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Vaccination Coverage in Patients with Idiopathic Inflammatory Central Nervous System Demyelinating Diseases at Siriraj Hospital, a Single-center Experience

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ABSTRACT

Objective: Individuals with Idiopathic Inflammatory Central Nervous System Demyelinating Diseases (CNS-IIDDs) have an elevated risk for infection. Vaccination is key to reducing infection. This study aimed to determine vaccination coverage, the adverse effects of vaccination, and general vaccination knowledge in the patients.

Methods and Methods: A single-center cross-sectional study in the Multiple Sclerosis Clinic at Siriraj Hospital, Thailand, was performed using the designed questionnaires.

Results: Of 100 participants, 90% were female, with a mean (SD) age of 46.2 (12.9). Overall, all received compulsory vaccine coverage. For optional vaccines, the coverage was lower-than-expected, with rates of 3%, 4%, and 3% for human papilloma virus, pneumococcal, and zoster vaccines, respectively. Only 28% of participants received the 2021/2022 seasonal influenza vaccine. The only factor associated with the uptake of the influenza vaccination was the participants' health coverage. By asking questions to evaluate general vaccination knowledge, two questions related to vaccination and immuno-suppressive agents received the highest percentage of 'not sure' responses.

Conclusion: Vaccination coverage was lower than expected among Thai CNS-IIDDs patients, both for optional and seasonal influenza vaccines. Vaccination in these groups of patients should be encouraged to prevent potential infections.

Keywords: vaccination, idiopathic inflammatory central nervous system demyelinating diseases, multiple sclerosis, neuromyelitis optica spectrum disorder, myelin oligodendrocyte glycoprotein antibody disease (Siriraj Med J 2023; 75: 538-545)

INTRODUCTION

Idiopathic Inflammatory Central Nervous System Demyelinating Diseases (CNS-IIDDs) encompass multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD), and myelin oligodendrocyte glycoprotein antibody disease (MOGAD). Treatment in CNS-IIDDs mainly relates to immunosuppressive (IS) or disease-modulating agents (DMDs) aiming at reducing neuroinflammation. On the other hand, those agents increase the risk of infection among patients.[1] The retrospective study reported the overall rate of infection in MS patients receiving infused, injectable, and oral medications of 37.3%, 36.8%, and 38.7%, respectively, with sinusitis, upper respiratory tract infection, and upper urinary tract infection being the leading infection causes.[2] A recent randomized controlled trial also showed an increased risk of infection, particularly in lower respiratory tract infections and herpes virus infections, among MS patients taking fingolimod.[3] According to the retrospective cohort study, patients with MS were more likely to be hospitalized and die of influenza than individuals without MS.[4] Although immunization would be key to reducing the infection

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rate in susceptible patients, it is overlooked and needs more attention.

Different countries vary in vaccine requirements for young, healthy children, such as the Bacillus Calmette and Guerin (BCG) is a mandatory vaccine in Thailand. Also, most Asian countries, including Thailand, offer obligatory vaccination against Japanese Encephalitis. According to the Thai Expanded Program on Immunization (EPI), the compulsory vaccination includes BCG, hepatitis B, diphtheria-tetanus-pertussis, oral polio (OPV), Measles-mumps-rubella vaccine (MMR), Japanese encephalitis (JE), Haemophilus influenzae (Hib) vaccine and optional vaccines are varicella (VZV), human papillomavirus, hepatitis A (HAV), pneumococcal (PPSV), meningococcal (MPSV), zoster, and influenza vaccine.[5] A recent systematic review and updated practice guideline on immunization in MS recommend that patients with MS follow all local vaccine standards and receive the influenza vaccination annually, and clinicians should counsel MS patients about treatment-specific vaccination guidance according to prescribing information.[6] Likewise, The Royal College of Physicians of Thailand (RCPT) recommends annual influenza vaccine uptake, particularly for patients receiving ISs.[5] In the past decade, the Thai FDA has approved more DMD/ISs to use in CNS-IIDDs, and those agents need particular pre-vaccination guidelines regarding specific drug use.

Few studies have evaluated immunization in patients with MS, usually focused on the influenza vaccine. In Canada, fewer than 40% of MS patients received the influenza vaccine in 2015.[7] In North America, 74.1% of study participants received the seasonal influenza vaccine. The compulsory and optional vaccine uptake was also lower than desired in the MS population compared with public health recommended targets. They also assessed patients' attitudes about vaccination and reported misconceptions about immunization safety in the context of MS.[8] In Italy, the study sharing a real-world, single-center experience was conducted. They reported that before new therapy started, 87.1% of the MS patients completed immunization, including MPSV, PPSV, and Hib vaccines.[9] Since 2019, the COVID-19 pandemic has drawn people's attention to the importance of vaccination. Several studies about COVID-19 vaccination in MS patients have emerged. Achiron et al. proved the safety of the COVID-19 BNT162b2 vaccine for MS patients. They found no significant increased risk of relapse activity after vaccination.[10] For NMOSD and MOGAD, fewer studies on immunization currently exist. A study from China suggested that it was safe to provide NMOSD patients receiving IS/DMD inactivated or viral protein vaccines. In contrast, all live vaccines were prohibited in patients receiving IS/DMDs.[11]

In Thailand, the vaccination uptake in patients with CNS-IIDDs has not been reported. We aimed to determine lifetime coverage of compulsory and optional vaccines, including seasonal influenza, COVID-19 vaccine, adverse effects from vaccination, and general vaccination knowledge in CNS-IIDDs patients at our center.

MATERIALS AND METHODS

Using our questionnaires, we conducted a single-center, retrospective, cross-sectional study in the Multiple Sclerosis and Related Disorders Clinic at Siriraj Hospital, a university-based hospital in Thailand, in May 2022.

Participants

We consecutively recruited patients who were routinely follow-up at the Multiple Sclerosis and Related Clinic at Siriraj Hospital. The experienced coordinator asked patients to self-answer the designed questionnaire. They were included if they (1) were at least 18 years of age, (2) fulfilled diagnostic criteria for each specific CNS-IDDS regarding MS[12], NMOSD[13], MOGAD[14], acute transverse myelitis[15], or idiopathic or recurrent optic neuritis including chronic relapsing inflammatory optic neuropathy (CRION).[16] They were excluded if they refused to take the questionnaire or could not provide information about vaccination. The study's protocol was approved by the Siriraj Institutional Review Board (COA no. Si 263/2021). All patients had written informed consent.

The sample size for this study was determined with a desired confidence level of 95% and a margin of error of 10%. The calculated sample size was 78 participants. To account for non-response and incomplete questionnaires, we increased the sample size by approximately 20%. Therefore, a total of 100 participants were recruited for the study to ensure sufficient statistical power and precision in estimating the population characteristics.

Questionnaires

We created a survey questionnaire containing 6 main categories: basic information, lifetime vaccination uptake, influenza vaccination uptake, previous side effects from vaccination, general knowledge about vaccination, and COVID-19 vaccination. Most questions were closed-ended questions with either yes/no questions or multiple choice. All patients were asked by the same experienced coordinator to self-answer the designed questionnaire. For those with visual impairment, the coordinator also helped them mark the answer on the questionnaire.

The questionnaire took approximately 3-7 minutes to complete.

The questionnaire included age, gender, education level, region of residence, average monthly income, health coverage, and underlying diseases or previous health problems. We categorized age groups as < 35, 35-50, 50-65, or >65 years old. Education level was categorized as below junior high school or junior high school and above. We classified the region of residence into Bangkok metropolis and vicinity and others. Average monthly income was reported as <20,000, 20,000-50,000, and >50,000 baht. We categorized health coverage as Universal Coverage (UC), Social Security (SS), Civil Servant Medical Benefits (CSMB), State enterprise, or self-pay. The underlying disease was recorded as ''yes'' with the disease name or ''no.'' For detail about the participants' diseases; we reviewed each participant's diagnosis and current treatment from our hospital's electronic medical record.

Participants reported whether they had ever received vaccines for any of the following: HAV, HBV, Hib, human papilloma (HPV), MMR, MPSV, PPSV, rabies, Tetanus, Tetanus-Diphtheria-Pertussis (Tdap), VZV, zoster vaccine, both inside and outside Siriraj Hospital, where responses were yes or no. For the influenza vaccine, we mainly focused on the most recent season, the 2021/2022 influenza vaccine, to assess current behaviors. To evaluate recent trends in influenza vaccine coverage, we needed data on influenza vaccination from 2016 to 2020. We also obtained information about previous side effects of vaccination. Understanding of vaccination was tested using the seven questions we designed to evaluate general knowledge about vaccination in the participants. The responses were yes, no, or not sure. For the COVID-19 vaccine, participants reported whether they had received any COVID-19 vaccine, which platforms of vaccine, and their possible side effects. For those who had not received the COVID-19 vaccine, we asked if they wished to get vaccinated and their opinions about the COVID-19 vaccine.

Statistical analysis

We performed statistical analysis on PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). Mean, and standard deviation was reported for normally distributed continuous variables and median and interquartile range for skewed data. Categorical variables were reported as percentages. We also evaluated factors associated with influenza vaccination uptake using Pearson's chi-squared statistics. The evaluated factors included gender, age group, education level, region of residence, average monthly income, and health coverage. P-value < 0.05 indicated statistical significance.

RESULTS

Participants characteristics

One hundred participants were recruited, with 90% being female. The mean age of 46 (SD 18-69) years. The third quarter was junior high school and above. Most of the participants lived in Bangkok's metropolis (67%). Up to 74% had an average monthly income of less than 20,000 baht. Health coverage was SS (30%), UC (28%), State enterprise (17%), and CSMB (10%), respectively. Only 15% of the participants were self-pay.

The most recruited participants were NMOSD (56%), followed by MS (30%), MOGAD (6%), idiopathic or relapsed TM (6%), and idiopathic or recurrent ON (2%), respectively. All received at least one agent of either IS or DMD. Sixty-nine percent of the participants had concomitant diseases (Table 1).

Vaccination Coverage

All compulsory vaccine coverage, including HBV, MMR, tetanus, and Hib, was 100%. For optional vaccines, the most commonly received were rabies (12%), followed by VZV (5%), PPSV (4%), HPV (3%), zoster (3%), and Tdap (3%). None received the MPSV and HAV vaccines (Fig 1).

The influenza vaccine coverage increased after 2017, but after the pandemic of COVID-19, the influenza vaccination seemed to drop slightly (Fig 2b). All influenza vaccine exposure was less than a third in the past 5 years. Only 28% received the seasonal influenza vaccine during 2021-2022. Of those, 36.4% (95% CI 26.64-46.21) were older than 65 years, 25.9% (95%CI 17.73-35.73) were 50-65 years of age, 28.9% (95%CI 20.35-38.92) were 35-50 years, and 23.5% (95%CI 16.02-33.57) were < 35 years of age (Fig 2a).

Nearly a third (27%) of the participants experienced vaccination-related side effects, including local site injection reaction (22%), low-grade fever (9%), myalgia (4%), and chills (3%). Only 1% of the participant had a severe allergic reaction. Using Pearson's chi-squared statistics, the participants' health coverage was the only factor related to influenza vaccination coverage.

General vaccination knowledge

We also asked questions regarding general knowledge about vaccination. The response rates were true, false, and not sure. More than 80% of the participants know that there are live-attenuated and inactivated vaccines, and vaccination can reduce the severity of pliable infectious

TABLE 1. Clinical and demographic characteristics of participants.

Parameters	Participants (n = 100)
Age at the time of survey (years), mean (SD)	46.2 (12.9)
Female, n	90
Diagnosis, n	
Neuromyelitis optica spectrum disorder	56
Multiple sclerosis	30
Myelin oligodendrocyte glycoprotein antibody disease	6
Idiopathic or relapsed transverse myelitis	6
Idiopathic or recurrent optic neuritis	2
Education level, n	
Below junior high school	25
Junior high school and above	75
Region of residence, n	
Bangkok metropolis and vicinity	67
Others	33
Average monthly income (baht), n	
< 20,000	74
20,000-50,000	20
> 50,000	6
Health coverage, n	
Universal Coverage (UC)	28
Social Security (SS)	30
Civil Servant Medical Benefits (CSMB)	10
State Enterprise	17
Self-pay	15
Underlying disease, n	
Yes	31
No	69
Immunosuppressive or immunomodulatory agents status, n	100
Mycophenolate mofetil	35
Prednisolone	69
Azathioprine	60
Interferon β-1a	5
Rituximab	13

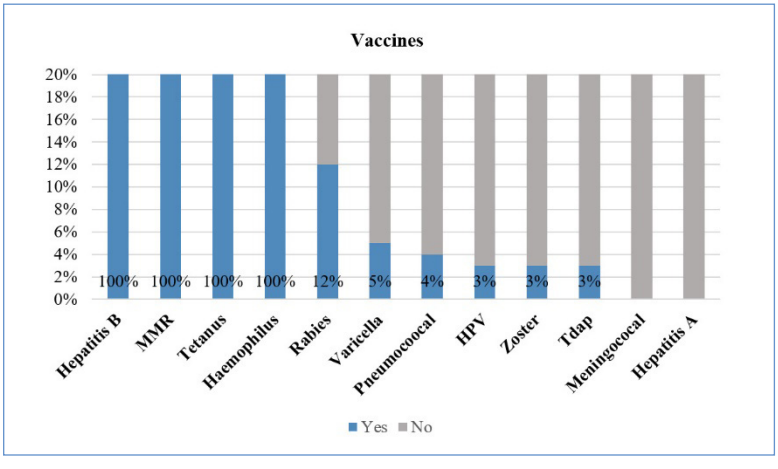

Fig 1. Prior compulsory and optional vaccines exposure in Thai patients with demyelinating diseases.

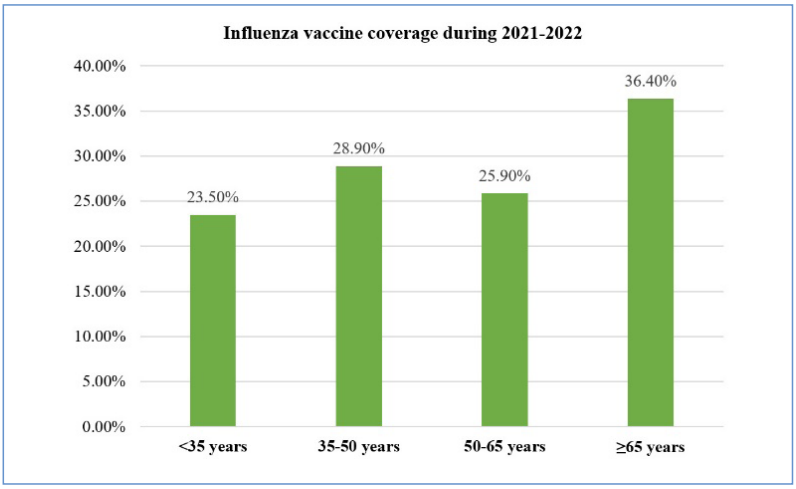

Fig 2a. Frequency of the 2021-2022 influenza vaccine coverage stratified by age.

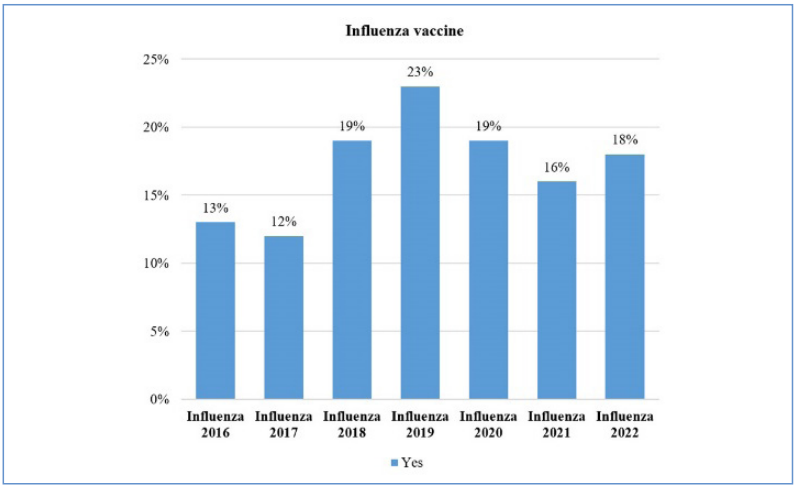

Fig 2b. Frequency of the influenza vaccine coverage during 2016-2022.

diseases accordingly. Also, only a third convince that vaccination could prevent infectious diseases. Fifty-six percent know that the influenza vaccine should be vaccinated annually. Surprisingly, most do not seem aware that a live-attenuated vaccine could not be given to patients receiving an IS, and IS could reduce the effectiveness of vaccines (Table 2).

COVID-19 vaccination

Ninety-one percent received the COVID-19 vaccination. Among the five COVID-19 vaccine platforms, CoronaVac (Sinovac), ChAdOx1 nCoV-19 (AstraZeneca), BBIBP-CorV (Sinopharm), BNT162b2 nCoV-19 (Pfizer), and mRNA-1273 SARS-CoV-2 (Moderna), the most commonly received was ChAdOx1 nCoV-19 (54%) (95%CI 43.74-64.01), followed by BNT162b2 nCoV-19 (39%) (95%CI 29.40-49.26), CoronaVac (25%) (95%CI 16.87-34.65), BBIBP-CorV (17%) (95%CI 10.22-25.81), and mRNA-1273 SARS-CoV-2 (13%) (95%CI 7.10-21.20). Of the remaining 9 percent who did not get vaccinated, of whom 55% did not want to get vaccinated.

Among all the participants receiving the COVID-19 vaccine, a leading side effect was local site injection reactions (60.4%), followed by flu-like symptoms. In general, the COVID-19 side effects did not last longer than a few days after injection. We will report the detail of COVID-vaccination in CNS-IIDDs elsewhere separately.

DISCUSSION

This cross-sectional study of vaccination coverage in CNS-IIDDs in a single center showed that the compulsory vaccine coverage was 100%; however, the frequency of the optional vaccine was lower than expected, mostly less than 30%. The seasonal influenza vaccination has persisted low in the past five years, especially during the pandemic of COVID-19. The achievement (91%) is reached for the mandatory COVID-19 vaccine campaign in the country during the pandemic of COVID-19.

Our study showed that all participants received compulsory vaccines according to Thai EPI guidelines, including MMR, HIB, and hepatitis B. The coverage is much more than 74.1%, 88.5%, and 32.3% of those reported in North America, respectively.[8] Our participant, whose mean age was 46.2 years, was born after the WHO EPI launching, while the participants in the North American study, whose mean age was 61.8 years, were born before

TABLE 2. General vaccination knowledge and responses.

General vaccination knowledge and responses	Participants (n = 100)
Vaccination could definitely prevent the occurrence of infectious diseases.	
True	33
False	52
Not sure	15
Vaccination could reduce the severity of vaccine-preventable infections.	
True	89
False	3
Not sure	8
There are a live-attenuated vaccine and an inactivated vaccine.	
True	84
False	7
Not sure	9
The live-attenuated vaccine could not be given to patients receiving an immunosuppressive drug.	
True	40
False	19
Not sure	41
Immunosuppressants could reduce the effectiveness of vaccines.	
True	40
False	19
Not sure	41
The influenza vaccine should be given annually.	
True	56
False	19
Not sure	25
The influenza vaccine can cause flu illness.	
True	38
False	39
Not sure	23

the recommendation came out. This reason possibly resulted in an increasing vaccination after establishing the WHO EPI in 1974.[17]

For optional vaccines, the coverage differs by study region. The North American study reported 61.2% of PPSV and 41.2% of zoster vaccination coverage[8], while those in the Italian study were 86.7%, respectively.[9] Our study showed surprisingly lower-than-expected rates of 3%, 4%, and 3% for HPV, PPSV, and zoster vaccination coverage, respectively. Since some vaccines, such as the zoster vaccine for individuals above 60 years of age or the HPV vaccine for individuals aged 9-26, have specific age group recommendations, the interpretation of vaccination coverage for these vaccines should be approached with caution.

We then focus on VZV vaccination. RCPT recommended completing the varicella vaccination course before starting ISs if the screening VZV-IgG antibody is negative. Also, the Thai Clinical Practice Guidelines for MS and NMOSD mandate screening VZV serostatus before initiating any DMTs or ISs[18], and it is required to have vaccination with proven immunity before starting any DMTs for MS, such as fingolimod, cladribine, alemtuzumab, etc.[19]

The low immunization in this optional vaccination coverage might be explained by the negligence of both participants and medical personnel and by the collateral effect of the COVID-19 pandemic. The participants' economic status might also affect their decision for immunization, especially for high-cost vaccines such

as the VZV vaccine. There should be a high alarm for underrate vaccination, especially in the specialist clinic susceptible to varicella infection.

The North American study revealed that the 2019/2020 seasonal influenza vaccination coverage ranged from 59.1% among MS patients aged 18-24 to 79.9% for those aged older than 65 years, and factors including postsecondary education and higher household income were associated with the influenza vaccination.[8] Compared with our Thai study, only 28% of participants received the 2021/2022 seasonal influenza vaccine. The only factor associated with influenza vaccination was a type of health coverage, CSMBs. Although the influenza vaccination rate was higher than in the previous study in 2012, which was 15.2% among the population with chronic diseases[20], it was far from expected. However, we could see an increasing influenza vaccination rate from 2016 to 2019, then a drop-down during 2019 and 2020, perhaps delayed or omitted vaccination during the COVID-19 outbreak. Healthcare providers and policymakers should focus on the low seasonal influenza vaccination rate in Thailand. Also, the annual influenza vaccination campaign and education about the efficacy of vaccination, in particular patients in need, should be done.

Based on the study findings, it was observed that vaccination coverage for optional vaccines and influenza vaccines among CNS-IIDDs patients remains low. To utilize this data for patient care purposes, it would be beneficial to design individualized vaccination record booklets or develop electronic systems within hospitals that provide vaccination schedule reminders for each patient. Additionally, creating informative materials to raise awareness about the benefits of vaccination could significantly increase vaccination rates. These measures are likely to be advantageous in increasing vaccination rates among CNS-IIDDs patients.

Focusing on adverse reactions, our study showed that the most common adverse reaction after vaccination was an injection site reaction with pain, followed by fever 22% vs. 9%, which was similar to the North American study.[8]

For the SARS-CoV-2 vaccination, the vaccination rate was 91% up until May 2022. The viral vector-based platform, ChAdOx1 nCoV-19, was our participants' most widely used vaccine (54%), followed by BNT162b2 nCoV-19 (39%), similar to a previous Thai study in 2021.[21] Of those, 60.4% developed pain at the injection site, followed by fever and headache, but none showed a severe adverse reaction. Among the 9% who did not receive COVID-19 vaccination, 55.5% did not get vaccinated because of doubtfulness in vaccine efficacy.

Our study on vaccination among Thai CNS-IIDDs patients was pioneering, but it had limitations. Firstly, the sample size was small due to resource constraints and the rarity of these diseases in the Thai population. This small sample size may have impacted the generalizability of our findings to a broader population. Secondly, we relied on participant recall for vaccination information as there is no standardized vaccination book in Thailand, introducing the possibility of recall bias. Future studies should aim for larger, more diverse samples and explore alternative data collection methods to mitigate these limitations. Addressing these limitations enhances the credibility of our findings and provides direction for future research.

CONCLUSION

In summary, the present study showed that vaccination was lower than expected among patients with CNS-IIDDs, both for optional and seasonal influenza vaccines. Without vaccination coverage, patients have an increased risk for possibly preventable infections. Vaccination in these groups of patients should be encouraged. Medical personnel may play a significant role in guiding patients about the importance of immunization and education about each specific vaccination needed in different conditions.

Financial Disclosures

None

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Habitual Snoring in Pediatric Thalassemia Disease; Prevalence, Quality of Life and Risk Factors

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ABSTRACT

Objective: To compare the prevalence of HS and quality of life in non-transfusion dependent thalassemia (NTDT) and Transfusion dependent thalassemia (TDT) patients and to identify risk factors associated with HS in pediatric thalassemia.

Materials and Methods: We conducted a cross-sectional study of pediatric thalassemic patients aged from 6 months - 18 years between January 2020 and October 2020, at Thammasat University Hospital, Thailand.

Results: There were 141 thalassemia patients (35 TDT and 106 NTDT), aged 7 months-18 years, 73 (51.8%) were male. Sixty-eight patients (48.2%) reported snoring; 28 patients (19.9%) had HS; the remaining 40 patients (28.4%) had simple snoring. The prevalence of HS was not significantly different between TDT and NTDT group (6 (17.1%) VS 22 (20.8%); *P*= 0.527). Quality of life assessed by OSA-18 score was not significant difference between TDT and NTDT groups (51.3 ± 18.8 VS 45.7 ± 11.4; *P*=0.141). The associating risk factors for the development of HS after multivariate logistic analysis were nasal congestion, and male gender, with an adjusted OR of 5.3 and 3.0, respectively.

Conclusion: Prevalence of HS was increased in children with thalassemia. Factors such as nasal congestion and male gender were strongly associated with HS in this population. The quality of life assessment using the OSA-18 questionnaire indicated that thalassemia children generally exhibited a good quality of life. Additionally, our study observed relatively low serum ferritin levels in comparison to previous studies. The standard care provided for TDT patients, includes regular blood transfusion and effective iron chelation, may contribute to slowing down the degree of nasopharyngeal narrowing in thalassemia patients.

Keywords: Habitual snoring; obstructive sleep apnea; thalassemia; OSA-18 (Siriraj Med J 2023; 75: 546-554)

INTRODUCTION

Thalassemia disease is a common inherited hematologic disorder caused by several mutations which affect the hemoglobin synthesis. It is highly prevalent in Southeast Asia.[1-2] In Thailand, about one half of the population likely carries some sort of thalassemia gene.[3-4] It is estimated about 10,000 new cases emerge each year. The clinical spectrum of thalassemia ranges from asymptomatic to severe anemia and can cause serious complications. Ineffective erythropoiesis causes osteoporosis and expansion of marrow space in the skull and facial bone and cause extramedullary hematopoiesis (EMH). Furthermore, compensatory lymphoid hyperplasia from frequent infection by encapsulated organisms are predisposed to upper airway obstruction, especially during sleep. There have been some reports about higher prevalence of obstructive sleep apnea (OSA) in children with beta-thalassemia than healthy children.[5-9] The data also reported that adenotonsillar lymphoid hyperplasia and high serum ferritin level were associated with the occurrence of OSA.

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The majority of the complications of thalassemia major are related to iron overload, bone marrow expansion and EMH, but EMH is more prevalent in thalassemia intermedia who are non-transfusion-dependent thalassemia (NTDT) when compared to transfusion-dependent thalassemia (TDT).[5] To our knowledge, there has been no study to compare the prevalence of OSA and quality of life in both NTDT and TDT groups. Polysomnography (PSG) is the gold standard for diagnosis of OSA, but it is associated with several disadvantages, particularly in children. As a result, a timely diagnosis of OSA is not always possible. Habitual snoring is a hallmark for OSA.[10-12] A few reports have shown that habitual snoring was a strong risk factor of OSA in children with sickle cell anemia[13] and one report showed a trend of habitual snoring was increased in OSA.[8] For a resource constrained environment, history of habitual snoring may be guided for OSA screening. The aims of our study are to compare the prevalence of habitual snoring and quality of life in NTDT and TDT patients. The secondary outcome is to identify risk factors associated with habitual snoring in pediatric thalassemia.

MATERIALS AND METHODS

We conducted a cross-sectional study of pediatric thalassemic patients aged from 6 months - 18 years between January 26th 2020 and October 14th 2020, at Thammasat University Hospital, Thailand. The approval for the study was granted by the ethics committee of Thammasat University (MTU-EC-PE-1-270/63). Assent and/or informed consent forms were obtained from patient > 7 years old and all parents/guardians respectively. Children or guardians who did not understand Thai adequately or could not provide sleep data or who were cases with incomplete medical records were excluded. Clinical information was collected through a manual chart review as follows: demographic data, underlying medical conditions, nutritional status, clinical history and previous treatment of thalassemia, history during sleep and snoring, OSA-18 questionnaire, physical examination for adenoid and tonsils size, nasal congestion and hepato-splenomegaly, and laboratory data (hemoglobin and serum ferritin level).

Operational definition

Habitual snoring (HS) is defined as the presence of loud snoring at least 3 nights per week while snoring less than 3 nights per week is defined as simple snoring (SS).[7,14,15] Transfusion-dependent thalassemia (TDT) refers to the group of patients who require regular blood transfusions for survival from early life, including severe forms of hematological phenotypes of beta-thalassemia.[16] Non-transfusion dependent thalassemia (NTDT) refers to the mild group of patients or thalassemia intermedia who do not require frequent blood transfusion for survival. Obesity, for children < 5 years of age was defined as weight-for-height > 3 standard deviations (SD) above the World Health Organization (WHO) Child Growth Standard median.[17-18] Children and adolescents aged between 5 – 15 years were defined as obese if BMI-for-age was > 2 SD above the WHO Growth Reference median.[17-19] The OSA-18 score is an 18-questionnaire that uses a Likert-type scoring system to evaluate the quality of life for 2-18 years children with OSA. It has been validated as both evaluative and discriminative in Thai pediatric OSA.[20] The OSA-18 consists of 18 items grouped in 5 domains of sleep disturbance, physical suffering, emotional distress, daytime problem and caregiver concerns. On the basis of this information, a summary score is calculated that ranges from 18 (no impact on quality of life) to 126 (major negative impact). A value at or above 60 is considered abnormal.[21]

Statistical analyses

Data were analyzed using STATA for Windows v14.0. Clinical characteristics and laboratory results for continuous data were reported as mean and standard deviation (SD) or median with interquartile range (IQR); categorical data were reported as the frequency with percentage. Independent Student-t test, Wilcoxon rank-sum test, and Kruskal-Wallis test were used to compare continuous data; nominal data analysis used a Chi-square test: P-value < 0.05 was considered statistically significant. Risk factors associated with HS were analyzed using univariate and multivariate logistic regression. For univariate analysis, crude odds ratios (OR) and 95% confidence intervals (CIs) were used to consider the strength of factors associated with HS. Factors with a $p < 0.20$ or clinical significance in literature review were then entered into a multiple logistic regression model. A value of $p < 0.05$ was considered to indicate statistically significant differences, and adjusted OR and their 95% CIs were reported to consider the strength of association.

RESULTS

A total of 141 thalassemia patients (35 TDT and 106 NTDT) were enrolled. Approximately 73 patients (51.8%) were male. The median age was 8.9 years (range 7 months-18 years). Most patients were between the ages of 5-15 years. Underlying comorbidities were found in 39 children (27.7%) and there was allergic rhinitis in 13 (9.2%) children. Forty-seven patients (33.3%) had

history of passive smoking. Half of the patients were in a medium income family. For screening cognitive function, we found that the median mathematic grade was 3 and found significant lower in TDT than NTDT group (3 VS 2.5 with *P*=0.002). Sixty-eight patients (48.2%) reported snoring; 28 patients (19.9%) were HS, the remaining 40 patients (28.4%) had simple snoring. The prevalence of HS was not significantly different between TDT and NTDT group (6 (17.1%) VS 22 (20.8%); *P*= 0.527). Twenty-five patients (71.4%) in TDT group had desferrioxamine for iron chelation therapy.

Most patients had normal nutritional status; only 11 patients (7.9%) were obese and 7 patients (4.9%) had malnutrition. On physical examination, nasal congestion and tonsil enlargement were found more readily in TDT than NTDT group but of no statistical significance. Mean hemoglobin level was 9.18±1.13 g/dL, there was no significant difference between groups. The median serum ferritin level was significantly higher in TDT than NTDT group (1315 VS 62; *P*< 0.001). The detail of clinical data is shown in Table 1.

Regarding the OSA-18 questionnaire score, the mean OSA-18 score was 46.9±13.2, with no significant difference between TDT and NTDT groups (51.3 ± 18.8 VS 45.7 ± 11.4; *P*=0.141). The fifth domain (caregiver concern) revealed the highest mean total score (11.7) whereas, the fourth domain (effects on daytime function) showed the lowest mean total score (8.1). Comparison between the TDT and NTDT groups revealed that only physical symptoms including rhinorrhea and difficult in were significant higher in TDT than NTDT group. The details are shown in Table 2.

TABLE 1. Comparison of clinical data between TDT and NTDT patients.

Clinical data	All (N=141)	TDT (N= 35)	NTDT (N=106)	*P*-value
Age (year); mean (IQR)	8.9 (5.3-12.8)	10.3 (7.3-13.8)	8.3 (5-12.6)	0.127
Age group; N (%)				0.237
< 2 years	9 (6.4)	2 (5.7)	7 (6.6)	
2 years - < 5 years	24 (17.0)	4 (11.4)	20 (18.9)	
5 years - <10 years	49 (34.8)	10 (28.6)	39 (36.8)	
10 years - < 15 years	39 (27.7)	15 (42.9)	24 (22.6)	
≥ 15 years	20 (14.2)	4 (11.4)	16 (15.1)	
Male gender; N (%)	73 (51.8)	20 (57.1)	53 (50)	0.463
Nutritional status; N (%)				
Malnutrition	7 (4.9)	3 (8.6)	4 (3.8)	0.263
Obesity	11(7.9)	2 (5.7)	9 (8.6)	0.586
Passive smoking; N (%)	47 (33.6)	15 (42.9)	32 (30.5)	0.179
Median mathematic grade; median (IQR)*	3 (2.5-4)	2.5 (2-3)	3.5 (3-4)	0.002
GPA; mean (IQR)	3.5 (3-3.8)	3.2 (2.6-3.7)	3.5 (3.2-3.9)	0.095
Snoring prevalence; N (%)				0.527
Habitual snoring	28 (19.9)	6 (17.1)	22 (20.8)	
Simple snoring	40 (28.4)	8 (22.9)	32 (30.2)	
Underlying disease; N (%)	39 (27.7)	8 (22.9)	31 (29.3)	0.464
Allergic rhinitis; N (%)	13 (9.2)	3 (8.6)	10 (9.4)	0.878
Nasal congestion; N (%)	37 (26.6)	13 (37.1)	24 (23.1)	0.103
Tonsil enlargement; N (%)	9 (6.5)	3 (8.6)	6 (5.8)	0.560
Median hemoglobin level (g/dL); median (IQR) *	9.1(8.5-9.6)	8.8 (8-9.1)	9.3 (8.7-10)	<0.001
Median serum ferritin level (ng/ml); median (IQR) *	76 (73-294)	1315 (608-1650)	62 (38-95)	< 0.001

*P <0.05

Abbreviations: NTDT, non-transfusion dependent thalassemia; TDT, transfusion-dependent thalassemia; GPA, Grade point average

TABLE 2. The comparison of OSA-18 scores between HS in TDT and NTDT patients.

OSA-18 questions	All (N=28)	TDT(N=6)	NTDT(N=22)	*P*-value
Sleep disturbance	9.9±2.8	10.3±1.9	9.8±3.1	0.265
Loud snoring	4.3±1.0	4.7±1.2	4.1±0.9	0.514
Breath holding /pause	1.3±0.8	1.5±0.8	1.3±0.8	0.890
Choking or grasping	1.9±1.2	1.7±0.8	2.0±1.2	0.292
Fragmented sleep	2.3±1.3	2.5±1.0	2.3±1.4	0.474
Physical symptoms	8.9±3.8	10.3±5.7	8.5±3.1	0.068
Mouth breathing	2.5±1.3	3.2±1.5	2.3±1.3	0.679
Frequent URIs	2.1±1.1	1.7±1.2	2.2±1.1	0.745
Rhinorrhea*	2.6±1.5	3.2±2.4	2.4±1.2	0.026
Dysphagia*	1.7±.1.2	2.3±.2.2	1.6±.0.8	0.002
Emotional symptoms	8.3±3.7	8.0±3.7	8.4±3.8	0.913
Mood swing or tantrums	2.6±1.5	2.5±1.4	2.6±1.6	0.694
Aggressive or hyperactivity	2.6±1.4	2.3±1.8	2.7±1.3	0.410
Discipline problems	3.1±1.5	3.2±2.3	3.1 ±1.3	0.071
Daytime function	8.1±2.9	10±3.7	7.6±2.5	0.216
Daytime drowsiness	2.3±1.4	2.0±2.0	2.4±1.2	0.131
Poor attention span	2.7±1.4	3.0 ±1.7	2.6±1.4	0.559
Difficult awakening	3.1±1.7	5.0±1.9	2.6±1.3	0.251
Caregiver concerns	11.7±4.3	12.7±5.8	11.4±3.9	0.257
Worried over child health	4.5±1.3	5.2±1.3	4.3±1.3	0.901
Concerned not enough air	2.7±1.6	2.8±2.1	2.7±1.5	0.329
Caregiver missed activities	2.1±1.3	2.3±1.5	2.0±1.2	0.522
Caregiver frustration	2.4±.1.4	2.3±1.8	2.4±.1.3	0.422
Total OSA-18 score; mean ± SD	46.9 ± 13.2	51.3 ± 18.8	45.7 ± 11.4	0.141

*$P < 0.05$

Abbreviations: NTDT, non-transfusion dependent thalassemia; TDT, transfusion-dependent thalassemia

Regarding associated factors for HS by univariate logistical regression models, the most relevant risk factor was age group 5-10 years (OR 9.8) followed by nasal congestion (OR 4.6). Other risk factors were underlying disease (OR 2.9), male gender (OR 2.3), history of passive smoking (OR 1.7), tonsil enlargement (OR 1.3) and Serum ferritin level ≥ 1,000 ng/ml (OR 1.2. The unadjusted OR with 95% CIs of possible risk factors for HS are demonstrated in Table 3.

After multivariate logistic analysis to adjust the associating risk factors for the development of HS, we adjusted for all factors that had an odds ratio above 1 and a P < 0.20 (Table 3). The remaining factors that affected HS were nasal congestion and male gender with adjusted OR 5.3 and 3.0 respectively. The results are shown in Table 4. However, severity of thalassemia was not a significant predictor of HS both in crude and adjusted analysis OR 0.7 (95%CIs 0.22-1.93).

DISCUSSION

In our pediatric thalassemia population, 48% of patients reported to have snored in the previous year, 19.9 % had habitual snoring. The estimated prevalence of HS was high compared to a report of 6.9-8.5% in general Thai school-aged children.[22,23] However, several studies reported prevalence of HS in general children around 2.4-45%.[24-29] The prevalence rates vary according to study design, and study population characteristics, such

TABLE 3. Univariate analysis of the risk factors for the development of habitual snoring.

Clinical data	HS (N=28)	Non-HS (N=113)	OR (95% CI)	*P*-value
Age group; N (%) *				0.014
< 2 years	1(3.6)	8 (7.1)	2.3 (0.18-29.84)	
2 years - < 5 years	4 (14.3)	20 (11.7)	3.7 (0.59-23.07)	
5 years - <10 years	17 (60.7)	32 (28.3)	9.8 (1.87-51.57)	
10 years - < 15 years	2 (7.1)	37 (32.7)	1.0	
≥ 15 years	4 (14.3)	16 (14.2)	4.6 (0.72-29.78)	
Male gender; N (%) *	19 (67.9)	54 (47.8)	2.3 (0.95-5.61)	0.057
Obesity; N (%)	2 (7.1)	9 (8.0)	0.9 (0.178-4.35)	0.875
Passive smoking; N (%)	12 (42.9)	35 (31.3)	1.7 (0.70-3.88)	0.245
Median mathematic grade	3.3 (2-4)	3.0 (2.5-4)		0.749
Mathematic grade ≥ 3; N (%)	12 (66.7)	50 (73.5)	0.7 (0.24-2.20)	0.057
Severity of thalassemia; N (%)	6 (17.1)	22 (20.8)	0.8 (0.29-2.15)	0.642
TDT	6 (21.4)	29 (25.7)		
NTDT	22 (78.6)	84 (74.3)		
Underlying disease; N (%) *	13 (46.4)	26 (23.0)	2.9 (1.19-7.03)	0.013
Desferrioxamine therapy; N (%)	4 (14.8)	21 (19.8)	0.7 (0.21-2.27)	0.553
Nasal congestion; N (%) *	14 (53.9)	23 (20.4)	4.6 (1.78-11.71)	<0.001
Tonsil enlargement; N (%)	2 (7.7)	7 (6.2)	1.3 (0.24-6.49)	0.780
Median hemoglobin level (g/dL)	9.1 (8.5-9.7)	9.1 (8.5-9.6)		0.865
Hemoglobin level <10 g/dL; N (%)	22 (78.6)	88 (77.9)	1.0 (0.35-2.631)	0.937
Median ferritin level (ng/ml)	79 (40-167)	74 (45-351)		0.830
Ferritin level ≥ 1,000 ng/ml; N (%)	4 (14.8)	14 (13.1)	1.2 (0.35-3.84)	0.815

*OR >1 with $P < 0.20$

Abbreviations: NTDT, non-transfusion dependent thalassemia; TDT, transfusion-dependent thalassemia

TABLE 4. Multivariate analysis of the risk factors for the development of habitual snoring.

Variable	Coefficient OR (95%CI)	*P*-value
Age group	0.9 (0.57-1.36)	0.563
Male gender	3.0 (1.10-8.19)	0.032*
Underlying disease	2.6 (0.99-6.72)	0.051
Nasal congestion	5.3 (2.01-13.95)	0.001*

*$P < 0.05$

adjusted for age group, sex, underlying disease and nasal congestion

as age, ethnicity, environment and the description and perception of HS. Our findings of a high prevalence of habitual snoring in thalassemia disease were in accordance with the finding of Sritippayawan et al[7] who reported a high prevalence of HS and OSA in children with severe beta-thalassemia. To the best of our knowledge, there have been scanty reports regarding the prevalence of HS in childhood thalassemia. Several studies reported high prevalence of OSA or sleep disturbance in children with thalassemia major.[8-9,30] Although PSG was the gold standard method for diagnosing OSA, resulting in stronger clinical significance, there are still limitations due to incorporating young children and sparse availability of long waiting lists and high costs. Simple screening tools are needed for children. HS was a subjective assessment but a recent study showed a significant association between parental reported HS and objectively measured pathologic snoring.[31-33] It may be implied that HS must be paid close attention and should be considered that HS is an at-risk population.

The mechanism of these findings has not been well established. Kapelushnik et al[5] reported the association of pediatric OSA and thalassemia intermedia whereby they proposed EMH in the nasopharyngeal airway to be the possible cause of OSA. Sritippayawan et al[7] also reported that most pediatric OSA with thalassemia major cases had adenotonsilar hypertrophy and needed surgery. Finally, the finding from adenotonsillar lymphoid tissue showed lymphoid hyperplasia without evidence of EMH. The authors suggested lymphoid hyperplasia was the cause of nasopharyngeal airway narrowing and OSA as in the general pediatric population. The mechanism of lymphoid hyperplasia is unknown. Several mechanisms have been proposed, including repeated adenotonsillar infection or compensatory lymphoid hyperplasia in response to splenectomy or increase systemic inflammatory response from evidence of high serum ferritin in severe thalassemia cases.[5-9]

In this study, we found no significant difference in the prevalence of HS in TDT and NTDT group, although there was higher serum ferritin in TDT group. Sritippayawan et a[7] reported a higher average serum ferritin level in the OSA group than the non-OSA group. They suggested those who had more severe OSA tended to have a higher serum ferritin level than those who had a milder disease. That result was inconsistent with our study. It may be due to the mean serum ferritin level in our study being lower than their study. The mean serum ferritin level in our TDT group was 1,257 ng/ml while their study reported the mean serum ferritin level of 4,606 ng/ml in moderate to severe OSA group and 2,554 ng/ml in mild OSA group. Tarasiuk et al[6] also studied the sleep disruption among beta-thalassemia and congenital dyserythropoietic anemia. Their patients had low serum ferritin (413.7 ng/ml) and no evidence of OSA was found among any of their patients. Regular blood transfusion would have kept EMH at a minimum[34] but in NTDT as chronic anemia would still provide a stimulus for bone marrow expansion and hence a risk for airway obstruction.[35] Regular blood transfusion with adequate iron chelation may protect the patient from developing sleep order breathing.

For OSA-18 score questionnaire, its clinical usefulness for distinguishing the severity of pediatric OSA is unclear and conflicting[21,36-37] but the usefulness in determining the factor most affecting the quality of life of children with OSA was mentioned.[41] In our study, we found the mean OSA-18 score was 46.9±13.2. The result was quite low when compared to previous reports for quality of life in pediatric OSA (53-60 in non-severe OSA and ≥ 60 in severe OSA).[20-21,39] It represented a good quality of life of our thalassemia patients. This finding was consistent with Sinlapamongkolkul et al,[40] who demonstrated a better health-related quality of life (HRQoL) score of their pediatric thalassemia patients when compared to the previous decade. They stated that these findings may represent a better standard of care because the prevalence of high serum iron ferritin level was quite low (14% of patients had serum ferritin > 1,000 ng/ml) in their study when compared to the previous decade.

In our study, there was no statistically significant difference in the total score of the OSA-18 score between NDTD and TDT but we the data showed the trend of OSA-18 score in TDT was higher that NDTD group. However, we did notice a trend indicating that the OSA-18 scores were higher in the TDT group compared to the NTDT group. It is important to note that the lack of statistical significance in our findings may be attributed to the limited sample size. Therefore, further research with a larger sample size is warranted to explore this trend in more detail. Moreover, while the OSA-18 questionnaire proved to be a reliable tool for assessing the subjective aspects of OSA-related quality of life, it is essential to consider various factors that might influence the scores. These factors may include family income, guardian education, parents' perceptions, and emotional well-being, which could potentially impact the overall score. Interestingly, in our study, we observed that parents in the NTDT group exhibited higher affluence and education levels compared to the TDT group. These differences in socioeconomic status might explain the higher scores in the caregiver concern domain within the NTDT group.

Nevertheless, it is important to highlight that these scores were relatively low when compared to general pediatric OSA score.

Regarding associated factors for HS, we revealed that HS was more prevalent in age group 5-10 years than in older children. This finding can be attributed to age-dependent increases in volumetric lymphoid/cephalometric ratio, which typically peak between 2-8 years of age.[41] Our results align with previous studies that have reported similar age-related trends in HS prevalence.[15,28,42-43] However, some studies have not shown a clear trend in this regard.[44] The gender difference in the prevalence of HS among children has been variable in the literature. While several studies have reported a higher occurrence of HS in males,[15,25-26, 28,42-43] others have not found a significant gender difference.[22,24-25,46-47] Passive smoking has been identified as an important risk factor for HS in multiple studies.[15,22,28,42-43,46,48-49] Cigarette smoke induces endotoxin, resulting in a potent inflammatory reaction, mucosal swelling and increase mucous production due to goblet cell proliferation lead to nasopharyngeal narrowing.[50] Our study also found this association but no statistical significance. Furthermore, our study identified a strong association between HS and respiratory problems, including allergic rhinitis and nasal congestion. These findings are consistent with previous research.[15,26, 28,43,46,51-52] The mechanism of these findings has not been clarified, but it has been proposed that inflammatory processes resulting from allergic rhinitis or respiratory tract infections can increase airway resistance. Additionally, early exposure to respiratory viruses may induce neuro-immunomodulatory changes in adenotonsillar tissue, thereby contributing to snoring and HS.[53] Interestingly, our study did not find an association between high serum ferritin levels and HS, in contrast to a previous study reporting a negative correlation between serum ferritin and obstructive sleep apnea (OSA).[7] One possible explanation for this discrepancy could be that effective regular blood transfusion and iron chelation therapy in our study population helped maintain normal serum ferritin levels and minimize nasopharyngeal airway narrowing.

Previous studies have shown the relationship between disordered cognitive functions and anemia of any cause, including thalassemia major.[54,55] Several mechanisms were proposed, including cumulative small injuries to the central nervous system resulting from hemolysis or repeated blood transfusions, which can lead to iron overload in the brain or neurotoxicity associated with lifelong chelating therapy. All of these factors may contribute to brain dysfunction.[56,57] Monastero R et al[55] stated neuropsychological tests were significantly impaired in patients with beta-thalassemia major, particularly in those exhibiting signs of hemosiderosis but there was no correlation between desferrioxamine doses, hemoglobin and ferritin levels. Nevruz O et al[58] also reported potentials of cognitive impairment in patients with thalassemia minor. They hypothesized that chronic hemolysis may play a role in the etiology of neurological findings. Furthermore, several studies have identified an association between HS and adverse behavioral and academic outcomes, even in the absence of intermittent hypoxia[28,59-62] This may be due to increased sleep fragmentation. Consequently, thalassemia patients with sleep disorders may experience impaired cognitive function. In our study, cognitive function was assessed by screening mathematic grades as a simple measure to reflect cognitive abilities. We found significantly lower mathematic grades in TDT group. However, when focusing the association with HS, we did not observe a correlation. It is essential to recognize that our study results were based on screening questions, which may have lower reliability. In addition, some previous studies have reported no association between snoring and cognitive deficits in preschool children.[61,62] Therefore more reliable tools should be further investigated. Bottom of Form

Our study had some limitations; firstly, we were unable to perform PSG, which is considered a standard method for diagnosis OSA, due to budgetary constraints. This may have limited our ability to accurately assess the presence and severity of OSA in the study population. Additionally, both HS and OSA-18 questionnaire were recognized by the reliance on a subjective measure, which could introduce rater biases. Potential bias could occur from parental over-reporting of sleep associated problems among children. However, a recent study showed a significant association between parental reported HS and objectively measure pathologic snoring.[31-33] While the absence of PSG hindered the accuracy of OSA diagnosis, our data still indicated a trend to support the increased prevalence of sleep disturbance in thalassemia children compared to the general population. This suggests that HS in pediatric thalassemia should be closely monitored and recognized as an at-risk population for sleep-related issues. Furthermore, in our study did not find any significant associations for many variables in HS. However, this lack of significant findings may be attributed to the limited sample size utilized in the study. Therefore, further research with a larger sample size is necessary to explore this trend in greater detail and obtain more robust conclusions.

CONCLUSION

Our study reviewed a higher of HS in thalassemia children. Several factors demonstrated strong associated with HS including nasal congestion, and male gender. The quality of life of our thalassemia children assessed by OSA-18 was generally good, together with low serum ferritin levels when compared to previous studies. The implementation of standard of care protocol for TDT patients, including regular blood transfusion and effective iron chelation, may contribute to the attenuation of nasopharyngeal narrowing in thalassemia patients. The current authors believe that parent reported HS can be used as a simple indicator for early diagnosis and appropriate medical intervention for OSA.

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Conflict of Interest Statement

The authors declare no conflict of Interest.

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Characteristics, Outcomes and Bed Utilization of 15-to-18-Year-Old Adolescents in a Pediatric Intensive Care Unit in Thailand

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ABSTRACT

Objective: There is a trend toward expanding pediatric age range. At the borderline age of 15-18 years, the characteristics of patients requiring intensive care admission in low- and middle-income countries are unknown. Our institution recently changed the cut-off age for pediatric care from 15 to 18 years. The objective of this study was to determine the characteristics, outcomes and bed utilization of patients aged 15-18 years admitted to pediatric intensive care unit (PICU) after this change.

Materials and Methods: This is an observational study at a tertiary medical PICU. Patients aged 15-18 years admitted to PICU in 2019-2020 were eligible. Medical records were reviewed.

Results: There were 1030 PICU admissions from all age groups. Fifty-two patients aged 15-18 years were admitted, with a total of 68 admissions. Eighty-seven percent had chronic conditions. The most common acute conditions were septic shock and infection, the most common chronic conditions were systemic lupus erythematosus (SLE) and hematologic malignancies. Forty-seven percent required mechanical ventilation, 36% required vasoactive medications and 27% required continuous renal replacement therapy. PICU mortality rate in patients aged 15-18 years old was significantly higher than that in the younger age group (13.24% vs 3.64%, $p = 0.002$). Hospital mortality rate was 22%.

Conclusion: Patients aged 15-18 years requiring PICU admission had high prevalence of chronic conditions and high mortality risk. Special attention should be given to the care of this group. The most common acute conditions were septic shock and infection. The most common chronic conditions were SLE and hematologic malignancies.

Keywords: Mortality; epidemiology; diagnosis (Siriraj Med J 2023; 75: 555-559)

INTRODUCTION

The pediatric age range varies across different countries. In the US, the cut-off age for pediatric inpatient care ranges between 15 and 18 years.[1] In the UK, this cut-off is generally 16 years.[2,3] In Thailand, this cut-off is 15 years for most hospitals. There has been a trend toward increasing the pediatric age range.[4] In fact, the American Academy of Pediatrics issued a policy toward eliminating the age limit for pediatric care in 2017.[5] Diseases affecting adolescents are likely to be different from those affecting younger children and may be more similar to those affecting young adults. Moreover, the prevalence of chronic conditions in adolescents and young adults is currently high.[6] Pediatric residency training generally puts an emphasis on child health supervision, growth, development and childhood diseases, but training in

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adult diseases and chronic diseases may be inadequate. With the expanding age range, pediatricians will likely encounter different disease characteristics from what they have been trained for during residency.

Pediatric intensive care unit (PICU) is an important patient care setting because its patients are critically ill and require appropriate emergency treatment. Therefore, it is important to know the disease characteristics of patients in the expanding pediatric age range – 15 to 18 years old – who are admitted to intensive care units (ICU), so that pediatric training can be tailored to serve this age group as well. To our knowledge, ICU data of patients in this age range from low- and middle-income countries are lacking. Our institution changed the cut-off age for pediatric care from 15 to 18 years on 1 Jan 2019. The primary objective of this study was to determine the characteristics and clinical outcomes of patients aged 15 to 18 years old admitted to PICU after this change. With the expanding age range, it is expected that an increasing number of patients will be admitted to PICU, but the magnitude of the increase is unknown in our healthcare setting. Therefore, the secondary objective was to determine PICU bed utilization of patients aged 15 to 18 years old using admission numbers and bed-day approach.[7]

MATERIALS AND METHODS

This is an observational study conducted in patients aged 15 to 18 years old who were admitted to PICU at Siriraj Hospital, Bangkok, Thailand from 1 Jan 2019 to 31 Dec 2020. Siriraj Hospital PICU is a tertiary-level general medical PICU. The study was approved by Siriraj Institutional Review Board (COA no. Si 099/2020) on 3 Feb 2020. Informed consent was obtained from legal guardians for cases with prospective data collection from 3 Feb 2020 onward. Patient assent was also obtained for cases where patients were able to communicate and give assent.

All patients aged 15 to 18 years old admitted to PICU between 1 Jan 2019 and 31 Dec 2020 were eligible. There were no exclusion criteria. Patients' medical records were reviewed. The collected data included age, sex, length of PICU stay, length of hospital stay, PICU mortality, hospital mortality, diagnoses (acute and chronic) and invasive treatment received in PICU. Data from PICU admission database were also reviewed for length of PICU stay and PICU mortality in all patients admitted to PICU.

Primary outcomes were diagnoses, PICU mortality and hospital mortality rates. The secondary outcome was PICU bed utilization measured by admission numbers and bed-days.[7] In patients who had multiple PICU admissions, diagnosis data from multiple admissions were unified to avoid duplication of diagnosis counts. Patients who were electively admitted to PICU only for one-day procedure e.g., dialysis catheter insertion, were excluded from diagnosis data analysis because these patients were not part of the critically-ill population of interest. Chronic condition was defined as a medical condition that could be expected to last at least 12 months.[8] Bed-days were calculated from the sum of the length of stays of patients admitted to PICU during the study period.

Categorical variables were presented as counts and percentages, and they were analyzed with Fisher's exact test. Shapiro-Wilk test was used to determine the normality of continuous variables. Continuous variables were presented as median and interquartile range (IQR) and were analyzed with Mann-Whitney *U* test. Statistical significance was defined as P value < 0.05. Statistical analysis was performed with PASW Statistics 18 (SPSS Inc., Chicago, IL).

RESULTS

There were 1030 PICU admissions from all age groups during the 2-year study period. There were 962 admissions of patients aged 0 to 14 years old. There were 52 patients aged 15 to 18 years old admitted to PICU, with a total of 68 admissions (6 patients were admitted twice, 1 patient was admitted 3 times, 1 patient was admitted 4 times and 1 patient was admitted 6 times). Out of these 52 patients, there were 7 patients who were electively admitted only for a one-day procedure, leaving 45 patients for analysis. Among these 45 patients, 39 (87%) had chronic conditions. Among 39 patients with chronic conditions, 26 patients were admitted to PICU due to progression of their underlying chronic conditions, 13 patients were admitted to PICU due to acute illness (mostly infectious diseases) on top of preexisting chronic conditions. Table 1 shows the most common acute and chronic conditions and disease groups. The most common acute condition requiring PICU admission was septic shock. Infection was the most common acute disease group. The most common chronic condition was systemic lupus erythematosus (SLE). Oncology was the most common chronic disease group. The details of diagnoses of each patient are shown in the supplementary appendix.

Patient demographic characteristics, treatments and outcomes are shown in Table 2. Forty-four percent were female. In terms of intensive care therapies, 47% required invasive mechanical ventilation, 36% required vasoactive medications, and 27% required continuous

TABLE 1. The most common acute and chronic conditions and disease groups among PICU patients aged 15 to 18 years old.

Acute conditions (N = 45)	Acute disease groups (N = 45)	Chronic conditions (N = 39)	Chronic disease groups (N = 39)
Septic shock 6 (13%)	Infection 11 (24%)	Systemic lupus erythematosus 5 (13%)	Oncology 14 (36%)
SVC obstruction 3 (7%)	Neurology 9 (20%)	Acute myeloid leukemia 3 (8%)	Rheumatology 5 (13%)
Acute liver failure, dengue shock syndrome, diabetic ketoacidosis 2 (4%) each	Gastroenterology 6 (13%)	Acute lymphoblastic leukemia 3 (8%)	Genetics, neurology 4 (10%) each

SVC = superior vena cava

TABLE 2. Patient demographic characteristics, treatments and outcomes.

Characteristics	Results (N = 45)
Age (years) – median (interquartile range)	16 (15.38, 17.04)
Female	20 (44%)
Chronic conditions present	39 (87%)
Source of pediatric intensive care unit admission	
Ward	17 (38%)
Emergency department	10 (22%)
Operating room or procedure room	10 (22%)
Transferred from other hospitals	8 (18%)
Invasive treatment received	
Invasive mechanical ventilation	21 (47%)
Vasoactive medications	16 (36%)
Continuous renal replacement therapy	12 (27%)
High-frequency oscillatory ventilation	1 (2%)
Extracorporeal membrane oxygenation	2 (4%)
Plasmapheresis	3 (7%)
Leukapheresis	1 (2%)
Angiogram and embolization	2 (4%)
Hospital mortality	10 (22%)
Hospital length of stay (days) – median (interquartile range)	20 (9.5, 42)

renal replacement therapy. The overall hospital mortality rate was 22%. The mortality rate was highest in patients who were admitted to PICU due to acute illness on top of preexisting chronic conditions at 38% (5 out of 13 patients). The mortality rate in patients who were admitted to PICU due to progression of their underlying chronic conditions was 15% (4 out of 26 patients), while mortality rate in patients without chronic conditions was 17% (1 out of 6 patients). However, these differences in mortality rates did not reach statistical significance (P value = 0.247).

PICU mortality rate in adolescents aged 15 to 18 years old was 9/68 (13.24%). PICU mortality rate in patients aged 0 to 14 years was 35/962 (3.64%). PICU mortality was significantly higher in adolescents aged 15 to 18 years old compared to the younger age group; odds ratio = 4.040, 95% confidence interval (1.855, 8.799), P value = 0.002. Lengths of PICU stays were not different between adolescents aged 15 to 18 years (median = 2 days, IQR = 1, 6) and the younger age group (median = 2 days, IQR = 1, 5; P value = 0.651).

During the 2-year study period, the number of PICU bed-days of all patients was 5073. The number of PICU bed-days of adolescents aged 15 to 18 years old was 384. The number of PICU bed-days of patients in the younger age group was 4689. PICU bed-days of adolescents aged 15 to 18 years old accounted for 7.57% of total bed-days and was equal to 8.19% of those in the younger age group.

DISCUSSION

The data from our cohort of adolescent PICU patients aged 15 to 18 years old showed that the most common acute condition was septic shock, and the most common acute disease group was infection. This is not surprising, given that septic shock and infection are commonly encountered in PICUs.[2,9] A large multicenter cohort study showed that the most common diagnosis categories of patients aged 16 to 19 years old admitted to adult ICUs and PICUs in the UK were trauma, respiratory, overdose, neurology, cardiovascular and infection.[2] This is similar to our data except for trauma and overdose. There were no trauma patients in our cohort because our setting is a medical PICU in an urban area where trauma cases are not frequently transferred in. The prevalence of overdose in our cohort was low for unknown reasons. However, it is possible that the types of drugs abused differ in different countries. The most common drugs of abuse in Thailand are cannabis and methamphetamine.[10-12] These drugs do not usually cause respiratory depression requiring endotracheal intubation and ICU admission, in contrast to other drugs, such as opioids, which can cause respiratory depression necessitating endotracheal intubation and ICU admission.

Regarding chronic conditions, it is notable that the most common chronic condition in our cohort was SLE. SLE is generally viewed as a disease mainly affecting women of childbearing age[13], but in fact, 10 to 20% of SLE patients are diagnosed during childhood.[14] SLE causes inflammation and dysfunction in multiple organ systems, and childhood-onset SLE is more aggressive than adult-onset SLE.[15] SLE patients who require ICU admission have a high mortality rate, ranging from 18.4 to 78.5% with a median of 29.6% in a recent systematic review.[16] Considering this fact along with the high prevalence of SLE in our cohort, it is important for pediatric intensive care and pediatric residency training to also focus on managing severe SLE and its complications. In terms of disease groups, oncology was the most common chronic disease groups in our cohort. This is probably because our hospital is a tertiary referral center. Pediatric oncologic patients who require PICU admission have mortality risk as high as 15 to 40%[17], higher than other PICU populations. This probably contributed to the high mortality in our cohort.

The findings that our cohort of adolescents aged 15 to 18 years old had a high hospital mortality rate (22%) and had a significantly higher PICU mortality rate than the younger age group are important. This was probably because of the very high prevalence of chronic conditions in our cohort (87%), and the fact that some chronic conditions are associated with high PICU mortality as mentioned above. Overall, adolescent patients aged 15 to 18 years old with chronic conditions who require ICU admission should be seen as having a higher risk of mortality, and special attention should be given to the care of these patients. Given the high prevalence of chronic conditions seen in our cohort, pediatric training should be tailored to cover common chronic conditions encountered in adolescents. Pediatric nursing education also needs tailoring to serve this adolescent patient population. Many chronic conditions can cause problems in multiple organ systems and necessitate involvement from multiple subspecialties. With this increased complexity, patient care collaboration between multiple subspecialties cannot be overemphasized. If appropriate, a dedicated care coordinator team can facilitate collaboration and communication between multiple subspecialties and families in the care for such complex patients.

In terms of bed utilization in this study, PICU admission number in patients aged 15 to 18 years old

accounted for 6.6% of the total admission number and was equal to 7.07% of admission number in the younger age group. PICU bed-days in this age group accounted for 7.57% of total bed-days and was equal to 8.19% of bed-days in the younger age group. For a tertiary medical PICU with similar setting to ours, if the age cut-off is expanded from 15 to 18 years, one may approximately expect a 7.07% increase in admission number and an 8.19% increase in bed-days.

There are some limitations to this study. Being a single center study conducted in a tertiary medical PICU limits generalizability, and this study has a small sample size. Some disease populations are likely under-represented, such as trauma and surgical patients. The PICU mortality comparison between age groups in this study was analyzed without correction for severity score because this score was not routinely collected at the time that this study was conducted. Nevertheless, the finding that large proportions of patients required invasive treatments implies that our cohort had high disease severity.

In conclusion, this study found that adolescents aged 15 to 18 years old requiring PICU admission had high prevalence of chronic conditions and high mortality risk. Special attention should be given to the care of this group. The most common acute conditions were septic shock and infection. The most common chronic conditions were SLE and hematologic malignancy. Pediatric intensive care training should also be tailored to serve this population of expanding pediatric age range.

Conflict of interest

All authors have no conflict of interest to disclose.

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Correlation between Manual Vacuum Aspiration and Endometrial Cell Sampler in Abnormal Uterine Bleeding

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ABSTRACT

Objective: Office endometrial biopsy using an endometrial cell sampler is an accepted method of obtaining endometrial tissue for histopathologic evaluation in women with abnormal uterine bleeding (AUB). Manual vacuum aspiration (MVA) is considered an alternative method, but data specific to the use of MVA is limited. This study aimed to evaluate the efficacy of MVA compared to endometrial cell sampler for diagnosing causes of AUB.

Materials and Methods: This prospective study enrolled women aged ≥35 years who presented with AUB during August 2015 to June 2016. For each patient, endometrial biopsy using an endometrial cell sampler was first performed followed by MVA. Correlation of endometrial histopathology between methods were analyzed using Kappa statistic. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were evaluated.

Results: Of the 162 patients enrolled, the data from 151 women were analyzed. Correspondence of histopathologic finding between tissue obtained from endometrial cell sampler and MVA was 72.8% (Kappa: 0.51). Correspondence of histopathologic finding between tissue obtained from MVA and the final most severe pathology used for treatment decision was 84.1% (Kappa: 0.72). MVA diagnosed all cases of malignancy, but endometrial cell sampler missed one case of malignancy. The overall sensitivity, specificity, PPV, and NPV of MVA was 84.5%, 100%, 100%, and 91.2%, respectively.

Conclusion: The histopathologic findings of MVA were in good agreement with those of endometrial cell sampler, and MVA had high accuracy for diagnosing endometrial pathology. MVA is suggested as a reliable alternative procedure for endometrial biopsy in women with AUB.

Keywords: Abnormal uterine bleeding; endometrial biopsy; endometrial sampling; manual vacuum aspiration
(Siriraj Med J 2023; 75: 560-566)

INTRODUCTION

Abnormal uterine bleeding (AUB) is a common gynecologic problem that affects women of all ages, and that is responsible for a large proportion of visits in outpatient gynecologic practice.[1] AUB was reported as the most common gynecological endocrine abnormalities presenting among new patients in Siriraj Hospital.[2] Endometrial tissue for histopathologic evaluation is the standard investigation in indicated women with AUB, especially in women with risk factors for endometrial cancer. There are a number of techniques that can be employed to obtain endometrial tissue, including hysteroscopy, uterine curettage, and various endometrial sampling devices.[1,3,4]

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Uterine curettage has been a mainstay method for obtaining endometrial tissue for endometrial pathology evaluation in patients with AUB, but this technique has largely been replaced by the use of endometrial sampling devices, which achieve the same objective via a well-tolerated, less invasive, office-based procedure that has less complications. Endometrial sampling devices were reported to yield accuracy comparable to that of uterine curettage for diagnosis of endometrial pathology in patients with AUB.[1,5-10] However, some drawbacks of endometrial sampling devices had been reported as insufficient sample and limited capacity in diagnosis of focal endometrial lesion such as endometrial polyp.[11-15]

Manual vacuum aspiration (MVA), which is a procedure that employs the use of a manual vacuum aspirator, a cannula, and a vacuum syringe, is a widely used in obstetric procedure for termination of early pregnancy. Compared to the standard procedure (uterine curettage), MVA could provide several advantages such as less pain, fewer complications, less invasive, and office-based procedure. However, MVA is not yet widely used due to limitation of supporting data.[16,17] Previous studies found similar tissue adequacy and similar histopathologic finding between MVA and uterine curettage.[18-21] Moreover, MVA was reported to have 86.4-96% sensitivity and 100% specificity for diagnosing endometrial pathology.[19-23] MVA has, therefore, been proposed as an alternative technique for obtaining an endometrial biopsy in women with AUB.

Despite the fact that MVA and endometrial sampling devices are both noninvasive office-based techniques, few studies have compared diagnostic performance between the two methods. Two previous studies reported that the two techniques showed comparable tissue adequacy and diagnostic accuracy[17,23]; however, those studies did not compare the two procedures in the same woman. Moreover, the mechanism of MVA instrument may create a higher degree of negative suction pressure than endometrial sampling devices which could improve the limitation of these devices in sampling focal endometrial lesion. Accordingly, the aim of this study was to prospectively evaluate the diagnostic efficacy of MVA compared to endometrial cell sampler for investigating the causes of AUB.

MATERIALS AND METHODS

This prospective study was conducted during August 2015 to June 2016 at the Department of Obstetrics and Gynecology of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Ethical approval was obtained from the Siriraj Institutional Review Board (SIRB) (COA no. Si 237/2015), and written informed consent to participate was obtained from all study women.

Thai women aged 35 years or more presenting with symptom of AUB at outpatient gynecology unit were eligible for inclusion. Women who were currently pregnant, who had known abnormal cervical cytology, or who were currently using any type of hormonal therapy were excluded. Any enrolled participant with a failed procedure, which was identified as failure to pass the instrument into the uterine cavity, was withdrawn from the study.

All procedures were performed in an outpatient setting by a single well-trained and experienced gynecologist (CS). Participants were placed in the dorsal lithotomy position after voiding. The perineum was prepped and draped using sterile technique. A sterile bivalve speculum was then gently inserted into the vagina. A Wallach® Endocell™ Endometrial Cell Sampler (Cooper Surgical, Inc., Trumbull, CT, USA) was then inserted through the endocervix and into the endometrial cavity. The entire endometrial cavity was sampled by gently moving the device in a 360-degree arc back and forth at least 2 times. That tissue was collected in a container. Finally, an MVA cannula ranging from No. 3 to No. 12 was titrated until the size of the cannula properly fit the size of the endocervical canal in order to create the proper negative pressure for each participant and to improve the effectiveness of MVA, and then the cannula was passed into the endometrial cavity. The number of the largest cannula used was recorded. Negative pressure or vacuum was generated using an Ipas MVA Plus® Aspirator (DKT Women Care Global, London, United Kingdom), and then the aspirator was connected to the cannula. The MVA was moved gently at least two complete 360-degree arcs back and forth within the endometrial cavity. The tissue obtained via MVA was then collected in a second container. Any complications that developed during any procedures were recorded.

The endometrial tissue collected by endometrial cell sampler and MVA was computer randomized into containers labeled 'Endometrium A' or 'Endometrium B' in order to blind the pathologist to the method of sampling. A single specialized gynecologic pathologist (MW) evaluated tissue adequacy and interpreted the histopathologic results. Endometrial glands and stroma both needed to be present in the endometrial tissue specimens as criteria for determining 'tissue adequacy'. In specimens from menopausal women, the term 'tissue adequacy' was substituted with the term 'atrophic endometrium'. The pathology results from endometrial cell sampler and MVA were compared. All specimens were classified

into 4 groups, including 1) inadequate specimen, 2) physiologic changes, 3) benign pathology, or 4) malignant pathology. 'Inadequate specimen' was defined as tissue presence with an absence of endometrial gland and/or endometrial stroma. 'Physiologic changes' was defined as the presence of inactive endometrium, proliferative endometrium, secretory endometrium, glandular and stromal breakdown, or atrophic endometrium. 'Benign pathology' was defined as the presence of endometritis or endometrial polyp. 'Malignant pathology' was defined as the presence of endometrial hyperplasia or endometrial cancer. The final diagnosis in each patient was the most severe pathology from either endometrial cell sampler or MVA. Further management was planned according to the most severe pathology.

Sample size calculation and statistical analysis

The sample size was calculated using data from a previous study that reported a Kappa statistic of 0.56 and a proportion of abnormal endometrial pathology of 35%.[20] Using a sensitivity of 87.7%, a specificity of 100%, and a level of confidence of 95%, the minimum number of enrolled patients was 137. Assuming a 10% dropout rate for any reason, the final number of participants to be recruited was 151.

All statistical analyses were performed using PASW Statistics version 21 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used to summarize patient baseline characteristics. Agreement of endometrial pathology between endometrial cell sampler and MVA, and between MVA and the final most severe pathology was analyzed using Kappa statistic with a value closer to 1.0 indicating better agreement. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MVA for diagnosis of the most severe pathology was calculated. A *p*-value less than 0.05 was considered to indicate statistical significance.

RESULTS

One hundred and sixty-two patients were prospectively enrolled in this study; however, 11 of those patients were withdrawn from the study due to failed procedure. The data from the remaining 151 patients were included in the final analysis. Baseline patient characteristics are shown in Table 1. The mean age and mean body mass index were 47.6±8.5 years and 26.0±4.5 kg/m^2, respectively. The most frequently used MVA cannula sizes were No. 3 (21.9%), No. 4 (34.4%), and No. 5 (33.8%).

Endometrial cell sampler and MVA obtained 95.4% and 86.8% tissue adequacy, respectively (p=0.002). Table 2 shows the histopathologic results compared between MVA and endometrial cell sampler. Correspondence of pathologic finding between methods was 72.8%, and pathological agreement was moderate (Kappa: 0.51). Pathologic results from MVA showed inadequate specimen in 15 cases of 'Physiologic change' pathology obtained from endometrial cell sampler. MVA diagnosed all malignant pathology (n=4), but endometrial cell sampler misdiagnosed 1 case of endometrial cancer

There was high correspondence of pathologic finding (84.1%) and substantial agreement (Kappa: 0.72) between MVA and the final most severe endometrial pathology that was used to guide management (Table 3). The accuracy of MVA for diagnosis of the final most severe endometrial pathology is shown in Table 4. The overall sensitivity, specificity, PPV, and NPV was 84.5% (95% confidence interval [CI]: 72.6-92.7), 100%, 100%, and 91.2% (95%CI: 83.9-95.9), respectively. No serious complication occurred in this study.

DISCUSSION

Endometrial tissue biopsy is an investigation that is employed to evaluate for endometrial pathology in indicated women with AUB. Outpatient endometrial sampling devices have become a method of choice for this purpose. Thus, endometrial cell sampler, Wallach® Endocell™ Endometrial Cell Sampler, was considered to be the standard procedure in this study. MVA is considered to be an alternative and effective endometrial biopsy method. However, few studies have investigated the efficacy of endometrial tissue collection for evaluation of endometrial pathology compared between MVA and endometrial cell sampler, and no study has compared these two biopsy collection modalities in the same woman. In this study, we directly compared the diagnostic efficacy of an endometrial sampling device (i.e., Wallach® Endocell™ Endometrial Cell Sampler) and MVA. Our results demonstrated MVA to have high accuracy for diagnosing endometrial pathology, and showed high correspondence of pathologic findings between MVA and endometrial cell sampler.

In our study, tissue adequacy identified by pathologist was 95.4% from endometrial cell sampler and 86.8% from MVA, which was statistically significantly different between methods. Previous studies reported 85-98% tissue adequacy from endometrial sampling devices[5-7,10,14,17,23] and 81-99% from MVA.[17-19,23,26] This difference in tissue adequacy between methods among studies could result from different factors, such as study design, the types of devices used, the characteristics of study participants, and differences in the level of operator experience. In contrast to the finding of the present study, the previous

TABLE 1. Baseline characteristics of the study population (N=151).

Characteristics	Mean ± SD or n (%)
Age (years)	47.6±8.5
Body mass index (kg/m^2)	26.0±4.5
Parity	
0	42 (27.9%)
≥1	109 (72.1%)
Menopausal status	
Premenopause	112 (74.2%)
Menopause	39 (25.8%)
Education	
Primary school	34 (22.5%)
High school	43 (28.5%)
Bachelor's degree or higher	74 (49.0%)
Occupation	
Housewife/unemployed	34 (22.5%)
Employee	29 (19.2%)
Government officer	33 (21.9%)
Private business owner	32 (21.2%)
Other	23 (15.2%)
Presence of family history of cancer	
Breast cancer	14 (9.3%)
Colorectal cancer	12 (7.9%)
Ovarian cancer	1 (0.7%)
Other type of cancer	28 (18.5%)
MVA cannula number	
3	33 (21.9%)
4	52 (34.4%)
5	51 (33.8%)
≥6	15 (9.9%)

Abbreviations: SD, standard deviation; MVA, manual vacuum aspiration

TABLE 2. Histopathology obtained from MVA and endometrial cell sampler (N=151).

		Endometrial Cell Sampler				
		Inadequate pathology	Physiologic change	Benign pathology	Malignant pathology	Total
MVA	Inadequate pathology	5 (3.3%)	15 (9.9%)	0 (0.0%)	0 (0.0%)	20 (13.2%)
	Physiologic change	2 (1.3%)	71 (47.0%)	9 (6.0%)	0 (0.0%)	82 (54.3%)
	Benign pathology	0 (0.0%)	14 (9.3%)	30 (19.9%)	0 (0.0%)	44 (29.1%)
	Malignant pathology	0 (0.0%)	0 (0.0%)	1 (0.7%)	4 (2.6%)	5 (3.3%)
	Total	7 (4.6%)	100 (66.2%)	40 (26.5%)	4 (2.6%)	151 (100%)

Abbreviation: MVA, manual vacuum aspiration

TABLE 3. Histopathology obtained from MVA and the final most severe endometrial pathology (N=151).

		The final most severe endometrial pathology				
		Inadequate pathology	Physiologic change	Benign pathology	Malignant pathology	Total
MVA	Inadequate pathology	5 (3.3%)	15 (9.9%)	0 (0.0%)	0 (0.0%)	20 (13.2%)
	Physiologic change	0 (0.0%)	73 (48.3%)	9 (6.0%)	0 (0.0%)	82 (54.3%)
	Benign pathology	0 (0.0%)	0 (0.0%)	44 (29.1%)	0 (0.0%)	44 (29.1%)
	Malignant pathology	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (3.3%)	5 (3.3%)
	Total	5 (3.3%)	88 (58.2%)	53 (35.1%)	5 (3.3%)	151 (100%)

Abbreviation: MVA, manual vacuum aspiration

TABLE 4. Accuracy of MVA for diagnosis of the final most severe endometrial pathology (N=151).

		The final most severe endometrial pathology		
		Benign or malignant pathology	Inadequate pathology or physiologic change	Total
MVA	Benign or malignant pathology	49 (32.5%)	0 (0.0%)	49 (32.5%)
	Inadequate pathology or physiologic change	9 (6.0%)	93 (61.6%)	102 (67.5%)
	Total	58 (38.4%)	93 (61.6%)	151 (100%)

Abbreviation: MVA, manual vacuum aspiration

2 studies that compared endometrial sampling devices and MVA[17,23] found no significant difference in tissue adequacy between methods. One possible explanation for our significantly different tissue adequacy between groups may be that we directly compared both methods in each woman, whereas previous studies did not directly compare both techniques in the same participant. In our study, endometrial cell sampler was used before MVA, so it could be argued that normal and pathologic endometrial tissue is more available and easier to harvest during the endometrial cell sampler procedure, with less tissue being available for harvest during MVA.

Our results showed 72.8% correspondence of pathologic finding with moderate pathologic agreement between endometrial cell sampler and MVA. Moreover, MVA showed 84.1% correspondence of pathologic finding between MVA and the final most severe endometrial pathology used to guide the treatment, which is considered a high level of correspondence. Our result indicates that MVA has comparable efficacy to endometrial cell sampler for investigation and diagnosis of the cause of AUB. Previous studies compared the concordance of pathologic results between either endometrial sampling devices or MVA with uterine curettage or hysterectomy. Tissue obtained from endometrial sampling devices showed 86-94% concordance of pathologic results with uterine curettage[7,9,14], while tissue obtained from MVA showed 63-64% concordance of pathologic finding with uterine curettage or hysterectomy.[20,21,26]

No previous study has directly compared diagnostic accuracy between MVA and endometrial sampling devices. Only one study compared efficacy between MVA and endometrial sampling device, but it did not directly compare both procedures in the same woman. The result of that previous study showed comparable sensitivity and specificity between the two evaluated methods, and the diagnostic efficacy of MVA was 86.4% sensitivity and 96% specificity, which was comparable to our result.[23] Several studies had previously compared the diagnostic efficacy of either MVA or endometrial sampling devices with uterine

curettage. Endometrial sampling devices were reported to have high sensitivity and specificity for diagnosing various endometrial pathologies, such as endometrial hyperplasia, endometrial carcinoma, endometritis[5-7,9], and MVA demonstrated high sensitivity and specificity for diagnosing several endometrial pathologies[19-22], which is consistent with our result. Our study also showed high accuracy of MVA for diagnosing endometrial cancer, while endometrial sampling device misdiagnosed one case of malignancy. However, we could not evaluate the efficacy of MVA for diagnosing endometrial hyperplasia due to there being no endometrial hyperplasia cases in our study.

MVA could produce higher degree of negative suction pressure in the cannula and syringe compared to office endometrial sampling devices. This higher vacuum may lead to more effective tissue retrieval compared to endometrial sampling devices, especially in focal endometrial lesion, and this was reported to be a limitation of endometrial sampling devices.[12,27] Moreover, we used the titration technique and selected the MVA cannula with the best fit to the endocervical canal in order to create the proper negative pressure for each participant and to improve the effectiveness of MVA.

Study strengths and limitations

The strengths of this study include its prospective design, direct comparison of the histopathologic diagnosis from both methods in each woman. All procedures were performed by one gynecologist, and all endometrial specimens were evaluated by one gynecologic pathologist. In addition, the gynecologic pathologist was blinded to the specimen collection method, which reduced bias of histopathology assessment.

This study also has some mentionable limitations. First, the study design may not fully reflect the effectiveness of MVA because MVA was performed following endometrial cell sampler, which was considered to be the standard procedure in this study. Second, we had a small number of endometrial carcinoma and no cases of endometrial hyperplasia. Further study regarding the cost-effectiveness of MVA should be investigated to support the benefit of using MVA as an alternative outpatient endometrial biopsy method in women with AUB in low-resource settings.

CONCLUSION

MVA had high correspondence of pathologic findings with endometrial cell sampler and with the final most severe endometrial pathology that was used to guide management. The results of this study suggest MVA as a reliable alternative minimally invasive outpatient procedure for obtaining an endometrial biopsy in women with AUB.

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Conflict of interest declaration

All authors declare no personal or professional conflicts of interest, and no financial support from the companies that produce and/or distribute the drugs, devices, or materials described in this report.

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The Effects of Storage Time at 2–8 Degrees Celsius on the Stability of von Willebrand Factor in Thawed, Platelet-Poor Plasma

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ABSTRACT

Objective: To investigate VWF stability in thawed plasma by comparing immediately thawed samples with plasma stored at 2–8 °C for 24–96 hours.

Materials and Methods: Plasma from healthy subjects with normal coagulation times and VWF panels was stored at -20 °C for one week. After thawing (at 0 hours), VWF:antigen (VWF:Ag), VWF:glycoprotein Ib binding assay (VWF:GPIbM), and VWF:collagen binding assay (VWF:CB) were assayed. The remaining plasma was stored at 2–8 °C and assayed at 24, 48, 72, and 96 hours. Differences between levels at baseline and 24, 48, 72, and 96 hours were deemed significant when P was < 0.05.

Results: Thirty-five samples were enrolled, with 25 from healthy subjects (VWF:Ag levels > 0.50 kIU/L). Median levels (interquartile range) were as follows: VWF:Ag = 0.91 (0.72–1.06) kIU/L; VWF:GPIbM = 0.85 (0.69–1.04) kIU/L; and VWF:CB = 0.78 (0.62–0.97) kIU/L. VWF:Ag remained stable for 72 hours, while VWF:GPIbM decreased significantly after thawing. VWF:CB declined after 48 hours at 2–8 °C. Similar stability trends were observed in 10 additional samples from VWD patients (VWF:Ag = 0.42 (0.36–0.46) kIU/L).

Conclusion: VWF:Ag and VWF:CB are stable in thawed plasma for 72 hours. VWF:GPIbM is less stable and should not be kept longer than 24 hours. Immediate testing of VWF:GPIbM after thawing is recommended.

Keywords: Stability; Thawed plasma; VWF:Ag; VWF:CB; VWF:GPIbM (Siriraj Med J 2023; 75: 567-574)

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, resulting from quantitative or qualitative abnormalities of von Willebrand factor (VWF). Currently, a provisional diagnosis of VWD requires both clinical and laboratory criteria to be met. The clinical criteria include the presence of abnormal bleeding symptoms with or without a familial history of VWD. The laboratory criteria involve the presence of abnormal quantitative or qualitative VWF assays.[1,2] Bleeding symptoms can be assessed empirically or preferably through systematic evaluation using scoring systems such as the bleeding assessment tool of the International Society on Hemostasis and Thrombosis.[3] If the bleeding score exceeds the normal cutoff value, further investigations are recommended.[3] VWF panel assays include a quantitative measurement (VWF:antigen; VWF:Ag) and functional assessments of platelet- and collagen-binding abilities.[4]

A definitive diagnosis of VWD and its subtypes relies on accurate results from individual tests. For instance, the possibility of VWD subtypes 2A, 2B, or 2M is suggested by a ratio of < 0.7 between each of the following factors

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and VWF:Ag: VWF:ristocetin cofactor (VWF:RCo), VWF:glycoprotein Ib binding by ristocetin (VWF:GPIbR), VWF:glycoprotein Ib binding by multimer analysis (VWF:GPIbM), and VWF:collagen binding (VWF:CB).[4]

Various external and environmental factors, such as age, blood group, and concurrent inflammation, can interfere with test results.[5,6] Additionally, pre-analytical processes, including blood collection, sample storage methods (immediate plasma spinning or whole blood), and storage temperature, can significantly impact the outcomes.[7] Given the limited number of laboratories capable of performing VWF assays, samples are often sent to referral laboratories. Immediate plasma spinning is recommended by Magnette et al due to the crucial role of pre-analytical processes.[7]

If assays can be conducted within 4 hours, plasma should be stored at 20–28 °C. Otherwise, freezing the plasma at -20 to -80 °C until testing is advised.[7,8] During transportation from a blood collection center to a referral laboratory, frozen plasma should be kept in dry ice.[8] Nevertheless, there is a risk of partially melted plasma inadvertently reaching the referral laboratory. Furthermore, frozen plasma, which requires three assays, is typically collected and transported in a single tube. In practical terms, conducting all three procedures on the same day may not be feasible, and the practice of repeated thawing-refreezing-thawing for testing on different days is not recommended.[9] As a result, thawed plasma is usually stored at 2–8 °C until the tests can be conducted. However, the stability of VWF in plasma over an extended duration remains uncertain.

We compared the stability of VWF:Ag, VWF:GPIbM, and VWF:CB in thawed plasma that was obtained from patients with VWD and healthy controls and stored at 2–8 °C for up to 96 hours.

MATERIALS AND METHODS

Plasma samples

The research received approval from the Institutional Review Board (COA no. Si 575/2017). Between November 2017 and September 2019, a total of 35 participants were enrolled, comprising 25 healthy subjects and 10 patients with either VWD type 1 or type 2A. Briefly, 1-milliliter samples were collected from 3.2% citrated plasma. Following blood collection, platelet-poor plasma (PPP) was obtained by centrifugation at 2,000 g for 15 minutes at room temperature. All samples from healthy subjects exhibited normal prothrombin time, activated partial thromboplastin time, VWF level, and function. To simulate typical sample transportation from other hospitals, each 1-milliliter sample was stored at -20 °C for a week before the experiment, with a time interval of within 4 hours between blood collection and plasma freezing. Thawed samples were immediately subjected to VWF panel assays. One hundred microliters of the remaining plasma from each sample were aliquoted into individual Eppendorf tubes and stored at 2–8 °C for 24, 48, 72, and 96 hours, with additional VWF panel assays conducted at those specific time points.

VWF assays

Quantitative analysis of VWF:Ag was performed using enzyme-linked immunosorbent assay, following the method described by Ingerslev.[10] The platelet-binding function of VWF:GPIbM, as described by Bodo et al, was assessed by coating plastic beads with gain-of-function recombinant glycoprotein Ib (Innovance VWF Ac).[11] The addition of plasma, serving as a source of VWF, initiated aggregation of the plastic beads, with the percentage of light transmission directly correlating with the platelet-binding capacity of VWF.[11] Finally, VWF:CB was measured through enzyme-linked immunosorbent assay, where a microtiter plate was coated with human collagen type 3 (SouthernBiotech).[12] The optical density directly correlated with the binding affinity of VWF to collagen.[12]

Statistical analyses

VWF panel results for continuous variables following a normal distribution are presented as the means and standard deviations. Nonnormally distributed continuous variables are reported as medians with interquartile ranges. Categorical variables are summarized as the number and percentage of samples. We defined the threshold of allowable bias of VWF to be 6.9% (calculated as the VWF level at 0 hours minus allowable errors). Decreases in the measured values of VWF that exceeded 6.9% were considered clinically significant for VWF instability.[13]

To compare the VWF results of thawed plasma stored at different time points with the defined threshold, a paired t-test was used for normally distributed outcomes. The Wilcoxon signed-rank test was employed for two related samples with a nonnormal distribution. The mean percentage change of each VWF test was evaluated in comparison to the threshold level across different time points using repeated-measures ANOVA.

Statistical significance was defined as a P value of < 0.05 for all performed tests. The analyses were conducted using PASW Statistics (version 18; SPSS Inc, Chicago, IL, USA).

RESULTS

Twenty-five samples were collected from normal

subjects, with a mean age of 40±18 years. Among these samples, 20 out of 25 (80%) were obtained from female individuals. Immediately after blood collection and prior to freezing the PPP, the VWF:Ag, VWF:GPIbM, and VWF:CB levels of each sample were above 0.50 kIU/L. After thawing the PPP, immediate assays (conducted at 0 hours) revealed median levels of VWF:Ag, VWF:GPIbM, and VWF:CB of 0.91 (0.72–1.06), 0.85 (0.69–1.04), and 0.78 (0.62–0.97) kIU/L, respectively (Table 1).

Significant decreases were observed in VWF:Ag levels, declining from 0.91 kIU/L at 0 hours to 0.67 kIU/L at 96 hours ($P < 0.001$; Table 1). Furthermore, at 96 hours, 80% of the samples had VWF:Ag levels below the threshold value. Regarding the VWF:GPIbM assay, a rapid decline in stability was observed after 24 hours, with levels decreasing from 0.85 kIU/L (0 hours) to 0.73 kIU/L (24 hours), yielding a *P* value of 0.001 (Table 1). More than 90% of the samples stored at 4 °C for 96

TABLE 1. Levels of VWF:Ag, VWF:GPIbM, and VWF:CB in thawed plasma from 25 healthy individuals at 0 hours and after storage at 2–8 °C for 24, 48, 72, and 96 hours.

Storage time after thawing (hours)	Amount (median and IQR) (kIU/L)	Number of samples with VWF lower than threshold (%)	Amount of decrease from threshold (median and IQR) (kIU/L)	% decrease from threshold (mean and 95% CI) (%)	*P*
VWF:Ag (N=25)					
0	0.91 (0.72 to 1.06)	-	-	-	-
Threshold (hour 0-allowable error)	0.85 (0.67 to 0.99)	-	-	-	-
24	0.86 (0.66 to 1.10)	9 (36)	0.05 (-0.03 to -0.14)	-0.71, (-7.62, 6.2)	.834
48	0.83 (0.65 to 1.04)	13 (52)	-0.004 (-0.11 to -0.11)	-4.64, (-13.24, 4.01)	.279
72	0.82 (0.71 to 1.05)	12 (48)	- 0.02 (-0.07 to -0.20)	-1.37, (-10.87, 8.13)	.769
96	0.67 (0.52 to 0.87)	20 (80)	-0.14 (-0.24 to -0.03)	-22.44, (-30.04, -14.85)	**<0.001**
VWF:GPIbM (N=25)					
0	0.85 (0.69 to 1.04)	-	-	-	-
Threshold (hour 0-allowable error)	0.79 (0.64 to 0.97)	-	-	-	-
24	0.73 (0.63 to 0.93)	15 (60)	-0.04 (-0.06 to -0.02)	-14.464 (-22.296, -6.632)	**0.001**
48	0.73 (0.62 to 0.93)	15 (60)	-0.01 (-0.11 to -0.02)	-14.747 (-22.357, -7.138)	**0.001**
72	0.69 (0.57 to 0.87)	18 (72)	-0.07 (-0.20 to -0.03)	-20.965 (-28.629, -13.301)	**<0.001**
96	0.66 (0.53 to 0.86)	23 (92)	-0.10 (-0.25 to -0.02)	-26.190 (-35.185, -17.195)	**<0.001**
VWF:CB (N=25)					
0	0.78 (0.62 to 0.97)	-	-	-	-
Threshold (hour 0-allowable error)	0.73 (0.58 to 0.90)	-	-	-	-
24	0.74 (0.61 to 0.90)	11 (44)	0.02 (-0.1 to -0.13)	-4.111 (-13.249, 5.027)	0.362
48	0.77 (0.61 to 0.97)	13 (52)	-0.004 (-0.1 to -0.21)	-1.647 (-13.061, 9.766)	0.768
72	0.69 (0.61 to 0.82)	15 (60)	-0.04 (-0.16 to -0.12)	-10.169 (-20.846, 0.507)	0.610
96	0.61 (0.51 to 0.74)	20 (80)	-0.10 (-0.20 to -0.04)	-19.580 (-29.223, -9.937)	**<0.001**

Abbreviations: 95% CI, 95% confidence interval; IQR, interquartile range; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor: antigen; VWF:CB, von Willebrand factor: collagen binding assay; VWF:GPIbM, von Willebrand factor: glycoprotein Ib binding assay

hours displayed VWF:GPIbM levels below the defined threshold.

In contrast, VWF:CB remained stable for up to 96 hours of storage, with levels declining significantly from 0.78 kIU/L at 0 hours to 0.61 kIU/L at 96 hours ($P < 0.001$; Table 1). Twenty of the 25 samples (80%) contained VWF:CB levels below the threshold value at 96 hours (Table 1).

Another set of experiments involved 10 plasma samples obtained from patients diagnosed with VWD type 1 or type 2A. The median levels of VWF:Ag, VWF:GPIbM, and VWF:CB at 0 hours were 0.42 (0.36–0.46), 0.20 (0.16–0.33), and 0.25 (0.19–0.53) kIU/L, respectively (Table 2). The VWF:Ag level experienced a significant decrease from the defined threshold after 48 hours of storage, declining from 0.42 kIU/L to 0.23 kIU/L at 72 hours ($P < 0.001$; Table 2). Similarly, the level of VWF:GPIbM dropped from 0.20 kIU/L to 0.13 kIU/L at 48 hours ($P < 0.001$; Table 2). All patients exhibited decreased levels of VWF:Ag and VWF:GPIbM below the threshold value after 48 hours. Regarding VWF:CB, stability was observed up to 48 hours, with the median level declining to 0.19 kIU/L at 72 hours and to 0.12 kIU/L at 96 hours ($P = 0.003$ and < 0.001, respectively; Table 2). All patients displayed VWF:CB levels below the threshold level at 96 hours.

TABLE 2. Levels of VWF:Ag, VWF:GPIbM, and VWF:CB in thawed plasma from 10 patients with von Willebrand disease at 0 hours and after storage at 2–8 °C for 24, 48, 72, and 96 hours.

Storage time after thawing (hours)	Amount (median and IQR) (kIU/L)	Number of samples with VWF lower than threshold (%)	Amount of decrease from threshold (median and IQR) (kIU/L)	% decrease from threshold (mean and 95% CI) (%)	*P*
VWF:Ag (N=10)					
0	0.42 (0.36 to 0.46)	-	-	-	-
Threshold (hour 0-allowable error)	0.39 (0.34 to 0.43)	-	-	-	-
24	0.42 (0.26 to 0.55)	4 (40)	0.01 (-0.03 to -0.06)	-5.60 (-20.43, 9.22)	0.414
48	0.33 (0.25 to 0.56)	6 (60)	-0.01 (-0.11 to -0.02)	-9.53 (-33.60, 14.54)	0.394
72	0.23 (0.15 to 0.40)	10 (100)	-0.14 (-0.17 to -0.04)	-39.17 (-52.25, -26.08)	**<0.001**
96	0.20 (0.15 to 0.26)	10 (100)	-0.19 (-0.21 to -0.15)	-54.80 (-63.31, -46.29)	**<0.001**
VWF:GPIbM (N=10)					
0	0.20 (0.16 to 0.33)		-	-	-
Threshold (hour 0-allowable error)	0.18 (0.15 to 0.30)		-	-	-
24	0.15 (0.09 to 0.23)	8 (80)	-0.06 (-0.16 to -0.003)	-27.57 (-58.12, 2.98)	0.072
48	0.13 (0.08 to 0.18)	9 (90)	-0.07 (-0.18 to -0.04)	-43.78 (-60.03, -27.53)	**<0.001**
72	0.10 (0.05 to 0.15)	10 (100)	-0.09 (-0.21 to -0.06)	-58.53 (-71.91, -45.15)	**<0.001**
96	0.06 (0.04 to 0.12)	10 (100)	-0.11 (-0.22 to -0.10)	-69.67 (-80.60, -58.73)	**<0.001**
VWF:CB (N=10)					
0	0.25 (0.19 to 0.53)	-	-	-	-
Threshold (hour 0-allowable error)	0.24 (0.17 to 0.49)	-	-	-	-
24	0.33 (0.21 to 0.45)	4 (40)	-0.03 (-0.13 to -0.01)	9.19 (-14.31, 32.70)	0.399
48	0.29 (0.18 to 0.46)	4 (40)	0.02 (-0.07 to -0.02)	4.07 (-21.09, 29.22	0.723
72	0.19 (0.12 to 0.34)	8 (80)	-0.05(0.01 to -0.16)	-29.81 (-46.76, -12.85)	0.003
96	0.12 (0.05 to 0.21)	10 (100)	-0.17 (0.08 to -0.29)	-64.09 (-76.77, -51.41)	**<0.001**

Abbreviations: 95% CI, 95% confidence interval; IQR, interquartile range; VWD; von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor: antigen; VWF:CB, von Willebrand factor: collagen binding assay; VWF:GPIbM, von Willebrand factor: glycoprotein Ib binding assay

DISCUSSION

Quality assurance systems in laboratories typically encompass various processes, such as blood collection, sample storage, laboratory methods, analyses, and reporting.[8] Although standard recommendations for pre-analytical processes in VWF assays are well established, they may not always be fully followed due to various limitations.[7]

Referral laboratories commonly encounter partially melted plasma during transportation from blood collection centers, leading to inevitable sample rejection. Furthermore, conducting all von Willebrand factor (VWF) assays on the same tube of frozen plasma within a single day is often impractical. However, repeating a blood collection is often impractical, and in certain instances, the assays may still need to be performed upon request from the external laboratory. Since the duration of sample storage can affect thrombin generation[14], the ideal situation would be to conduct assays within 4 hours at the blood collection center.

The Clinical Laboratory Standards Institute recommends centrifuging citrated whole blood (WB) immediately after collection to separate PPP. The PPP should be kept at room temperature, and assays should be conducted within 4 hours. If that is not possible, the PPP should be stored at -80 °C until ready for analysis.[15,16] Frozen plasma intended for long-term storage or transportation to referral laboratories should be maintained at temperatures ranging from -20 to -80 °C.

A study by Zhao et al demonstrated that frozen plasma stored at -80 °C remained stable for a year in terms of fibrinogen, thromboplastin time, and prothrombin time. However, activated partial thromboplastin time remained stable for only 6 months, while Factors VIII and IX remained steady for only 1 month.[17] After thawing frozen plasma, assays should be conducted immediately.[15] However, there is limited research on the stability of VWF in thawed plasma.

The diagnosis of VWD relies on accurate laboratory test results, particularly for subtype-classification tests. However, various challenges can affect these assays, especially during the transportation of samples from blood collection centers to referral laboratories.

Limited studies have investigated the stability of VWF in different scenarios. Improper sample preparation can alter both the quantity and function of VWF. For instance, Favaloro et al demonstrated that VWF:Ag in whole blood (WB) or platelet-poor plasma (PPP) remained stable at room temperature (20–25 °C) for up to 6 days.[18] Unfortunately, frozen or thawed plasma and VWF activity were not assessed in their study. Zürcher et al reported that VWF:Ag and VWF:RCo in WB or PPP were stable at room temperature (2 °C in winter and 17–29 °C in summer) for up to 2 days.[19] However, the stability of frozen or thawed plasma was not investigated. Other studies by Gosselin et al and Linskens et al found that VWF:RCo remained stable for 16 and 48 hours, respectively, when plasma was immediately centrifuged after collection and stored at 22–28 °C.[20,21]

The aforementioned studies have shown that VWF stored in either WB or PPP remains stable at room temperature for up to 2–6 days. However, it is important to note that this finding may not apply to tropical countries, where room temperatures can reach 33–36 °C during the summer. Despite this, it is worth mentioning that most laboratories in Thailand still adhere to the guidelines set forth by the Clinical Laboratory Standards Institute for the storage and transportation of samples to referral laboratories for VWF assays.[7]

Furthermore, the studies conducted thus far have focused on the stability of VWF in WB or immediately spun plasma, without considering frozen and thawed plasma. Limited studies have specifically examined the stability of VWF in frozen and thawed plasma. One study demonstrated that VWF:Ag remained stable for up to 6 days after the plasma was thawed and stored at 4±2 °C, which is similar to our findings.[22] Unfortunately, no functional assays were conducted in the earlier investigation.

Regarding long-term storage, most previous studies primarily investigated thawed fresh frozen plasma or thawed lyophilized plasma. Several studies have shown that the VWF:Ag of thawed fresh frozen plasma remains stable for up to 6 days at 4 °C.[22-24] However, those studies did not assess the platelet or collagen binding activities of VWF. Furthermore, the studies focused on VWF:RCo as the platelet binding activity of VWF, without considering VWF:GPIbR or VWF:GPIbM. Table 3 summarizes the stability of VWF with various preparations and storage conditions from previous studies.

In our study, we observed that VWF:Ag and VWF:CB exhibited stability in thawed plasma obtained from normal subjects when stored at 2–8 °C for up to 72 hours prior to testing. However, we noted that VWF:GPIbM displayed lower levels of stability under the same storage conditions. We hypothesize that the cold temperature during freezing at -80 °C might affect platelets present in PPP and impair VWF function, as previous studies have shown that ice can damage platelets and impair VWF when WB is stored on ice.[25-27] Considering the stability of thawed plasma from VWD patients, whose initial VWF values were lower than normal, VWF:Ag and VWF:CB appeared to be less stable than in normal subjects. Interestingly, VWF:GPIbM tended to be more

TABLE 3. Studies on the stability of von Willebrand factor with different preparations and storage conditions

Authors	VWF of interest	Types of samples	Storage temperature	Maximum stability duration
Studies on samples with a short-term storage after blood collection				
Favaloro EJ et al[17]	VWF:Ag	WB	RT (20-25 °C)	6 days
		PPP	RT (20-25 °C)	6 days
Zürcher M et al[18]	VWF:Ag and VWF:RCo	WB	RT (2 °C in winter and 17-29 °C in summer)	2 days (stable both VWF:Ag and VWF:RCo)
		PPP	RT (2 °C in winter and 17-29 °C in summer)	2 days (stable both VWF:Ag and VWF:RCo)
Gosselin RC et al[19]	VWF:RCo	PPP	Frozen in -70 °C freezer Dry ice	16 hours (stable both in freezer or dry ice)
Linskens EA et al[20]	VWF:RCo	PPP	RT (temperature not stated)	48 hours
Favaloro EJ et al[25]	VWF:Ag and VWF:CB	WB	22 °C	3.5 hours (stable both VWF:Ag and VWF:CB)
			0-4 °C (on ice)	3.5 hours (unstable both VWF:Ag and VWF:CB)
Böhm M et al[26]	VWF:Ag and VWF:RCo	WB	0-4 °C (on ice)	6 hours (unstable both VWF:Ag and VWF:RCo)
			RT (temperature not stated)	6 hours (stable both VWF:Ag and VWF:RCo)
		PPP	0-4 °C (on ice)	6 hours (stable both VWF:Ag and VWF:RCo)
			RT	6 hours (stable both VWF:Ag and VWF:RCo)
Studies on frozen samples with a long-term storage				
von Heymann C et al[21]	VWF:Ag	Fresh frozen plasma		4 °C 6 days
Buchta C et al[22]	VWF:Ag	Frozen solvent/detergent-treated plasma		4 °C 6 days
Schoenfeld H et al[23]	VWF:Ag	Lyophilized plasma		4 °C 6 days

Abbreviations: PPP, platelet poor plasma; RT, room temperature; VWF:Ag, von Willebrand factor: antigen; VWF:CB, von Willebrand factor: collagen binding assay; VWF:GPIbM, von Willebrand factor: glycoprotein Ib binding assay; VWF:RCo, von Willebrand factor: ristocetin cofactor assay; WB, whole blood

stable in VWD patients than in normal subjects. We speculate that the low initial VWF levels may mask the effect of storage time on VWF stability.

Based on the results of the study, we recommend the immediate assay of thawed plasma for all VWF panels. In cases where simultaneous assays are not feasible, priority should be given to the VWF:GPIbM assay due to its observed instability after thawing. Furthermore, we suggest that blood collection centers divide plasma into smaller aliquoted samples (200 µL/sample) before freezing. Each individual test can then be performed using a separate aliquot, either at the blood collection center's laboratory or at an off-site referral laboratory. This approach eliminates the need for repeated freezing and thawing of the original bulk sample. Last, if the initial results from a referral laboratory indicate values lower than the normal range, we recommend collecting a fresh plasma sample directly from the patient rather than transferring a sample from the blood collection center. The tests should be repeated before making a definitive diagnosis of VWD and its subtype.

A limitation of this study is the small number of samples from VWD patients. Further studies with a larger sample size are needed to draw definitive conclusions.

CONCLUSION

Transporting blood samples to referral laboratories for VWF assays poses challenges. Despite established transportation standards, there is still a risk of partially melted plasma reaching the referral laboratories, and sample rejection may not always be feasible. It has been observed that VWF:Ag and VWF:CB in thawed plasma can remain stable for up to 72 hours, whereas VWF:GPIbM displays less stability.

List of abbreviations

PPP, platelet-poor plasma; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor:antigen; VWF:CB, von Willebrand factor:collagen binding assay; VWF:GPIbM, von Willebrand factor:glycoprotein Ib binding by multimer analysis; VWF:GPIbR, VWF:glycoprotein Ib binding by ristocetin; VWF:RCo, VWF:ristocetin cofactor; WB, whole blood

Declarations

Ethics approval and consent to participate

This study was authorized by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University (approval no: 420/2560/EC4).

Consent for publication

This manuscript has been approved by all authors. A copy of the consent document is available for review from the Editor-in-Chief of Siriraj Medical Journal.

Availability of data and materials

The data sets used during the study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest related to this study.

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Authors' contributions

All authors designed the study. YN collected and analyzed all data and drafted the manuscript. TB performed all of the VWF assays, while TR1, TR2 and YN read and revised the manuscript.

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Therapeutic Penetrating Keratoplasty for Severe Fungal Keratitis in a Thai Tertiary Care Center

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ABSTRACT

Objective: To evaluate the outcomes of therapeutic penetrating keratoplasty (TPK) performed for severe fungal keratitis.

Materials and Methods: Medical records of all patients who underwent TPK in Siriraj Medical Center between April 2010 and July 2020 were culled, and those in which fungal pathogens were definitively identified were studied. Patient records with follow up less than three months were excluded. Patient demographic data, outcome measures and complications following TPK were recorded. The primary outcome was eradication of the fungal infection. Secondary outcomes were preservation of anatomical integrity, graft survival and achievement of visual acuity (VA) greater than or equal to 3/60.

Results: Sixty patients met the study criteria and were included in the analysis. The mean patient age was 56 (range: 23-79) years, and most patients were men (46, 77%). The mean follows up time was 30.87 months (range1.61-122.71). Fifteen eyes (25%) sustained corneal perforation before undergoing TPK. Graft survival was 30% at 1 year, 18% at 5 years, and 11% at 10 years. The most common organism was fusarium (23 patients, 38%). The median duration from presentation to surgery was 14 (8-21) days. Disease eradication was achieved in 44 patients (73%) and VA better than 3/60 was achieved in 14 (23%). Anatomical integrity was maintained in 46 (76%) eyes. Repeat PKP was performed in 15 patients (25%), most commonly for recurrent infection.

Conclusion: TPK offers a good chance of disease eradication and maintenance of anatomical globe integrity and is a reasonable therapeutic option in patients with severe fungal infection.

Keywords: Therapeutic penetrating keratoplasty; fungal keratitis; fungal ulcer (Siriraj Med J 2023; 75: 575-583)

INTRODUCTION

Infectious keratitis is a vision-threatening condition and the leading cause of corneal blindness worldwide. Bacteria, fungi, viruses and protozoa are all well recognized pathogens, and severe cases can progress to endophthalmitis or corneal perforation with devastating results. Fungal keratitis represents a particular treatment challenge due to its typically middling response to medical therapy.

In warm climates, filamentous fungal species, most notably Fusarium and Aspergillus, are the most common types of fungal pathogens.[1,2] In cooler climates, yeast species predominate, typically Candida.[3] Most patients have a recognized history of corneal trauma or contact lens wear. In general, the first line management of fungal keratitis is with topical and systemic anti-fungal medications. The number of available and approved topical agents is small, and most are azoles, the exception being natamycin, a polyene compound.[4] Periodic corneal debridement may improve drug penetration and is often performed as an adjunctive therapeutic procedure.

Therapeutic penetrating keratoplasty (TPK) is commonly performed to treat very severe fungal keratitis,

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as it logically reduces the sheer bulk of infectious organisms and replaces lysed and lysing corneal tissue.[5,6] In general, the goal in such circumstances is saving the globe. While there seems to be a general consensus that this approach is effective, available data and details remain incomplete. We undertook this study to shed additional light on the existing repository of data regarding outcomes following TPK for severe fungal keratitis.

MATERIALS AND METHODS

This was a retrospective observational study conducted at Siriraj Hospital, a 2200 bed tertiary health care center in Bangkok, Thailand. The study was approved by the Research Committee, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 412/2020) and was performed in accordance with the Declaration of Helsinki. The requirement for informed consent was waived by the Research Committee.

Medical records were reviewed for patients who underwent TPK for a diagnosis of fungal keratitis between April 2010 and July 2020. Records were included for analysis if definitive identification of fungal organisms was achieved by either potassium hydroxide (KOH) smear, fungal culture, in-vivo confocal microscopy (IVCM), or histopathologic analysis of the cornea button resected at the time of TPK. The decision to proceed with TPK was made at the discretion of the attending physician. Mostly in Siriraj Hospital, TPK was done in severe fungal corneal ulcer (size >6 mm.) with rapid progression and poor response to medical therapy. Additionally, TPK was performed when the cornea was perforated or when Pythium was identified as a pathogen. Patients were excluded from analysis if fungi were not identified, or if follow-up duration following TPK was less than three months.

Preoperative clinical data were recorded including patient age, gender, underlying systemic disease, contact lens use, history of predisposing corneal trauma, characteristics of the corneal ulcer, causative pathogens, best corrected visual acuity (BCVA), in Log MAR format, intraocular pressure (IOP), and the presence of perforation. Corneal ulcers were categorized according to the size of the infiltrate in the longest dimension. Severe ulcers were >6 mm, moderate ulcers were 2-6 mm, and small ulcers were <2 mm. Postoperative data collection included BCVA, IOP and surgical complications.

Outcomes data included eradication of infection, graft survival as defined by no recurrence of fungal infection and no graft failure regardless of cause (including rejection or other reasons for endothelial failure), anatomical survival as defined by preservation of the globe intact without phthisis bulbi or the need for enucleation or evisceration, and functional success as defined by BCVA better than or equal to 3/60. Where TPK was repeated, data collection after the second procedure was limited to these outcomes.

Statistical analysis

Data were analyzed using SPSS (Statistical package for social sciences) software. version 18.0 (SPSS Inc., Chicago, IL, USA). Mean (± standard deviation) and median (along with interquartile range, IQR) values were used to describe the parametric and nonparametric data, respectively. Frequency and percentage were used to describe the data.

Fisher's exact test and Mann-Whitney U test were used to analyze predictive factors associated with the success of an outcome, p-value < 0.05 was considered statistically significant. Survival of the graft and anatomical survival of the globe were represented using Kaplan–Meier curves.

RESULTS

Patient demographic data

During the study period, 60 patients underwent TPK for fungal keratitis at Siriraj Hospital. Forty-six patients (77%) were male. The mean age at presentation was 56 years (range 23-79 years). The mean follows up time was 30.87 months. The median follows up time was 17.2 months, with a range of 1.61 to 122.71 months. A total of 10 patients were followed up for a period of 5 years, while 1 patient was followed up for a duration of 10 years. Forty keratitis (67%) were moderate in size, and 18 (30%) were severe. Fifteen (25%) keratitis sustained corneal perforation before TPK was performed.

Predisposing factors

A history of ocular surface trauma was obtained in 47 patients (78%). Of these 47 patients, 21 (45%) recalled trauma with plant material, while eight (17%) gave a history of insect-induced trauma, and seven (15%) recalled soil-contaminated trauma. Two patients (3.3%) reported a history of contact lens wear just prior to developing corneal symptoms.

Causative pathogens

Fusarium was identified in 23 (38%) ulcers, aspergillus was identified in seven (12%) and pythium in seven (12%). Both Candida and Penicillium were identified in three (5%). Thirteen fungal pathogens (21%) were visualized by IVCM but could not be characterized.

Preoperative management

Preoperatively, most patients were treated with combine 2 topical antifungal medications; topical amphotericin B and topical fluconazole every 1 hour around the clock, although this regimen varied somewhat during a few years of the study period, and topical natamycin 5% suspension and topical voriconazole 2% hourly were used in the later part of the study.[7] The intrastromal and intracameral injection with fluconazole or voriconazole also considered in the case of deep keratitis or fungal ball in anterior chamber. Systemic oral itraconazole or oral voriconazole daily were used according to the culture result.[8] Corticosteroids by any route were not used in any patients, preoperatively. The average duration between admission and TPK was 14 (8-21) days.

Surgical procedures

All TPK procedures were performed under general anesthesia. Intraoperatively, care was taken to size the graft 0.5–1 mm greater than the widest dimension of the infiltrate. Following trephination and resection of the infected portion of host cornea, the anterior chamber and iris surface were irrigated with amphotericin B or voriconazole to remove exudates and hypopyon, and the graft margin was inspected carefully to ensure that no necrotic tissue remained. The average graft size was nine (8-10) mm. Each resected cornea button was divided into two roughly equal parts, one of which was further subdivided for culture on blood agar, chocolate agar, Sabouraud's dextrose agar, and Thioglycollate broth agar. The second part was sent in formalin 1% for histopathological analysis.

Fifty-eight (97%) eyes underwent TPK alone, two (3%) eyes underwent TPK combined with extracapsular cataract extraction, no patients sustained expulsive choroidal hemorrhage or other serious intraoperative complications.

Postoperative management

Postoperatively, patients were treated with topical natamycin 5% suspension and topical voriconazole 2% every hour, combined with oral itraconazole or voriconazole. The dose was adjusted depending on clinical response, assessments being made daily with attention to signs of recurrent infection. Topical and oral antiglaucoma medications were added as necessary in patients with IOP elevation. Topical corticosteroids may be used after 1 month if there was no sign of any fungal recurrence.

Postoperative findings and outcomes

During the first two postoperative weeks (acute phase), suture related complications were noted in 35 (58%) eyes, and recurrent fungal infection was noted in 19 (32%). Subsequently (late phase), graft failure was noted in 25 (42%) eyes, glaucoma in 16 (27%) and cataract in 4 (7%). These results are shown in Table 2.

Among the 60 eyes included in this study, 19 eyes (32%) experienced recurrence of infection following TPK. Of these, eight eyes (14%) required repeat TPK as a treatment approach, while nine eyes (15%) eventually underwent enucleation, and four eyes (7%) underwent evisceration. There are two patients that underwent more than one procedure. One patient underwent enucleation following secondary TPK, while another patient underwent

TABLE 1. Complications of TPK.

Phase	Complications	Number* (%)
Acute (<2 weeks)	Suture related	35 (58)
	Recurrence of infection	19 (32)
	PED	9 (15)
	Endophthalmitis	2 (3)
Chronic (>2 weeks)	Graft failure	25 (42)
	Glaucoma	16 (27)
	Graft rejection	10 (17)
	Cataract	4 (7)
	Phthisis	1 (2)

PED, Persistent Epithelial Defect ; *some patient may have more than one complication; % from total N = 60

TABLE 2. Factors association with eradication of the disease.

Factors		Success (n)	Failure (n)	P-value
Gender	Male	34	12	1.00
	Female	10	4	
Contact lens use	No	43	15	0.47
	Daily	0	1	
	Monthly	1	0	
Trauma details	No	8	5	0.95
	Contaminated water	4	1	
	Agriculture products	16	5	
	Soil	5	2	
	Insects	6	2	
	Others	5	1	
Perforation	No	33	12	1.00
	Yes	11	4	
Pathogen	*Fusarium*	19	4	0.15
	Aspergillus	3	4	
	Pythium	3	4	
	Candida	2	1	
	Purpureocillium lilacinum	1	0	
	Exserohilium spp.	1	0	
	Penicillium spp.	3	0	
	Culvuria	1	1	
	Unidentified	11	2	
Size of ulcer	Grade 1 (<2mm.)	2	0	0.75
	Grade 2 (2-6 mm.)	30	10	
	Grade 3 (>6mm.)	12	6	
Duration admission to surgery(days)		15	8	0.056
Graft size	≤ 8mm.	9	9	0.42
	> 8mm.	28	14	

spp., several species
n : number of patients

evisceration after secondary TPK. Graft survival at one year following TPK was 30%, decreasing to 18% at 5 years and 11% at 10 years. These results are shown using Kaplan-Meier curve in Fig 1. Anatomical survival was achieved in 76% of eyes at one year, decreasing to 70% at 5 years and remaining at that level for 10 years as shown in Fig 2. Overall, 14 eyes (23%) achieved BCVA > 3/60. Visual results are shown in Fig 3.

Of the 15 eyes that sustained corneal perforation prior to TPK, graft survival was observed in 7 (47%) eyes and anatomical survival was observed 11 (73%) eyes at five years.

Repeat TPK was performed in 15 (25%) patients. Of this number, the indications for repeat TPK were fungal infection in eight (56%), graft failure in four (25%), graft rejection in two (13%) and one (6%) graft melting. Three of the repeat TPK procedures were in eyes that had sustained corneal perforation before the first TPK; one of these repeat TPKs was for recurrent infection and two were for graft failure.

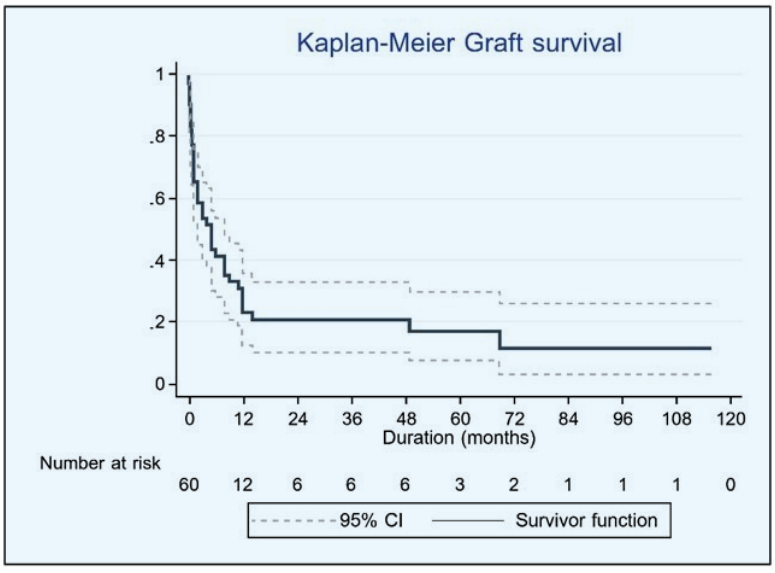

Fig 1. Kaplan-Meier graft survival rate

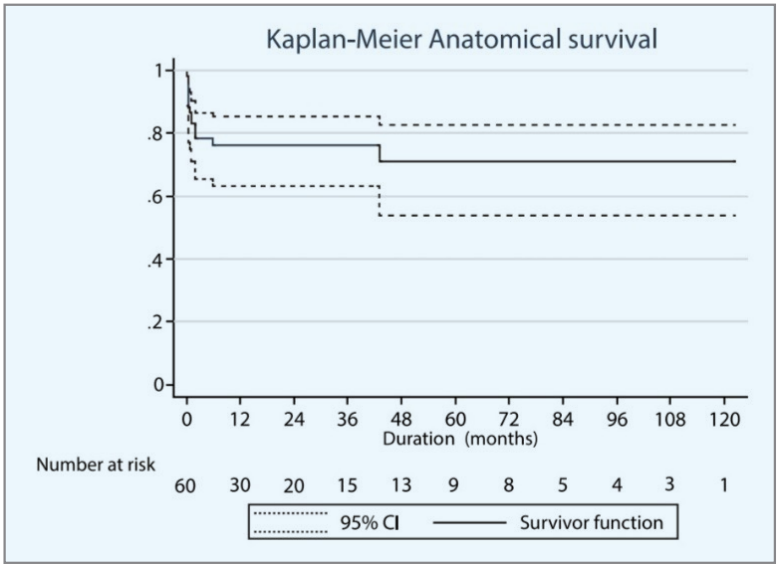

Fig 2. Kaplan-Meier anatomical success rate.

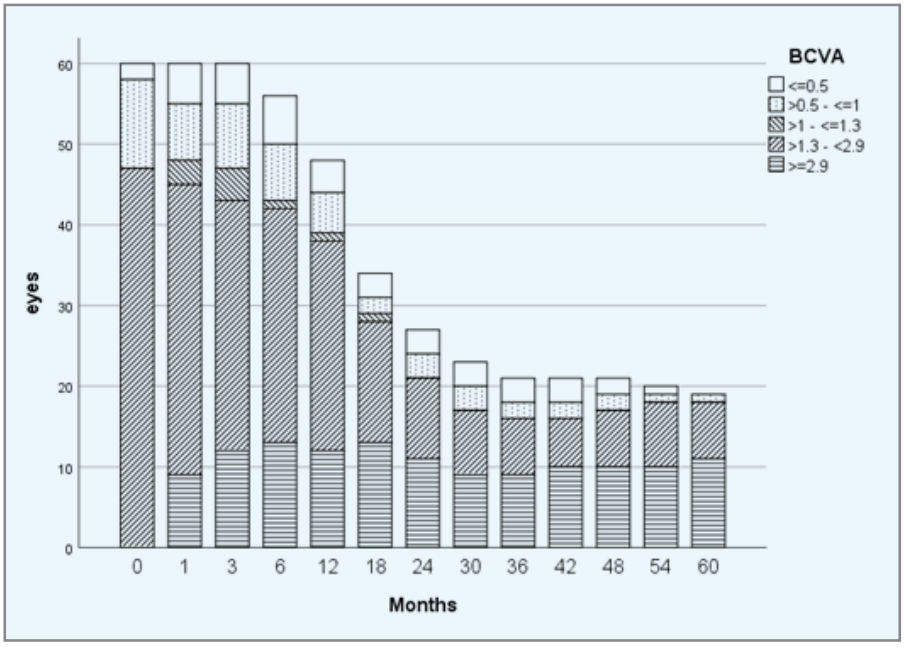

Fig 3. BCVA pre and post operation.

Factor association with the functional outcome

Graft size less than 8 mm, and preoperative IOP below 21 mmHg were statistically correlated with improved functional outcome. Moreover, we found that Candida, *Purpureocillium lilacinum* and *Penicillium spp.* were associated with better functional outcome. No other clinical findings were statistically significantly associated with disease eradication. These results are shown in Table 3.

DISCUSSION

In this retrospective 10 years study of 60 patients with fungal keratitis, a single TPK procedure resulted in eradication of the infection in 73% of patients. Fusarium was the most identified pathogen, followed by Aspergillus and Pythium species. Ultimately, anatomical preservation of the globe was achieved in 76%, while graft survival was 30% at 1 year. BCVA was 3/60 or better in 23% of affected eyes. Corneal perforation had occurred prior to TPK in 25% of cases and anatomic survival was achieved in nearly three fourths of these.

Our epidemiologic findings were quite similar to those of prior studies of fungal keratitis.[5,6,9-19] The mean patient age was 56 years, and the majority were males. Trauma was the most common predisposing risk factor (78%), especially plant-induced trauma (45%), quite as with other studies in Asian developing countries.[15-18] Fusarium has consistently ranked as the most common

TABLE 3. Factors association with the functional outcome.

Factors		Success (n)	Failure (n)	P-value
Gender	Male	11	35	1.000
	Female	3	11	
Contact lens use	No	14	44	1.000
	Daily	0	1	
	Monthly	0	1	
Trauma details	No	3	10	0.780
	Contaminated water	1	4	
	Agriculture products	4	17	
	Soil	3	4	
	Insects	1	7	
	Others	2	4	
Perforation	No	9	36	0.300
	Yes	5	10	
Pathogen	*Fusarium*	2	21	0.015*
	Aspergillus	0	7	
	Pythium	2	5	
	Candida	2	1	
	Purpureocillium lilacinum	1	0	
	Exserohilium spp.	0	1	
	Penicillium spp.	2	1	
	Culvuria	1	1	
	Unidentified	4	9	
Duration admission to surgery(days)		14	46	0.220
Pre operative IOP				
	Normal < 21 mmHg.	4	2	0.031*
	High ≥ 21 mmHg.	10	44	
Graft size	≤ 8mm.	9	9	0.003*
	> 8mm.	5	37	

spp., several species; Statistically significant difference when *p< 0.05
n : number of patients

fungal keratitis pathogen in warm climates, and our results followed this pattern.

No fungal pathogen could be isolated or observed on histopathologic analysis in 13 eyes (21%) despite our strong clinical impression along these lines and observation of fungal elements on IVCM. We assume this diagnostic shortfall is attributable to fungal organisms being present insufficient quantities to enable detection by culture or histopathologic analysis, the deeper of the lesion than corneal scraping or the medications that the patients received before coming to our tertiary center. In any case, the sensitivity of fungal culture is limited, often cited at approximately 44%.[20] So efforts at microbiologic detection and characterization are often disappointing.[21-24]

The rate of pre-TPK corneal perforation in our patients was 25% (15 eyes) which is less than other studies of this therapeutic modality. Studies in Thailand[25], India[26,27], Japan[28] and Nepal[5] showed rates of 51.9%, 56%, 62.2% and 71%, respectively. We assume this represents the degree to which patient s have access to tertiary care facilities. Anatomical success was ultimately achieved in 73% of perforated eyes. Corresponding with previous studies, anatomical success in perforated eyes was 61.9% in Thailand[25] and 82.5% in China.[29] TPK effected eradication of the fungal infection in 73% of eyes, maintenance of globe integrity in 76% at one year and 70% at 10 years follow-up. These results suggested that TPK had high beneficial advantage to eradicate and protect the globe in the cases of refractory fungal medication including the perforated eyes. Functional success was judged to have been achieved in 23% of patients. These rates approximate or-in the case of functional success--fall a bit short of those in studies by Raj[26] and Bajracharya.[5] We suspect our lower functional success reflects differences in three important factors: 1) patient population--most obviously that bacterial keratitis was not included in the current study; 2) precisely how functional success was determined and difference in definition; and 3) the quality of donor corneas. Thailand has a more or less chronic shortage of donor corneas, and those used in salvage procedures like TPK had generally lower endothelial cell counts than grafts reserved for more promising cases.

Functional outcomes in this study were significantly associated with three factors: 1) the identified pathogen; 2) preoperative IOP; and 3) graft size. Candida, *Purpureocillium lilacinum* and *Penicillium spp*. were associated with better functional outcomes. Whether this observation is incidental or reflects actual lower virulence of these organisms remains unclear. Preoperative IOP <21 mmHg predicted a better functional outcome in our patients, a finding that suggests that elevated IOP reflects more advanced corneal infections, as has been previously suggested.[30] Larger graft sizes are well recognized to be more likely to sustain rejection,[12,26,31] in the other hand, the larger graft reflects the more severe of the keratitis. In the current study 8mm was the cutoff, above which functional outcome was less likely. Our median graft size was 9 mm, which presumably affected our patients' graft survival rate. Taken together, these factors imply that the TKP since in the earlier stage of the disease may give the better the functional survival rate. However, regrafting in the recurrence fungal infection or failed graft still give the benefit for saving the globe.

This study showed that our patients' most common postoperative complication was suture related (58%) and fungal recurrence of infection (35%) in the first two weeks postoperatively. Recurrence rates in similar studies have ranged from 26% to nearly 53%.[5,9,26] Late phase complications were most commonly graft failure (42%) and glaucoma (27%). This rate of postoperative glaucoma was considerably higher than that colleagues have reported, a finding we suspect may be related to severity of fungal corneal ulcer case and post operative PAS induced by large graft size and post operative inflammation.

The most problematic pathogen in our patients was Pythium[32,33] which can be differentiated from fungal keratitis by its characteristic appearance on slit-lamp examination, smear, culture, and histopathology. It is rapid progression and quiet not response to medical treatment. In this study, two of seven patients underwent evisceration or enucleation. Nevertheless, TPK effected anatomic success in 7 of these patients and only 2 required regrafting, a relatively good outcomes rate than previous study. It suggests that early TPK may be in patients' interest when this Pythium is detected.

The limitations of the study include the retrospective study. Comparisons between corneal ulcer studies are always complicated by interobserver variability: clinical assessments are largely subjective, a fact which naturally limits the degree to which findings, including ours, can be generalized. Prevailing circumstances also impacted our study in that donor cornea availability was limited, meaning the timing of TPK was not entirely a question of surgeon preference and donor tissue was often suboptimal, and we strongly suspect this impacted our graft survival and functional outcomes. This study effort benefits from a relatively long follow-up period and contributes to the rather limited fund of data on TPK in the setting of fungal keratitis.

CONCLUSION

TPK is a reasonable option in severe fungal keratitis which are refractory to medical treatment, resulting in preservation of the globe when such an outcome seems otherwise unlikely.

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Disclosure

The authors report no conflicts of interest in this work.

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The Relationship between Primary Caregivers' Psychosocial Factors and Self-esteem in Children and Adolescents with ADHD: An Exploratory Cross-sectional Study

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ABSTRACT

Objective: This study examined the correlation between primary caregivers' psychosocial factors and self-esteem in children and adolescents with attention-deficit/hyperactivity disorder (ADHD).

Materials and Methods: A cross-sectional study involving primary caregivers and their children with ADHD, aged 8-15, was conducted from September 2022 to February 2023. The children's self-esteem was assessed using the Five-Scale Test of Self-Esteem for Children. Primary caregivers' psychosocial factors were assessed using the Attitude of Parenting Questionnaire, Parenting Style and Dimension Questionnaire, Patient Health Questionnaire-9, and Generalized Anxiety Disorder 7-item. Descriptive statistics and multivariable linear regression were used to determine the associations among variables.

Results: The study included 66 pairs of children and adolescents with ADHD and their primary caregivers. The study found 53% of caregivers screened positive for depression, while 16.7% screened positive for anxiety. Almost all caregivers (90.9%) adopted an authoritative parenting. The mean self-esteem score in participants with ADHD was 39.23±8.99. Younger caregivers, those with an education level below a bachelor's degree, higher monthly income, positive screening for depression, and lower attitudes toward parenting scores were significantly associated with low self-esteem scores in their children.

Conclusion: Age, education level of caregivers, and monthly family income were significantly correlated with the self-esteem of children and adolescents with ADHD. The attitudes of caregivers towards parenting and depression in caregivers also impacted self-esteem of children with ADHD under their care. Thus, strategies aimed at promoting positive caregiving attitudes, regular screening of caregivers for depression, and providing appropriate treatment are recommended to enhance self-esteem in children and adolescents with ADHD.

Keywords: Attention-deficit/hyperactivity disorder; self-esteem; caregiver; psychosocial factor; depression (Siriraj Med J 2023; 75: 584-591)

INTRODUCTION

Attention Deficit/Hyperactivity Disorder (ADHD) is a common neurodevelopmental disorder among children and adolescents, with a global pooled prevalence ranging between 5.3%-7.2%.[1] The primary cause of ADHD is a deficit in the brain's ability to regulate concentration, inhibition, and the organization of activities. The three hallmark symptoms of ADHD include inattention, impulsivity, and

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hyperactive behavior, exhibited to a greater degree than typical children of the same age. The severity of ADHD varies among individuals; the Diagnostic and Statistical Manual, Fifth Edition (DSM-5) has established criteria for classifying ADHD severity as "mild", "moderate", or "severe" based on ADHD-related behaviors and the impact on academic and social functioning.[2]

ADHD symptoms can contribute to several negative outcomes for children and adolescents, including academic failure, problems with peer acceptance, and strained family relationships. These repercussions often result in lower self-esteem among the affected children and adolescents. Those with a negative perception of their abilities tend to struggle when dealing with stressful events, which can lead to the development of maladaptive coping strategies.[3] A study by Mazzone et al.[4] found correlation between low self-esteem and poorer outcomes in ADHD treatment, as well as an increase in mental health issues. Additionally, a study by Glass et al.[5] found that children and adolescents with low self-esteem had a higher prevalence of behavioral and mental problems.

Encouraging high self-esteem in children and adolescents to can help promote more effective treatment outcomes, develop proper problem-solving skills, and decrease mental and behavioral problems. Consequently, recent studies have focused more on factors associated with the self-esteem of ADHD patients to devise better treatment plans. Factors linked to low self-esteem include severe ADHD symptoms[6,7], side effects from ADHD medication[8], severe aggressive behaviors[9] and depression.[8] However age, gender, average grade points, and intelligence quotient (IQ) showed no correlation with the self-esteem of these children.[10]

Caregivers play a crucial role in promoting superior treatment outcomes for patients with ADHD, as they are primarily responsible for managing patients' behavior, medication intake, and providing assistance and mental support when patients encounter difficulties, such as learning and forming relationships. Support from caregivers promotes increased self-esteem in children and adolescents, leading to an improved prognosis for ADHD.

Although parents play a significant role in fostering self-esteem in patients with ADHD, no studies have analyzed the association between caregivers' psychosocial factors and the self-esteem of ADHD patients. Therefore, the objective of our study is to explore the caregivers' psychosocial factors, their attitudes towards child care, and their parenting styles which influence the level of self-esteem in children and adolescents.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study was conducted amongst dyads of ADHD patients and their caregivers at Siriraj Hospital to investigate the correlation between caregivers' psychosocial factors and self-esteem of children and adolescents with ADHD undergoing treatment. Using an estimation for a medium effect size on major variables at 80% statistical power and 5% maximum type I error[11], we calculated a required sample size of 66 pairs for this study. The inclusion criteria contain pairs of a primary caregiver and a patient with ADHD, aged 8 to 15, receiving ADHD treatment at Siriraj Hospital. ADHD patients who had comorbidities with intellectual disability, autism spectrum disorder or other psychological conditions such as depression, anxiety or schizophrenia were excluded.

Data collection

Ethical approval was granted by the Siriraj Institutional Review Board (COA no. Si 661/2022 (IRB1)). Data were gathered from September 2022 to February 2023. Eligible children and teenagers with ADHD, along with their parents were thoroughly informed about this study. After providing informed consent, they completed paper-based questionnaires. This study utilized five questionnaires: The Five-Scale Test of Self-Esteem for Children, The Attitudes of Child Care Questionnaire, Parenting Styles and Dimensions Questionnaire, Patient Health Questionnaire-9, and Generalized Anxiety Disorder 7-item. If participants were unable to read the questionnaire themselves, the research team read it to them.

Measurements

Demographic information form

This form recorded demographic and clinical data of both the primary caregiver and their children with ADHD. The collected patient's information compromised gender, age, and grade point average (GPA). The primary caregiver's data included gender, age, marital status, educational level, and monthly household income. The patient's clinical information, including type of ADHD, severity of ADHD symptoms, and comorbidities, was procured from the patient's medical record review and evaluation by their attending physician. Severity of ADHD was designated by attending physicians according to the DSM-5 criteria[2] as follows:

- Mild: Few symptoms beyond those required number for diagnosis are present, and symptoms result in minor impairment in social or school settings.

- Moderate: Symptoms or functional impairment between "mild" and "severe" are present.
- Severe: Many symptoms beyond the number needed for diagnosis are present; symptoms result in marked impairment in social or school settings.

The Five-Scale Test of Self-Esteem for Children, Thai-version (Thai-FSC)[12]

The Thai FSC, a child-rated questionnaire comprising 36-items, is a practical instrument for effective assessment of self-esteem in children aged 6 to 18. It exhibits good internal consistency (Cronbach's alpha coefficients = 0.60-0.78). Total scores range from 0 to 72 points, and while the questionnaire does not have a cut-off point, a higher FSC score indicates better self-esteem.

The Attitudes of Parenting Questionnaire[13]

To evaluate caregivers' attitudes towards child care, we employed the Attitudes of Parenting Questionnaire. In this questionnaire, caregivers are self-responders to 40 items addressing understanding and acceptance of their children, comprehension of their children's disease and treatment, and the level of support they receive from their community. The total score extends from 30 to 180 points, and while the questionnaire lacks a cut-off score to categorize attitudes, a high score indicates a positive parenting attitude. The questionnaire has strong internal consistency (Cronbach's alpha coefficients = 0.84).

Parenting Styles and Dimensions Questionnaire, Thai-version (Thai-PSDQ)[14]

The Thai-PSDQ evaluates parenting styles. Here, primary caregivers rate the frequency of certain behaviors towards their child based on 32 statements, using a five-point Likert scale (ranging from "Never" = 1 to "Always" = 5). For example, parents rate how much they encourage their children to freely express themselves, even when disagreeing with the parents. The questionnaire explores different parenting styles with 15 items on authoritative parenting, 12 on strict authoritarian parenting, and five on permissive parenting. To obtain an overall authoritative, authoritarian, and permissive score for each parenting style, an average of the items relevant to each style was computed, with total scores for each parenting style ranging from one to five. A caregiver is considered to predominately practice the parenting style with the highest average score. Authoritarian parents typically exhibit rational, issue-oriented behavior when directing their child's activities, while evaluating both autonomous self-will and disciplined conformity. Authoritarian parents strongly influence their child's behavior and attitudes, adhering strictly to a pre-established code of conduct. These parents generally adopt a non-collaborative approach, do not encourage verbal discourse, and expect their child to submit to their perspectives. In contrast, the permissive parenting style is marked by a non-punitive and affirmative attitude towards the child's impulses, desires, and actions. These parents rarely impose strict control and seldom enforce conformity to externally-defined standards.[15] PSDQ demonstrated internal consistency of was 0.86 for authoritative, 0.82 for authoritarian, and 0.64 for permissive parenting styles.

Patient Health Questionnaire-9, Thai-version (Thai PHQ-9)[16]

The Thai PHQ-9 was employed to evaluate depression in primary caregivers. This tool requires caregivers to rate the frequency of experiencing any of the listed problems over the preceding two weeks by responding to nine statements on a 4-point Likert scale (ranging from "Not at all" = 0 to "Nearly every day" = 3). The combined scores ranged from 0 to 27, with higher scores indicating more severe depression. Previous research on the Thai PHQ-9 in the general Thai population revealed that a summed score of 9 or greater suggested a major depressive disorder, boasting a sensitivity of 0.84 and specificity of 0.77.

Generalized Anxiety Disorder 7-item, Thai-version (GAD-7, Thai-version)[17]

The GAD-7, Thai version, was used in this study to measure symptoms of generalized anxiety disorder (GAD). The scale consists of seven items, corresponding to the DSM-V symptom criteria for GAD. Primary caregivers indicate the frequency of experiencing any of the listed problems over the past two weeks on a 4-point Liker scale (ranging from "Not at all" = 0 to "Nearly every day" = 3). The total possible score ranges from 0 to 21, with higher scores indicating more severe GAD symptoms. A GAD-7 score of ≥ 5 serves as a threshold for identifying GAD, exhibiting a sensitivity and specificity of 0.89 and 0.82, respectively.

Data analysis

Data was processed using SPSS Statistics Program, Version 26 (IBM Corp, Armonk, NY). Descriptive analysis was conducted to compute the frequency and percentage of categorical data, along with the mean ± standard deviation (SD). As our study aims to explore the connection between psychosocial variables of primary caregivers and their children's self-esteem scores, we examined all

potential factors of both children and their caregivers through multivariable linear regression, irrespective of the statistically significant results of univariate linear regression for each variable. In the multivariable linear regression, a p-value of less than 0.05 was considered statistically significant.

RESULTS

From September 2022 to February 2023, we followed up with 73 children aged 8-15 diagnosed with ADHD at Siriraj Hospital. Among these children, 4 had intellectual disabilities and 3 had autism spectrum disorder, and were thus excluded based on our exclusion criteria. Consequently, 66 children were eligible to participate in this study. All 66 pairs of children and adolescents with ADHD and their primary caregivers agreed to participate in this study. The average age of these children and teenagers with ADHD was 12.09 ± 1.83 years, and 65.2% were males. The most prevalent type of ADHD was the combined type (54.5%), and mild ADHD symptoms were observed in 65.2% of the participants. Comorbidity in the form of learning disorders, was present in 62.1% of cases (Table 1).

The primary caregivers had an average age of 47.86 ± 5.56 years. The majorities were females (80.3%), and mothers made up 68.2% of the caregivers. About 81.8% were married, and over half held a bachelor's degree or higher (68.2%). A monthly family income exceeding $885 was reported by 69.7% of participants. Nearly all caregivers (90.0%) employed an authoritative parenting style and exhibited an average of childcare attitude score of 125.85 ± 19.75. Moreover, 53% were screened positive for depression, and 16.7% for generalized anxiety disorder (Table 1).

The average self-esteem score among all participants was 39.23 ± 8.99. Univariable linear regression analysis (Table 2) revealed a positive correlation between self-esteem scores and both a high GPA ($p < 0.001$) and the hyperactive/impulsive type ($p = 0.015$). In contrast, more severe ADHD symptoms and the presence of comorbidity were negatively correlated with self-esteem scores, with p-values of <0.001 and 0.022, respectively. Among caregiver factors, older age ($p = 0.001$), an educational level of bachelor's degree or higher ($p < 0.001$), a high parental attitude score ($p < 0.001$), and an authoritative parenting style ($p = 0.009$) were positively correlated with self-esteem. Meanwhile, higher family income, positive depression and anxiety screenings were negatively correlated with participants' self-esteem, with p-values of 0.004, <0.001 and 0.002, respectively.

TABLE 1. Demographic characteristics of children and adolescents with ADHD and their primary caregivers.

Demographic characteristics	Descriptive results
Child	
Gender, male (%)	43 (65.2)
Age[a]	12.09 (1.83)
GPA[b]	2.55 (2.10, 3.33)
ADHD type, n (%)	
Combined type	36 (54.5)
Inattentive type	19 (28.8)
Hyperactive/impulsive type	11 (16.7)
Severity ADHD, n (%)	
Mild	43 (65.2)
Moderate	23 (34.8)
Comorbid, n (%)	25 (37.9)
Total self-esteem score[a]	39.23 (8.99)
Primary caregiver	
Gender, male (%)	13 (19.7)
Age[a]	47.89 (5.56)
Marital status, n (%)	
Single	3 (4.5)
Married	54 (81.8)
Divorce/separate	9 (13.6)
Educational level, n (%)	
Below bachelor's degree	21 (31.8)
Bachelor's degree and above	45 (68.2)
Monthly household income, n (%)	
<885 US dollars[+]	20 (30.3)
≥885 US dollars[+]	46 (69.7)
Parenting style, n (%)	
Authoritarian	6 (9.1)
Authoritative	60 (90.9)
Positive depression screening, person (%)	35 (53)
Positive anxiety screening, person (%)	11 (16.7)
Child care attitude score[a]	125.85 (19.74)

Data presented as number (percentage), [a]Data presented as mean (SD), [b]Data presented as median (IQR)
Abbreviations: GPA = grade point average, ADHD = attention-deficit/hyperactivity disorder
[+] 1 US dollar = 33.92 bahts

TABLE 2. Univariable linear regression analysis for association between demographic characteristics and total self-esteem scores.

Demographic characteristics	Regression coefficient (95% CI)	P-value
Child		
Gender		
Male	Reference	
Female	-2.82 (-7.44, 1.80)	0.228
GPA	9.87 (7.64, 12.10)	<0.001*
ADHD type		
Combined type	Reference	
Inattentive type	0.24 (-4.68, 5.16)	0.922
Hyperactive/impulsive type	7.45 (1.47, 13.43)	0.015*
Severity ADHD		
Mild	Reference	
Moderate	-10.49 (-14.36, -6.62)	<0.001*
Comorbid		
No	Reference	
Yes	-5.20 (-9.60, -0.79)	0.022*
Primary caregiver		
Gender		
Male	Reference	
Female	-2.50 (-8.06, 3.07)	0.374
Age	0.62 (0.25, 1.00)	0.001*
Marital status		
Single	-9.89 (-21.54, 1.76)	0.095
Married	2.19 (-4.11, 8.48)	0.490
Divorce/separate	Reference	
Educational level		
Below bachelor's degree	Reference	
Bachelor's degree and above	9.48 (5.33, 13.64)	<0.001*
Monthly household income		
<885 US dollars+	Reference	
≥885 US dollars+	-6.78 (-11.24, -2.24)	0.004*
Parenting style		
Authoritarian	Reference	
Authoritative	9.97 (2.63, 17.30)	0.009*
Positive depression screening	-12.89 (-15.98, -9.80)	<0.001*
Positive anxiety screening	-9.00 (-14.54, -3.46)	0.002*
Child care attitude score	0.40 (0.34, 0.45)	<0.001*

*Statistically significant at p-value < 0.05

Abbreviations: GPA = grade point average, ADHD = attention-deficit/hyperactivity disorder

+ 1 US dollar = 33.92 bahts

To explore parental psychosocial factors associated with participants' self-esteem, we analyzed all demographic characteristics of both parents and subjects using multivariable linear regression (Table 3). Older age and higher education positively correlated with the self-esteem score of children and teenagers with ADHD (p = 0.002 and < 0.001, respectively). In comparison, higher family income was negatively correlated with self-esteem scores ($p < 0.001$). An increase of 1 point in child care attitude score corresponded to an increase of 0.17 points in participants' self-esteem score (95% CI 0.10, 0.23; $p < 0.001$). Furthermore, positive depression screenings remained negatively correlated with participant self-esteem (β = -5.58; 95% CI -7.22, -3.95; $p < 0.001$).

DISCUSSION

Our study determined that numerous factors associated with primary caregivers were linked to the self-esteem of children and adolescents with ADHD. Caregivers who demonstrated positive parental attitudes tended to have patients with better self-esteem. This finding is consistent with a study by Khaleque et al. (2013), which suggested that better parental attitudes towards child care, coupled with appropriate acceptance and responsiveness to their children, had a medium effect size on the children's self-esteem.[18] This information shows the influence of positive individual and family relationships on the development of self-esteem.

On the other hand, caregivers with depression were associated with low self-esteem in children and teenagers. This result aligns with the study by Krauss et al. (2021) which found that caregiver's depression negatively predicted child self-esteem.[19] Parents with depression tend to create a stressful and uncomfortable atmosphere within the household, causing children to hesitate initiating social interaction with others and leading to a perception of reduced self-worth. Another explanation for the impact of caregiver depression on

TABLE 3. Multivariable linear regression analysis for association between primary caregiver's psychosocial factors and the child's mean total self-esteem scores.

Primary caregiver's psychosocial factors	Regression coefficient (95% CI)	P-value
Categorical variable		
Gender		
Male	Reference	
Female	-2.65 (-4.67, 0.63)	0.093
Marital status		
Single	-1.64 (-6.32, 3.05)	0.486
Married	-2.30 (-4.46, 0.13)	0.089
Divorce/separate	Reference	
Educational level		
Below bachelor's degree	Reference	
Bachelor's degree and above	8.91 (4.47, 13.34)	<0.001*
Monthly household income		
<885 US dollars+	Reference	
≥885 US dollars+	-8.27 (-12.52, -4.01)	<0.001*
Parenting style		
Authoritarian	Reference	
Authoritative	0.22 (-2.70, 2.27)	0.862
Positive depression screening	-5.58 (-7.22, -3.95)	<0.001*
Positive anxiety screening	-2.43 (-4.99, -0.13)	0.062
Numerical variable		
Age	0.22 (0.08, 0.35)	0.002*
Child care attitude score	0.17 (0.10, 0.23)	<0.001*

*Statistically significant at p-value < 0.05

+ 1 US dollar = 33.92 bahts

child self-esteem is genetic redisposition. Low self-esteem is considered an early symptom of depression in children and adolescents and is thought to be inherited from parents to a degree of 60-70%.[20]

Furthermore, our research revealed that caregivers' age positively correlated with the self-esteem of children and teenagers with ADHD. This outcome is consistent with Jendreizik's study, which found that older caregivers were associated with more effective proper parenting, understanding, and acceptance towards their children in the prognosis and treatment of ADHD.[21] Caregivers' positive attitudes towards children contribute to better self-esteem in patients under their care.

Our study found that a higher education level in caregivers is associated with elevated self-esteem in children. Caregivers with more advanced education are more likely to have a deeper understanding of ADHD and the skills to address behavioral problems. This finding aligns with the study by Parker and Benson (2004) which stated that caregivers who pay attention to their children's problems and appropriately handle such situations appropriately correlated positively with children's self-esteem.[22] In addition, caregivers with higher level of education tend to be more attentive and supportive of their children's education pursuits, which aligns Flouri (2006) who suggested that increased attention and encouragement from caregivers towards their child's education can enhance a child's self-efficacy and subsequently raise self-esteem.[23]

Interestingly, our study revealed that higher family income correlates negatively with the self-esteem scores of children and teenagers with ADHD. This contradicts previous studies that reported poverty as a factor contributing to a caregiver's emotional stress and impaired parenting behavior, leading to lower self-esteem in children.[24] We hypothesize that caregivers with higher incomes may tend to invest more time in their careers than on their child care, possibly experiencing more workplace stress, which may result in caregiver-child conflict. A strained relationship between caregivers and children could potentially have a negative impact on the children's self-esteem.[25]

There have been several studies focusing on the sociodemographic factors of patients with ADHD that are associated with their self-esteem.[3,10] However, fewer studies have investigated the influence of caregivers on the self-esteem of children and teenagers, despite caregivers playing a critical role in monitoring patient behavior and supporting patients with daily struggles. As a result, this study is pioneering in Thailand as it explores caregiver psychosocial factors affecting the affect the self-esteem of children and adolescents with ADHD. Our findings should prompt could urge healthcare providers working with children and adolescents with ADHD to focus on caregivers' attitudes towards child care along with parental depression screening. These factors significantly influence patients' self-perception and can contribute to improved outcomes in ADHD treatment.

Limitations

Our study had several limitations. First, although the statistical power of our study is acceptable, the participant group consisted of only 66 children and adolescents aged 8 to 15 with ADHD. All of whom received treatment at the pediatric developmental clinic at Siriraj Hospital. Therefore, the generalizability of our results may be limited. Future studies are recommended to gather data from multiple centers and larger sample sizes to increase applicability of our findings. Second, our study did not capture some variables potentially linked to the self-esteem of children and teenagers, such as the quality of caregiver-child relationships and family financial debt. These factors warrant exploration in a subsequent research. Last but not least, self-esteem scores collected from children and adolescents may possibly be biased due to potential self-overestimation. These scores were derived solely from self-report questionnaires, without the inclusion of additional investigations of social function. Future studies should consider broadening the data to include these investigations.

CONCLUSION

The caregivers' age, education level, and monthly family income had significant correlations with the self-esteem of children and teenagers with ADHD. Moreover, caregivers' attitudes towards child-rearing and the presence of depression in caregivers also influenced the self-esteem of patients with ADHD under their care. Enhancing caregivers' awareness and understanding of ADHD symptoms and treatment, promoting self-acceptance and self-value among patients, encouraging a positive attitude towards child-rearing, and screening and managing caregiver depression can boost children's self-esteem, contributing to more successful ADHD treatment outcomes.

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Dental Students' Perspectives and Learning Experiences during the Covid-19 Outbreak: A Qualitative Study

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ABSTRACT

Objective: The present study aimed to explore the perspectives and learning experiences of undergraduate dental students during the Covid-19 pandemic using a qualitative approach.

Materials and Methods: The current study used a qualitative focus group approach based on the Consolidated Criteria for Reporting Qualitative Research checklist. Three focus groups were carried out using a piloted interview topic guide. A convenient sampling was adopted to include undergraduate year 3 to year 5 dental students who had attended regular face-to-face session before the pandemic. Each session consisted of 6 to 8 participants who were randomly assigned, and the sessions lasted for about 30 to 45 minutes. The interviews were recorded and transcribed verbatim. Content analysis using a inductive approach was employed to the focus group data. All the final codes were refined and agreed by all members of the research team.

Results: Four main themes with their respective subthemes were identified through the coding process, namely change in study life balance, online learning, interpersonal relationship, and concern for future.

Conclusion: Dental students faced numerous challenges during the Covid-19 pandemic, both mentally and academically. Academicians must reconsider and re-evaluate the curriculum, including the mode of delivery, as total eradication of the virus is not likely to be possible in the foreseeable future.

Keywords: Coronavirus; Covid-19; health profession; focus groups; undergraduate (Siriraj Med J 2023; 75: 592-598)

INTRODUCTION

On 11th March 2020, the World Health Organization (WHO) declared the coronavirus disease 2019 (Covid-19), or known as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a pandemic considering the infection's level of transmission and severity.[1] Since then, COVID-19 has caused millions of fatalities worldwide, challenged the government with a historically difficult issue in the face of intense social, economic, and budgetary demands, with a profound impact on every aspect of human existence.[2] As a consequence of the virus's rapid transmission, most countries have imposed stringent measurements and required the public to wear a mask and maintain a safe distance physically.[3,4] Furthermore, countries across the globe enacted nationwide lockdowns in an effort to restrict the infection from spreading.[5,6] As a result of the pandemic, educational institutions were compelled to shut down, prompting academics to devise alternative methods to continue their teaching and learning sessions.[7]

Dental schools were no exception to this interruption as dentistry is among the courses deemed as high risk

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of infection due to the nature of clinical training and the close proximity to patients' oral cavities during dental treatment.[2,8] The three primary components of dental education are lecture or problem-based learning (PBL), simulation or laboratory practical, and clinical training, which is the most crucial component in the dental curricula.[9] Ever since the COVID-19 outbreak, most of these dental educational activities were moved from face-to-face instruction to online or e-learning via digital platforms. Such an action was required to protect the health and wellness of employees, students, and patients and to maintain the educational system. E-learning is defined as a system of learning that uses electronic media, typically over the internet.[10] It is worth noting that the incorporation of information technology in the educational system has completely transformed the way individuals learn.

Over the years, e-learning has been demonstrated to possess remarkable success in teaching and learning for medically related disciplines, including dentistry.[11,12] Nonetheless, e-learning was one of the instructional methods used by dental schools in Malaysia even before the Covid-19 outbreak. It started to gain popularity and replaced all other teaching and learning methods during the Covid-19 pandemic. Undoubtedly, the unusual COVID-19 pandemic expedited the shift to online education, but it was still unclear whether academic staff and students were prepared for these changes. Nevertheless, study exploring the learning perceptions of dental students in Malaysia during the Covid-19 pandemic is still scarce in the literature. To address this gap, the current study aimed to explore the perspectives and learning experiences of undergraduate dental students during the Covid-19 pandemic using a qualitative approach.

MATERIALS AND METHODS

Study design

The current study used a qualitative focus group methodology according to the Consolidated Criteria for Reporting Qualitative Research (COREQ) checklist.[13] Ethical approval was obtained from the AIMST University Human & Animal Ethics Committee (Ref No: AUHEC/FOD/2022/18). Three focus groups were carried out using a piloted interview topic guide.

Participants recruitment

A convenient sampling was adopted in the current study of which undergraduate year 3 to year 5 dental students were emailed with the participant's information sheets to invite them to take part in the study. The reason for choosing year 3 to year 5 students was that they had previously attended a regular face-to-face session before the COVID-19 outbreak. The participants' information sheets outlined that the participation was voluntarily, the focus group would be video-recorded, and the participants were free to withdraw from the study at any point of time before the data was published.

Data collection

A topic guide was developed and underpinned by research evidence on the learning experiences of students during the Covid-19 pandemic.[14-16] The content of the topic guide was further validated by two experts in dental education. All respondents gave written consent prior to the focus group session, and they were given the opportunity to discuss any doubts they encountered with the researchers. Between June and July 2022, three focus group discussions took place using Zoom Video Communication Software. Each session consisted of 6 to 8 participants who were randomly assigned, and the sessions lasted for about 30 to 45 minutes.

Two facilitators were present in each session, with WWT facilitated the conversation and GSSL contributed ideas. The two facilitators were known by the student participants as faculty members, but they were not actively engaged in their academic learning. During the focus group discussions, only the facilitators and participants were present with no other third parties. Data collection was halted after all investigators agreed that no new themes emerged.

Data analysis

Data obtained from all three focus groups were transcribed verbatim. Participants were given the chance to cross-check the findings and make comments following transcription. Content analysis using a inductive approach was employed to the focus group data. New codes were added in an inductive manner of which two investigators (WWT and GSSL) coded the data independently. Any disagreement in the codes was resolved through discussion among all investigators. All the final codes were refined and agreed by all members of the research team.

RESULTS

A total of 65 invitations were distributed, but only 20 students agreed to participate in the focus group discussions. Four major themes with their respective subthemes were identified: *Changes in study-life balance, Online learning, Interpersonal relationship, and Concern for future*. These themes were illustrated in Fig 1.

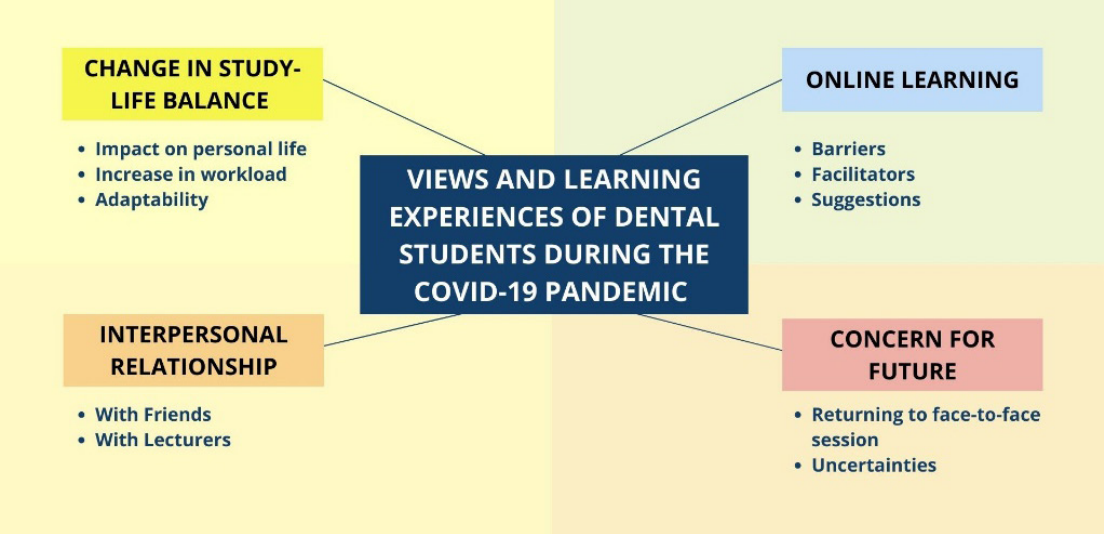

Fig 1. Four major themes on views and learning experiences of dental students during the COVID-19

Theme 1: Change in study-life balance

Subtheme 1: Impact on personal life

Most participants experienced deterioration in mental health during the Covid-19 pandemic. They were not allowed to go out for sports, nor were they allowed to meet up with their friends.

F2P1: *"You can't do sports, you can't go out visiting, you can't go out to eat proper food in dinner...nothing, you're just stuck at home..."*

F3P3: *"…I would say it was severely declining (mental health), especially when it started, because I'm an extrovert. And uhm… I need human interaction… I need my people."*

F3P9: *"I did feel lonely, sometimes I did feel depressed a bit."*

Their daily routine was only to attend online classes. The students were stressful, less motivated and some of them felt overwhelmed by the situation.

F3P4: *"All my roommates went back home and only me and one of my housemates were here. And it was really depressing because the only thing you've got is to attend the classes, go to the cafe eat, come back."*

F3P2: *"…for me, it was less motivating because I was not focusing at all during class…"*

F3P8: *"I had a very hard time coping to the point where I was sent into counseling... We had like, a lot to catch up on and it was just overwhelming."*

On the contrary, some participants coped exceptionally well during the Covid-19 pandemic. They enjoyed the extra time that they had with family at home.

F2P4:" I'm enjoying the life… we have extra time staying in our hostel or in our house."

F2P4: *"I found myself quite enjoyable at that time because I think I have a lot of family bonding time."*

Subtheme 2: Increase in workload

The participants also experienced an increase in academic workload as they had to attend multiple online classes per day.

F3P4: *"We have to sit in front of the laptop for almost like five to six hours because we have four to five classes per day."*

F2P1: *"I feel online classes are very draggy, probably because of the lack of human interaction probably because lecturers probably don't know if we understand or not."*

It was also highlighted that the organization of the schedule was messy due to the uneven distribution of classes and frequent rescheduling of classes.

F1P2: *"The distribution of the lecture is bad as well..."*

F2P1: *"...rescheduling happens a lot, so we don't get to plan our week according to how we want."*

Subtheme 3: Adaptability

Most participants had a hard time to adapt to the COVID-19 situation.

F2P1: *"I was very used to physical learning process so when it came to online it was hard for me to adapt."*

F2P3: *"…I find myself really hard to set a schedule to make myself study during COVID..."*

As time goes by, the participants were able to adapt better, and they managed to find a way to cope with the situation.

F2P3: *"...I sort of found a solution gradually as time goes on."*

Theme 2: Online learning

Subtheme 1: Barriers

One of the biggest barriers faced by the participants during online learning was difficulty to focus. Since the classes were all conducted online, they got distracted easily by their surroundings and other online web pages.

F2P1: *"… you kind of lose the focus you had during that class, and you get distracted and yeah and sometimes certain students' mic are on, and the sessions just get repeatedly distracted."*

F3P3: *"since we're using a device, it's quite easy to get distracted, because we have multiple tabs on..."*

F3P7: *"…there are other distractions because I have like three devices, three devices on the table."*

Screen time fatigue was also cited as a big challenge as they had to sit in front of the computer screen for long hours.

F1P2: *"My eyes are quite sensitive to blue light. Even if I turn on the blue light filter, it's quite taxing on my eyes after prolonged staring on screen."*

F3P7: *"It's a long time, long time in front of screen."*

Poor connectivity and technical issues also acted as a barrier to online classes resulting in the postponement of classes.

F3P2: *"They need to upgrade their Wi-Fi system, because of poor connections, the student cannot have the lecture, and they have to postpone the lecture to a later date."*

F2P3: *"Sometimes lecturers didn't know how to use the Zoom, and then all the technical errors, and... by the time 20, 30 minutes have gone, we've lost our interest."*

Subtheme 2: Facilitators

When it comes to the facilitators of online learning, the participants liked the extra time and comfort that they had, when they attended online classes at home.

F3P7: *"…you can just wake up late and just go for online class."*

F2P3: *"I have time to actually look into more material and resources because I have the excess time."*

F3P5: *"I guess it's the comfort also. For me being at home..."*

The recordings for online classes were also very useful for them as they could rewatch them anytime they wanted.

F2P5: *"One of the best things for online lectures. There is a recorded video so we can watch it again."*

F3P4: *"We got the recordings, and you can replay how many times you want."*

Subtheme 3: Suggestions

In terms of suggestions to improve online learning, the participants hoped that the university would improve on the connectivity issue to ensure uninterrupted online learning experiences.

F3P8: *"I feel like the connectivity issues in our school needs rectification."*

F3P4: *"I would suggest improving the quality of Wi-Fi."*

Apart from that, most participants hoped that the lecturers would do more demonstration for online practical sessions to enhance their understanding of the subject matter.

F3P2: *"I hope that lecturers can demonstrate… maybe like, do something on a model and then show us on how the procedure really goes."*

F3P4: *"I will recommend the lecturers to do a video demonstration..."*

Theme 3: Interpersonal relationship

Subtheme 1: With Friends

The participants missed the human interaction, and some felt anxious to meet their friends.

F2P1: *"… I felt anxious meeting my friends."*

F3P9: *"I think because of the pandemic, we lost that human interaction, that connection is really important."*

Subtheme 2: With Lecturers

The participants claimed that they did not know a lot of the lecturers as they moved on to new academic year and a lack of understanding between lecturers and students also arose.

F2P1: *"…we did not know who they (lecturers) are and our only interactions with them was through online classes."*

F3P6: *"…lack of understanding between…maybe between us and the lecturers..."*

However, some lecturers did show empathy and care towards the students who stayed at the campus during the lockdown period.

F3P9: *"Few of the lecturers reached out to us and asked how we are doing..."*

Theme 4: Concern for future

Subtheme 1: Returning to face-to-face session

Some participants expressed their concern about returning to face-to-face sessions due to the fear of being infected with the virus.

F2P5: *"But still the Covid is around so gathering in a classroom, in a compacted area can increase the risk of getting infected with Covid."*

While others were grateful when the university gradually reopened and allowed students to come back in batches.

F3P9: *"When they (schools) allow us to come back batch by batch. I was so happy and was so grateful."*

Subtheme 3: Uncertainties

Participants faced a lot of uncertainties about their future. Many feared that they would not be able to graduate on time, due to the missed clinical sessions and poor patient flow.

F2P1: *"…You don't know how long it's going to prolong. How many clinical sessions you're missing."*

F2P2: *"The fear of will I graduate? When will I graduate? Because the patient flow was terrible…I get stressed."*

F3P9: *"How am I going to finish quota… there was a dreadful period."*

DISCUSSION

The aim of the present study was to explore the perspectives and learning experiences of undergraduate dental students during the Covid-19 pandemic using a qualitative focus group approach. Focus group discussions were used in the current study to address the research gaps since employing focus group study can provide information about participants' attitudes, feelings, beliefs, motivations, and experiences in a situation where these aspects are conditional and diverse.[17] Therefore, focus group discussions' participatory and discursive nature appeared to best represent these characteristics.

Undeniably, the sudden strike of the Covid-19 pandemic has impacted the normal delivery of classes, affecting the learning process of millions of students worldwide. One of the major challenges faced by the participants was the change in study-life balance, where many of them experienced a negative impact on their personal lives. Indeed, a series of movement control orders were implemented by the Malaysian government to restrict the movement of the citizen during the pandemic,[18] and students were confined to their houses or hostels. They were forbidden from attending the schools for extracurricular and academic activities as well as restricted from socializing with their friends in campus. Thus, a high possibility that they might experience depression, loss of motivation, and a sense of general overwhelm. Furthermore, the present findings are in accordance with previous research showing that students generally experienced a deterioration in their mental health throughout the pandemic period.[19-21]

Most of the students in the current study also highlighted the increase in academic workload during the COVID-19 pandemic due to the increased number of online lectures every day. Since no physical practical and clinical sessions were allowed, only lecture classes could be scheduled for the students to occupy their academic calendar. Several universities even converted practical sessions to online delivery mode during the Covid-19 pandemic.[22,23] However, due to the nature of dental profession that relies heavily on clinical competence and practical abilities, such a strategy to shift face-to-face practical session to online mode may not be feasible for dental students.[6,9]

Furthermore, the present findings are in accordance with previous research showing that students generally experienced a deterioration in their mental health throughout the pandemic period. When universities reopen, they must develop a comprehensive plan to address their students' potential mental health needs. A previous study has found that the Covid-19 pandemic has alarming implications for individual mental health and social functioning, increasing the risk of developing mood and anxiety disorders as well as elevated hyperarousal symptoms.[24]

The present results showed that students faced numerous barriers during online learning such as difficulty to focus, screen time fatigue, technical error, and connectivity issues. These barriers were similar to other studies conducted worldwide.[21,25,26] It should be highlighted that the pre-requisite for online classes is a good internet connection. However, many students residing in rural areas of the country could face challenges in obtaining internet coverage and stable connection.[27]

However, due to the nature of dental profession that relies heavily on clinical competence and practical abilities, such a strategy to shift face-to-face practical session to online mode may not be feasible for dental students. This is evident in another study conducted in Malaysia where only 49.7% of the students were satisfied with the clinical knowledge delivered through online classes.[28] Moreover, another possible explanation could be students and lecturers were not equipped with the necessary skills and equipment for online lectures which resulted in frequent technical errors. A previous study found that technological barrier was cited as the top three barriers faced by students in virtual learning during the Covid-19 pandemic.[29] Participants also advocated adding additional video demonstration to compensate for the practical sessions that were missed, which is consistent with a previous study that suggested to reduce the academic load and adding more clinical videos to case-based learning sessions in improving the overall learning experience.[21]

Additionally, the present study found that students missed the human interaction during online learning, which is congruent with a previous study.[21] Some of them were also anxious to meet their friends due to the prolonged online learning. Furthermore, it has been reported that

the limited interactions between students and teachers during online learning in the midst of the pandemic may have contributed to the difficulty students experienced in communicating with their teachers and the possibility of misunderstandings emanating.[26] Nonetheless, it can still be argued that after dental students gradually resume back to physical classes during the endemic phase in the near future, their communication skills might improve and that their anxieties about communicating with friends or even lecturers and patients might be reduced.

All participants reported varying degrees of concerns for their future such as completing their clinical requirements, graduating on time, and returning for face-to-face session, as the virus is still around. Kharma *et al.*[30] discovered that 85% of dental students felt stressed and anxious to return to dental school for training during the Covid-19 pandemic. Similarly, another study conducted among Malaysian dental students showed that only 32.6% of students were ready to attend the faculty classes after school reopen.[28] Dental students are now faced with additional stress due to the fear of being contacted with the disease upon returning to school and resumption of clinical training.[28] Thus, dental schools need to adopt strict safety guidelines to safeguard the health and wellbeing of students and staff during this crucial moment.[31]

Several limitations were noted in the present study. First, the findings from the current qualitative research do not demonstrate a causal relationship and should not be generalized to the wider population due to a small sample size. Instead, the present study aimed to understand and explore in depth on the perspectives and learning experiences of dental students during the Covid-19 pandemic. Second, as the study was only carried out after the Malaysian government declared Covid-19 an endemic, it is crucial to consider how well the participants were able to recall their learning experiences during the pandemic phase.

Thus, dental schools need to adopt strict safety guidelines to safeguard the health and wellbeing of students and staff during this crucial moment. For instance, dental schools may refer to the guidelines for dental education during the COVID-19 pandemic published by the Association for Dental Education, Asia Pacific (ADEAP), after thorough evaluation of the condition in the Asia Pacific region.[31] To generalize the findings to all dental students in Malaysia, future research should employ a quantitative method and involve dental students from various dental schools across the country. Nevertheless, the present findings add more data to the literature in improving the understanding of how dental students perceived their learning experiences during the Covid-19 pandemic.

CONCLUSION

Within the limitation of the present study, dental students faced numerous challenges during the Covid-19 pandemic, both mentally and academically. Although online teaching and learning served as the only medium for knowledge transfer during this period, it still could not completely replace the conventional method as dentistry relies heavily on practical skills and clinical competencies. Nevertheless, the abrupt closure of universities worldwide owing to the Covid-19 pandemic provides an opportunity for cultural revolution in the education sector. Academicians must reconsider and re-evaluate the curriculum, including the mode of delivery, while bearing in mind the inequalities students may confront in accessing resources, since total eradication of the virus is not likely to be possible in the foreseeable future.

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Conflict of interest

The authors declare no conflict of interest.

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Court-Type Thai Traditional Massage for Patients with Intractable Peripheral Neuropathic Pain: a Randomized Controlled Trial

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ABSTRACT

Objective: Neuropathic pain management involves both pharmacological and non-pharmacological interventions. Despite this, no prior research has demonstrated the efficacy of court-type Thai traditional massage (CTTM) for neuropathic pain relief. This study aimed to investigate the potential benefits of CTTM in alleviating neuropathic pain.

Materials and Methods: A preliminary single-blind randomized controlled trial was conducted on 28 participants with peripheral neuropathic pain, who were equally assigned to 2 groups. Both groups received standard drug treatment; however, the intervention group additionally received CTTM and hot herbal compression, while the active control group only received HHC. The adjuvant treatments were administered twice weekly for 4 weeks (V1-V8). A follow-up was conducted 4 weeks posttreatment (V9). Outcome measures were assessed at V1, V4, V8, and V9 using a numerical rating scale and the Thai versions of the Neuropathic Pain Symptom Inventory, the Brief Pain Inventory, and the EQ-5L-5D health questionnaire.

Results: The data revealed that the intervention and active control groups had statistically significant differences in their pain intensity scores ($P < 0.001$), total neuropathic pain intensity scores ($P = 0.001$), and utility of health scores ($P = 0.007$) during the follow-up period. When comparing outcomes between V1 and V8, the groups exhibited significant differences in pain reduction ($P = 0.003$) and quality of life ($P = 0.027$).

Conclusion: This study provides initial evidence supporting the potential benefits of CTTM in alleviating peripheral neuropathic pain and improving quality of life. Future research should further investigate the application of CTTM in managing peripheral neuropathic pain conditions.

Keywords: Complementary therapies; massage; pain intensity; peripheral neuropathic pain; quality of life; randomized controlled trial (Siriraj Med J 2023; 75: 599-611)

INTRODUCTION

Peripheral neuropathic pain arises from lesions or diseases affecting the somatosensory nervous system.[1] Comprehensive clinical pain assessment of neuropathic pain encompasses severity, characteristics, medication usage and potential side effects, the impact of mobility and usual activities. For example, chronic low back pain patients with peripheral neuropathic pain were associated with higher disability than the patients without neuropathic pain.[2] A crucial outcome of neuropathic pain

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management is patient-reported pain relief or intensity reduction, individual adverse effects, and utilization of rescue medication. Although numerous drug classes are employed in pharmacological pain treatment, various nonpharmacological strategies are utilized to alleviate pain especially alternative medicine practices such as acupuncture, massage therapy, and behavior modification.[3]

Court-type Thai traditional massage (CTTM), a therapeutic technique that originated in Thailand, aims to facilitate patient healing and rehabilitation. This massage approach is characterized by the application of pressure using fingers and hands along specific massage lines and points on the body. Practitioners manipulate posture and angles to regulate the direction and intensity of pressure during massage.[4]

Several prior clinical trials have investigated the effects of CTTM on pain and psychological conditions. Traditional Thai massage (TTM) reduced spasticity and enhanced limb functions in elderly stroke patients, with results comparable to conventional physical therapy programs.[5] Patients with osteoarthritis experienced increased walking speed and improved quality of life following CTTM treatment.[6] A study comparing the efficacy of CTTM and amitriptyline for chronic tension-type headache patients demonstrated a significant decrease in pain severity.[7] CTTM outperformed topical diclofenac in enhancing the quality of life and shoulder functionality while decreasing pain intensity in patients with frozen shoulders.[8] Furthermore, CTTM effectively alleviated upper trapezius myofascial pain syndrome.[9] The effects of CTTM on electroencephalograms indicated a significant increase relaxation in patients with scapulocostal syndrome.[10] However, no study has yet examined the efficacy of CTTM in patients with neuropathy. Consequently, the present study aimed to investigate the impacts of CTTM on pain relief, physical and emotional functions, and quality of life in patients with peripheral neuropathic pain.

MATERIALS AND METHODS

Study design

This study was a preliminary single-blind, parallel-group, randomized controlled trial. Before the research commenced, it was approved by the Siriraj Institutional Review Board on September 18, 2019 (COA no. Si 649/2019) and registered in the Thai Clinical Trials Registry on August 1, 2021 (TCTR20210801006). Participants with intractable peripheral neuropathic pain were recruited from the pain clinic at Siriraj Hospital. All participants provided informed consent before commencing the study. The total duration of the investigation was 8 weeks.

Participants

The inclusion criteria for this study required patients to be over 18 years of age and diagnosed with peripheral neuropathic pain by the grading system for neuropathic pain diagnosis.[11] Additionally, patients were required to present with Neuropathic Pain Questionnaire 4 (DN4)[12] scores ≥ 4 and numerical rating scale (NRS) pain scores ≥ 4 at recruitment. Before study participation, patients' treatment progress during the preceding 3 months had to be stable, and they needed to be able to visit Siriraj Hospital at least twice a week. Exclusion criteria encompassed patients with surgery planned within 3 months, a history of uncontrollable psychiatric disorders, open wounds in the painful area, addiction to alcohol or drugs, pregnancy, or any contraindications specified in this study, such as a fever exceeding 38.5 °C or skin disease on the affected area. The research and participant assignments were conducted at the Ayuraved Applied Thai Traditional Medicine Clinic.

The criteria for the withdrawal of participants from the study were 1) voluntary withdrawal at any time due to inability to comply with study requirements or other reasons (e.g., experiencing side effects), 2) physician-advised withdrawal for a patient's health or well-being, and 3) loss to follow-up.

Interventions

The intervention ("M") group received standard neuropathic pain treatment and twice-weekly adjuvant treatment with CTTM and hot herbal compression (HHC) for 4 weeks. The active control ("H") group received standard treatment and twice-weekly HHC for 4 weeks. The treatment phase spanned the first to the eighth visits (V1-V8). A follow-up was conducted at the ninth visit (V9), held 4 weeks after the conclusion of treatment. A consort diagram of the study is depicted in Fig 1. Four applied traditional Thai massage (ATTM) practitioners with over 5 years of experience administering the interventions. The pressure the ATTMs could exert with their hands was determined to ensure that there would be consistent pressure levels across treatments. Each participant group was assigned 2 ATTM practitioners, who alternately treated the patients in their assigned groups throughout the study.

The CTTM treatments were categorized into 2 distinct massage patterns targeting the upper and lower extremity areas, corresponding to the patients' s pain locations. The first pattern focused on the upper extremity region, encompassing the upper back and upper limbs. The second pattern addressed the lower extremity region (the lower back and lower limbs). The duration of the

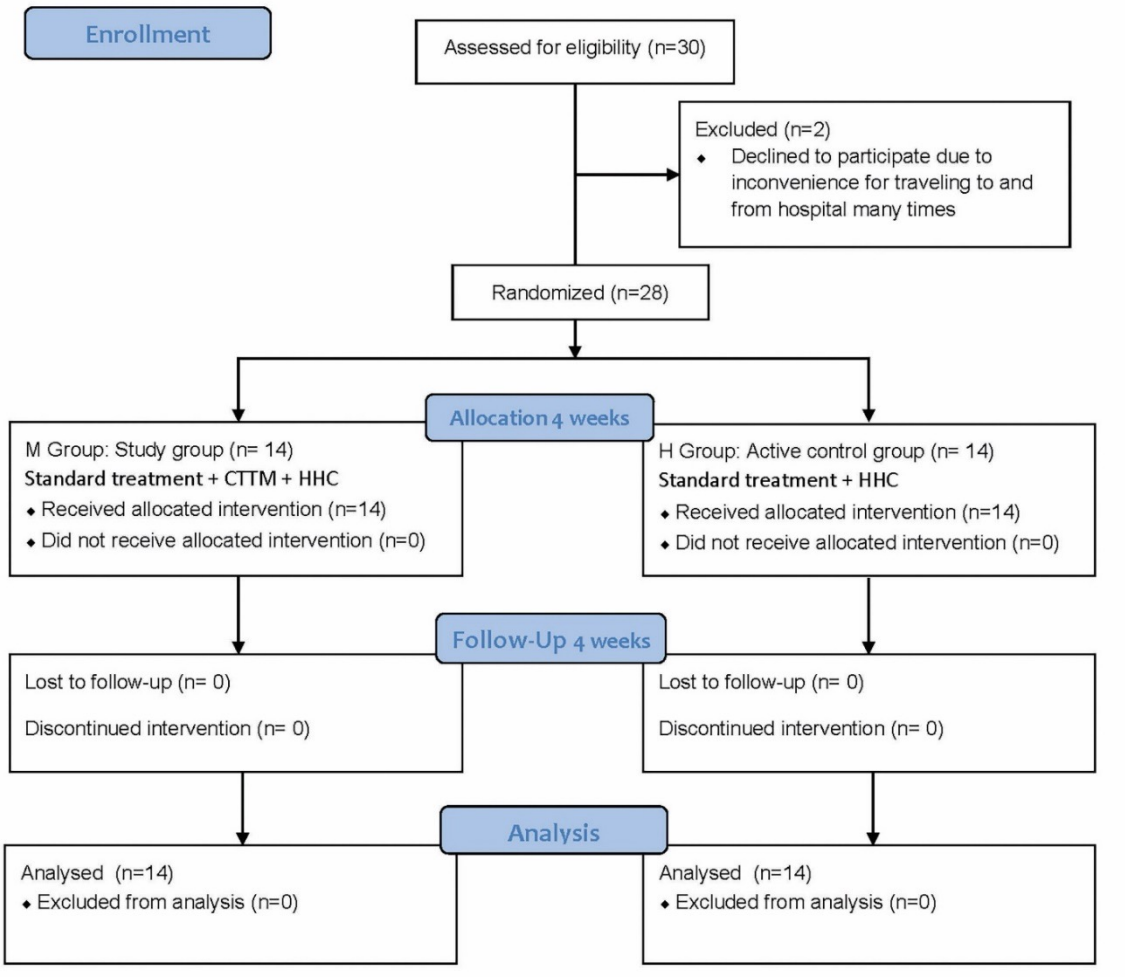

Fig 1. CONSORT diagram of the study
Participants were randomly divided into 2 groups by the randomization method. (1) Study group (M group): received treatment according to the standard neuropathic pain protocol plus adjuvant treatment (court-type Thai traditional massage [CTTM] and hot herbal compress [HHC] twice a week for 4 weeks). (2) Active control group (H group): treated according to the standard neuropathic pain protocol and HHC twice a week for 4 weeks. Allocation was a treatment phase of twice weekly (V1-V8); follow-up occurred after a 4-week rest period at the end of treatment (V9).

massage treatment was about 45 minutes. Both groups received HHC following the same patterns for about 15 minutes. Fig 2 illustrates the massage lines and points utilized in each pattern.

The compression balls utilized in the HHC were specially produced by the Ayurved Siriraj Manufacturing Unit of Herbal Medicine and Products, adhering to good manufacturing practice guidelines. The primary ingredients of the dry balls were *Zingiber montanum* (Koenig) Link ex Dietr., *Curcuma longa* Linn., *Curcuma zedoaria* (Berg) Roscoe., *Cymbopogon citratus* (De ex Nees) Stapf., and borneol, along with other components, amounting to a total of 150 grams per piece. Before application on patients, the balls were heated to a temperature of 43 to 45 °C.

Measured outcomes

The primary outcome was pain intensity reduction. It was assessed with numerical rating scales (NRS)[13,14] and the Thai versions of the Neuropathic Pain Symptom Inventory (NPSI-T).[15] Pain severity was measured 4 times (at V1, V4, V8, and V9) as an average in the past 24 hours. The NRS of pain severity was also assessed before and after each treatment session. The secondary outcomes were pain-related interferences and the patients's quality of life. This was self-assessed with the Brief Pain Inventory (BPI-T) and EQ-5D-5L at V1, V4, V8, and V9.

NRS is the 11-point scale asking participants to select a number from 0 to 10 that best represents their pain intensity, with 0 meaning "no pain at all" and 10 meaning "pain as bad as it could be." The participant's NRS score is the number they select.

The NPSI-T is a self-assessment tool with 12 items (Q1-Q12). Total intensity pain scores range from 0 to 100 and are the sum of the scores for each item other than Q4 and Q7. Subgroups of NPSI are divided into 5 clinical domains and calculated following questions: superficial spontaneous pain (Q1), deep spontaneous

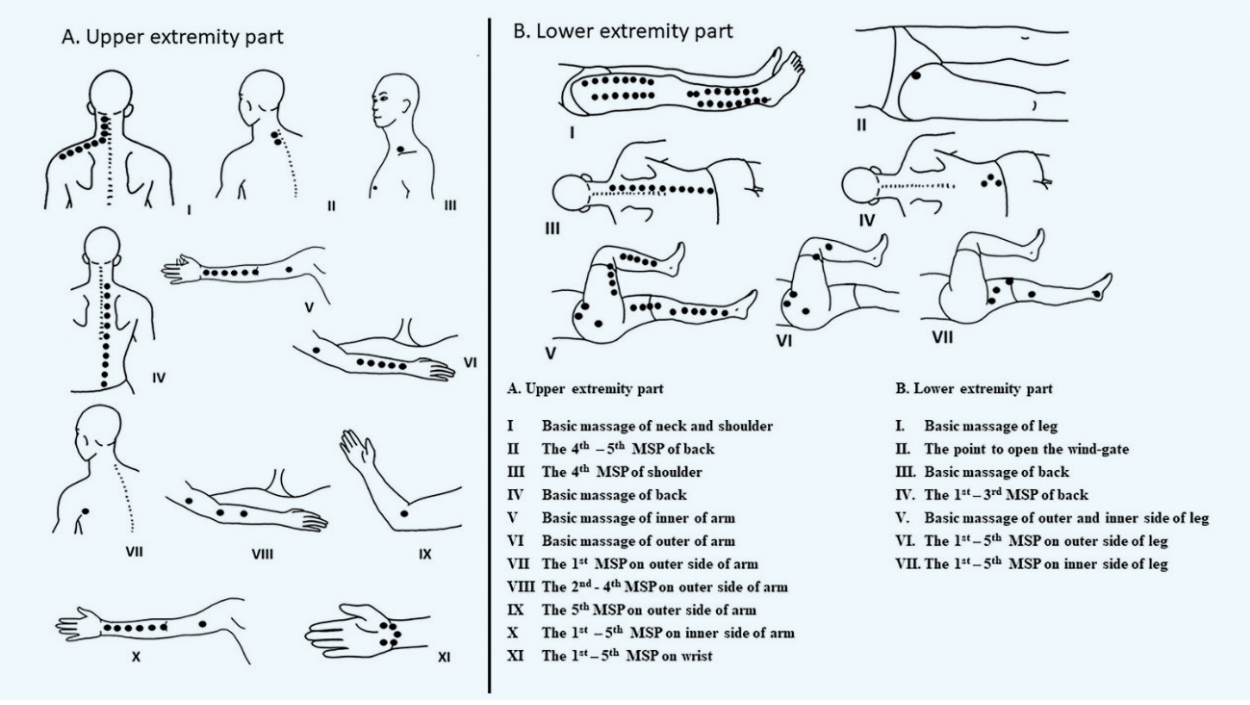

Fig 2. The basic court-type Thai traditional massage line and point and the major signal point of massage in the M group

pain ([Q2+Q3]/2), paroxysmal pain ([Q5+Q6]/2), evoked pain ([Q8+Q9+Q10]/3), and paresthesia/dysesthesia ([Q11+Q12]/2). The study demonstrated the validity and reliability of the NPSI-T for assessing neuropathic pain in Thai patients.[14]

The BPI-T is a self-report measure used to rapidly assess the severity of pain and its impact on functioning. It is a reliable and valid instrument for assessing chronic pain.[16,17] The BPI-T has 9 items to evaluate the severity of pain and the effect of this pain on daily functioning. By using a 0 to 10 scale, our patients were asked to rate 1) their worst, least, average, and current pain intensity, 2) the perceived effectiveness of their current treatments, 3) the degree to which pain interferes with general activities, mood, walking ability, normal work, relationships with people, sleep, and enjoyment of life.

The EQ-5D-5L is a quality-of-life assessment tool recommended by the EuroQol Group as the preferred method for evaluating utility. For Thailand, these coefficients were studied by interviewing sample comprising 1,207 people and health-related quality of life measurement property testing and its preference-based score in the Thai population.[18] In a comparison of the Thai EQ-5D-5L and EQ-5D-3L value sets, the EQ-5D-5L scored higher.[19] The first part of the EQ-5D-5L assessment encompasses 5 health dimensions: mobility, self-care, usual activities, pain, and anxiety. Each dimension features 5 severity levels, ranging from "no problem" to "extreme problem." The second part consists of directly evaluating health status via the Visual Analog Scale (EQ-VAS), with scores from 0 to 100 (0 representing the worst health state and 100 representing the best health state). The utility score is based on the responses in the first section, using a country-specific utility score table that reflects the person's preference regarding their health. The score ranges from 0 to 1, with 0 meaning death and 1 meaning perfect health.[18]

Sample size and randomization

Sample size calculation: The establishment of the sample size for testing two independent means was based on an n4Studie calculation and derived from a pilot study with a sample size of 10. The size for each group was determined to be 25. An adjustment was then made for interim analysis (N per group = 25 * 1.11 = 27.75), with the resulting sample size rounded to 28.

Randomization: The patients were assigned into 2 groups by a computer-generated program that utilized the block-of-4 randomization method. The sealed envelope method was also employed to maintain anonymity by using assigned numbers.

Blinding: A separate ATTM, who was not involved in the treatments and was blinded to the patient groups, conducted treatment evaluations. The practitioner inquired about the participants' pre- and post-treatment pain levels and checked their vital signs. The ATTM also assessed the patients with the NPSI-T, the BPI-T, and the EQ-5D-5L health questionnaire at V1, V4, V8, and V9.

Statistical analyses

Analyses were conducted using IBM SPSS Statistics for Windows, version 22 (IBM Corp, Armonk, NY, USA), with a two-sided *P* value of ≤ .05 considered statistically significant. Continuous data are reported as the means ± standard deviations, while categorical data are presented as percentages (%). Normality analysis was performed using the Shapiro-Wilk test. Comparisons were made with repeated measures ANOVA to compare means within and between groups for NRS, NPSI-T, BPI-T, and EQ-5D-5L. The unpaired t-test was employed to calculate the differences in the mean values at V1 and V8.

RESULTS

Study participants

This study involved 28 patients diagnosed with peripheral nerve injury, peripheral nerve compression or radiculopathy with peripheral neuropathic pain. The characteristics of the participants in the M and H groups are presented in Table 1. The 2 groups had no significant differences in their demographic profiles or neuropathic pain baselines (DN4, initial average NRS, level of pain severity, pain duration, and area of pain). Most with the participants were diagnosed of lumbosacral radiculopathy. During eight weeks of the study, all participants were continued the same prescribed gabapentinoids, weak opioids, psychological and physical therapy as prior the study individually.

Treatment outcomes

NRS assessments of pain

Both groups showed a statistically significant decrease in pain severity between before and after each treatment session (Fig 3A). The average NRS pain scores showed significant differences between both groups and within the M group but not the H group, according to repeated measures ANOVA at V1, V4, V8, and V9; which were 6.3 ± 1.8, 3.8 ± 1.7, 3.6 ± 1.8 and 4.1 ± 2.1 in M group ($P < 0.001$) and 5.4 ± 1.6, 4.5 ± 1.4, 5.2 ± 1.8 and 4.2 ± 2.3 in H group ($P = 0.067$), respectively (Table 2 and Fig 3B). There was a significant decrease in the M group's average NRS pain scores when comparing V4, V8, and V9 to V1 (Fig 3C). The M group also exhibited a clinically significant decline in pain severity from V1 to V8.

Thai version of EQ-5D-5L

The EQ-VAS of EQ-5D-5L displayed no statistically significant difference between groups (Fig 3D). In contrast, the utility score revealed a statistically significant difference between both groups ($P = 0.007$) and within the M group ($P = 0.004$; Table 2 and Fig 3E).

BPI-T assessments of pain

The mean BPI-T pain severity scores were compared between groups. The M group exhibited lower pain levels than the H group in the worst and average pain assessments, with statistically significant differences (Table 2, Fig 4A, and Fig 4C). Furthermore, the M group demonstrated differences in average pain levels within the group when comparing V4 and V8 with V1, as well as differences in worst pain levels within the group when comparing V4 with V1. Pain interference in all aspects of the BPI-T showed no difference when comparing between and within groups (Table 2 and Fig 4D - 4J).

NPSI-T assessments of pain

NPSI-T is an appropriate tool for assessing neuropathic pain.[15,20] Our study results revealed a significant difference in NPSI-T score reductions between groups and in the M group's total intensity score from V1 to V8. The total intensity pain scores between groups (Fig 4K) demonstrated statistically significant differences ($P = 0.001$). The intensity pain scores of the M group also decreased significantly ($P = 0.005$). Subscores of NPSI-T for all 5 types of pain showed no differences within a group (Table 2 and Fig 4L - 4P).

DISCUSSION

Although all 28 patients had chronic intractable with peripheral neuropathic pain, and were diagnosed peripheral nerve injury, peripheral nerve compression or radiculopathy with peripheral neuropathic pain. CTTM was found to significantly relieve pain for patients in the M group compared to those in the H group. The results also indicated the difference in the degree of reduction in pain severity levels. The total NPSI-T and utility scores of EQ-5D-5L demonstrated a statistically significant decrease in pain and increased quality of life at V8 in the M group.

This study's clinical outcomes were based on subjective assessments covering pain severity, quality of life, and pain interference. Although the VAS, NRS, and BPI-PS are widely used, no evidence unequivocally demonstrates their superiority in measuring pain.[21] This study utilized NRS to assess pain severity and pain relief. Previous researches support this approach.[11,13]

Evidence of the efficacy of CTTM

A previous study showed a median pain score reduction of 4.5 VAS units using aromatherapy massage for neuropathic pain in diabetic patients.[22] Another study found that neuropathic pain decreased 2 to 4 weeks after using lavender oil in aromatherapy massage without

TABLE 1. Comparison of demographic data of the participants.

Variable	M group (N = 14)	H group (N = 14)	*P*
Sex[a] Male	7 (50%)	4 (29%)	0.440
BMI group[a]			
Underweight	0	1 (7%)	
Normal weight	5 (36%)	5 (36%)	
Pre-obesity	3 (21%)	6 (43%)	0.119
Obesity class I	1 (7%)	2 (14%)	
Obesity class II	5 (36%)	0	
Age[b] (years)	58.9 ± 13.8	60.4 ± 15.7	0.781
Age group[a]			
30–60	8 (57%)	6 (43%)	0.450
> 60	6 (43%)	8 (57%)	
Underlying disease			
Diabetes mellitus	1	3	
Hyperlipidemia/heart	4	2	
Gout	0	2	N/A
Thyroid	1	1	
Hypertension	7	6	
DN4[b]	5.4 ± 1.1	5.1 ± 1.1	0.489
DN4	5 (4,8)	5 (4,7)	N/A
Initial NRS (average pain)[b]	6.3 ± 1.8	5.4 ± 1.6	0.191
Initial pain severity[a]			
Mild (1-3)	1 (7%)	3 (21%)	
Moderate (4-6)	6 (43%)	6 (43%)	0.513
Severe (7-10)	7 (50%)	5 (36%)	
Pain duration[a] (years)			
< 1	2 (14%)	2 (14%)	
1–3	1 (7%)	6 (43%)	0.104
> 3–10	9 (64%)	6 (43%)	
> 10	2 (14%)	0 (0%)	
Area of pain[a]			
Upper extremity	2 (14%)	2 (14%)	
Lower extremity	3 (21%)	5 (36%)	0.555
Lower extremity & lower back	9 (64%)	7 (50%)	
Diagnosis			
Peripheral nerve injury	2 (14%)	1 (7%)	
Peripheral nerve compression	1 (7%)	2 (14%)	N/A
Radiculopathy			
Cervical level	0	2 (14%)	
Lumbosacral level	11 (79%)	9 (64%)	
Drug			
Antidepressants	7	3	
Sodium-channel blockers	2	2	
Gabapentinoids	14	14	N/A
Weak opioids	9	10	
Strong opioids	2	1	
Topical agents (Capsaicin, analgesic)	0	3	

Data are presented as number (%), mean ± SD, or median (min, max)
[a]*P* values between groups were calculated by the chi-squared test.
[b]*P* values between groups were calculated by unpaired t-test.

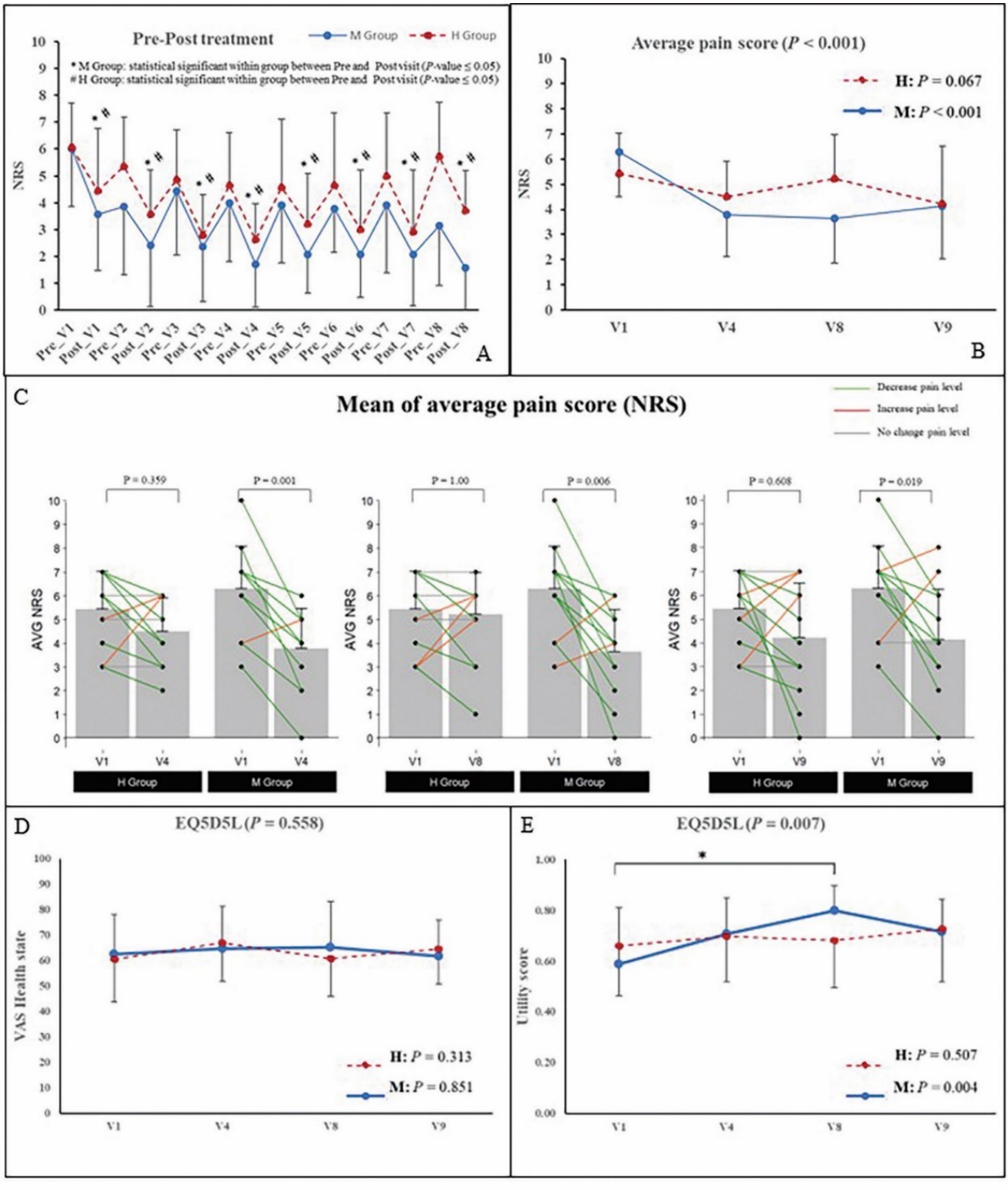

Fig 3A. Comparison of the NRS pain scores before and after treatment sessions within groups by paired t-test for sessions V1 through V8.
* Statistical significance within the M group, comparing before and after each treatment session ($P < 0.05$).
# Statistical significance within the H group, comparing before and after each treatment session ($P < 0.05$).
Fig 3B. Average NRS pain score over 24 hours
Fig 3C. Average NRS pain score over 24 hours, comparing V4, V8, and V9 with V1 between and within groups by mixed bar chart and line chart, to show the trends for each time and patient group.
Fig 3D. Comparison of the mean differences within groups and between groups at V1, V4, V8, and V9 of VAS health state of EQ 5D 5L
Fig 3E. Comparison of the mean differences within groups and between groups at V1, V4, V8, and V9 of utility score of EQ 5D 5L.
*$P < 0.05$ indicates statistical significance within the M group.

TABLE 2. Comparison within groups and between groups at V1, V4, V8, and V9.

Parameters	GR	Mean ± SD V1	V4	V8	V9	P^a Within Group	Between Group	Diff V1-V8	95% CI Lower	Upper	P^b
NRS	M	6.29 ± 1.77	3.79 ± 1.67	3.64 ± 1.78	4.14 ± 2.11	**< 0.001**	**< 0.001**	2.64*	0.70	4.59	**0.003**
	H	5.43 ± 1.60	4.50 ± 1.40	5.21 ± 1.76	4.21 ± 2.29	0.067		0.21	-1.10	1.52	
NPSI-T Total	M	25.21 ± 15.41	17.14 ± 14.87	15.07 ± 9.97	17.64 ± 7.78	**0.005**	**0.001**	10.14*	1.12	19.16	0.403
	H	28.57 ± 15.02	19.43 ± 11.92	21.64 ± 11.63	13.49 ± 13.49	0.081		6.93	-0.60	14.46	
NPSI-T Superficial	M	1.50 ± 2.68	0.79 ± 2.01	0.79 ± 2.08	0.64 ± 1.74	0.294	0.184	0.71	-0.86	2.29	0.819
	H	2.71 ± 2.89	1.71 ± 2.76	1.79 ± 2.72	1.79 ± 3.12	0.563		0.93	-1.47	3.33	
NPSI-T Deep	M	3.32 ± 2.01	2.32 ± 2.36	1.93 ± 2.29	2.68 ± 1.68	0.135	**0.029**	1.39	-0.32	3.11	0.838
	H	4.43 ± 3.00	2.71 ± 2.64	3.21 ± 1.31	3.18 ± 2.64	0.189		1.21	-0.84	3.27	
NPSI-T Paroxysmal	M	2.54 ± 2.72	1.57 ± 2.50	1.43 ± 1.65	1.50 ± 1.65	0.158	**0.033**	1.11	-0.66	2.87	0.791
	H	2.57 ± 2.27	1.46 ± 2.60	1.25 ± 1.77	2.32 ± 2.87	0.179		1.32	-0.44	3.08	
NPSI-T Evoked	M	2.17 ± 2.34	1.43 ± 1.92	1.67 ± 2.09	1.74 ± 2.12	0.335	0.080	0.50	-1.15	2.15	0.389
	H	2.33 ± 2.24	1.90 ± 1.60	2.36 ± 1.79	1.00 ± 1.23	0.099		-0.02	-0.87	0.83	
NPSI-T Paresthesia	M	2.75 ± 2.29	2.14 ± 2.53	1.29 ± 1.22	1.71 ± 1.82	0.189	0.197	1.46	-0.05	2.98	0.293
	H	2.43 ± 2.91	1.82 ± 2.22	1.93 ± 1.63	1.89 ± 2.78	0.803		0.50	-1.84	2.84	
EQ 5D 5L Utility score	M	0.587 ± 0.224	0.709 ± 0.142	0.798 ± 0.100	0.716 ± 0.128	**0.004**	**0.007**	-0.211*	-0.411	-0.011	**0.027**
	H	0.660 ± 0.196	0.699 ± 0.179	0.682 ± 0.187	0.728 ± 0.208	0.507		-0.022	-0.173	0.130	
EQ 5D 5L VAS health state	M	62.43 ± 15.75	64.64 ± 16.58	65.36 ± 17.92	61.79 ± 14.22	0.851	0.558	-2.93	-16.24	10.38	0.635
	H	60.43 ± 16.65	67.14 ± 15.41	60.71 ± 14.91	64.64 ± 13.79	0.313		-0.29	-10.97	10.40	
BPI-T worst	M	7.50 ± 1.61	5.36 ± 2.450	5.93 ± 2.09	6.36 ± 1.50	**0.018**	**0.004**	1.57	-0.08	3.22	0.356
	H	7.07 ± 1.77	6.14 ± 1.29	6.29 ± 1.82	5.64 ± 2.79	0.123		0.79	-0.87	2.44	
BPI-T least	M	3.14 ± 0.52	3.00 ± 0.54	2.79 ± 0.48	2.57 ± 0.47	0.669	0.219	0.36	-1.30	2.01	0.730
	H	3.93 ± 0.52	3.00 ± 0.50	3.86 ± 0.53	2.93 ± 0.74	0.162		0.07	-1.58	1.73	

TABLE 2. Comparison within groups and between groups at V1, V4, V8, and V9. (Continue)

Parameters	GR	Mean ± SD V1	V4	V8	V9	P^a Within Group	Between Group	Diff V1-V8	95% CI Lower	Upper	P^b
BPI-T	M	6.00 ± 1.88	3.93 ± 1.82	4.14 ± 1.99	4.86 ± 1.51	**0.010**	**0.002**	1.86*	0.117	3.60	0.127
average	H	5.29 ± 1.68	4.50 ± 1.40	4.79 ± 1.76	4.14 ± 2.28	0.206		0.50	-1.24	2.24	
BPI-T	M	5.21 ± 3.07	3.93 ± 3.10	4.36 ± 2.65	4.86 ± 2.35	0.577	0.286	0.86	-1.95	3.66	0.879
activity	H	5.36 ± 2.27	4.07 ± 2.70	4.71 ± 2.92	4.43 ± 2.82	0.527		0.64	-2.16	3.45	
BPI-T	M	4.14 ± 2.68	2.71 ± 3.00	4.07 ± 3.05	3.79 ± 3.12	0.217	0.140	0.07	-2.34	2.48	0.637
mood	H	4.36 ± 2.68	3.36 ± 3.08	3.71 ± 2.87	2.64 ± 3.50	0.317		0.64	-1.77	3.06	
BPI-T	M	4.50 ± 3.25	4.07 ± 3.63	4.21 ± 3.53	4.43 ± 2.47	0.944	0.132	0.29	-2.02	2.59	0.390
walking	H	6.00 ± 2.00	4.64 ± 2.95	4.71 ± 2.92	3.43 ± 3.67	0.018		1.29	-1.02	3.59	
BPI-T	M	4.07 ± 2.97	3.79 ± 3.58	3.71 ± 3.41	4.64 ± 2.73	0.751	0.469	0.36	-2.19	2.91	0.292
normal work	H	5.36 ± 2.37	4.14 ± 3.42	3.64 ± 2.41	3.57 ± 3.03	0.228		1.71	-0.83	4.26	
BPI-T	M	3.64 ± 3.79	2.29 ± 2.79	2.21 ± 2.42	2.79 ± 3.07	0.318	0.309	1.43	-1.19	4.05	0.515
relations	H	3.36 ± 2.92	2.79 ± 2.97	2.79 ± 2.61	2.21 ± 3.07	0.692		0.57	-2.05	3.19	
BPI-T	M	4.86 ± 3.63	5.00 ± 3.19	4.43 ± 2.85	4.64 ± 3.63	0.948	0.473	0.43	-2.04	2.90	0.526
sleep	H	4.29 ± 2.76	2.57 ± 2.74	3.07 ± 2.56	2.86 ± 3.28	0.096		1.21	-1.25	3.68	
BPI-T	M	4.57 ± 2.98	3.36 ± 2.87	4.00 ± 2.80	4.36 ± 2.71	0.421	0.172	0.57	-1.69	2.84	0.615
enjoyment	H	2.46 ± 2.99	4.00 ± 2.56	4.07 ± 3.02	3.93 ± 2.98	0.330		1.14	-1.12	3.41	

[a]Repeated measures ANOVA and adjustment for multiple comparisons (using the Bonferroni method) were used to calculate the *P* values within and between groups. * Significant *P* values within groups ≤ 0.05 (compared between V8 and V1).

[b]Unpaired t-test was used to calculate P values between groups, with means of difference between V1 and V8.

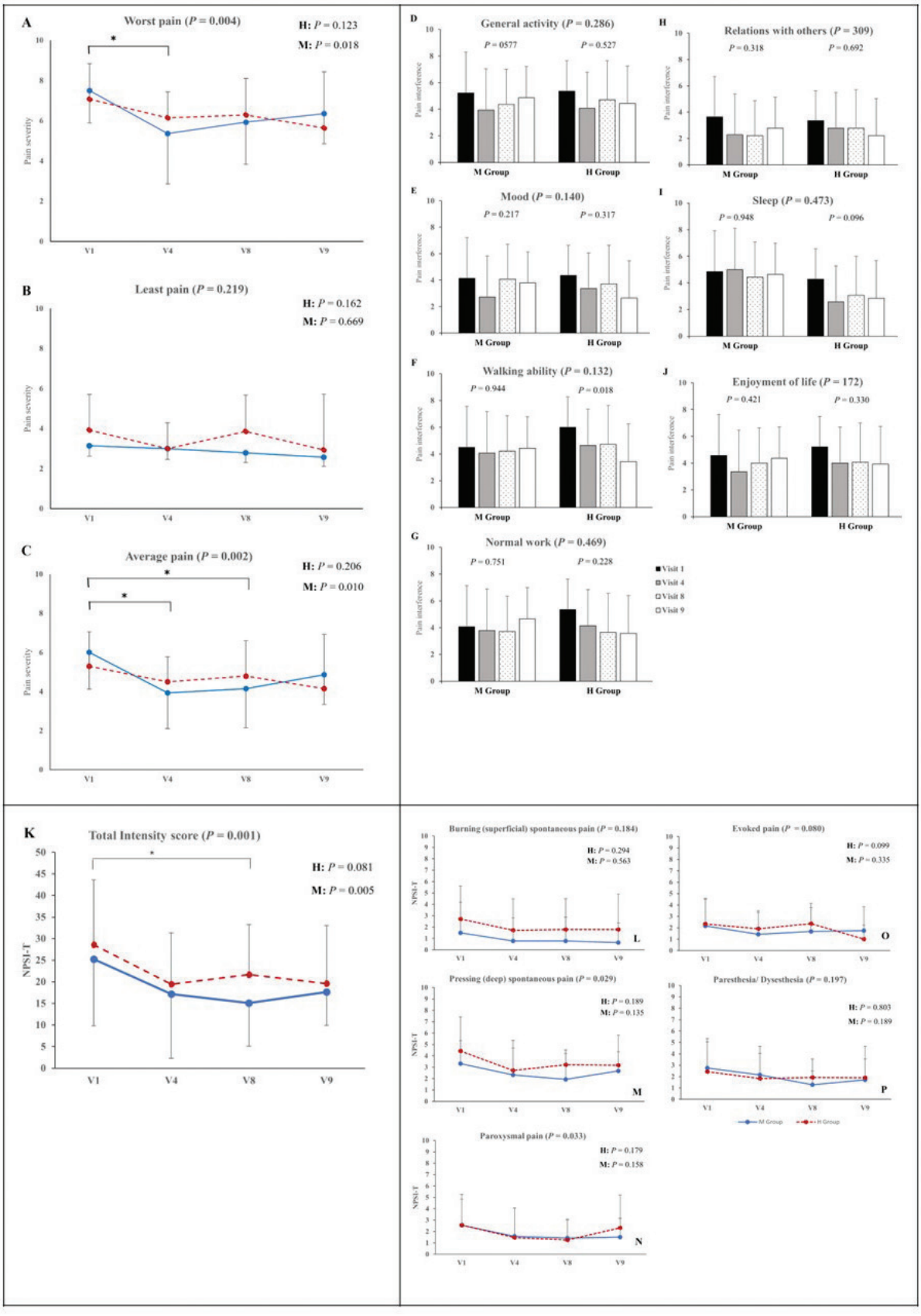

Fig 4A-4C. Pain severity (during the preceding 24 hours), assessed by BPI-T, at V1, V4, V8, and V9.
Fig 4D-4J. Pain interference (during the preceding 24 hours), assessed by BPI-T, at V1, V4, V8, and V9.
Fig 4K. Total intensity scores of the NPSI-T at V1, V4, V8, and V9
Fig 4L-4P. Subscores of the NPSI-T at V1, V4, V8, and V9
P values calculated within groups and between groups by repeated measures ANOVA and adjustment for multiple comparisons (using the Bonferroni method).
*$P < 0.05$ indicates statistical significance within the M group.

side effects.[23] In addition, acupuncture may alleviate neuropathic pain from spinal cord injuries. Acupuncture treatment significantly improved present pain, general pain, pain unpleasantness, and coping compared to baseline values in one study.[24] While evidence for CTTM in neuropathic pain treatment is limited, a systematic review of 13 randomized control trials demonstrated acupuncture's effectiveness in diabetic neuropathy, Bell's palsy, and carpal tunnel syndrome.[25] Some studies also showed that acupuncture and another massage therapy effectively treated neuropathic pain after spinal cord injuries.[26]

CTTM, a deep-pressure massage, lacks previous evidence for neuropathic pain treatment. However, the M group in our investigation experienced continuous reductions and statistically significant decreases in average pain scores for the first two weeks (V4) and apparently sustained to four weeks (V8) in the treatment period. However, the pain score in V9, slightly increased from V8 in the M group, reflecting that CTTM might have only a short-term effect on pain reduction. The massage, therefore, may be considered an adjunctive treatment in refractory peripheral neuropathic pain.

Potential mechanisms of CTTM for pain relief

Animal model studies have outlined various peripheral and central pathophysiological processes after nerve injury, indicating the basis for neuropathic pain mechanisms. Neural plasticity, involving changes in neuronal function, chemistry, and structure, produces the altered sensitivity characteristics of neuropathic pain.[27,28] Animal studies have demonstrated multiple neuropathic pain mechanisms. Still, these mechanisms may not apply to humans when describing massage effects because pain correlated with patterns and impacts on quality of life are also affected by the biological, emotional, and social perspectives.

CTTM has been found to relieve pain in tension headaches[7] and muscle pain of the neck, shoulder and back[29,30], decrease spasticity, increase functional ability, and improve the quality of life of elderly stroke patients.[5] Deep massage can stretch muscles, helping to break down subcutaneous adhesions and prevent fibrosis[31], potentially leading to improved sensory feedback from muscle spindle receptors. Furthermore, the repetitive cutaneous stimulation provided by massage may reduce pain through the gate control theory.[32] Massage manipulation has been shown to result in similar patterns of change in skin temperature and blood flow in the upper and lower extremities.[33,34] CTTM stimulates blood and lymphatic circulation and the sympathetic nervous system by exerting pressure on the skin and muscles. Consequently, the flow of nutrients to tissues is enhanced, and the excretion of toxins and residual substances within the body improves.[4] Another possible explanation for the pain reduction induced by CTTM involves gate control theory. Under this theory, CTTM stimulates pressure receptors by exerting pressure on the skin and muscles, inhibiting the transmission of pain receptors at the spinal cord or the "gate".[35-37] However, the mechanism of CTTM for relieving peripheral neuropathic pain is still not fully elucidated. Limitations of this study include the relatively small sample size and the considerable variation in participant types and settings.

CONCLUSION

No prior published review of evidence or protocols on the efficacy and safety of CTTM for treating neuropathic pain exists. This study could serve as a preliminary investigation into the effectiveness of CTTM for adjuvant treatment in relieving pain and improving the quality of life for patients with peripheral neuropathic pain. Future studies should explore the frequency of treatment per month and the use of CTTM to treat specific types of peripheral neuropathic pain.

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Conflicts 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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