4, 7.1)	6.4 (6.2, 7.2)	0.949

*Mean (standard deviation) and P value by analysis of variance. †Median (interquartile range) and P value by Kruskal–Wallis test.
Abbreviations: ANTS: anaesthetists' non-technical skills, MCQ: multiple choice question, SAQ: short answer question, OSCE: objective structured clinical examination

TABLE 3. Correlation between midterm examination scores of anaesthesia training, residents' competency as evaluated by staff, and multiple choice question ANTS scores.

Test	Pretest ANTS (r)	*P* value	Posttest ANTS (r)	*P* value
Multiple choice questions	0.140	0.349	-0.001	0.995
Short answer questions	-0.008	0.959	-0.242	0.100
OSCE	0.218	0.141	-0.173	0.245
Knowledge score	-0.058	0.698	0.376	0.009
Skill competency	0.054	0.720	0.140	0.347
Attitude score	0.014	0.924	-0.246	0.096

P value by Pearson's correlation coefficients (r), OSCE: Objective structured clinical examination.

modification between sex and midterm MCQ scores in the pretest MCQ scores (P<0.05). Three potential biasing variables (PGY, knowledge score, and attitude) of the total effect of midterm examinations indicated by the DAG (Fig 2A) were included as the minimally sufficient adjustment set with midterm examination variables (MCQ*sex/SAQ/OSCE) (Table 4). Among males, MCQ scores had a negative correlation with pretest ANTS scores (*P*=0.024). Regardless of sex, SAQ scores had a negative correlation with pretest ANTS scores (*P*=0.033) whereas OSCE was positively correlated with pretest ANTS scores (*P*=0.038).

Analysis of posttest MCQ scores

Eleven variables (sex, PGY, self-study, resident roles, knowledge score, skill competency, attitude, pretest ANTS score, MCQ score, SAQ score, and OSCE) suggested by the previous literature review were related to ANTS scores.[4,5,7]

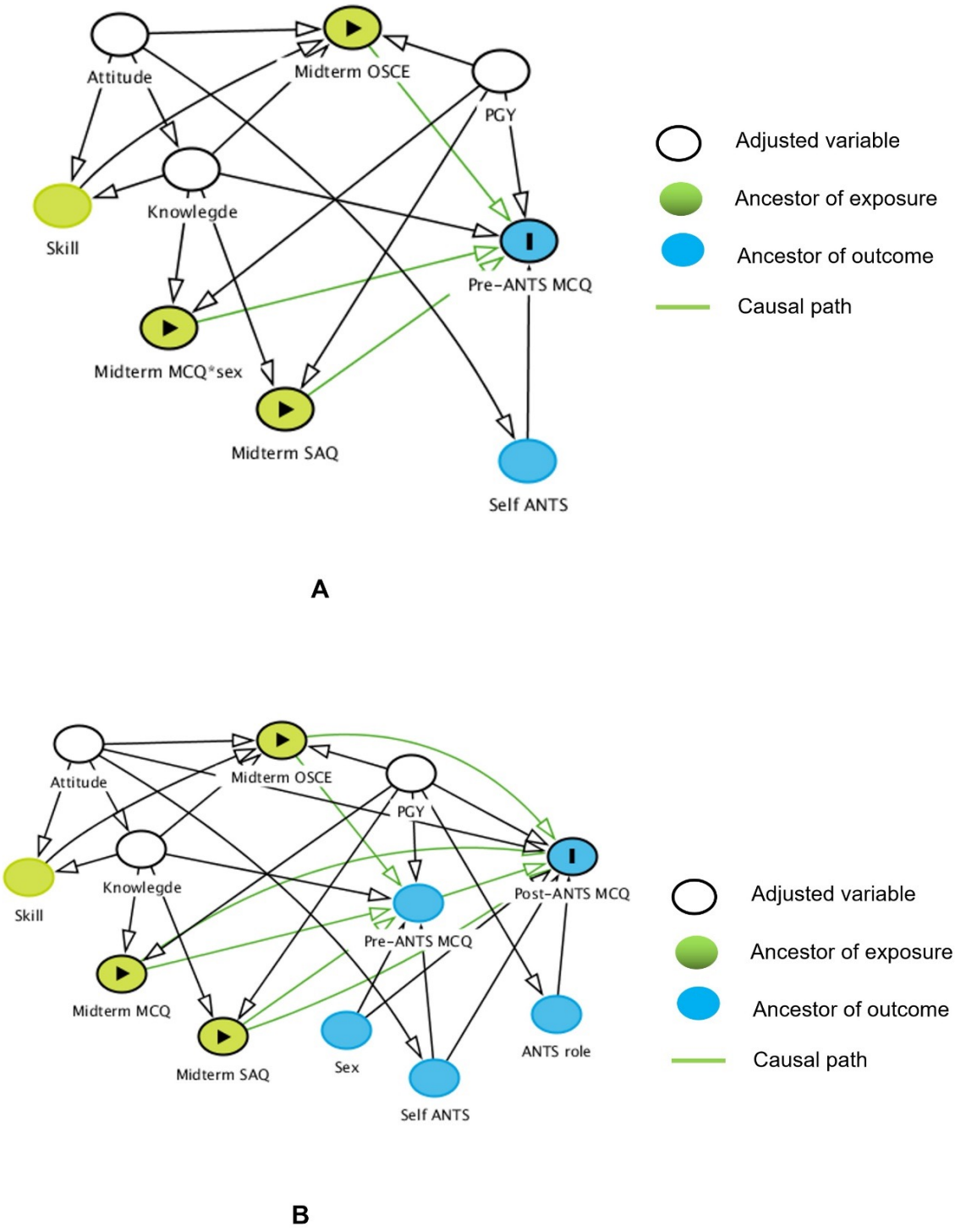

Fig 2. Hypothesised causal relationship between midterm examinations and pretest (A) and posttest (B) non-technical skill scores.
Note: PGY: post-graduate year, ANTS: anaesthetists' non-technical skills, MCQ: multiple choice question, SAQ: short answer question, OSCE: objective structured clinical examination.

There was no evidence of effect modification between sex and any other variables in relation to the midterm examinations in the posttest MCQ model. Three potential biasing variables (PGY, knowledge score, and attitude) of the total effect of midterm examinations indicated by the DAG (Fig 2B) were included as the minimally sufficient adjustment set with midterm examination variables (MCQ/SAQ/OSCE) (Table 4). The midterm MCQ scores showed a positive correlation with ANTS posttest scores (P=0.007) whereas the SAQ scores showed a negative correlation with ANTS posttest scores (P=0.004).

TABLE 4. Multiple linear regression by total effect model predicting relative probability of pretest and posttest ANTS scores based on midterm examinations.

Main exposure variables	Pretest ANTS score† Crude β (95% CL)	Adjusted β (95% CL)	*P* value*
MCQ score	0.07 (0, 0.14)	0.14 (0.04, 0.24)	0.006
Male (ref= female)	8.14 (-0.11, 16.4)	8.04 (-0.07, 16.15)	0.052
MCQ score: male	-0.12 (-0.23, 0)	-0.13 (-0.24, -0.02)	0.024
SAQ score	0 (-0.07, 0.07)	-0.11 (-0.21, -0.01)	0.033
OSCE score	0.5 (-0.17, 1.18)	1.02 (0.06, 1.98)	0.038
r of final model	0.38		
Main exposure variables	**Posttest ANTS score‡ Crude β (95% CL)**	**Adjusted β (95% CL)**	***P* value***
MCQ score	0 (-0.07, 0.07)	0.14 (0.04, 0.24)	0.007
SAQ score	-0.07 (-0.16, 0.01)	-0.16 (-0.27, -0.05)	0.004
OSCE score	-0.51 (-1.37, 0.36)	-0.25 (-1.29, 0.8)	0.634
r of final model	0.52		

*F-test. β: Beta coefficients, CL: confidence limits, MCQ: multiple choice question, SAQ: short answer question, OSCE: objective structured clinical examination, ref: reference.

†Minimally sufficient adjusted set suggested by a directed acyclic graph: post-graduate year of anaesthesia residents, knowledge score, attitude competency.

‡Minimally sufficient adjusted set suggested by a directed acyclic graph: post-graduate year of anaesthesia residents, knowledge score, attitude competency.

DISCUSSION

We examined whether technical skills in anaesthesia training using midterm MCQ/SAQ/OSCE assessments were associated with non-technical skill knowledge based on the pretest/posttest MCQ ANTS simulation workshop. According to the one-day workshop with three ANTS scenarios for each midterm examination, only one or two residents, and not all, could participate in each workshop. The rest were observed in the observer room. Instead of assessing their ANTS behavior one-by-one in the workshop, the debrief session, which was attended by all the residents, was performed to ensure a better and understanding of ANTS scenarios. Therefore, an assessment of ANTS knowledge using MCQs was feasible and helped the residents understand more about the ANTS elements incorporated in their learning by performing them daily. Therefore, the residents gradually gained non-technical skills during the training. Since we used two prospective cohorts (2017 and 2019), eight residents who were PGY1 in 2017 become PGY3 in 2019. Approximately 17% (8/48) of the residents might have been familiar with pretest MCQ ANTS. To minimise this bias, PGY was one of the adjusted sets for confounders in the final multivariate model of pretest and posttest MCQ ANTS. Our findings from Table 3 reveal that the relationship between MCQ/SAQ/OSCE scores and pretest/posttest MCQ ANTS scores (univariate analysis) were low ($r<0.3$), but the final multivariate linear regression models revealed a significant relationship not only between midterm examinations and pretest ANTS scores ($r=0.38$) but also between midterm examinations and posttest ANTS scores ($r=0.52$).

This proves that the relationship between technical and non-technical skills in anaesthesia is not simply linear due to the environment and many other circumstances during training, such as sex, PGY, self-study, residents' competencies, and residents' roles during ANTS workshop. This non-linear network can help researchers interpret the complex relationships within it.[10,11] Therefore, DAG was performed to construct the causal relationship between the two anaesthesia skills and to minimise confounding variables. We used midterm examinations (MCQ/SAQ/OSCE) to represent technical skill in anaesthesia training, which was similar to what was done in Raksamani's study.[12] The difference was that the MCQs, which comprised all four ANTS categories (SA/TM/TW/DM), was implemented instead of assessing ANTS behaviours among residents to correspond with non-technical skills. Moreover, to minimise the measurement bias, technical skills, as assessed by midterm examinations and the ANTS workshop, were performed in the same evaluation period (Fig 1).

Before the ANTS workshop, we found that OSCE had a positive correlation with pretest MCQ ANTS scores. This may be attributable to the fact that OSCE demonstrates clinical skills/competency in a standardised medical scenario, which represents routine clinical practise.[13] Thus, this skill may have been applied in the scenario in the pretest MCQ before the workshop. Furthermore, our results revealed that midterm MCQ scores had a positive correlation with pretest MCQ scores but, among males, it had negative correlation. Regardless of sex, the reason for a positive correlation between the midterm MCQ scores and pretest ANTS MCQ scores could be similar types of questions. However, compared to females, MCQ scores revealed a reverse association with pretest MCQ scores among males. A previous study revealed that males had higher pretest MCQ scores than females.[7] Therefore, it was found that sex could somehow affect the correlation between midterm MCQ scores and pretest ANTS scores and was considered a common confounding variable in other models.[14,15]

After the ANTS workshop, there was no effect modification between the midterm examinations and posttest ANTS scores. We found midterm MCQ scores to have an obvious positive correlation with posttest ANTS scores ($P<0.007$). Perhaps, the ANTS lecture before the workshop promoted ANTS basic knowledge simultaneously, while enhancing ANTS skills through different case scenarios (SA/TW/TM/DM) during the workshop and debriefing sessions. This possibly led to a higher positive correlation between midterm MCQ scores and posttest ANTS score. A previous study revealed that participants or observers can gain similar knowledge of ANTS based on the pretest/posttest MCQ scores.[7]

The reasons we implemented the 20-item MCQs to represent non-technical skills in anaesthesia training were as follows: first, multiple choice tests can be an effective and simple way to measure learning.[16] Second, assessments can be completed quickly, providing students with prompt feedback. Third, MCQs can assess knowledge and cognition and cover large topics in the curriculum.[17] Moreover, the application of MCQs can vary from simple recall to problem solving. Well-written MCQs can go beyond testing rote facts and might measure higher cognitive abilities.[18] However, from our results (Table 2), it was found that the posttest MCQ ANTS scores among residents (14.3) improved compared to pretest scores (12.6) but not greatly. This might be because the nature of four-answer choices makes it difficult to decide the best answer among four ANTS categories from each ANTS situation, especially an ANTS training beginner. Therefore, we discussed the appropriate answer with the residents after the posttest to stimulate higher cognitive function during clinical practise.

Surprisingly, SAQ scores revealed a negative correlation with pretest MCQ ANTS scores, and this finding was confirmed by posttest MCQ ANTS scores when SAQ scores indicated a more significant negative correlation. This could be because SAQs need to be related to a common or specific topic depending on the objective of the curriculum subjects whereas the ANTS MCQ test is broader and non-specific to a particular component of ANTS. Moreover, SAQs are more similar to essay questions that tend to be more difficult than MCQs since there are more cues to stimulate memory and because recall rather than recognition is required.[19] A good attitude, which was our most important confounder, is necessary in anaesthesia training and could be the key to becoming proficient in both technical and non-technical anaesthesia skills, especially the learners' attitudes towards medical ethics education.[20]

Strengths and limitations of the study

We formed a multivariate analysis model using DAG to adjust indicated confounders in the final model in order to determine whether the midterm examinations were independent predictors of the outcomes. The limitations of the study are as follows: first, the assessment of ANTS knowledge was used instead of ANTS behavioural skills of each resident owing to the limitation of time for the midterm ANTS workshop. Second, because we used two cohorts from two different years, the periods of training might have affected the midterm examination scores because the training environment changes with time.

CONCLUSION

Our findings revealed that technical skills based on midterm MCQ and OSCE assessments were associated with non-technical skill knowledge in anaesthesia. An assessment of ANTS knowledge during the workshop can help residents comprehend detailed ANTS information and foresee adverse situations in advance. Residents can gain non-technical skills by performing routine anaesthesia practise by collaborating with other anaesthesia personnel (nurse anaesthetists/anaesthesia technicians/anaesthesia staffs/surgeons) daily. A good attitude, which was our most important confounder, is necessary in anaesthesia training and could be the key to becoming proficient in both technical and non-technical anaesthesia skills. A further research regarding non-technical skill knowledge and non-technical skill behaviour of all residents should be performed to improve our ANTS training workshop.

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Anxiety, Depression and Cognitive Emotion Regulation Strategies in Psychiatric Patients during the COVID-19 Pandemic

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ABSTRACT

Objective: The coronavirus disease 2019 (COVID-19) pandemic likely impacted emotional regulation and mood states. The present study aimed to investigate the perceived risk, stigma, and emotional regulation strategies of psychiatric patients, as well as the association between these characteristics, cognitive emotion regulation strategies (emotional suppression and cognitive reappraisal), and anxiety and depressive symptoms during the COVID-19 pandemic.

Materials and Methods: The present cross-sectional study of 282 patients with anxiety and mood disorders was conducted during the COVID-19 pandemic in 2020. Participants completed questionnaires that investigated anxiety (hospital anxiety and depression scale [HADS]), depression (patient health questionnaire [PHQ-9]), and cognitive emotion regulation strategies (emotion regulation questionnaire [ERQ]). Descriptive statistics were used to assess the data. The t-test, chi-square test, and Mann-Whitney U test were used to compare the differences between the two groups, with cut-off scores of 11 in the HADS and nine in the PHQ-9.

Results: Most participants were female (78.4%), and the median age was 31 years. A total of 23.8% of participants reported having anxiety symptoms, and 24.8% and 54.3% of participants reported having depression on the HADS-D and PHQ-9, respectively. HADS-A, HADS-D, and PHQ-9 were found to be significantly associated with emotional regulation style (P=0.002, P=0.005, P=0.006) but not with perceived risk and stigma. Patients with anxiety or depression tended to use expressive suppression more often than cognitive reappraisal.

Conclusion: This study found that mood states were associated with cognitive emotional regulation strategies during the COVID-19 pandemic. Patients with anxiety or depression tended to use expressive suppression more often than cognitive reappraisal.

Keywords: COVID-19; coronavirus; cognitive emotional regulation; anxiety; depression (Siriraj Med J 2022; 74: 857-864)

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) spread rapidly following its emergence in 2019 in Wuhan, Hubei, China.[1] The World Health Organization (WHO) designated the coronavirus disease a public health emergency after only a few months, and the number of verified cases and associated fatalities continues to increase.[2] The present pandemic presents a serious worldwide problem. Thailand was the first country outside of China to diagnose a case of COVID-19 on January 17, 2020.[3]

The current global crisis has affected healthcare systems, global economic outlooks, and the delivery of education, as well as people's daily lives, habits, and physical and mental health, whereby psychological distress due to a fear of infection, depression, anxiety, and stigma have been reported.[4,5] Anxiety and fear may have a negative impact on one's mental health, particularly those with psychological vulnerability. Furthermore, other pre-existing social, cultural, and economic vulnerabilities can also play a part.[6] When a stressful event occurs, certain groups, such as women, children and the elderly, are at greater risk of complications.[7]

Patients with psychiatric disorders are also vulnerable. When a large stressor or unexpected event occurs, psychiatric symptoms may be worsened or prolonged.[8] Previous research in Thailand found that outpatients with a mental health condition had a strong understanding of COVID-19, good preventive practices, and a low level of anxiety about COVID-19.[9]

An individual's emotional regulation, which can be defined as "the process that individuals influence which emotions they have, and how they experience and express these emotions", appears to contribute to a stress reaction.[10] The current study concentrated on the two most important emotion management strategies: cognitive reappraisal and emotional suppression. Cognitive reappraisal necessitates the adaptive application of cognitive effort to modify a situation's evaluation, which alters the emotional response before it fully occurs. Conversely, expressive suppression is a response-focused technique that involves a behavioral attempt to inhibit the display of an already occurring emotional response.[11,12] During the COVID-19 pandemic, previous studies revealed that emotional suppression was associated with poorer psychological well-being, such as increased depressive symptoms, psychological distress, and reduced emotional well-being.[13-15]

To the best of our knowledge, concerns regarding mood symptoms and emotional regulation in patients with psychiatric disorders during the pandemic have not been thoroughly studied in Thailand. Most research has focused on the overall population or a specific community during the pandemic. The current study has two goals: 1) to investigate the psychological impact, perceived risk, stigma, and emotional regulation strategies of patients with psychiatric problems during the COVID-19 pandemic, and 2) to investigate the relationship between the characteristics, emotion regulation strategies, anxiety, and depressive symptoms. The authors expect that those psychiatric patients who employ emotion regulation techniques that favor emotional suppression over cognitive reappraisal will have higher PHQ-9 and HADS scores.

MATERIALS AND METHODS

Participants

The current study included psychiatric patients who visited the outpatient unit of the psychiatric clinic at Siriraj Hospital in April and May 2020. Participants had to be at least 18 years old, diagnosed with a mood or anxiety disorder, have a good comprehension of the Thai language, and be willing to participate in the study. Individuals with severe or emergency medical or psychiatric disorders, such as unstable vital signs, psychotic symptoms, or suicidal behavior, were excluded.

The study was approved by the Siriraj Research Affairs and Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 659/2020). The study was performed in accordance with the principles of the Declaration of Helsinki. Some parts of the questionnaire obtained for the present study were translated and conducted under the approval of the "Preventive measure and psychology stress on COVID-19" across Japan, Korea, Taiwan, and Thailand" (REC Number: 202003HS002).

Procedure and measurement

The study team recruited 282 patients with mental disorders according to the inclusion criteria listed above. The participants completed a questionnaire that assessed their demographic data and questionnaire sets. To comply with the COVID-19 pandemic policy, the authors offered a paper-based questionnaire with a set of envelopes to be returned by post and a paper with a quick response code for an online questionnaire. Participants were free to answer in any way that they saw fit. The questionnaires were completed anonymously.

Instruments

The questionnaire consisted of three sections. The first section collected demographic information about the respondents, including sex, age, occupational status, and educational status. The second section consisted

of questions about perceived risk and stigma that applied the questions from the questionnaire of the project "Preventive measure and psychology stress on COVID-19 by medical staff and students across Japan, Korea, Taiwan, and Thailand". The third section included three structured scales: the Thai version of the patient health questionnaire (PHQ-9), the hospital anxiety and depression scale (HADS), and the emotion regulation questionnaire (ERQ).

Patient health questionnaire (PHQ-9)

The PHQ-9 is a self-report of depressive symptoms comprised of nine items based on the DSM-IV diagnostic criteria for major depressive episodes.[16] Lotrakul et al. translated the original version to Thai and used it as a screening tool for depression. With a cut-off score of nine or higher, the Thai version of the PHQ-9 exhibited acceptable internal consistency (Cronbach's alpha=0.79).[17]

Hospital anxiety and depression scale (HADS)

The HADS is a questionnaire used for screening anxiety and depressive disorders.[18] The HADS consists of 14 items, including the HADS-Anxiety Subscale (HADS-A) and the HADS-Depressive Subscale (HADS-D). A score equal or greater than 11 indicated anxiety or depression. The Thai version of the HADS-A has a sensitivity and specificity of 100% and 86%, respectively, and the HADS-D has a sensitivity and specificity of 85.7% and 91.3%, respectively.[19]

Emotion regulation questionnaire (ERQ)

Gross and John developed the ERQ, a 10-item self-reported questionnaire intended to examine respondents' inclination to manage their emotions in two styles: cognitive reappraisal (six items) and expressive suppression (four items).[11] Higher subscale mean scores suggest greater use of a specific emotion control style. Khumrod and Soonthornchaiya translated and then evaluated the Thai version of the ERQ to assess its validity and reliability (2017). In the Thai version, the content validity index of the ERQ was 0.80, and its reliability was 0.82.[20]

Statistical analysis

The data were analyzed using SPSS (version 21.0; IBM SPSS, Armonk, NY, USA). Descriptive statistics were used to characterize the demographic data, agreement of perceived risk, and stigma among all participants. Descriptive analyses included the means, standard deviations, medians, and interquartile ranges (for non-normally distributed data). Using the HADS cut-off score of 11 and the PHQ-9 cut-off score of nine, the data were classified into two groups: the participants who had anxiety or depression (HADS-A ≥ 11, HADS-D ≥ 11, or PHQ-9 ≥ 9) and those who did not. The t-test, chi-square test, and Mann-Whitney U test were conducted to compare the differences in characteristics, ERQ, perceived risk, and stigma between the two groups. Pearson's correlation was used to investigate the correlation between HADS-A, HADS-D, and PHQ-9. A probability level of $p < 0.05$ was considered to be statistically significant.

RESULTS

Basic characteristics

This cross-sectional study included 282 participants. Of these, 221 (78.4%) were females, and 61 (21.6%) were males. The median age was 31 years (median [(Q1-Q3) = (25-41)]). the employment rate was 251 (89.0%), and the majority of participants (229; 81.2%) had graduated from college or university (Table 1). The primary diagnoses were depressive disorders, bipolar disorder, and anxiety disorder or insomnia disorder in 45.7%, 14.9%, and 39.4%, respectively. Overall, 23.8% of participants reported having anxiety symptoms (HADS-A positive; HADS-A ≥ 11), while 24.8% and 54.3% of participants reported having depression according to the HADS-D (HADS-D ≥ 11) and PHQ-9 (PHQ-9 ≥ 9), respectively. The median age of the participants with anxiety and depression was significantly different between the two groups (HADS-A, p<0.001; HADS-D, p=0.002; PHQ-9, p<0.001). Participants who were HADS-A, HADS-D, or PHQ-9 positive were younger than those who were negative. No other differences were observed between the two groups (Table 1).

Emotional regulation style: Comparison between styles by HADS and PHQ-9

Participants preferred cognitive reappraisal over expressive suppression (72.3% vs 27.7%) (Table 2). When anxiety symptoms were used to compare emotional regulation styles, the HADS-A was found to be significantly associated with emotional regulation style (P=0.002; OR [95% CI] = 2.51 [1.39-4.52]). Participants who were anxious (HADS-A positive) preferred expressive suppression (42.4%), whereas those who were not anxious (HADS-A negative) preferred cognitive reappraisal (72.3%). HADS-D was significantly associated with emotional regulation style in participants with depression (P=0.005; OR [95% CI] = 2.29 [1.27-4.13]). Participants with depression (HADS-D positive) preferred expressive suppression (40.9%), whereas those without depression (HADS-D negative) preferred cognitive reappraisal (76.8%). The PHQ-9 was also significantly associated with emotional

TABLE 1. Characteristics of psychiatric patients grouped by the hospital anxiety and depression scale-anxiety subscale (HADS-A), depression subscale (HADS-D), and patient health questionnaire-9 (PHQ-9)

Characteristics	Total n=282	HADS-A Negative n = 215 (76.2%)	HADS-A Positive n = 67 (23.8%)	HADS-A P-value	HADS-A OR (95%CI)	HADS-D Negative n = 212 (75.2%)	HADS-D Positive n = 70 (24.8%)	HADS-D P-value	HADS-D OR (95%CI)	PHQ-9 Negative n = 129 (45.7%)	PHQ-9 Positive n = 153 (54.3%)	PHQ-9 P-value	PHQ-9 OR (95%CI)
Sex				0.397	0.74 (0.37-1.49)			0.702	0.88 (0.45-1.71)			0.234	0.71 (0.40-1.25)
Male	61 (21.6)	49 (22.8)	12 (17.9)			47 (22.2)	14 (20.0)			32 (24.8)	29 (19.0)		
Fetamale	221 (78.4)	166 (77.2)	55 (82.1)			165 (77.8)	56 (80.0)			97 (75.2)	124 (81.0)		
Age (years); Median (Q1-Q3)	31.0 (25-41)	33.0 (26-43)	26.0 (22-35)	<0.001*		33.0 (26-43)	27.5 (23-35.25)	0.002*		35.0 (28-44.75)	28.0 (23-36)	<0.001*	
Occupation				0.465	1.36 (0.59-3.12)			0.145	1.79 (0.81-3.95)			0.224	1.61 (0.74-3.51)
Employed	251 (89.0)	193 (89.8)	58 (86.6)			192 (90.6)	59 (84.3)			118 (91.5)	133 (86.9)		
Unemployed	31 (11.0)	22 (10.2)	9 (13.4)			20 (9.4)	11 (15.7)			11 (8.5)	20 (13.1)		
Educational status				0.774				0.977				0.622	
Primary school	10 (3.5)	9 (4.2)	1 (2.4)			7 (3.3)	3 (4.3)			6 (4.7)	4 (2.6)		
Middle school	17 (6.0)	13 (6.0)	4 (6.0)			13 (6.1)	4 (5.7)			8 (6.2)	9 (5.9)		
High school	26 (9.2)	20 (9.3)	6 (9.0)			20 (9.4)	6 (8.6)			14 (10.9)	12 (7.8)		
College or University	229 (81.2)	173 (80.5)	56 (83.6)			172 (81.1)	57 (81.4)			101 (78.3)	128 (83.7)		

* Mann-Whitney U test

TABLE 2. Emotional regulation style in psychiatric patients grouped by the hospital anxiety and depression scale-anxiety subscale (HADS-A), depression subscale (HADS-D), and patient health questionnaire-9 (PHQ-9)

Emotional regulation style	Total n=264	HADS-A Negative n = 198 (75.0%)	HADS-A Positive n = 66 (25.0%)	HADS-A P-value	HADS-A OR (95%CI)	HADS-D Negative n = 198 (75.0%)	HADS-D Positive n = 66 (25.0%)	HADS-D P-value	HADS-D OR (95%CI)	PHQ-9 Negative n = 119 (45.1%)	PHQ-9 Positive n = 145 (54.9%)	PHQ-9 P-value	PHQ-9 OR (95%CI)
Emotional regulation style				0.002	2.51 (1.39-4.52)			0.005	2.29 (1.27-4.13)			0.006	2.20 (1.24-3.88)
Cognitive reappraisal	191 (72.3)	153 (77.3)	38 (57.6)			152 (76.8)	39 (59.1)			96 (80.7)	95 (65.5)		
Expressive suppression	73 (27.7)	45 (22.7)	28 (42.4)			46 (23.2)	27 (40.9)			23 (19.3)	50 (34.5)		

regulation style (P=0.006; OR [95% CI] = 2.20 [1.24-3.88]). Participants with depression (PHQ-9 positive) used expressive suppression (34.5%), whereas participants without depression (PHQ-9 negative) used cognitive reappraisal (80.7%).

Perceived risk and stigma

Regarding perceived risk, 58.2% (n=163) participants agreed with the statement "It is possible that I might be infected by COVID-19," 63.0% (n=177) agreed with the statement "It is possible that my neighbors, colleagues, and friends might be infected by COVID-19," and 87.5% (n=246) agreed with the statement "It is possible that others might be infected by COVID-19".

Regarding stigma, 11.0% (n=31) of participants agreed that they felt fear/afraid, 25.3% (n=71) avoidance, 3.2% (n=9) kept their diagnosis a secret, 9.6% (n=27) felt embarrassed, and 10.3% (n=29) stigma.

The HADS-A, HAD-D, and PHQ-9 scores were not associated with the agreement of all perceived risk and stigma items (all P>0.05; Table 3).

TABLE 3. Perceived risk and stigma

	Agree n (%)	Disagree n (%)	HADS-A P-value	HADS-A OR (95%CI)	HADS-D P-value	HADS-D OR (95%CI)	PHQ-9 P-value	PHQ-9 OR (95%CI)
Perceived risk								
It is possible that I might be infected by COVID-19	163 (58.2)	117 (41.8)	0.999	1.00 (0.57-1.75)	0.834	1.06 (0.61-1.83)	0.085	0.66 (0.41-1.06)
It is possible that my neighbors/colleagues/friends might be infected by COVID-19	177 (63.0)	104 (37.0)	0.953	1.02 (0.58-1.79)	0.242	1.39 (0.80-2.41)	0.576	0.87 (0.54-1.42)
It is possible that others might be infected by COVID-19	246 (87.5)	35 (12.5)	0.568	0.78 (0.32-1.86)	0.907	1.05 (0.47-2.36)	0.154	0.60 (0.29-1.22)
Stigma								
Fear/Afraid: I will be afraid to let people know if I may have been infected with COVID-19	31 (11.0)	250 (89.0)	0.786	0.89 (0.38-2.09)	0.448	1.43 (0.56-3.65)	0.963	0.98 (0.46-2.08)
Avoidance: If I suspect I may have been infected with COVID-19, I will not think about it until I become unwell/sick	71 (25.3)	210 (74.7)	0.534	1.23 (0.64-2.35)	0.677	0.88 (0.48-1.62)	0.925	0.97 (0.57-1.67)
Keeping a secret: If I suspect I may have been infected with COVID-19, I will keep it a secret	9 (3.2)	272 (96.8)	1.000	1.10 (0.22-5.42)	0.695	0.65 (0.16-2.69)	1.000	0.96 (0.25-3.63)
Embarrassment: I will feel embarrassed if others know that I may have been infected with COVID-19	27 (9.6)	254 (90.4)	0.835	1.11 (0.43-2.87)	0.419	1.51 (0.55-4.16)	0.598	0.81 (0.36-1.80)
Stigma: I will lose friends if I tell them I may have been infected with COVID-19	29 (10.3)	252 (89.7)	0.156	0.56 (0.24-1.26)	0.919	1.05 (0.43-2.57)	0.097	0.50 (0.22-1.15)

DISCUSSION

The present study investigated the psychological impact, perceived risk, stigma, and emotional regulation strategies of patients with psychiatric problems during the COVID-19 pandemic and the association between the characteristics, emotion regulation strategies, and psychiatric symptoms.

Anxiety and depression

Regarding the first objective, the results showed that about a quarter of participants reported having depressive or anxiety symptoms using the HADS-A or HADS-D, and about half reported having depression using the PHQ-9. There was a significant difference in the median age of patients with anxiety and depression. While the other features were similar, the participants with anxiety or depression were younger. Although the number of participants with anxiety differed between the two questionnaires, HADS-D and PHQ-9, we discovered a moderately significant correlation (r=0.767, P<0.01) between both questionnaires (Table 4). The results of the two questionnaires differ because the HADS-D concentrates on emotions and interests, whereas the PHQ-9 assesses neurovegetative symptoms. Other than age, we could not find an association between mood states (depression and anxiety), sex, and employment.

The current study's participants were comparable to that of a previous study, which enrolled outpatients with a mental health condition from university hospitals.[9] However, the objectives of this study were different. The earlier study concentrated on the participants' COVID-19 knowledge, preventive behaviors, and anxiety levels, whereas the current study included emotion control, perceived risk, and stigma.

Perceived risk and stigma

According to the perceived risk in the three statements, the majority of the participants (87.5%) agreed with the perceived risk of COVID-19 infection in others. Approximately half (58.2%) of the participants reported a risk of infection. This could imply that believing others are afflicted is simpler than believing oneself. Participants may have been confident in their preventive behaviors toward COVID-19, which is consistent with a recent study that found that almost half of the participants had a low-risk perception of COVID-19.

Regarding stigma, around a quarter of the participants agreed with the statement of avoidance, while "keeping a secret" received the least agreement. This could be because most participants did not see the illness as an issue that needed to be concealed. Avoidance is one of the defense mechanisms used by neurotic patients to deal with worry. We hypothesized that anxiety and sadness are related to perceived danger or stigma. However, no association was found between perceived risk and stigma in participants with high anxiety (HADS-A ≥ 11), depression (HADS-D ≥ 11), or PHQ-9 ≥ 9. There are several possible explanations for this, including 1) because the data were collected during the country's second wave of the pandemic (around April-May 2020), which did not have a high prevalence rate compared to Europe, most people felt they were not at a high risk of being infected; and 2) at that time, most people may have known about COVID-19 and how to deal with it. As a result, the perceived threat or stigma may have been lower.

TABLE 4. Correlation between HADS and PHQ-9

	HADS-A	HADS-D	PHQ-9
HADS-A		0.705**	0.797**
HADS-D	0.705**		0.767**
PHQ-9	0.797**	0.767**	

**. Correlation is significant at the 0.01 level (2-tailed).

Emotional regulation strategies

According to the results, participants preferred cognitive reappraisal over expressive suppression. Anxiety and depression were significantly related to emotional regulation style, and participants who were anxious or depressed preferred expressive suppression. Emotional regulation is a complex mental process controlled by various elements such as mental health, mood state, and life satisfaction.[21] Effective emotion regulation is crucial for effective functioning in a dynamic context. Previous studies have shown that greater use of cognitive reappraisal and less use of expressive suppression are associated with more positive outcomes in the domains of mood, well-being, and social functioning.[22] A previous meta-analysis showed a significant positive correlation between cognitive reappraisal and a positive indicator of mental health, while expressive suppression showed a negative correlation with a positive indicator of mental health. In addition, this meta-analysis found that cultural values may influence individual emotion regulation strategies.[23] A previous study revealed that regulating emotions using cognitive reappraisal was associated

with greater resilience (i.e., the ability to seek enjoyable activities and social support and feelings of hope and resourcefulness).[24] Cognitive reappraisal is a technique that may be learned and utilized to mitigate the impact of stress on an individual's well-being, including stress induced by a pandemic.

The current study revealed that patients with psychological problems are especially susceptible and in need of care, particularly during the current COVID-19 pandemic. Psychological therapy focusing on emotional regulation skills could be used to support them cope with the COVID-19 pandemic. Even though face-to-face interventions are not possible during the pandemic, psychological support via the Internet and phone can ensure that patients with a psychiatric condition receive the care they require. It appears that people are now better equipped to deal with COVID-19. The current study sought to better understand the perceptions and emotional control styles of people suffering from psychiatric diseases. This increases preparedness to deal with mental health issues in the event of future pandemics.

Strengths and limitations

The present study has several strengths and limitations. To the best of our knowledge, this is the first study in Thailand to investigate emotion regulation styles and the relationship between these factors and anxiety and depression in people with mental health problems. This study also included a large number of patients with mood and anxiety disorders, which could be investigated using an emotional regulation lens. However, this study has significant drawbacks. First, this study was conducted at a single site and included only outpatients with mood and anxiety disorders, which may have resulted in a selection bias, and the representativeness of the population was limited. Inpatients may have more active symptoms, and their reactions may differ. Second, not all psychiatric disorders were included. Therefore, our findings may not be representative of other psychotic problems. Furthermore, the symptoms were established through self-report rather than face-to-face questioning, which may have resulted in a reporting bias. However, during the pandemic, these methods proved useful for data collection. Next, because the data were collected only once during the second wave of the pandemic, which was around April-May 2020, we could not examine the change or trend of the response during the pandemic. Therefore, the data should be carefully interpreted because it may only reflect the perception and behavior toward COVID-19 of the population at that specific point in time. Although we observed an increase in negative feelings and emotional regulation among mental patients, as noted previously in the introduction and results of the manuscript, it is difficult to regulate external factors that influence each participant's perception of COVID-19. Each participant has a unique viewpoint on COVID-19 based on how they feel about various aspects, such as health, costs, employment, etc. Furthermore, we could not conclude that patients with depression and anxiety disorders tended to use expressive suppression during the COVID-19 pandemic any more than normal because only one time point of data was collected.

Future recommendations and implications

In contrast to other examples, such as HIV infection, it can have a direct impact on specific groups of people, such as minorities or vulnerable groups, which may not be socially acceptable. COVID-19, on the other hand, can have an individual influence in cluster outbreaks like those in pubs and clubs, or on groups that can easily be identified as spreaders. The participants in this study were not infected with COVID-19 at the time of the study. As a result, this could assist to rule out strong thoughts about being infected, such as being labeled, feeling guilty, and so on. However, in the current study, external influences and one's own views about the individual perspective were difficult to control. In order to address this challenge and obtain a clearer answer to the study, there may be several ways to design for additional research, such as: 1) inquiring into psychosocial issues that directly affect individual subjects for a better understanding of individual details, or 2) incorporating psychosocial variables into the analysis.

Further research across different sites and over time is necessary to understand the impact of the emerging disease pandemic on psychiatric problems and emotional control. Further research should cover additional mental illnesses, particularly in psychotic patients and inpatients who are more prone to stress. Furthermore, comparing people with and without psychiatric diagnoses should be considered in future studies. Nevertheless, the authors believe that the findings of this study contribute significantly to the literature on the experience of patients with psychiatric illnesses during the emerging disease pandemic. Furthermore, the association between emotion regulation strategies and mental health was investigated, which provides a foundation for future research and may lead to the development of useful interventions.

CONCLUSION

The results of the current study revealed that about a quarter to a half of the participants reported depressive

or anxiety symptoms. There was a significant difference in the median age of patients with anxiety and depression; participants with anxiety or depression were younger. Anxiety and depression were found to be associated with emotion regulation strategies. Patients with anxiety or depression tended to use expressive suppression more often than cognitive reappraisal. Understanding mood states and cognitive emotion regulation strategies will aid the future promotion of psychosocial interventions during pandemics.

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Potential conflicts of interest

None.

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Clinical Characteristics and Treatment Outcomes of Patients with Primary Ocular Adnexal Lymphoma at Siriraj Hospital

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ABSTRACT

Objective: Malignant lymphoma represents the most common primary orbital malignant neoplasm.

Materials and Methods: We retrospectively reviewed data and analyzed clinical characteristics and treatment outcomes of patients with primary ocular adnexal lymphoma at Siriraj Hospital, a tertiary health-care and teaching center in Bangkok, Thailand between January 2004 and June 2017.

Results: From the total of 94 patients with primary ocular adnexal lymphoma, 77 cases (81.9%) were indolent, of which extranodal marginal zone lymphoma (EMZL) was the most common (76.6%). Aggressive subtype accounted for 17 cases (18.1%), consisting of diffuse large B-cell lymphoma (9.6%), mantle cell lymphoma (4.3%), and extranodal NK/T cell lymphoma (3.2%). Plasmacytic differentiation was found in half of EMZL and 4 cases of EMZL had association with IgG4-related disease. Most of the patients presented with proptosis (54.3%), followed by a palpable mass (42.6%). Most patients had Ann Arbor stage I (66.3%) with zero ECOG performance status (91.1%). Chemotherapy was the main treatment for both indolent and aggressive lymphomas. The overall response rate and complete response rate were 88.6% and 68.7%, respectively. The 5-year progression-free survival (PFS) and overall survival (OS) rates were 60.1% and 84.2%, respectively. The indolent group had better overall (92.4% vs 69.2%) (P=0.01) and complete response rates (73.1% vs 50%) (P=0.01) than the aggressive group.

Conclusion: Histopathological subtypes and clinical stages of lymphoma are the best indicators of prognosis and treatment outcomes. Chemotherapy was an effective treatment modality for both indolent and aggressive lymphoma subtypes with better treatment outcomes in the indolent group.

Keywords: Primary ocular adnexal lymphoma; extranodal marginal zone lymphoma with plasmacytic differentiation; IgG4-related disease; clinical characteristics; treatment outcomes (Siriraj Med J 2022; 74: 865-873)

INTRODUCTION

Lymphomas are malignant tumors of B or T lymphocytes and, in rare cases, of natural killer (NK) cells. They are divided into Hodgkin lymphoma and non-Hodgkin lymphoma (NHL). Ocular adnexal lymphoma refers to a malignant lymphoproliferative disease that involves the conjunctiva, lacrimal gland, eyelid, or orbit.[1] Despite the uncommon orbital involvement by NHL, it represents the most common primary orbital and ocular adnexal malignant neoplasm.[1–3] Lymphomas are clinically divided

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into two groups. The first group, low-grade lymphoma, includes extranodal marginal zone lymphoma (EMZL) of mucosa-associated lymphoid tissue, follicular lymphoma, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma. The second group, high-grade lymphoma, is composed of diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, T-cell lymphoma, and extranodal NK/T cell lymphoma. Most type of ocular adnexal lymphoma is low-grade B-cell NHL. EMZL is the most common subtype, followed by follicular lymphoma, DLBCL, and mantle cell lymphoma.[4–10] Uncommon ocular adnexal lymphoma variants include plasmablastic lymphoma, extrannodal NK/T cell lymphoma, small lymphocyte lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, plasmacytomas, and peripheral T-cell lymphoma, NOS.[4–10] There are many clinical manifestations of ocular adnexal lymphoma. Disease within the lacrimal glands and eye lids usually presents as palpable masses. In case that the disease extends behind the globe, then pain, proptosis, ophthalmoplegia, and globe displacement may be present. Localized plaque (termed a "salmon patch") on the conjunctiva could be presented as an early sign of a conjunctival lymphoma. Orbital imaging typically shows a poorly defined mass that molds to surrounding structures without direct invasion or bony erosion.[11] Suspicion of the diagnosis-either as a result of clinical or radiological findings—should lead to a confirmatory biopsy. Once a definite diagnosis is established, patients are referred for treatment by a multidisciplinary team composed of an ophthalmologist, a hemato-oncologist, a pathologist, and a radiation therapy specialist.

The purpose of this study was to evaluate the clinical characteristics and treatment outcomes of patients with primary ocular adnexal lymphoma at Siriraj Hospital. Deeper understandings gained through this research may contribute to improvements in multidisciplinary therapeutic planning.

MATERIALS AND METHODS

The protocol for this study was approved by the Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 212/2018). Medical records were reviewed to collect data on all patients diagnosed with primary ocular adnexal lymphoma between 1 January 2004 and 30 June 2017 (13 years) at Siriraj Hospital, a tertiary health-care and teaching center in Bangkok, Thailand. The collected data related to the patients' demographics, clinical and pathological assessments, disease stage, treatment modalities, and outcomes. Demographic data consisted of age, sex, provincial address, and underlying diseases. As to the clinical assessment data, they detailed the clinical presentations, affected anatomical locations, laterality, time to diagnosis, and serological findings for lactate dehydrogenase levels and the statuses of the human immunodeficiency, hepatitis B, and hepatitis C viruses. The pathological assessment data comprised the biopsy site, date, and histological subtypes of the lymphomas which were independently reviewed by an experienced ocular pathologist (MU) and hematopathologist (SS). The disease stages were based on the Ann Arbor stage at the time of diagnosis, the Eastern Cooperative Oncology Group (ECOG) performance status, the number of extranodal sites involved, and the International Prognostic Index. In terms of treatment-modality, the details comprised type (chemotherapy, radiotherapy, surgery, immune-modulating therapy, or a combination of such treatment), the number of cycles of each treatment method, the kind of chemotherapy, and the salvage therapies employed for patients with recurrence. The treatment outcome assessments evaluated the responses to treatment, disease status, and current patient-status, all of which were based on WHO criteria. The outcome categories used were "complete response" (disappearance of the lesion), "partial response" (a decrease of ≥ 50% of the longest diameter of the tumor), "stable disease" (a decrease of < 50% or an increase of < 25%), and "progressive disease" (an increase of ≥ 25%). All treatment outcomes were evaluated at the end of treatment, whereas the disease status was appraised at the final follow-up.

Progression-free survival (PFS) was defined as the length of time following the completion of the primary cancer treatment during which a patient remained free of the complications or events that the treatment was intended to prevent or delay. In comparison, overall survival (OS) was defined as the length of time between the date of diagnosis and death from any cause.

Quantitative data were analyzed with descriptive statistics. Univariable analyses were performed with SPSS for Windows, version 16.0 (SPSS Inc., Chicago, Il, USA). A p-value of < 0.05 was considered statistically significant. OS was calculated from the date of diagnosis to the date of the last follow-up or death from any cause. PFS was measured from the date of diagnosis to the date of the last follow-up, relapse/progression, or death from any causes. The probabilities of OS and PFS were estimated using the Kaplan–Meier method and the log-rank test for survival comparison.

RESULTS

From 99 patients diagnosed with lymphoma, after pathological reviews by an experienced ocular pathologist (MU) and hematopathologist (SS), 5 cases were excluded

with 3 cases diagnosed with IgG4-related disease, one case of plasmacytoma and one case of B-cell acute lymphoblastic leukemia. A total of 94 patients with primary ocular adnexal lymphoma were collected which comprised 56 males (59.6%) and 38 females (40.4%). Their ages ranged from 39 to 99 years, with a median of 61.5 years. Median time to diagnosis was approximately 3 months, while the median follow-up duration was 33 months. Most patients presented with proptosis (56%), followed by a palpable mass (42%) and cellulitis (2%). The most commonly involved anatomical locations were the orbit (62%), conjunctiva (17%), lacrimal gland (15%), and eyelid (5%) (Table 1). For the immunophenotype, 90 cases (95.7%) were of B-cell and the remaining 4 cases (4.3%) were of T-cell or NK cell. In terms of subtypes, 77 cases (82%) were indolent, of which extranodal marginal zone lymphoma (EMZL) was the most common (76.6%), followed by follicular lymphoma (2.1%), small lymphocytic lymphoma (1.1%) and unclassifiable small B-cell lymphoma (1.1%). Of the 72 cases of EMZL, there were 37 cases (51.4%) with plasmacytic differentiation. Interestingly, there were 4 cases (5.5%) of EMZL with accompanying IgG4-related disease and 2 of them also showed plasmacytic differentiation. While the aggressive subtype accounted for 17 cases (18.1%), consisting of DLBCL (9.6%), mantle cell lymphoma (4.3%), extranodal NK/T cell lymphoma (3.2%), plasmablastic lymphoma (1.1%) and peripheral T-cell lymphoma, NOS (1.1%). We reviewed the one case diagnosed with unclassifiable small B-cell lymphoma; the tissue biopsy was too small and the patient lost to follow-up so that further clinical examination and tissue biopsy could not be performed. Regarding the other laboratory findings, most patients had normal lactate dehydrogenase levels (92%). Three patients (4%) were found positivity with the human immunodeficiency virus, two (2.7%) tested positive for hepatitis B, while three (4%) were positive for hepatitis C.

After definitive tissue diagnosis and disease staging, the treatments were planned. Of the 94 cases, 64 (68%) were treated at Siriraj Hospital, while the remainder (30; 32%) were referred to other hospitals to meet patients' preference or insurance-coverage requirements. Most patients had Ann Arbor stage I (66.3%) with zero ECOG performance status (91%). Of those, the International Prognostic Index of low risk (82.4%) was the most common (Table 1).

Regarding treatment modalities, 42 (67.2%) cases received chemotherapy and 11 cases (17.2%) received radiotherapy (Table 2). CVP regimen (cyclophosphamide, vincristine, and prednisolone; 59.5%) was the most common chemotherapy regimen, followed by CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisolone; 16.7%) and others. Seven cases (10.9%) received combination chemotherapy and radiotherapy, while a small number of patients (3.1%) underwent surgery only or were administered with an immune modulating agent (1.6%).

Analysis of the treatment outcomes revealed that the overall response rate and complete response rate were 88.6% and 68.7%, respectively. Most cases of indolent subtype were from the first Ann Arbor staging (76.4%) following by 11.1% of stage II, 6.9% of stage III and 5.6% of stage IV. In contrast to aggressive subtype, the staging was varied with 23.5% in stage I, 41.1% in stage II, 5.8% in stage III, and 29.4% in stage IV. The 5-year PFS and OS rates were 60.1% and 84.2%, respectively (Table 3). Subgroup analysis found that the indolent lymphoma group had a 92.4% overall response rate and a complete response rate of 73.1%. 5-year PFS and OS rates for the indolent group were 62.8% and 92.5%, respectively. In aggressive lymphoma types, the overall response rate was 69.2%, with a complete response rate of 50%. The 5-year PFS and OS rates were 50% and 65.9%, respectively. Fig 1A illustrates the Kaplan-Meier PFS rates for various lymphoma subtypes, while Fig 1B presents the Kaplan-Meier OS rates.

DISCUSSION

Results of the current study were compared with those of the study by Yen et al.,[12] which involved a review of the level 3 evidence of 27 studies on ocular adnexal lymphoma treatments. We grouped each of those 27 studies into regions to identify the characteristics of primary ocular adnexal lymphoma specific to each continent (Table 4). Of the 1,938 patients, females predominated slightly (54%)[12] but males predominated in our study (59.6%). The median age at diagnosis (60.1 years) was nearly the same as our study (61.5 years). Their study and our study found that orbit was the most commonly affected anatomical location. However, some other reports found that conjunctiva was the most common site of involvement, followed by orbit.[12] In general practice, orbital lesions are difficult for general ophthalmologists to biopsy so that many patients with proptosis and orbital lesions are referred from primary or secondary care hospitals to our hospital that is a tertiary care center and medical school. This may explain why orbit was the most commonly affected site for primary ocular adnexal lymphoma.

Distribution of the lymphoma subtypes in our study revealed that extranodal marginal zone lymphoma (EMZL) was the most frequent subtype (76.6%), followed by

TABLE 1. Demographic data, clinical assessment, pathological assessment, and stage of lymphoma.

Total patients		**94**
Sex	Male	56 (59.6%)
	Female	38 (40.4%)
Median age at diagnosis (Yr.)		61.5 (range, 39-99)
Median time to diagnosis (Mo.)		3 (range, 0.25-24)
Median time to follow-up (Mo.)		33 (range, 15-81)
Clinical presentation	Proptosis	51 (54.3%)
	Palpable mass	40 (42.6%)
	Eye pain	2 (2.1%)
	Cellulitis	1 (1.1%)
Anatomical location	Orbit	59 (62.8%)
	Conjunctiva	16 (17%)
	Lacrimal gland	14 (14.9%)
	Eyelid	5 (5.3%)
Immunophenotype	B cell	90 (95.7%)
	T/NK cell	4 (4.3%)
Type of lymphoma	Indolent	77 (81.9%)
	Aggressive	17 (18.1%)
Lymphoma subtype	1. Extranodal marginal zone lymphoma (EMZL)	72 (76.6%)
	- with plasmacytic differentiation	- 37/72 (51.3%)
	- with accompanying IgG4 related disease	- 4/72 (5.5%)
	2. Diffuse large B-cell lymphoma (DLBCL)	9 (9.6%)
	3. Mantle cell lymphoma	4 (4.3%)
	4. NK/T cell lymphoma	3 (3.2%)
	5. Follicular cell lymphoma	2 (2.1%)
	6. Small lymphocytic lymphoma	1 (1.1%)
	7. Unclassifiable small B-cell lymphoma	1 (1.1%)
	8. Plasmablastic lymphoma	1 (1.1%)
	9. Peripheral T-cell lymphoma	1 (1.1%)
Ann Arbor staging (total: 89)	I	59 (66.3%)
	II	15 (16.9%)
	III	6 (6.7%)
	IV	9 (10.1%)
Performance status (ECOG) (total: 79)	0	72 (91.1%)
	1	3 (3.8%)
	2	2 (2.5%)
	3	2 (2.5%)
International Prognostic Index (IPI) (total: 74)	Low risk (0-1)	61 (82.4%)
	Low-intermediate risk (2)	5 (6.8%)
	High-intermediate risk (3)	8 (10.8%)
	High risk (4-5)	-
LDH level (total: 75)	Normal	67 (91.8%)
	Higher than upper normal level	6 (8.2%)
HIV status (total: 75)	Positive	3 (4%)
	Negative	72 (96%)
HBV infection (total: 75)	Positive	2 (2.7%)
	Negative	73(97.3%)
HCV infection (total: 75)	Positive	3 (4%)
	Negative	72 (96%)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; LDH, Lactate dehydrogenase; NK, natural killer; SD, Standard deviation

TABLE 2. Treatment modalities.

Treatment modalities (total: 64)	64 (68%) from 94 cases
Chemotherapy	42 (67.2%)
Regimen	
- CVP	25 (59.5%)
- CHOP	7 (16.7%)
- R-CVP	2 (4.8%)
- Chlorambucil	5 (11.9%)
- Cyclophosphamide, prednisolone	2 (4.8%)
- R-BAC	1 (2.4%)
Radiotherapy	11 (17.2%)
Surgery	2 (3.1%)
Combined chemotherapy and radiotherapy	7 (10.9%)
- RT + CHOP	2
- RT + CVP	4
- RT + CP	1
Rituximab	1 (1.6%)
Observation	1 (1.6%)

Abbreviations: CHOP, Cyclophosphamide, doxorubicin, vincristine, and prednisolone; CP, Chlorambucil hydrochloride and prednisone; CVP, Cyclophosphamide, vincristine, and prednisolone; R-BAC, Rituximab, bendamustine, and cytarabine; R-CVP, Rituximab, cyclophosphamide, vincristine, and prednisolone; RT, Radiotherapy

TABLE 3. Treatment-outcome assessments.

Lymphoma type	N (total = 79)	ORR (%) (total = 79)	CR (%) (total = 64)	PFS (%)		OS (%)	
Indolent	66 (83.5%)	92.4 % (61/66)	73.1% (38/52)	3 yr – 75.2% 5 yr – 62.8%		3 yr – 96.5% 5 yr – 92.5%	
				CMT (N=34)	RT (N=11)	CMT (N=34)	RT (N=11)
				3 yr – 66.7%	3 yr – 100%	3 yr – 86.4%	3 yr – 100%
				5 yr – 50.1%	5 yr – 100%	5 yr – 78.6%	5 yr –100%
Aggressive	13 (16.5%)	69.2 % (9/13)	50.0 % (6/12)	3 yr – 50.0% 5 yr – 50.0%		3 yr – 65.9% 5 yr – 65.9%	
				CMT (N=8)	No RT cases	CMT (N=8)	No RT cases
				3 yr – 33.3%		only 1 death case	
				5 yr – 33.3%			
Both	79 (100%)	88.6% (70/79)	68.7% (44/64)	3 yr – 70.6% 5 yr – 60.1%		3 yr – 91.6% 5 yr – 84.2%	

Abbreviations: CMT, chemotherapy; CR, complete response rate; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; RT, radiotherapy

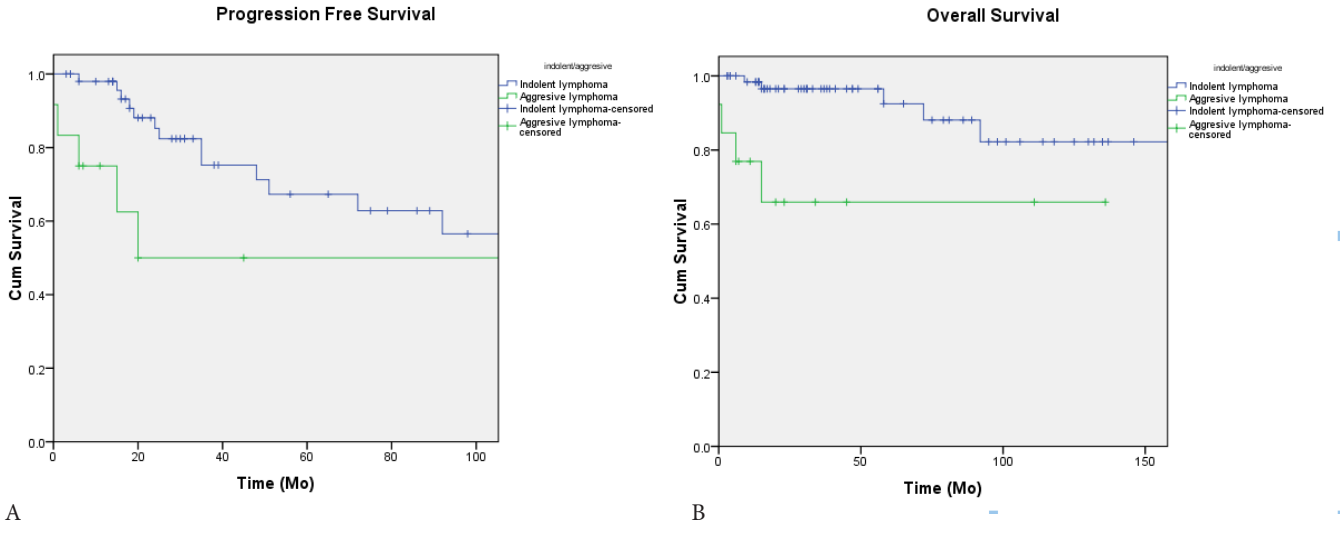

Fig 1. A) Kaplan-Meier progression-free survival (PFS) for the lymphoma subtypes; B) Kaplan-Meier overall survival (OS) for the lymphoma subtypes.

DLBCL (9.6%) and others. This distribution corresponds with other studies. Seresirikachorn et al. from northern Thailand reported EMZL as the most common (85.2%), followed by DLBCL (5.6%) and others.[13] Similarly, Yen et al. reported EMZL as the most common (75.2%), but followed by follicular lymphoma (7.9%) and DLBCL (6.1%).[12] A distinctive finding in our study is the recognition of plasmacytic differentiation in half of EMZL cases (51.4%). This phenomenon is in fact quite common in marginal zone lymphoma and it is expected to see in lymphoplasmacytic lymphoma while it is rare in mantle cell lymphoma.[14] Moreover, in our study, there were 4 cases (5.5%) of EMZL with accompanying IgG4-related disease and 2 of them also showed plasmacytic differentiation. As IgG4-related disease is quite common to produce ocular mass and, recently, a number of cases with ocular adnexal lymphoma of EMZL type have been reported to have accompanying IgG4-related disease.[15] It is possible that a subset of EMZL may arise in IgG4-related disease.[16] The benefit from the detection of plasmacytic differentiation in EMZL and other B-cell lymphomas is that the immunophenotype of restriction to cytoplasmic immunoglobulin light chain (light chain restriction) in the plasma cell component can be used to monitor the disease. For example, if EMZL with plasmacytic differentiation has lambda light chain restriction, evaluation of marrow staging or any subsequent tissue sample can determine whether any lambda light chain restriction exists in the plasma cells. The same light chain restriction in plasma cells as that found in the primary lesion is supportive for involvement by lymphoma.

The treatment of ocular adnexal lymphoma usually depends on its stage and the clinical judgement of the attending physician's. Although irradiation is the treatment of choice for stage I and II localized diseases, it is challenging because of the nearby radiosensitive structures, such as the lens, lacrimal gland, and retina, all of which may be adversely affected by radiation.[17] The majority of cases use radiotherapy either as a stand-alone treatment or in combination with chemotherapy or surgery; however, the present study found that chemotherapy, rather than radiation, was the primary treatment in 67.2% of cases. There are several reasons for this situation. Firstly, as Siriraj Hospital is a tertiary-care hospital, most of the patients were above stage I (33.7%), and many cases had the aggressive lymphoma. Furthermore, the data were collected in a 13-year retrospective period; during that time the radiation therapy was not as effective or safe as at present. Moreover, a long radiation-therapy waiting list coupled with the negative side effects on the local ocular structures would also have influenced physicians to select chemotherapy over radiotherapy. Even though nowadays radiotherapy universally gives very good outcomes for extranodal marginal zone lymphomas[12], the authors still performed the subgroup analysis to compare the outcomes of the extranodal marginal zone lymphoma patients who received chemotherapy versus radiotherapy. Both groups responded well to their treatment modalities, with no statistically significant difference between the outcomes of the two groups ($p = 0.311$).

In this study, the characteristic of lymphoma in terms of its presentation, anatomical location, and subtypes are similar to those found by other studies in Thailand, but with some small differences compared to Western countries (Table 4). When a comparison is made with various studies from Northern Thailand, Asian countries,

TABLE 4. Characteristic and treatment outcome of primary adnexal ocular lymphoma.

Region	Present study	Northern Thailand[13]	Asia[19-21]	Europe[3,22-26]	America[27-33]
No. of participants	94	54	47-114	52-192	48-353
Median Age (yrs)	61.5	61	46-62	58-65	59-68
Female (%)	40.4	57.4	42-57	54-63	37-68
Clinical presentation	Proptosis 54.3% Palpable mass 40%	Palpable mass 75.9% Proptosis 14.8%		Conjunctival lesion 32% Proptosis 27% Palpable mass 19%	Palpable mass 69%
Location of disease	Orbit 62.8% Conjunctiva 17% Lacrimal 14.9%	Lacrimal 46.3% Orbit 31.5% Conjunctiva 13%	Orbit 59-83% Conjunctiva 6-36% Lacrimal 3% Eyelid 1-8%	Orbit 40-69% Conjuncitva 17-36% Lacrimal 19% Eyelid 4-18%	Orbit 47-59% Conjunctiva 25-64% Lacrimal 20-25% Eyelid 10-24%
Lymphoma subtype	EMZL 76.6% DLBCL 9.6% MCL 4.3% NK/T 3.2% FL 2.1% SLL 1.1% PBL 1% PTCL 1% BL 2.1%	EMZL 85.2% DLBCL 5.6% PTCL 5.6%	EMZL 81-87% DLBCL 3-11% MCL 6.4%	EMZL 36%-88% LPL 23% FL 6-14% Immunocytoma 9% PTCL 1% B-LBL 1%	EMZL 57-89% FL 12-25% DLBCL 3-14% CLL/SLL 3-23%
Primary treatment type	CMT 67.2% RT 17.2%	RT 50% CMT 31.6%	RT 51-100% CMT RT+CMT	RT 68-100% RT+CMT 25%	RT 34-100% CMT 12-32%
Median F/U (yrs) PFS	2.75 LG 3 yr – 75.2% HG 3 yr – 50%	N/A LG 3 yr – 69.9% HG 3 yr – 42.9%	3.8-6.2 EFS 5 yr – 75-96%	4.4-9.9 EFS 5 yr MALT 73.6-88% EFS 5 yr LG - 68% EFS 5 yr HG 43-52%	2.7-6.8 5 yr – 55.9-97% LG 5 yr - 78% HG 5 yr - 50%
OS	LG 3 yr – 96.5% HG 3 yr – 65.9%	LG 3 yr – 92.5% HG 3 yr – 42.9%	5 yr – 89-96%	LG 5 yr - 78% HG 5 yr – 50%	5 yr – 69-98

Abbreviations: B-LBL: B lymphoblastic lymphoma, BL: Burkitt lymphoma, CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma, CMT: chemotherapy, CR: complete response rate, DLBCL: diffuse large B-cell lymphoma, EMZL: extranodal marginal zone lymphoma, EFS: event-free survival, FL: follicular lymphoma, HG: high grade, LG: low grade, LPL: lymphoplasmacytic lymphoma, MCL: mantle cell lymphoma, NK/T: NK/T-cell lymphoma, OS: overall survival, PBL: plasmablastic lymphoma, PFS: progression free survival, PTCL: peripheral T-cell lymphoma, RT: radiotherapy, SLL: small lymphocytic lymphoma

and Western countries where radiation has been used as the primary treatment, the PFS and OS results of the current investigation are not markedly different (Table 4). Analysis of the low-grade or indolent lymphoma group found a better prognosis than the high-grade or aggressive lymphoma subtype (5-year OS, 92.5% vs 65.9%; and 5-year PFS, 62.8% vs 50%, respectively). Yen et al. reported that the 5- and 10-year disease-free survival rates for EMZL (884 patients) were 86.4% and 78.7%, and that the 5- and 10-year OS rates were 93.8% and 84.9%, respectively.[12] In lymphoma other than EMZL (988 patients), the 5- and 10-year disease-free survival rates were 75.7% and 71.0%, and the 5- and 10-year OS rates were 78.9% and 73.5%, respectively.[12] EMZL is the most common type found in the orbit, and it generally shows the best prognosis. Follicular lymphoma patients usually have good long-term survival prospect. DLBCL shows an aggressive natural history but responds well to chemotherapy, whereas mantle cell lymphoma is usually very aggressive and often fatal.[23]

There were a number of limitations in our study. Firstly, it was a retrospective study, and many patients had been referred back for treatment by other hospitals; thus, complete data could not be elicited. Moreover, there were few cases of radiation therapy compared to the other treatment modalities, and only a small number of aggressive lymphomas. In order to evaluate the potential etiologic factors, a systematic, multicenter effort would therefore be needed to collect a large enough sample size. Further studies are needed to better understand each type of lymphoma, especially the aggressive type, which shows inferior survival rate.

CONCLUSION

Primary ocular adnexal lymphoma patients at Siriraj Hospital mostly presented with proptosis with an orbital lesion. Extranodal marginal zone lymphoma with an early stage and low ECOG was the most common subtype. Chemotherapy was still the main treatment utilized for both the indolent and aggressive lymphomas, in which the indolent group demonstrates better overall and complete response rates than the aggressive group. The histopathological subtype and clinical stage are the two best indicators of prognosis and treatment outcomes.

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Peripheral Atherosclerotic Profile in Type 1 Diabetic Patients: Lipid Ratios as a Predictive Marker of Asymptomatic Patients

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ABSTRACT

Objective: To investigate the relationship between Lipid ratios and asymptomatic peripheral artery disease (aPAD) in type 1 diabetic patients.

Materials and Methods: This cross-sectional study was performed among 223 diabetics. Patients were segregated with aPAD when their ankle-brachial index (ABI) was abnormal (ABI ≤ 0.90, or ABI > 1.20). Patients were segregated into the following groups (patients with normal ABI vs. patients with arterial stiffness). The association between lipid ratios and aPAD was analyzed using multivariate logistic regression analysis and the receiver operator characteristic curve.

Results: Our study reported a slight preponderance of females (108 males vs. 115 females), with a mean age of 30.70±9.69 years and a diabetes duration of 11.13±8.95 years. The prevalence of arterial stiffness was 38.11%. TC/HDL-C ratio was a significant predictor for atherosclerosis with a sensitivity of 77.3%, specificity of 62.5%, and diagnostic accuracy of 0.758%. The results revealed that the 4th quartile (odds ratio [OR]=12.52 [5.06-31.00], p=<0.001) of TC/HDL-C ratio was statistically higher in patients with arterial stiffness. Similarly, the last quartiles of LDL-C/HDL-C and TG/HDL-C ratio were higher in the arterial stiffness group (OR=3.70 [1.68-8.11], p=0.001; OR=4.74 [2.12-10.59], p=<0.001; respectively). In the arterial stiffness group, non-traditional lipid values were significantly higher in males compared to females.

Conclusion: Lipid ratios are correlated with aPAD in type 1 diabetic patients, and should thus be assessed in clinical decision-making and risk stratification on atherosclerotic cardiovascular disease.

Keywords: Ankle-brachial index; asymptomatic peripheral artery disease; atherosclerosis; lipid ratios. (Siriraj Med J 2022; 74: 874-882)

INTRODUCTION

Patients with type 1 diabetes (T1D) are highly predisposed to develop early atherosclerosis.[1] Asymptomatic peripheral artery disease (aPAD) is frequent in diabetics.[2] It is usually caused by atherosclerosis, in which an atherosclerotic plaque produces arterial stenosis or occlusion. As a consequence, blood flow to the affected limb is reduced. The majority of subjects are asymptomatic, although many suffer from occasional claudicating.[3] Diabetes-related peripheral neuropathy has a reported prevalence ranging from 15% to 86%,[4] with painful diabetic neuropathy described in around 26% of diabetic patients.[5] People with aPAD have a higher risk of diabetic foot amputation, myocardial ischaemia, and stroke, as well as

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mortality.[6] According to recent data, aPAD affects more than 200 million individuals globally.[7] Although current guidelines for screening of aPAD vary significantly,[8] the ankle-brachial index (ABI) as a routine examination to identify aPAD is highly recommended. Type 1 diabetic patients aged over forty have an aPAD prevalence of 20%, which rises to 29% over the age of fifty.[9]

Additionally, the clinical characteristics of aPAD in diabetic people differ from those reported in the general population, resulting in a worse prognosis.[10] Thus, an early detection is essential for diabetic patients, since aPAD is a significant risk factor for amputation, and is directly linked to cardiovascular diseases (CVDs).[11] Arterial stiffness, which may be regarded as a sign of subclinical atherosclerotic disease,[12] is an important parameter to study since it reflects CVD and mortality in diabetics and may serve as a valuable marker to prevent future vascular complications.[13]

Dyslipidemia is considered a risk factor for aPAD.[14] Conventional lipid measures, such as abnormal values of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), are prevalent risk factors for aPAD.[15] Specifically, numerous studies have highlighted that atherosclerosis develops and progresses under conditions of abnormal lipid values in diabetes. A high triglyceride (TG)/HDL-C ratio may be a favorable indicator of CVD.[17,18] Similarly, the total cholesterol (TC)/HDL-C ratio has also been linked to an increased risk of CVD.[19,20] The connection between the LDL-C/HDL-C ratio and aPAD has been well-established,[21] indicating that the LDL-C/HDL-C ratio was also a stronger marker for major CVD in a prospective observational study.[22]

As a result, identifying atherosclerotic risk factors in patients with T1D will ameliorate diabetes-based screening and preventive strategies in diabetics at higher risk for vascular complications. To that end, in this study, we sought to investigate the prevalence of arterial stiffness among diabetics in order to determine whether there is a link between aPAD and CVD, mainly subclinical atherosclerosis by evaluating lipid ratios in patients with T1D, as similar studies are currently scarce.

MATERIALS AND METHODS

Study design and sampling

The research was designed as a cross-sectional retrospective study that collected data from March 1, 2016, to February 2, 2020, on type 1 diabetic patients admitted to the Endocrinology Unit from an Academic Hospital in Sidi-Bel-Abbes, northwestern Algeria. The study was performed among 370 patients with T1D diagnosed in their pubertal phase (according to the WHO criteria). All the participants' files were revised for the following: medical history, other associated diseases, and complications such as diabetic retinopathy, neuropathy, and nephropathy.

The study included 223 patients with T1D who were above the age of 18, admitted to the hospital, and had no history of CVD. A total of 147 patients were excluded because they were younger than 18, lacked medical data, with missed informed consent (Fig 1).

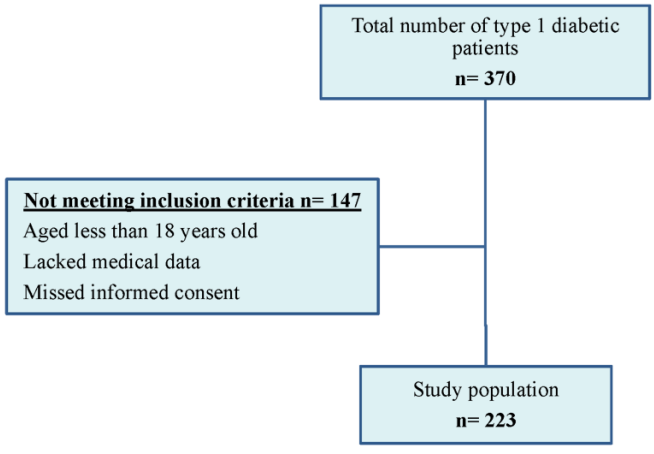

Fig 1. Flow chart of the study population

Data collection

All patients had a full anthropometric evaluation, which comprised height, weight, body mass index (BMI), and waist circumference measures. A sphygmomanometer was used to measure blood pressure in the supine position, followed by a second measurement (after a few minutes) in a standing position. Hypertension was defined as systolic blood pressure (SBP) of 140 mmHg or higher and diastolic blood pressure (DBP) of 90 mmHg or higher. Fasting blood glucose (FBG), glycated hemoglobin (HbA1c), urea, serum creatinine, urinary albumin excretion rate (UAER), and serum lipids (TC, HDL-C, LDL-C, and TG), as well as thyroid-stimulating hormone (TSH), were all collected from patients' medical records. Two noninvasive tests were used to assess the prevalence of aPAD (pulse examination, and ABI). Lower limbs were assessed for symptoms of aPAD, and peripheral pulses were measured at both dorsalis pedis and tibialis posterior arteries.[23] ABI was calculated using the lowest ankle SBP for each leg divided by the maximum brachial SBP to increase the positive test rate in a screening population.[24] ABI measurements were conducted using the Minidop ES 8 Mhz sonographic device.[25]

Normal (ABI = 0.91 to 1.20), decreased (ABI 0.90), and increased (ABI > 1.20) were the categories for ABI

results. This was considered to be comparable to arterial stiffness, using the following lipid ratios (TC/HDL-C, LDL-C/HDL-C, and TG/HDL-C) as markers of atherogenic risk. The presence of subclinical athérosclerosis was later evaluated in every patient.

Data management and analysis

Qualitative data were reported as percentages (%) and relative frequencies, and continuous variables were presented as mean ± standard deviation (SD) with associated 95 percent confidence intervals (95% CI). The Chi-square test was used for qualitative categorical factors and the Student *t*-test for quantitative data.

The association between ABI status and atherosclerosis, odds ratios (OR) and its 95% CI for lipid values were calculated utilizing the multivariate logistic regression analysis after adjusting across quartiles of lipid ratios. To establish the appropriate cut-off value and validity of lipid ratios, the receiver operator characteristic (ROC) curve was employed, and the area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed. When the *p*-value was less than or equal to 0.05 ($p \leq 0.05$), statistically significant differences were maintained. SPSS software was used to compute and analyze all data (SPSS 22, IBM Corporation; Chicago, IL. August 2013).

Ethical considerations

Written informed consent was maintained from all patients. This retrospective study was approved by the Ethics Committee of Academic Hospital with approval number AAU-5/2022.

RESULTS

The general features of the participants are summerized in Table 1. Two hundred and twenty three type 1 diabetic patients (108 males and 115 females) were admitted to the Diabetes Unit. Patients were arbitrarily segregated into the following groups based on their ABI whether <0.91 or >1.20 for arterial stiffness. Of the 223 diabetic patients, 138 (61.88%) patients had normal ABI while 85 (38.12%) patients had arterial stiffness. The mean age was 30.70±9.69 years, and the mean of diabetes duration was 11.13±8.95 years. The average age of diabetics with arterial stiffness was statistically higher than those with normal ABI (38.76±8.85 years *vs*. 25.73±6.24 years, $p<0.001$). The mean of duration diabetes was statistically increased in the arterial stiffness group compared to normal ABI group (16.05±9.67 years *vs*. 8.10±6.94 years, $p<0.001$) (Table 2).

Clinical features of the diabetics are presented in Table 2. In terms of anthropometric measurements, there was a higher statistical difference in body weight between the two groups (60.08 ± 11.82 kg and 63.60 ± 8.56 kg for the patients with and without normal ABI, respectively, $p=0.01$). Similarly, significant differences were noted in BMI ($p=0.02$), and waist circumference ($p<0.001$), while there was no significant difference in body height. Furthermore, statistically higher SBP and DBP values were reported in the arterial stiffness group compared to the normal ABI type 1 diabetic patients ($p<0.001$) (Table 2).

Remarkably, unlike FBG values, there was a statistical difference in HbA1c values (normal ABI: 10.06±2.51%, arterial stiffness: 10.79±2.25 %, $p=0.03$) between both groups on admission. Concerning lipid values, as shown in Table 2 when the *t*-test was applied to the TC, triglycerides, and HDL-C for the two groups, *p*-value was statistically significant ($p<0.001$, $p<0.001$, $p=0.01$; respectively). Moreover, all lipid ratios were statistically higher in diabetics with arterial stiffness ($p<0.001$).

To determine the optimal cut-off lipid ratios for detecting atherosclerosis, the ROC curve was used. The TC/HDL-C ratio was a reliable predictor of atherosclerosis. The best cut-off value was ≥ 4.0, with a sensitivity of 77.3%, specificity of 62.5%, a PPV of 71.2%, and a NPV of 53.6% with a diagnostic accuracy of 0.758 (Fig 2).

The multivariate regression between lipid ratio quartiles, as potent marker of atherosclerosis, revealed that the 4th quartile (OR=12.52[5.06-31.00]; $p<0.001$) of TC/HDL-C ratio was greater in the arterial stiffness group as indicated in Table 3. Likewise, in patients with arterial stiffness, the last quartile (4th) of the LDL-C/HDL-C, and the TG/HDL-C ratios were statistically higher (OR=3.70 [1.68-8.11], $p=0.001$; OR=4.74 [2.12-10.59], $p<0.001$; respectively).

As shown in Fig 3, all lipid ratios were significantly greater in men than females with arterial stiffness. Males with arterial stiffness had greater TC, LDL-C, and TG values as compared to females

DISCUSSION

Our study examined the clinical and biological characteristics in long-term patients with T1D with a broad range of ABI to discover the variables linked with the existence of aPAD in these patients and to assess whether the link of these findings with more established atherosclerosis could be validated. The gender distribution of diabetics revealed a slight preponderance of females (51.60%) over males (48.40%) with a sex-ratio of 0.93. In patients with arterial stiffness, the percentage of men was statistically higher than that of women (70.60 % *vs*.

TABLE 1. General characteristics of the type 1 diabetic patients.

Variables	All Patients n=223 Number (%)	Normal ABI n=138 Number (%)	Arterial Stiffness n=85 Number (%)	*P* value*
Gender, n (%)				<0.001*
Male	108 (48.40)	48 (34.80)	60 (70.60)	
Female	115 (51.60)	90 (65.20)	25 (29.40)	
Age groups, years				<0.001*
[18-29]	120 (53.80)	108 (78.20)	12 (14.10)	
[30-39]	65 (29.20)	23 (16.70)	42 (49.40)	
[40-49]	26 (11.70)	7 (5.10)	19 (22.40)	
[50-59]	11 (4.90)	0 (0.00)	11 (12.90)	
≥ 60	1 (0.40)	0 (0.00)	1 (1.20)	
Smoking history, n (%)				<0.001*
Male	39 (17.50)	12 (8.70)	27 (31.80)	
Female	--	--	--	
Prevalence of weight categories, n (%)				0.07
Underweight, BMI <18.5 Kg/m²	47 (21.10)	28 (20.30)	19 (22.40)	
Normal weight, BMI=18.5-25.0 Kg/m²	137 (61.40)	99 (71.70)	38 (44.70)	
Overweight, BMI=25.0-29.9 Kg/m²	29 (13.00)	9 (6.50)	20 (23.50)	
Obesity, BMI ≥30 Kg/m²	10 (4.50)	2 (1.50)	8 (9.40)	
Other associated diseases, n (%)				
Low visual acuity	65 (29.10)	29 (21.00)	36 (42.40)	0.001*
Diabetic retinopathy	41 (18.40)	17 (12.30)	24 (28.20)	0.02*
Diabetic nephropathy	19 (8.50)	2 (1.50)	17 (20.00)	<0.001*
Hypertension	28 (12.60)	5 (3.60)	23 (27.10)	<0.001*
Hypothyroidism	18 (8.10)	13 (9.40)	5 (5.90)	0.34
Anemia	66 (29.60)	42 (30.40)	24 (28.20)	0.72
Dyslipidemia	6 (2.70)	1 (0.70)	5 (5.90)	0.02*

(*) percentages were compared with Chi-square test, $p \leq 0.05$ was considered as significant.
Abbreviations: ABI; Ankle-Brachial Index, BMI; body mass index.

29.40 %, $p<0.001$). A cross-sectional study by Nattero-Chàvez et al[26] revealed that 92 patients with T1D had an abnormal ABI; of those, 59 (64.0%) were men, whereas, another study displayed that 73 patients with T1D had an arterial stiffness; of those, 48 (66.0%) were men.[26,27] Our research revealed a significant effect of age and diabetes duration on differences between ABI. The results are consistent with the literature where the most notable risk factors for arterial stiffness were age, and longer diabetes duration.[26-28] In this study, a significant difference between BMI in both groups was noted. These results are in accordance with reports from the literature showing that higher BMI values and obesity were significant risk factors for arterial stiffness.[26,27,29] Interestingly, retinopathy was reported to be linked with an increased prevalence of arterial stiffness, which was consistent with a prior retrospective study of diabetics.[30] In our research group, hypertension was diagnosed in 28 cases, and it was present in 23 (27.10 %) patients with arterial stiffness. Our results are consistent with prior research demonstrating that the

TABLE 2. Comparison of clinical features according to the absence or presence of arterial stiffness.

Variables	All patients n=223		Normal ABI n=138		Arterial stiffness n=85		*P* value*
	Mean±SD	95% CI	Mean±SD	95% CI	Mean±SD	95%CI	
Mean age (years)	30.70 ± 9.69	29.42-31.98	25.73 ± 6.24	24.68-26.78	38.76 ± 8.85	36.86-40.67	<0.001*
Diabetes duration (years)	11.13 ± 8.95	9.95-12.31	8.10 ± 6.94	6.93-9.27	16.05 ± 9.67	13.96-18.14	<0.001*
Age at 1st diagnosis (years)	19.65 ± 7.98	18.60-20.71	17.67 ± 7.35	16.44-18.91	22.87 ± 7.96	21.15-24.59	<0.001*
Body height (m)	1.66 ± 0.07	1.65-1.67	1.66 ± 0.07	1.65-1.68	1.66 ± 0.08	1.64-1.67	0.46
Body weight (Kg)	61.42 ± 10.81	60.00-62.85	60.08 ± 11.82	58.09-62.07	63.60 ± 8.56	61.76-65.45	0.01*
BMI (Kg/m²)	22.12 ± 3.66	21.64-22.60	21.54 ± 4.07	20.85-22.22	23.06 ± 2.64	22.49-23.63	0.002*
Waist circumference (cm)	85.00 ± 9.41	82.98-87.02	80.26 ± 9.18	77.25-83.28	88.75 ± 7.84	86.47-91.03	<0.001*
SBP (mmHg)	113.1 ± 12.6	111.4-114.8	110.1 ± 10.6	108.3-111.9	117.9 ± 14.1	114.8-120.9	<0.001*
DBP (mmHg)	66.4 ± 8.32	65.3-67.5	65.6 ± 7.56	64.3-66.9	71.8 ± 8.31	69.8-74.9	<0.001*
FBG (g/l)	3.00 ± 1.21	2.84-3.15	2.89 ± 1.25	2.68-3.10	3.17 ± 1.11	2.93-3.41	0.09
HbA1c (%)	10.33 ± 2.44	10.00-10.67	10.06 ± 2.51	9.62-10.49	10.79 ± 2.25	10.29-11.30	0.03
Hemoglobin (g/l)	12.28 ± 1.67	12.04-12.52	10.95 ± 1.29	10.60-12.24	12.79 ± 1.92	12.38-13.21	<0.001*
Total cholesterol (g/l)	1.46 ± 0.33	1.42-1.50	1.38 ± 0.27	1.33-1.43	1.59 ± 0.37	1.51-1.67	<0.001*
HDL-C (g/l)	0.46 ± 0.11	0.44-0.47	0.48 ± 0.08	0.46-0.49	0.42 ± 0.13	0.39-0.45	<0.001*
LDL-C (g/l)	0.87 ± 0.24	0.84-0.91	0.86 ± 0.21	0.83-0.90	0.89 ± 0.27	0.83-0.95	0.38
Triglycerides (g/l)	0.94 ± 0.61	0.86-1.02	0.86 ± 0.54	0.77-0.96	1.07 ± 0.68	0.93-1.22	0.01*
TC/HDL-C	3.37 ± 1.18	3.21-3.53	2.93 ± 0.68	2.81-3.04	4.09 ± 1.45	3.77-4.40	<0.001*
LDL-C/HDL-C	2.01 ± 0.72	1.92-2.11	1.84 ± 0.54	1.75-1.93	2.29 ± 0.87	2.10-2.48	<0.001*
TG/HDL-C	2.26 ± 1.89	2.01-2.51	1.82 ± 1.01	1.65-1.99	2.98 ± 2.64	2.41-3.55	<0.001*
Creatinine (mg/l)	12.22 ± 12.35	9.87-14.58	8.18 ± 2.65	7.54-8.83	18.83 ± 8.02	13.14-24.51	<0.001*
Urea (g/l)	0.37 ± 0.30	0.31-0.43	0.26 ± 0.14	0.22-0.29	0.56 ± 0.39	0.43-0.68	<0.001*
UAER (mg/24h)	152.15 ± 60.27	87.31-226.59	28.51 ± 6.83	9.52-33.98	216.04-112.83	128.43-339.62	<0.001*
TSH (µIU/mL)	7.13 ± 4.54	2.67-12.46	3.57 ± 2.86	3.10-5.94	12.20 ± 6.57	5.06-22.48	<0.001*

(*) means were compared with independent sample Student's *t*-test, a $p<0.05$ was considered as significant.

Abbreviations: SD; standard deviation, CI; confidence interval, ABI; Ankle-Brachial Index, BMI; body mass index, FBG; Fasting blood glucose, HbA1c; glycosylated hemoglobin, SBP; systolic blood pressure, DBP; diastolic blood pressure, TC; total cholesterol, HDL-C; high-density lipoprotein cholesterol, LDL-C; low-density lipoprotein cholesterol, TG; triglycerides, UAER; urinary albumin excretion rate, TSH; thyroid-stimulating hormone.

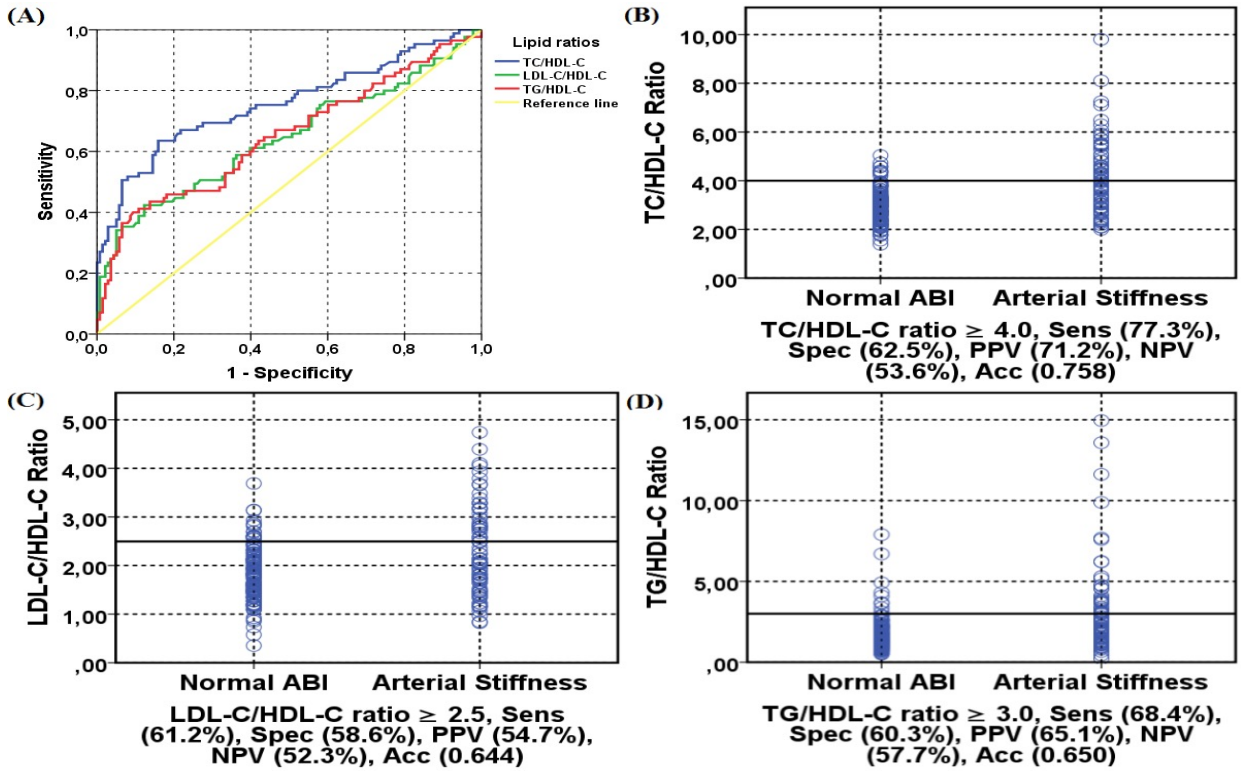

Fig 2. ROC curve to define the best cut-off lipid ratios to detect atherosclerosis.
Abbreviations: ABI; Ankle-Brachial Index, Sens; sensitivity, Spec; specificity, PPV; positive predictive value, NPV; negative predictive value, Acc; accuracy, TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; triglycerides, ROC; receiver operating characteristics.

TABLE 3. Multivariate analysis of the relationship between lipid ratios quartiles and ABI status in type 1 diabetic patients.

Variables	Normal ABI n= 183 Number (%)	Arterial stiffness n= 85 Number (%)	Odds ratio (95% CI OR)	*P* value*
TC/HDL-C ratio				
1st quartile (1.39-2.55)	41 (29.7)	12 (14.1)	**Reference**	---
2nd quartile (2.56-3.10)	45 (32.6)	12 (14.1)	0.91 [0.36-2.25]	0.84
3rd quartile (3.11-3.91)	40 (29.0)	17 (20.0)	1.45 [0.61-3.42]	0.39
4th quartile (3.92-9.80)	12 (8.7)	44 (51.8)	12.52 [5.06-31.00]	<0.001*
LDL-C/HDL-C ratio				
1st quartile (0.35-1.48)	37 (26.8)	18 (21.2)	**Reference**	---
2nd quartile (1.49-1.92)	41 (29.7)	15 (17.6)	0.75 [0.33-1.70]	0.49
3rd quartile (1.93-2.43)	40 (29.0)	16 (18.8)	0.82 [0.36-1.84]	0.63
4th quartile (2.44-4.74)	20 (14.5)	36 (42.4)	3.70 [1.68-8.11]	0.001*
TG/HDL-C ratio				
1st quartile (0.22-1.26)	39 (28.3)	16 (18.8)	**Reference**	---
2nd quartile (1.27-1.79)	41 (29.7)	15 (17.6)	0.89 [0.38-2.04]	0.78
3rd quartile (1.80-2.46)	39 (28.3)	17 (20.0)	1.06 [0.47-2.39]	0.88
4th quartile (2.47-14.95)	19 (13.8)	37 (43.5)	4.74 [2.12-10.59]	<0.001*

(*) multivariate logistic regression significant at $p \leq 0.05$.
Abbreviations: CI; confidence interval, OR; Odd ratio, Q; quartiles, ABI; Ankle-Brachial Index, TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; triglycerides.

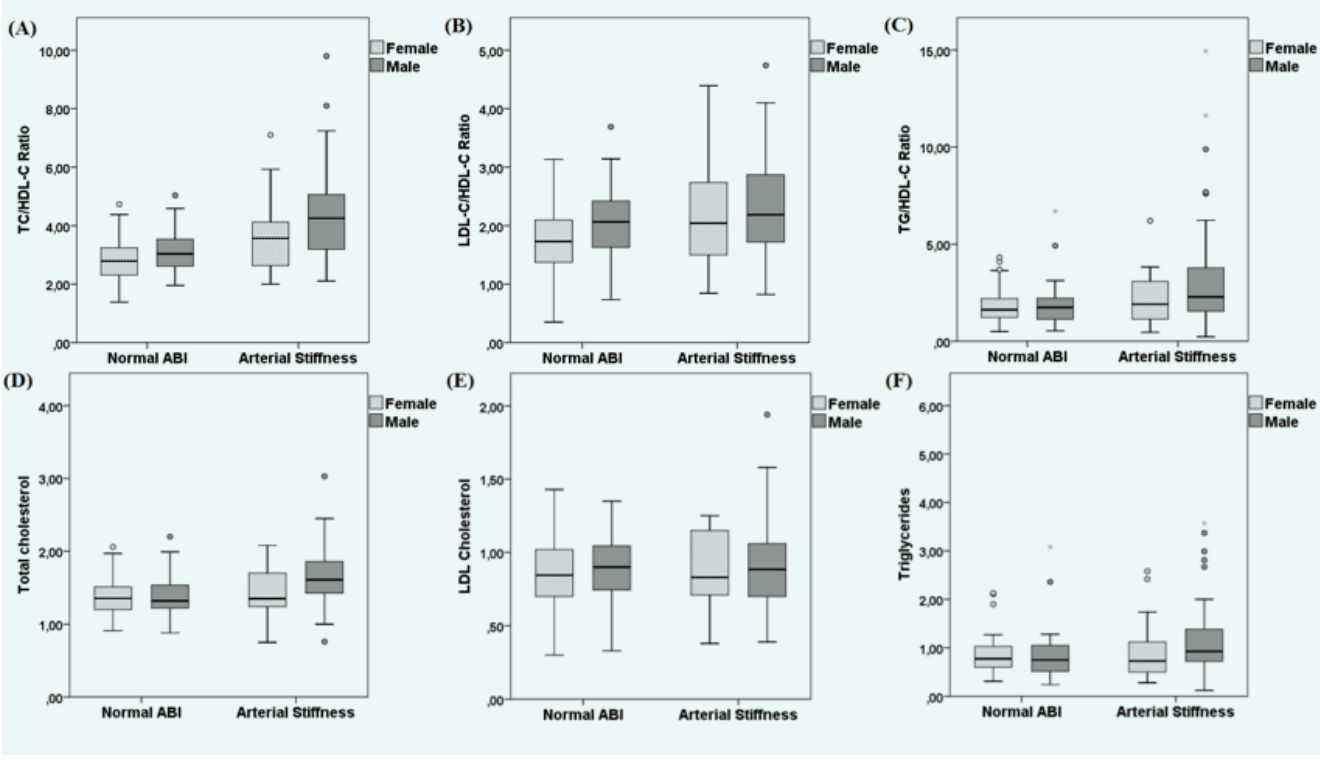

Fig 3. Comparison of lipid ratios levels between patients with and without arterial stiffness according to their gender
Abbreviations: ABI; Ankle-Brachial Index, TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; triglycerides.

incidence of resistant hypertension rises with advanced aPAD and that hypertension is the greatest risk factor for arterial stiffness.[26-28,29]

Additionally, consistent with the literature review,[31] we discovered that the risk of arterial stiffness is impending in these subjects with each incremental increase in HbA1c. Similarly, Adler et al[32] reported that every 0.9% increase in HbA1c was correlated with a 29% increased risk of incident aPAD in diabetics.[32] Our findings demonstrated a significant increase in hemoglobin values among the arterial stiffness group. Comparable results regarding higher hemoglobin concentrations were independently associated with abnormal ABI in the general population, indicating that a rise in this level may be linked with higher risk of atherosclerosis.[33] We also found that diabetics with increased ABI had an atherogenic lipoprotein profile, defined by greater plasma levels of TC and TG concentrations, and reduced HDL-cholesterol levels, these findings are in consistent with the literature.[26,29,30,32] In addition, we also highlighted a higher values of UAER (≥ 30 mg/24h) in diabetics with arterial stiffness, which may have increased the reliability of the results. Zander et al[29] confirmed that in patients with microalbuminuria and macroalbuminuria, aPAD was observed significantly more often.[30] An significant finding of our investigation was the association between elevated ABI and serum TSH levels in patients with T1D. Our results echo those of Zhao et al[34] who concluded that higher serum TSH values may be a predictor of arterial stiffness.[34]

Considering the substantial link between ABI and increasing values of TC/HDL-C, LDL-C/HDL-C, and TG/HDL-C in this research, we may presume that increased ABI values in individuals with T1D may be indicative of increased CVD risk. Gender disparity is one of the most notable aspects of CVD. Multiple studies demonstrated the influence of gender differences on the prevalence of atherosclerosis risk factors.[35,36] In our study, male participants with higher ABI values had dyslipidaemia, and increased values of lipid ratios that indicate atherosclerosis and stroke. Several studies have analyzed the relationship between TC/HDL-C ratio and arterial stiffness; this ratio has a good discriminating potential for predicting various CVD pathologies such as coronary diseases.[19] Furthermore, a clinical study identified the TC/HDL-C ratio to be the main lipid, or inflammatory indicator for incident aPAD with relative risk of 3.8 [95% CI 1.6–8.5] for patients with the highest TC/HDL-C ratio quartile compared to those with the lowest quartile.[20] On the contrary, minimal evidence suggests an association between LDL-C and incident aPAD. Hence, an inconsistent correlation between LDL-C and aPAD has been established in the studies that have

assessed the values of this lipoprotein.[21] A cross-sectional study included 1911 aPAD patients (467 male *vs* 1444 female) with a mean age of 80 years, indicating that each 1 mg/dL increase in LDL-C was correlated with a 1.8% increased risk of aPAD.[37] Conversely, according to the Women's Health Study, there was no association between LDL-C and incidence aPAD.[38] Based on our findings, the association of the TG/HDL-C ratio with elevated ABI levels and aPAD was stronger in diabetics with arterial stiffness. Thus, TG/HDL-C ratio might be a positive predictor of CVD even in the earliest stages of abnormal lipid metabolism.[17] Moreover, previous study reported that an increased TG/HDL-C ratio independently predicted a decrease in HDL-C and has been strongly linked to a higher risk of atherosclerosis and arterial stiffness.[18]

Like other studies, our research has some limitations. First, this was a cross-sectional retrospective study and, therefore, we cannot identify the severity of aPAD. Second, the lack of non-invasive tests other than ABI has limited our ability to assess arterial stiffness and aPAD prevalence in patients with normal ABI results. Our study also offers several strengths, including the employment of the ROC curve to investigate which lipid ratio is highly associated with arterial stiffness. This research also presented epidemiological evidence from a particular population-based study employing nationally representative data from a single ethnicity. According to our knowledge, this is currently the first study to establish the association between arterial stiffness and dyslipidemia in patients with T1D utilizing an atherogenic lipid profile in Algerian population.

To conclude, in our population of adult patients with T1D, arterial stiffness determined by ABI was related to higher lipid ratio values and subclinical atherosclerosis in a large proportion of patients independent of other conventional CVD risk factors. Therefore, non-invasive methods such as the ABI and the implementation of lipid ratios are highly recommended and may detect a subgroup of cases with undiagnosed aPAD who might benefit from early therapy of CVD risk factors.

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Effects of Different Durations of 9-Square Dance Exercise Versus Treadmill Exercise on the Physical Fitness and Quality of Life of Healthy Volunteers: A Pilot Randomized Controlled Trial

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ABSTRACT

Objective: To evaluate the impact of 9-square dance exercise (9SDE) on physical fitness and quality of life compared to traditional treadmill exercise (TME).

Materials and Methods: In total, 33 healthy volunteers (10 men, 23 women) were recruited and randomly assigned into three groups: 9 square dance exercise for 8 minutes (9SDE-8), 9 square dance exercise for 30 minutes (9SDE-30), or treadmill exercise (TME). Exercises were done three times a week for 12 weeks and physical fitness tests were performed for all the groups at weeks 0, 6, and 12. Participants were assessed using the European Quality of Life Measure 5 Domains and 5 Levels questionnaire (EQ-5D-5L).

Results: Significant improvements in cardiorespiratory endurance, leg strength, and flexibility were demonstrated in the 9SDE-30 group ($p<0.05$). There was no significant difference in physical fitness between the 9SDE-30 and TME groups. The 9SDE-8 group showed a significant improvement in utility in the EQ-5D-5L questionnaire ($p<0.05$), while the TME group showed a significant improvement in directly evaluated health status ($p<0.05$). 9SDE-30 and TME showed similar improvements in cardiorespiratory endurance and leg strength.

Conclusion: Considering its low-resource requirement and overall utility, coupled with its effectiveness in promoting cardiovascular fitness and leg strength, 9SDE represents a viable exercise alternative for those with limited time and resources.

Keywords: 9 square dance exercise; physical fitness; aerobic exercise; Thai traditional exercise; quality of life (Siriraj Med J 2022; 74: 883 -890)

INTRODUCTION

Exercise is defined as a form of physical activity that involves continuous movements in specific patterns for the purpose of maintaining or increasing physical fitness and overall wellness.[1] Aerobic exercise uses larger muscle groups and increases oxygen consumption.[2] The benefits of aerobic exercise include increased cardiorespiratory fitness and insulin sensitivity; besides its well-known benefits of preventing a myriad of chronic conditions, such as hypertension, heart disease, and metabolic syndrome.[3-4]

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Consequently, regular exercise is associated with increased physical and mental wellness and quality of life.[5-6]

Despite all the stated benefits, survey results from the department of physical education ministry of tourism and sports of Thailand revealed that in 2020, only 41.8% of the Thai population over 15 years old exercised or played sports regularly. The most frequently cited reasons for not exercising included not having enough time (54.34%), having underlying conditions that preclude exercise (8.93%), being unmotivated (6.91%), and inadequate urban living spaces (3.98%). In addition, the need for subsidies and support for purchasing sports equipment and the provision of facilities remained significant barriers to exercise, with 61.08% requiring support and 39.61% requiring additional facilities.[7] In that regard, exercise routines that are culturally based, such as Qigong or Tai Chi, represent good options and have been shown to decrease fatigue and improve the quality of life. In particular, traditional dances, such as folk or swing or square dances, can help to improve the physical and emotional states of an individual.[8]

Traditional dances as part of an exercise routine have been shown to offer varying degrees of cardiovascular, flexibility, and strength training compared to more modern modes of exercise. For example, after Indonesia adopted the traditional Legong dance as exercise. Griadhi et al. measured changes in aerobic capacity and muscle strength between Legong dancers and conventional aerobic training and found that the back and leg muscle strengths were better, when measured using a dynamometer, in the Legong group than in the aerobics group.[9] Other examples include a study comparing a Korean traditional dance group to a no exercise group, in which the Korean traditional dance group experienced statistically significant improvements in grip strength and flexibility, as measured by a back scratch test, and leg strength, as measured by the chair stand test.[10]

Thai culture, with its rich history of Thai traditional medicine practice that emphasizes the mind–body connection or Dhammanamai, also offers traditional dance as a form of exercise. Using its core principle, which stresses caring for one's body through purposeful movements, such as stretching and dance, the 9-square-dance exercise (9SDE) was conceived. This Thai traditional square dance requires continuous, coordinated walking movements and is considered to be an ideal type of exercise for balance and neuromotor coordination.[11-12] A study by Atipas et al. examined the effects of performing 9SDE, in conjunction with the 9-square step exercise (9SSE) in patients with balance disorders, such as vestibular neuritis, dizziness, and vestibulopathy. The group which practiced the dance exercise experienced an increase in the average composite equilibrium scores and a decrease in the abnormal equilibrium scores as well as a balance of symptom severity after 8 weeks.[13]

In addition, the routines for performing 9SDE require very little space, as a 3 feet by 3 feet area will suffice. Further, its low demand for specialized resources means that it can be carried out in situations where space is limited, such as for people living in urban housing or even in work offices. In addition, both its traditional nature and its versatility mean that it potentially has great appeal for the elderly, who may not be attracted to traditional exercise and who also may have limited means of travel. However, despite the very physically active nature of 9SDE, its ability to quantitatively increase physical fitness remains unexplored. Consequently, the purpose of this research was to quantify and compare the impacts of 9SDE with exercise performed on a treadmill on various aspects of physical fitness and quality of life.

MATERIALS AND METHODS

This study recruited 33 healthy volunteers aged between 18–59 years old with a body mass index (BMI) of 18.5–24.9 kg/m^2 who have no regular exercise habits, defined as exercising less than 2 times per week. Exclusion criteria were current or past smokers, regular alcohol use of more than 120 mg per day, difficulties with ambulation, or the use of any cardiovascular medications or nutritional supplements. Eligible volunteers were randomly assigned to one of three groups, namely 9 square dance exercise for 8 minutes (9SDE-8), 9 square dance exercise for 30 minutes (9SDE-30), or treadmill exercise (TME), by computer stratified randomization according to the consort diagram in Fig 1. The protocol for this study was approved by the Siriraj Institutional Review Board (COA no. Si 269/2018) and TCTR 20211010001. Informed consent was obtained from each participant prior to their enrollment in the study.

All three groups of participants were asked to perform their respective exercises 3 times per week for 12 weeks. They were supervised by experienced instructors and their exercise and any occurrence of adverse events were recorded in their personal logbook. The 9SDE-8 and 9SDE-30 groups were assigned to a specific room at the Ayurved Clinic of Applied Thai Traditional Medicine in Mahidol University where standard 9SDE squares had been prepared. The TME group conducted their exercise sessions at Siriraj Hospital fitness center at the Faculty of Medicine Siriraj Hospital, Mahidol University. All the exercise groups were closely supervised before, during, and after their exercise sessions. All participants had

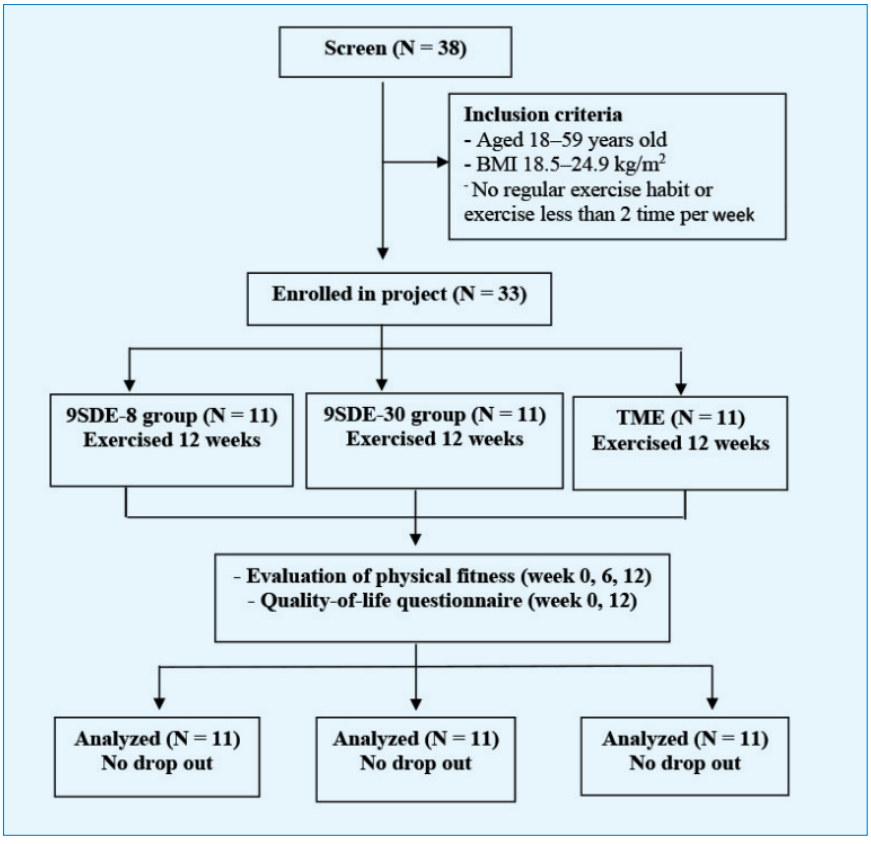

Fig 1. Consort diagram of the study

5–10 minutes of rest, followed by stretching exercises consisting of 8 exercise postures, comprising 2 arms and shoulder stretches and 6 back, legs, hips, and calves stretches with 3 repetitions of 10 seconds each. The warm-up and cool-down periods were 15 minutes.

The 9SDE was conducted according to the original protocol by Professor Dr. Ouay Ketusinh.[12] Participants were taught to perform a 9SDE exercise routine by a group of experienced applied Thai traditional medicine instructors. The 9-grid pattern for 9SDE was made by taping off a 120 × 120 cm area on the floor. The rhythm of the dance was 130 steps per minute. Participants were asked to perform the 9SDE routine according to the detail in Fig 2.[11] The 9SDE-8 group performed 4 minutes of exercise on both the left and right sides, whereas the 9SDE-30 group similarly performed 15 minutes of exercise on either side. After completing the exercise sessions, the sessions were recorded in each participant's personal logbook.

The TME group were advised and closely monitored by an experienced sport scientist. Before the first session, the participants were taught how to use the treadmill according to a TME protocol until the instructor was confident that they could execute the session safely and properly. Participants were told to increase the speed of the treadmill gradually according to the protocol in Table 1.[14] After completing the exercise, each participant had a cool-down period and then recorded the activity in his/her personal logbook.

The physical fitness of all the participants was evaluated at 0, 6, and 12 weeks from the start of the enrollment period. The evaluator was blind to the group assignment of each participant. The physical fitness test for all the participants included cardiorespiratory endurance, muscle strength, flexibility, and total body composition. Interpretations of the physical fitness tests were done according to the manual of tests and standard of physical fitness for Thai people.[15]

The self-assessed quality of life questionnaire was done at weeks 0 and 12 using the Thai version of the European Quality of Life Measure 5 Domains and 5 Levels (EQ-5D-5L) questionnaire, which was divided into 2 parts. The first part was a health satisfaction assessment across 5 health dimensions. This utility score ranged from +1 (healthy) to 0 (death) and -1 ("worse than death"). The second part for directly evaluating the health status was a direct measure of health, with a scale from 0 to 100, where 0 represents the worst health, and 100 is the best.[16]

Statistical analysis

Only those who attended >70% of the training sessions were included in the analysis. One-way ANOVA and Pearson chi-square test were used for analyzing the

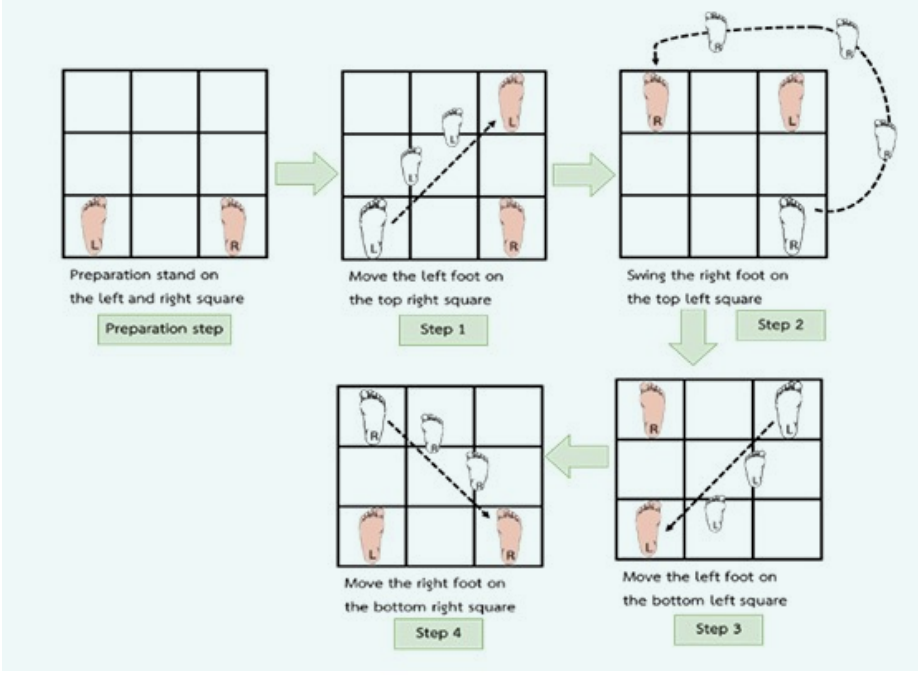

Fig 2A.

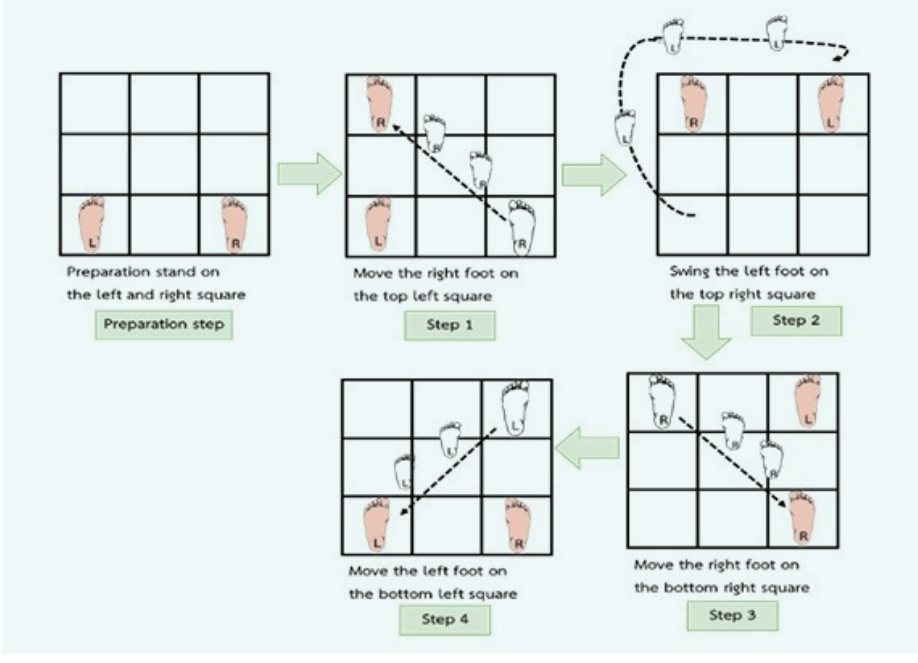

Fig 2B.

Fig 2. 9 Square dance exercises
Fig 2A. 9 Square dance exercises (Kao-ten) on the left side
Fig 2B. 9 Square dance exercises (Kao-ten) on the right side

TABLE 1. Treadmill exercise protocol

Time (minutes)	1	1-2	2-3	3-4	4-5	5-25	25-26	26-27	27-28	28-29	29-30
Speed (km/h)	3	3.5	4	4.5	5	5.5 ± 0.5	5	4.5	4	3.5	3

continuous and categorical variables, respectively. Post-hoc pairwise comparisons between the interventions were done. Changes from baseline to week 6 and week 12 post-intervention in each group were calculated as the mean and standard deviation. Repeated measure analysis of covariance (ANCOVA) was used to compare the between-group differences in the mean change from baseline to week 12. The statistical analysis was performed using SPSS ver 18.0 (SPSS Institute). The Kolmogorov–Smirnov test was used for the normal distribution of data, and p-value < 0.05 was considered statistically significant.

RESULTS

Overall, 33 participants attended more than 99.3% of their total exercise sessions. Women comprised 69.7% of the participants initially included in the study. The mean age of the participants was 32.42 ± 7.91 years old. The average BMI was 21.83 ± 1.82 kg/m^2. There were no significant differences in age and BMI between the groups. The mean age and BMI for each group are shown in Table 2.

Physical fitness

Physical fitness testing demonstrated within group differences in the step test for 9SDE-30 group from baseline to 6 and 12 weeks (8.64 ± 2.45 time/min, $p < 0.05$ and 13.27 ± 3.41 time/min, $p < 0.01$), and in the TME group at baseline to 6 and 12 weeks (8.09 ± 2.79 time/min, $p < 0.05$, and 14.00 ± 3.09 time/min, $p < 0.01$). Lung capacity changes were statistically significant in both the 9SDE-30 and TME groups from baseline to 12 weeks (-6.45 ± 1.25 cm^3/BW, $p < 0.01$ and -6.34 ± 2.17cm^3/BW, $p < 0.05$) respectively. Leg strength changes were also significant in both the 9SDE-30 and TME groups from baseline to 12 weeks (-0.26 ± 0.89 kg/BW, $p < 0.05$ and -0.55 ± 0.16 kg/BW, $p < 0.05$, respectively). All the groups also showed significant differences in the flexibility. The changes in physical fitness test parameters for each group of exercise at baseline, 6 and 12 weeks are shown in Fig 3.

The quality of life

The utility score for the 9SDE-8 group showed a significant difference (0.30 ± 0.03, $p < 0.01$) between baseline and week 12. The TME group showed a significant difference in directly evaluated health status (VAS) (13.55 ± 3.87, $p < 0.01$) between baseline and week 12, but there was no significant difference between 2 groups. The results from the EQ 5D 5L questionnaire are summarized in Table 3.

DISCUSSION

Our study demonstrated significant changes in cardiovascular fitness, lower body strength, and flexibility among participants in the 9SDE-30 and treadmill groups. The fact that both of the groups that required exercise duration of 30 minutes demonstrated more consistent cardiovascular changes helps to highlight the wisdom of American College of Sports Medicine (ACSM) 's

TABLE 2. Baseline characteristics of the participants: comparisons among the groups

Variable	9SDE-8	9SDE-30	TME	*P*-value
Participant (N)	11	11	11	
Gender (% women)	7 (63.6%)	8 (72.7%)	8 (72.7%)	0.866
Age (year)	32.55 ± 6.91	33.82 ± 11.08	30.91 ± 4.97	0.701
Min (year)	24	23	24	
Max (year)	44	59	38	
BMI (kg/m^2)	21.71 ± 1.96	21.36 ± 1.23	22.41 ± 2.14	0.397
Weight (kg)	57.37 ± 7.37	56.80 ± 6.24	59.68 ± 6.64	0.578
Height (cm)	162.35 ± 5.26	162.95 ± 8.33	163.18 ± 6.37	0.956

Values are presented as number (%) or mean ± standard deviation.
9SDE-8, 9 Square dance 8 minutes; 9SDE-30, 9 Square dance 30 minutes; TME, Treadmill exercise.

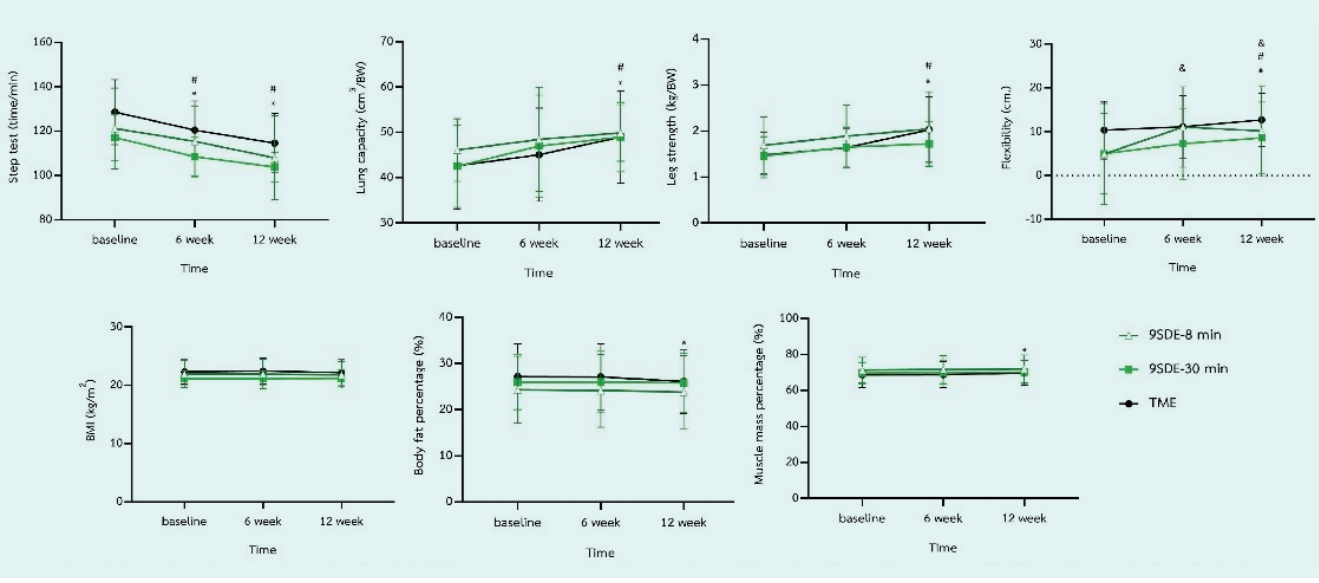

Fig 3. Comparisons between changes in the physical fitness parameters at baseline, 6 weeks, and 12 weeks. Superscripts represent significant changes from baseline in the 9SDE-8 group (&), 9SDE-30 group (#), and TME group (*). No significant between-group differences were demonstrated.

TABLE 3. EQ-5D-5L questionnaire results

Variable	Baseline	12-week	P-value Baseline to 12 weeks	P-value Baseline to group at 12 weeks
Utility				
9SDE-8	0.96 ± 0.04	0.99 ± 0.01	(0.009)**	
9SDE-30	0.98 ± 0.03	0.99 ± 0.03	(0.170)	0.666
TME	0.97 ± 0.04	0.98 ± 0.03	(0.103)	
Directly evaluated health status (VAS)				
9SDE-8	76.82 ± 15.54	82.73 ± 13.67	(0.096)	
9SDE-30	77.73 ± 9.58	79.55 ± 14.57	(0.717)	0.653
TME	64.09 ± 10.68	77.64 ± 10.27	(0.006)**	

Values are presented as the mean ± standard deviation.
9SDE-8, 9 Square dance 8 minutes; 9SDE-30, 9 Square dance 30 minutes; TME, Treadmill exercise.
** significant at $p \leq 0.01$.

recommendation that the optimal duration for exercise sessions should be at least 30 minutes in order to obtain the maximum health benefits.

This study showed that the 9SDE improved physical fitness including cardiorespiratory endurance, leg strength, flexibility, and lung capacity. The 9SDE form of exercise can lead to improved muscle strength because of the nature of its square-stepping routines. Other types of exercises that incorporate similar square-stepping routines can also lead to increased leg strength. For instance, Shigematsu et al. studied subjects who underwent square step exercise (SSE), while the participants in Ronnarithivichai et al.'s study used a 9-square table aerobic exercise with the addition of rubber ring stretching. Both studies reported significant increases in cardiorespiratory endurance and the leg strength of subjects after the study periods.[17-18]

Other exercise routines that utilize traditional dance have also been associated with an increase in flexibility. For instance, Douka et al. reported increased flexibility in subjects who took part in Greek traditional dance compared to before the exercise period ($p < 0.001$).[19] Similarly, Ronnarithivichai et al. found an increase in flexibility of 1.84 centimeters.[17] It is worth noting that these exercises were accompanied by both a warm-up and cool-down period. However, when none was provided, as in a study by Ghriadi et al., where young women were asked to perform aerobic exercises without any warm up or cool down, no change in flexibility was found.[9]

In this study, the 9SDE improved quality of life in the EQ-5D-5L questionnaire. Besides the significant improvements in physical health, there is extensive research regarding the association between traditional dance as a form of exercise and improvements in mental health and well-being. Traditional dance, such as Greek or Turkish folkloric dance, also tends to be an important part of cultural heritage, which can help individuals maintain social connections and promote a sense of community. One study demonstrated that Turkish folkloric dancing was associated with significant improvements in the SF-36 subscale, namely physical functioning, general health, and mental health.[20] In another study, Greek traditional dance performed by patients with chronic heart failure class II–III resulted in significant improvements in SF-36 physical health, mental health, and total score, as well as the life satisfaction inventory when compared to formal exercise training and a no exercise group. Moreover, only the Greek traditional dance group experienced significant changes in the intrinsic motivation inventory.[21]

Traditional dance as a form of exercise may also have special appeal for women. Most of our study participants were females and the adherence among this group was extremely high (99.3%). Publications pertaining to traditional dances as a source of exercise have also found that they are particularly attractive to women. Filippou et al. studied the attendance motives of participants in Greek traditional dancing classes and found that women were more likely to attend the activities and cited the main reasons as needing to leave the house and to have a break away from the monotony of everyday life.[22]

Our study has several limitations to note. The requirement for on-site exercises in all groups may have limited the participants to only those without regular working hours. The self-evaluation nature of the questionnaire also means that there may be recall biases. Future directions for this research include the possibility of conducting similar research for online 9SDE classes and study of the appropriate 9-square size in relationship to the height of the participants.

CONCLUSION

The use of 9SDE as an exercise routine can lead to an improvement in cardiorespiratory endurance and leg strength. Its traditional nature can also help improve the individual's sense of well-being, while also allowing a more feasible form of exercise for space and resource-limited individuals.

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Conflict of interest: All the authors declare they have no personal or professional conflicts of interest relating to any aspect of this study.

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Radiologist's Role in Artificial Intelligence Era

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ABSTRACT

Artificial intelligence (AI) in radiology is recently a rapidly growing subject. Much literature about AI in radiology has been launched within 5 years, as well as commercial AI companies. This phenomenon makes some old radiologists feel worried about losing their jobs, and junior doctors hesitate to choose radiology as a specialty. Currently, implementations of proprietary AIs in clinical practice are limited, with a default setting for a convenient human overwrite. The AIs in clinical imaging largely remain either investigational as part of clinical/pre-clinical trials or being developed for commercialized purposes. Radiologists have an important role in all AI processes from the beginning to the end and vital in training the machine, as well as to validate its added benefit for outcome prediction/prognostication. This article will discuss the importance for radiologists to develop, implement, and monitor AI in clinical imaging, together with some ethical considerations. We would like to encourage radiologists to use AI as an adjunct tool, to save time and have better performance.

Keywords: Artificial intelligence; performance; radiologist; radiology (Siriraj Med J 2022; 74: 891-894)

INTRODUCTION

Artificial intelligence (AI) is a human intelligence simulation on a computer. AI has been applied in many industries, including medicine. Generally, AI means that a computer can think and learn. Machine learning is a more advanced AI subset, and deep learning is a form of machine learning that model patterns in data as a complex, multilayered network. The advanced deep learning technique mimics the human brain behavior as we call neural networks. One of the famous neural networks is the convolution neural networks (CNN) used for complex tasks, such as pattern recognition, object detection, image segmentation, and other image processing-based problems, especially for medical data analysis.[1] However, this article used the term "AI" instead of "CNN", because of its familiarity.

Radiology is among the three recent major signs of progresses in AI apart from dermatology and robotics[2] because a major task in radiology involves many images.[3] AI does not only interpret images but also can help in many radiology workflow steps, e.g., scheduling, reporting, and billing.[4] However, most literature about AI in radiology have studied image interpretation task.[5] Literature related to implications of AI in radiology has been presented since 1994[1] and has been rapidly growing since 2018.[6] Performing AI in radiology looks promising. Rodriguez-Ruiz et al.[7] reported that AI had comparable accuracy with breast radiologists in breast cancer screening. This result causes anxiety among radiologists, as well as junior doctors that AI may replace them shortly.[6] This review aims to explain the importance of radiologists' role in AI production and implementation, the current situation, the process of working with AI, and its ethical considerations.

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Radiologists' roles in AI development

AI development uses a dataset with the diagnosis results from an expert, called "ground truth", regarding the systemic approach of a product that includes input, process, output and feedback. There are two sets of data, namely, (1) the training dataset to train the AI and (2) the testing dataset to evaluate the output. Supervised and unsupervised learnings are two AI training methods.[8] Nowadays, supervised learning is used in radiology.[1] The computer receives labeled data and learns and predicts the new input labels in supervised learning. The labeled data require radiologists to label, and its quality depends on the labeling of radiologist as such.[9] Other datasets, including validation and test sets, are fed again to validate after finishing the learning process. External validation by independent cohort and from real-life data from several institutions is advised.[10] Wataganara T.[11] suggested to supervise AI by human experts to minimize the overt sensitivity and false positive results. AI performance needs radiologists to also give feedback. This training method is dependent on the amount and variability of the labeled data. The more data, the better the performance, but with extensive labor as the drawback. The variability of labeled data has two meanings. First is the population variability in which the training images for a given task may be biased because of the sampled population.[12] Its generalizability also requires confirmation from radiologists. Second, labeled data quality variability, particularly, the quality of the image that is produced by different techniques or machines. This type of variability makes an unnecessary complexity for the computer to normalize data.[9] Unsupervised learning learns the dataset on the basis of data patterns without using ground truth and as in the developmental process.[13] Hybrid learning that uses partially labeled data and unlabeled data is the other future option.[11]

Radiologists' roles in AI implementation

AI has various vendors in the market.[14] Radiologists are the key persons who select the choices; however, they should listen to their administrators because it increases hospital costs. Additionally, information technology supports should be available. Moreover, one dataset is used to train limited specific tasks.[3] Thus, AI cannot detect uncommon diseases or other tasks.[15] As implied, hospitals will have higher costs for multiple tasks and radiologists will have time for the other tasks. A single AI application for multiple tasks has been scantly reported.[16] Concerning AI performance, radiologists should confirm AI results at the initial AI software implementation in their departments and continue monitoring.[17] Additionally, AI cannot correlate the exam with the patients' clinical results as radiologists can.[12] Hence, AI should be used in simple tasks but high in quantity while radiologist works on more sophisticated tasks. Hence, radiologists will have more time to pay attention to clinical correlation and interact with patients[18] and have more personalized imaging. Contrastingly, some hospitals cannot afford the cost of AI, especially in developing countries where radiologists' fee are not too high. Hospital administrators have to compare hiring human radiologists and AI cost-effectiveness.

Current situations

The four tasks of AI in diagnostic radiology include image classification, object detection, semantic segmentation, and instance segmentation[11], e.g., liver mass classification, microcalcification detection in mammography, and brain structure segmentation.[3] AI has been applied in radiological practice in various fields and modalities. Computed tomography (CT), magnetic resonance imaging, and plain radiographs are the most common modalities, whereas ultrasonography (US) and mammography are the least.[19] The operator-dependent styles and the lack of standardized US images are the major causes of slower AI progression in US than the other modalities.[20] The two most common fields are neuroradiology and chest radiology.[5] Regarding chest radiology, both radiographs and CT are interesting in the market because chest radiographs are the most common imaging in the hospitals, especially in the coronavirus disease-2019 (COVID-19) infection era.[21-23] Additionally, the amount of lung involvement in patients with COVID-19 for treatment planning and prognosis prediction is important for AI to have higher and faster accuracy in calculation.[21] Much AI research has launched recently; however, a few researchers have tested AI systems in real-world clinical settings,[16,24] and research about AI's true benefits to patients has not been exactly explored.[14]

Patients preferred AI in other areas than in medicine. Among medicine, some specialties were more trusted than others depending on subject areas and patient demographics. Dermatology received slightly more trust than radiology and surgery, whereas women, lower-educated persons, and non-Western immigrants showed less trust in AI.[2] In the radiologist community, those who preferred AI and who came up against it, talk about the same topic but different aspects. Radiologists who liked AI, are interested in the AI capabilities, whereas radiologists who disliked AI focused on ethical and legal aspects.[25] However, radiologists would like to know the training methods for AI and the standard used for

them give more trust to AI. This means to change AI to explainable/interpretable AI or to turn a black box into a glass box. Instead, the recent experiment found that seeing the model's prediction had no effect on people's behavior or might have adverse effects due to information overload.[26]

How to work with AI

Radiologist workflow usually begins with searching for abnormalities, interpreting the lesion, and making a tentative diagnosis. Diagnostic errors may occur at every step. The workflow could change in the presence of AI. Some radiologists use AI for the initial lesion detection and confirm the AI diagnosis if there is no discordant conclusion. Other radiologists work the same as they usually do and with AI to finally make sure that they do not miss any lesions. In other words, humans confirm AI or AI confirms humans. Both ways waste more time than radiologists alone, especially when conflicts occur between AI and humans. Surprisingly, literature has shown that the reading time decreased when the results were normal and increased when the results were abnormal.[14] Nevertheless, most radiologists were satisfied with AI assistance although it prolongs their report time.[16] Do remember, automation bias may occur and their skills may decline with regularly AI use[24], especially students who may lose the ability to learn.[10] Sand et al.[24], reported that radiologists should mention the sensitivity and specificity of AI systems in their reports, and the use of AI tools as well as its version should be mentioned in the patient's file.[10]

Ethical and legal considerations

The law about AI in radiology practice is not available yet.[1] Only half of the commercial AI has been approved by the Food and Drug Administration or Conformite Europeenne.[19] During mistakes, the responsibility of radiologists, administrators, or AI product providers is questioned. Generally, administrators and radiologists should share their ideas when selecting AI software, set steps for using AI, and define who takes the responsibility in each step before applying AI in routine practice. Biases should be considered because they can occur in all stakeholders.[27] AI developers want to make a profit and want their systems to be commercially used. Administrators may like the cheapest AI and thus pay less attention to AI performance. Researchers want their research to be published and may put result biases. Some radiologists, especially in areas with radiologist shortages, who have work overload may be happy to welcome AI to help with their work. Other radiologists who have a high income and high prestige in the hospitals would not prefer AI to share their situations. Both radiologist kinds may have biases on AI either support or blockage. Di Basilio et al.[23], demonstrated that the individuals who obtained direct on-the-job training on AI, had positive opinions on the use of AI. The regulatory approval process should be discussed and implemented at both the local and national levels. A recent proposal for the use of AI among French radiologists suggests that AI can be used to assist radiologist decision-making but not replace it.[10] Radiologists use AI to save time and enhance their performance.

CONCLUSION

Radiologists play important roles in both AI development and implementation. AI can imitate the human brain and thought, but it has no feeling like humans, which is an important part of patient care. Therefore, AI cannot replace the radiologist who uses it, but it may replace the radiologist who does not. Radiologists should be familiar with AI and let them help in their routine practices.

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The Impact of Lockdown during COVID-19 Pandemic on Physical and Mental Health of Adolescents

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ABSTRACT

The COVID-19 pandemic is a once-in-a-lifetime incident whose impact touched everyone from all walks of life. Such an unparalleled global event warranted unprecedented measures to mitigate the imminent public health catastrophe and protect risk groups. However, these actions have inevitably marginalized the physical and mental health of adolescents who were at a lower threat of adverse physical outcomes from COVID-19 infection. Restrictive public health measures resulted in disruption of routines from the closure of the school and public spaces, social isolation, loneliness, lack of engagement, and boredom. These impacts culminated in physical inactivity, sedentary lifestyle, eating disorders, and obesity and led to physical changes that have long-term implications. Equally, the substantial psychological stress of the pandemic resulted in an increased report of anxiety, depression, behavioral problems, and suicide attempts among adolescents in both previously healthy and those with pre-existing mental conditions. This narrative review provides a brief overview of the current evidence of the physical and mental impact of the pandemic lockdown on adolescent health and discussed interventional implications.

Keywords: COVID-19 pandemic; adolescent; physical health; mental health (Siriraj Med J 2022; 74: 895-902)

INTRODUCTION

Since its emergence as a global health crisis in December 2019, the COVID-19 pandemic has had a deep and far-reaching impact on every aspect of livelihood across all age groups. This highly contagious novel respiratory illness has caused a significant toll on the physical health of nearly 600 million people to date and claimed the lives of more than 6 million.[1] Much of the early research focused on morbidity and mortality among risk groups as it disproportionately affects the elderly population with comorbidities.[2] However, as the pandemic pans its course, there is an expanding recognition of the multi-facet mental and indirect physical consequences faced by other less physically vulnerable groups of the general population that were inadvertently overlooked initially.

Adolescence is a challenging phase of life which involves dealing with biological and physical bodily changes, as well as forging emotional maturity, mental health, social networking, and identity. It is during this unique transitional period that adolescents are subject to increased exposure to health-influencing events and behaviors that could shape long-term health outcomes.[3,4] The COVID-19 pandemic has brought new physical consequences and emotional stress that compound this fragile adolescent period via disruption to social opportunities, which resulted in isolation, loneliness,

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and deprivation of various routine services.[5] This has come as a result of the enormous effort invested by health authorities and governments to mitigate the rapid viral transmission through social and physical distancing directives, isolations and quarantines, and lockdowns as a last resort. While these measures served their purposes and facilitated alleviation of one specific health burden for the general population, they are inevitably without their costs to other aspects of physical and mental health for adolescents (Fig 1). This review article aimed to highlight the impact of the COVID-19 pandemic lockdown on the physical and mental health of adolescents, specifically focusing on physical inactivity, eating behaviors, obesity, malnutrition, and substance use in the physical domain, and psychological conditions and suicidal rate in the mental domain. The implication for intervention is also discussed. A search of medical databases, including PubMed, Google Scholar, and references in original and review articles were carried out for the following terms in the title and keyword: COVID-19, pandemic, adolescent, physical health, mental health, psychological impact, physical inactivity, weight, weight gain, overweight, obesity, eating behavior, eating disorder, food insecurity, food access, anxiety, depression, suicide, and substance use. Relevant articles focusing on the health impact on adolescents published between 2019 and 2022 were evaluated and summarized.

Impacts of COVID-19 pandemic lockdown on adolescents' physical health

Research has shown that adolescents were at a lower risk of developing severe COVID-19-related morbidity and mortality.[6] However, they are not exempted from impaired physical health as an indirect consequence of the pandemic and there are increasing reports highlighting several adverse physical outcomes among adolescents during lockdowns.

Impact of COVID-19 pandemic on physical inactivity

It is not difficult to envisage the effects of restrictive social measures on physical activity, and studies have shown a consistent reduction across all geographic regions with the closure of schools, parks, and public spaces. Several studies have reported a decrease in physical activity of up to 60%, and a recent meta-analysis showed a mean reduction of 20% among children and adolescents compared to the pre-pandemic period.[7-10] Unsurprisingly, moderate to vigorous activities were most affected (28%), while the impact on light activities was unclear. Additionally, the result was not affected by the baseline levels of physical activity, suggesting that the product of the lockdown was felt across the board, and not only in those with a previously active lifestyle.[10] The implication of such finding concerns the risk of obesity, given its prevalence is subsequently four times higher among physically

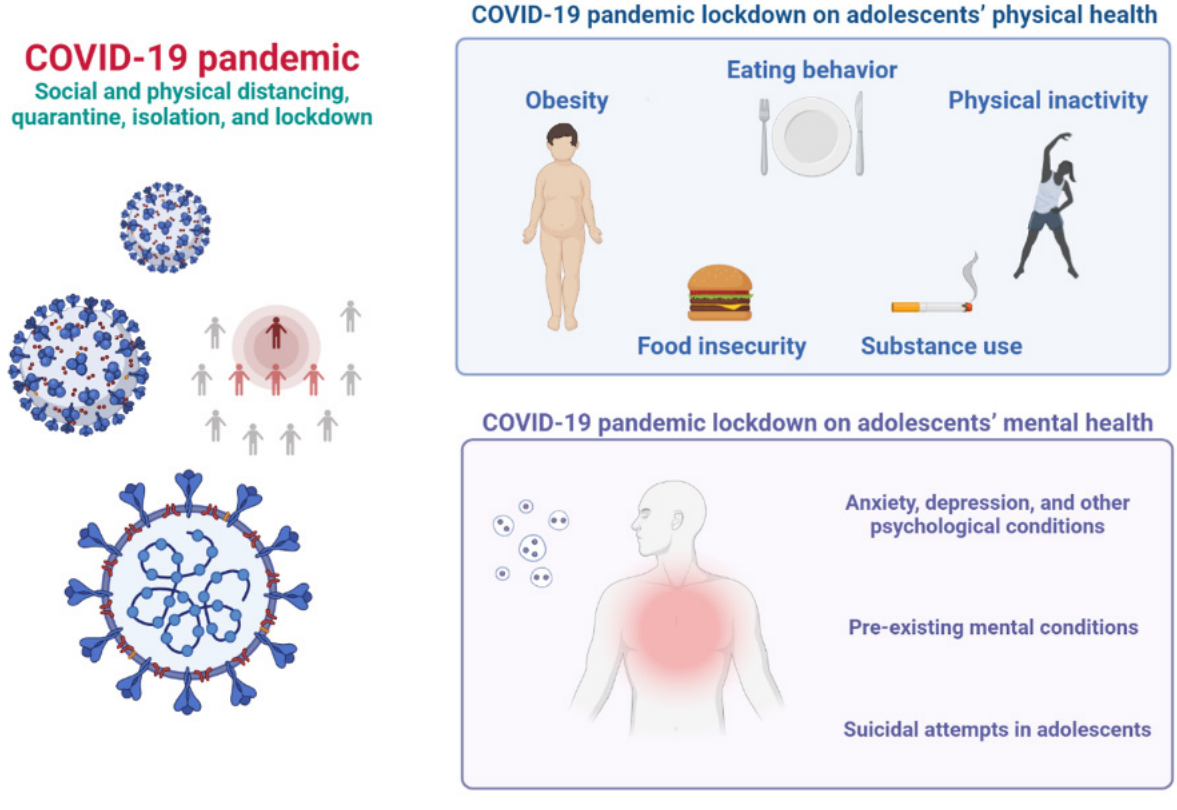

Fig 1. Impact of lockdown during COVID-19 pandemic on physical and mental health.

inactive individuals and is also linked to a myriad of adverse health consequences.[11]

Impact of COVID-19 pandemic on eating behaviors

Stringent social confinement also aggravates stress and boredom, which, in turn, influence obesogenic eating behaviors and eventual weight gain. Such behaviors are brought about via disruption to structured routines, increased screen time-associated snacking, and a change in available household food inventory from stockpiling large quantity of less healthy non-perishable goods in order to minimize social exposure.[12-15] The effect of altered organized schedules has previously been prototyped in summer recess-associated weight gain compared to during the school year due to lack of daily routines. Increased screen time, such as playing video games and watching television, is also associated with unhealthy eating habits and snacking.[13-17] A pilot cross-sectional survey of children and adolescents during the first lockdown in Italy illustrated the effect of social isolation and loneliness on unfavorable food intake by reporting an increase in comfort food consumption, namely sweet packaged snacks (34%), ice cream and desserts (32%), chocolate (32%), as well as bakeries (47%) and processed meats (25%). Consequently, 60% of the study participants gained weight during the study period.[18] This inclination was further supported by a study in 563 adolescents, which demonstrated a 45% surge in consumption of sweet, desserts, and high carbohydrate food at home, in adjunct to a 24% increase in total food intake.[13] Such phenomenon reinforced the association of social isolation and loneliness with eating disorders, particularly overeating and binge eating as a coping mechanism, contributing to weight gain and obesity via excess caloric balance.[5,19,20]

Impact of COVID-19 pandemic on obesity

With the pre-pandemic prevalence of overweight and obesity already at one-third among children and adolescents, the eminent alarming trajectory of this health concern – fueled by physical inactivity and eating behaviors – could only be undoubtedly anticipated.[21-23] A study in China, the first country to implement lockdown, evaluated the body weight status between pre-lockdown and during lockdown among high school students and found an increase in both body weight and body mass index (BMI). This resulted in a higher prevalence of overweight, from 26.7% to 30.4%, and obesity, from 16.1% to 19.3%.[24] A similar pre-pandemic and pandemic comparison study also replicated an increase in the overall prevalence of obesity from 13.7% to 15.4% among school children in 2020.[25] Two recent systematic reviews and meta-analyses have confirmed the association between COVID-19 and weight gain and obesity in adolescents.[26,27] La Fauci et al. identified 14 studies evaluating the effect of lockdown on obesity and found that only 4 (14.3%) studies reported unchanged weight distribution, while the remaining studies demonstrated an increase in either body weight or BMI scores.[27] In the study by Chang et al., the mean differences for weight gain and BMI was 2.67 kilograms (kg) and 0.77 kg/m^2, respectively. Interestingly, when subgroup analysis was performed, subjects with pre-existing type 1 diabetes mellitus or obesity did not reach statistical significance for weight gain and BMI change.[26] Although there is a possibility that these patients were already on a regimented diet, lifestyle modifications, and medication – therefore, were less affected by the loss of structured routines compared to healthy individuals – further exploration is needed to account for this disparity, given contradicting evidence in the literature among children and adolescents with pre-existing comorbidities exists.[28-30]

Altogether, the overall increase in body weight and BMI during the lockdown period should raise alarms for appropriate and timely interventions because the health implication of obesity is two folds. Firstly, at the face value, obesity is associated with an augmented risk of COVID-19 infection, disease severity, and mortality.[19,31,32] It is also associated with comorbidities linked to adverse COVID-19 prognosis, such as diabetes and hypertension.[33] Therefore, the consequence of a growing number of vulnerable populations during the ongoing pandemic is worrying. Secondly, a sedentary lifestyle can become permanent out of habitual familiarity as a "new normal" the longer the lockdown continues. This behavior could, in theory, aggravates pre-existing comorbidities or paves the way to new-onset metabolic syndrome and its complications, such as cardiovascular diseases, in the long term.[26]

Impact of COVID-19 pandemic on food insecurity and malnutrition

Good physical health also relies on adequate and optimum nutrition, and a growing number of studies have shown how the pandemic negatively impacted access to food for adolescents. It has been shown that vulnerability to food shortage was primarily the result of economic and financial hardship from parental unemployment. While the situation affected households of all socioeconomic status, poorer households were unsurprisingly at up to 46-fold risk of moderate to severe food insecurity. They also experienced a greater decline in meals with proteins.[34-37] A multinational data reported that 30-40% of adolescents in developing countries have reportedly

gone hungry during the first wave of the pandemic, resulting in a decrease in the number of meals with proteins from 1.07 to 0.77 as well as the consumption of other nutrient-dense food such as eggs, vegetables, and dairy.[35,36] The threatened food availability was further compounded by limited agricultural production, supply chain disruptions, and distribution and trade restrictions.[36] Ultimately, the impact of food insecurity and malnutrition on children and adolescents is far and wide beyond the immediate physical consequences of poorer general health, reduced cognitive performance, and higher risk of hospitalization, but also extends into mental health territory with increased risk of behavioral problems, anxiety, depression, and suicidal ideation.[38]

Impact of COVID-19 pandemic on substance use

Perhaps, as dire as it may sound, the only solace that could be taken from this tumultuous turmoil is the relatively stable rate of alcohol and substance use among youth during the pandemic.[39] Studies focusing either exclusively on a single substance or multiple substances have yielded incongruent results. Most studies evaluating alcohol use, smoking, cannabis use, and other drug use reported no change in overall use.[40] Interestingly, adolescents who disregarded social distancing and engaged in physical in-person interaction had an increment association with alcohol use (odds ratio [OR]=1.41), heavy drinking (OR=1.81), marijuana use (OR=1.39), and recreational drug use (OR=1.37).[41] This finding is unsurprising given that substance use usually occurs in a social context, and substance-using peer network is one of the strongest affiliated predictors.[42]

Impacts of COVID-19 pandemic lockdown on adolescents' mental health

The literature is increasingly emphasizing the impact of the COVID-19 pandemic on adolescent mental health, either directly as a consequence of the disease or indirectly from public health policies. The importance of such awareness could not be overstated because adolescents are at risk of enduring poor mental health outcomes from an unprecedented global event, yet they lack the range of coping mechanisms and mental resilience of adults. The result of a negative psychological impact could be permanent and fuels additional undesirable physical health outcomes long-term.[5,43]

Impact of COVID-19 pandemic on anxiety, depression, and other psychological conditions

Several studies have shown that children who tested positive or were quarantined for COVID-19 experienced separation stress and were more likely to develop psychological conditions such as anxiety, adjustment disorder, and post-traumatic stress.[3,44-47] Tangibly, coping with stress and grief from losing family members and friends to the disease can overwhelm an adolescent's mental resolve. The COVID-19 pandemic also prompted other psychological tolls from social isolation, loneliness, lack of engagement, and boredom attributable to restrictive public health policies. A recent systematic review and meta-analysis, which included 15 studies and 22,996 participants, found that 79.4% of children were negatively affected by the lockdown. The results showed a pooled prevalence estimate for anxiety (34.5%), depression (41.7%), irritability (42.3%), sleep disturbance (21.3%), inattention (30.8%), and excessive fear of infection, financial uncertainty, and food insecurity (22.5%).[48] Another systematic review demonstrated an overall consistent trend of higher anxiety and depression experienced by adolescents associated with the COVID-19 pandemic.[43] Additionally, there have been suggestions that disruption of daily routines and engagement may result in poor in-person rapport, attention seeking, clingy behavior, and parental dependence.[4]

Impact of COVID-19 pandemic on pre-existing mental conditions

The COVID-19 pandemic also wreaks havoc on children and adolescents with pre-existing mental health conditions because the lockdown deprived them of daily routines, medical care, and tailored services. As a consequence, there are emerging reports on impaired mental well-being in patients with mental comorbidities. Reports have shown aggravated intensity and frequency of behavioral problems in 35-41% of autistic patients. Parents of children with attention deficit and hyperactive disorder reported "emotional rollercoaster," aggressiveness, and sleep problems during lockdowns, and 34% of the patients exhibited clinical deterioration.[48,49] Children and adolescents with varying psychiatric problems also experienced worse anxiety, depressive symptoms, anger, sleep impairment, peer relationship, and general well-being than those with chronic somatic conditions and healthy individuals.[50] More research is needed to delineate the natural history of these observations as the pandemic becomes under control. Nonetheless, they highlight the collateral damage from the existing public health measures for the pandemic and an area of opportunity for future intervention.

Impact of COVID-19 pandemic on adolescent suicidal rate

The ultimate perturbing implication of the COVID-19 pandemic comes in the form of increased rate of suicidal

attempts in adolescents. Studies have steadily shown that the suicidal rate among youth escalated during the pandemic, especially in the early months when the lockdown was implemented as a countermeasure while vaccines were still in their infancy of research and development.[51-54] A study in Japan reported a 1.86-fold surge relative to the pre-pandemic level, and U.S. data pointed to a 31% increase in suicide attempts requiring emergency department visits. Notably, girls and those with mental illness were disproportionately affected, with 51% higher emergency visits and more than 5 folds mental illness-related suicides, respectively.[52,53] Although the suicide rate has since plateaued and decreased in the subsequent time-course of the pandemic, it remains elevated compared to pre-pandemic time.[52]

Implication for intervention

There is limited head-to-head evidence on the magnitude of the effects adolescents experienced compared to other age groups in the population to date per se. Furthermore, there is also a paucity of available data in the literature on the longitudinal natural history of the aforementioned pandemic-related physical and mental health impact on adolescents, given that the world only recently shifted its paradigm to "living with COVID-19". Therefore, it remains to be seen whether these health outcomes are long-lasting, require interventions, or are subject to subsidence with the easing of the lockdown.

Intervention for physical health

On the physical aspect, an Australian study reported that adolescents were less likely to meet moderate-to-vigorous physical activity compared to adults during the social restriction implemented in early 2020 (OR 1.26, 95% CI 1.01-1.57).[55] An inference that adolescents might have suffered more weight gain compared to adults could be drawn from a systematic review and meta-analysis by Bakaloudi et al. in which their weight gain exerted a driving effect for statistical significance on the summative weighted mean difference (WMD) when combined to the weight gain in the adult population (WMD 1.57, 95% CI 1.01-2.14).[56] It could be postulated that the loss of moderation to a sedentary lifestyle and eating habits secondary to hindered structured routines, social interactions, and playtime are likely responsible for the differences in the outcomes, given that these activities constitute a more significant proportion of their daily lives than adults. Therefore, the assertion that there is a pressing need to re-engage adolescents in healthy eating and a physically active lifestyle is well-founded. This is because current behaviors are the foundation for future adult habits, and it has been shown that maintaining sufficient physical activity is associated with reduced decline, chronic diseases, and deaths.[57] The immediate minimum goal would be to restore adolescents' physical activity back to pre-pandemic levels to prevent these lifetime consequences. The resumption of access to routine healthcare and re-allocation of resources that had worn thin by the pandemic can be the stepping stones in facilitating identification of those adversely affected and delivery of appropriate physical lifestyle interventions, on top of regular provision for preventive health maintenance (e.g., vaccination, sexual health) and management of pre-existing chronic diseases.

Intervention for mental health

Being an adolescent is a robust risk factor for poorer mental health in studies conducted in the pediatric population[58]; however, its predictive value is less pronounced when gauged with the general population. It appears that adolescents were just as equally affected psychologically by the pandemic lockdown as adults, as demonstrated in a meta-analysis by Prati et al. The study included 25 studies in adults and adolescents and found that age was not a significant contributor to the effect size of mental health impact.[59] A subsequent study and subgroup analysis also confirmed no evidence that the standardized mean change in mental health symptoms differed between adolescents and adults.[60] These results reiterate the challenges of the full-scale adulthood burden faced by adolescents with their limited coping mechanisms and mental resilience during their maturation phase. Moreover, the long-term evaluation of mental health impact from the pandemic lockdown is, unfortunately, less straightforward, partly because of the intersecting range of onset and the broad definitions for the neuropsychiatric domain of long-COVID syndrome.[61] Hence, distinguishing adolescents with long-term mental health conditions caused by COVID-19 infection – especially in those without symptoms initially – and those secondary to the pandemic remains challenging.[62] To the best of the authors' knowledge, there are no longitudinal studies assessing adolescents' mental health in the post-lockdown period available to date; therefore, more studies are needed to fill this knowledge gap in order to develop a tailored and structured action plan for adolescents for global events of similar scale hereafter.

Potential strategic implementation

In addition to fine-tuning public health policies that are more mindful of adolescents' holistic well-being, one promising strategic avenue to explore in preventing

the negative impact of any lockdown in the future is the implementation of telemedicine. Reports have suggested that digitally proficient adolescents increasingly embrace virtual technology as a mean to receive consultation and therapy.[63] The advantages of telemedicine, even before the COVID-19 pandemic, include decreased wait times, removal of geographic and travel barriers, and higher patient turnovers for physicians.[57,63] It also enables continuity of medical care in a contactless proxy to conventional in-person visits during the pandemic lockdown. Furthermore, the upside of this emerging technology in diagnosing, counseling, and treating adolescents with psychological conditions could be expansive. Telehealth offers the benefit of enhanced confidentiality in private settings and the removal of the social stigma of being seen seeking mental health consultation. There is also a growing evidence of the application of telepsychotherapy and self-guided therapy based on digital health platforms, which Garagiola et al. have reviewed extensively.[57] Nonetheless, as with virtual classrooms, questions remain on the accessibility for adolescents in disadvantaged households and communities who face barriers to internet infrastructures and necessary technological devices.

CONCLUSION

The COVID-19 pandemic posed an unparalleled global health crisis and brought about equally unprecedented public health measures; however, adolescents at lower risk of COVID-19 infection were unfortunately marginalized. Closure of schools and public spaces, social isolation, loneliness, lack of engagement, and boredom have left adolescents reeling from a myriad of physical and mental health consequences. The potential of these effects on long-term health is alarming. Therefore, the growing awareness of the physical and mental tolls on adolescents must be translated into resource allocation and policies by governing bodies, healthcare authorities, and stakeholders to provide appropriate and timely prevention and intervention to ensure the best health outlook of this "COVID-19 generation".

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