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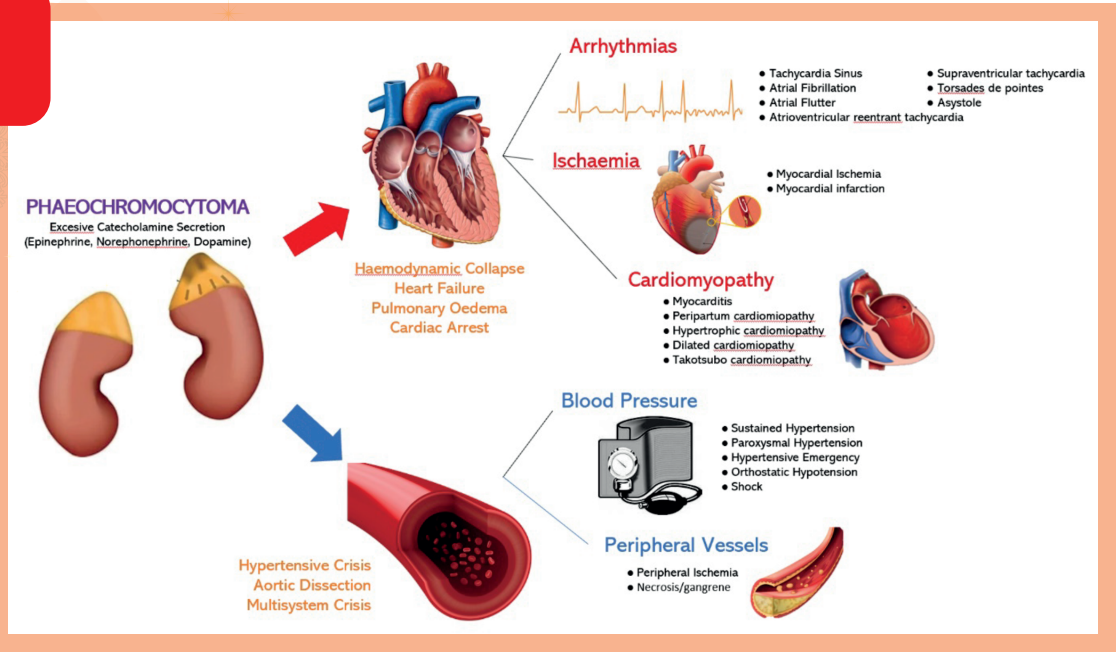

By Doonyaporn Wongsawaeng, et al.

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Goal-Directed Therapy to Improve Gross Motor Function and the Quality of Life of Children with Cerebral Palsy: A Randomized Controlled Trial

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ABSTRACT

Background: The multiplicity of interventions for the treatment of cerebral palsy (CP) can cause confusion about which are most suited to certain individuals. Hypothesis is that goal-directed therapy (GDT) can guide integrating therapies to improve clinical outcomes compared with conventional therapy (CT).

Materials and Methods: A prospective, assessor-blinded, randomized controlled trial was done with 23 children with CP (mean age, 4 years 4 months old; SD 1y4mo), who were divided into groups according to their level of gross motor function: GDT and CT. Both groups received 12 physiotherapy (PT) sessions and advice on daily home programs. The GDT group additionally had a team meeting to set a specific goal, and PT programs were shaped toward that goal. Assessments were done at baseline and after treatments, using the Thai-version Gross Motor Function Measure (GMFM-66), CP-Quality of Life (CP-QOL), caregiver burden, and home program compliance.

Results: After the treatments, the GDT group showed significant improvements in GMFM-66, CP-QOL, and caregiver burden, while the CT group revealed improvements in caregiver burden and some domains of the GMFM, including sitting and crawling & kneeling. Comparisons between groups found GDT was more effective than CT in improving GMFM-66 and CP-QOL. Home program compliance was higher in the GDT (69%) than the CT group (42%).

Conclusion: GDT demonstrated clear gains for children with CP regarding gross motor function and QOL improvements. Team communication toward a customized goal was crucial, empowering the children and their caregivers to comply with home programs to achieve the set goal.

Keywords: Cerebral palsy; goal-directed therapy; gross motor function; quality of life; caregiver burden (Siriraj Med J 2022; 74: 1-10)

INTRODUCTION

Cerebral palsy (CP) is one of the most common causes of childhood physical disability[1] and is caused by a non-progressive lesion to the immature brain.[2] This syndrome causes the dysfunctional control of movement and posture, while perception, vision, learning, and language are also frequently affected.[3] These characteristics can influence the child's ability to learn and perform everyday life activities[4], and also the quality of life (QOL) of patients and their caregivers are affected by these disabilities.[5] Treatment program should promote the improvement of the patient's motor function, and facilitate their participation in activities and adaptation to daily living, with the ultimate goal of improving their QOL.[6]

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Numerous treatments for CP are available, which can be overwhelming for patients and families, and even make it challenging for healthcare professionals to make a clinical decision on what is the most suitable treatment for each child. Recent systematic reviews provide evidence-based guidance for assessing interventions for children with CP, such as the Evidence Alert Traffic Light system.[7] Here, green-light-go interventions are inferred to be effective with a higher level of evidence, while yellow-light interventions show uncertain effects and require outcome measures to monitor progress, and red-light interventions are considered ineffective and therefore to be avoided.[7] However, this does not suggest that every child with CP should undergo all green-light interventions for best practice since CP has a heterogeneity of symptoms and clinical status. The key to success is doing the right things with the right child at the right time.[8]

Goal-directed therapy (GDT) approach could be a solution to this overwhelming situation. GDT focuses on comprehensive treatment strategies that serve as stepping stones to achieving individual goals, which along the way promote functional performance and a gradual independence in everyday life activities.[9,10,11] The approach consists of the identification of individual needs of both the child and the family, assessments of the child's performance and capacity, setting goals that are meaningful tasks and relevant despite the child's level of gross motor function[12], and the development of individual-tailored treatment programs.[13] Setting specific goals is a means to enhance awareness of the objectives of the therapy and can affect the treatment performance by focusing attention, directing effort, increasing motivation, and enabling the development of strategies to achieve the set goals.[13] There is evidence showing an improvement in basic motor abilities and self-care in young children with CP after undergoing GDT, and a decrease in their need for caregiver assistance for self-care and mobility.[13,14] However, in previous research, the research methodology either did not involve a randomized controlled trial (RCT) and the duration and frequency of treatment in the GDT group setting were three hours per session, five days a week, for a period of three weeks[14] or did and treatment was three days a week for three consecutive months.[13] Additionally, the treatment used in previous studies may be too onerous for patients in many developing countries because of certain socioeconomic problems and human resource issues[15], such as parents finding it inconvenient to bring their children to the hospital because of the cost of travel, lack of time, and limited number of therapists available.[16,17]

Consequently, an RCT was performed to compare GDT and conventional therapy (CT), with reducing the amount of interventions performed at the hospital and encouraging the use of home programs, compatible with the available resources. Currently, conventional therapy is mostly practiced through a physiatrist, who authorizes prescribed treatment programs via the medical records after assessment of a patient and conveyed to the respective rehabilitation team. In contrast, GDT includes a family-team meeting organized to set specific goal(s), with the treatment programs then shaped to meet the set goal. We hypothesized that even with both groups receiving the same amount of hospital programs, GDT would be more effective than CT for improving gross motor function, QOL, caregiver burden, and compliance with home programs.

MATERIALS AND METHODS

Study design

This prospective, assessor-blinded RCT was conducted between April 2018 and April 2019. The study protocol was approved by the Institutional Review Board (COA no. Si 675/2017), and registered with the Thai Clinical Trials Registry (registration no. TCTR20180419002). Forty-five children with CP from a pediatric rehabilitation unit of the university hospital were evaluated for eligibility (Fig 1), with written informed consent obtained from their parents of all participants. The participants were stratified by their severity of CP using Gross Motor Function Classification System (GMFCS)[18], as assessed by a study doctor, and then randomly allocated into either the intervention group (GDT) or control group (CT) by drawing random numbers from a sealed envelope. Computer-generated randomization with block sizes of 4 was used to create the random numbers. The participants were requested to maintain their other treatments, such as oral antispastic medications and orthoses that could be considered co-interventions; however, these could be changed as a medical necessity. The researcher regularly monitored and recorded whether additional therapies were used by the participants.

Participants

Eligible participants were children with CP, GMFCS levels I–IV, aged 1–6 years old, able to understand basic instructions and communicate pain or discomfort, and having caregivers with Thai language literacy who were able to complete the questionnaires. The exclusion criteria were specifically patients with uncontrolled epilepsy, history of fractures, serial casting, orthopedic surgery or chemoneurolysis intervention within 6 months

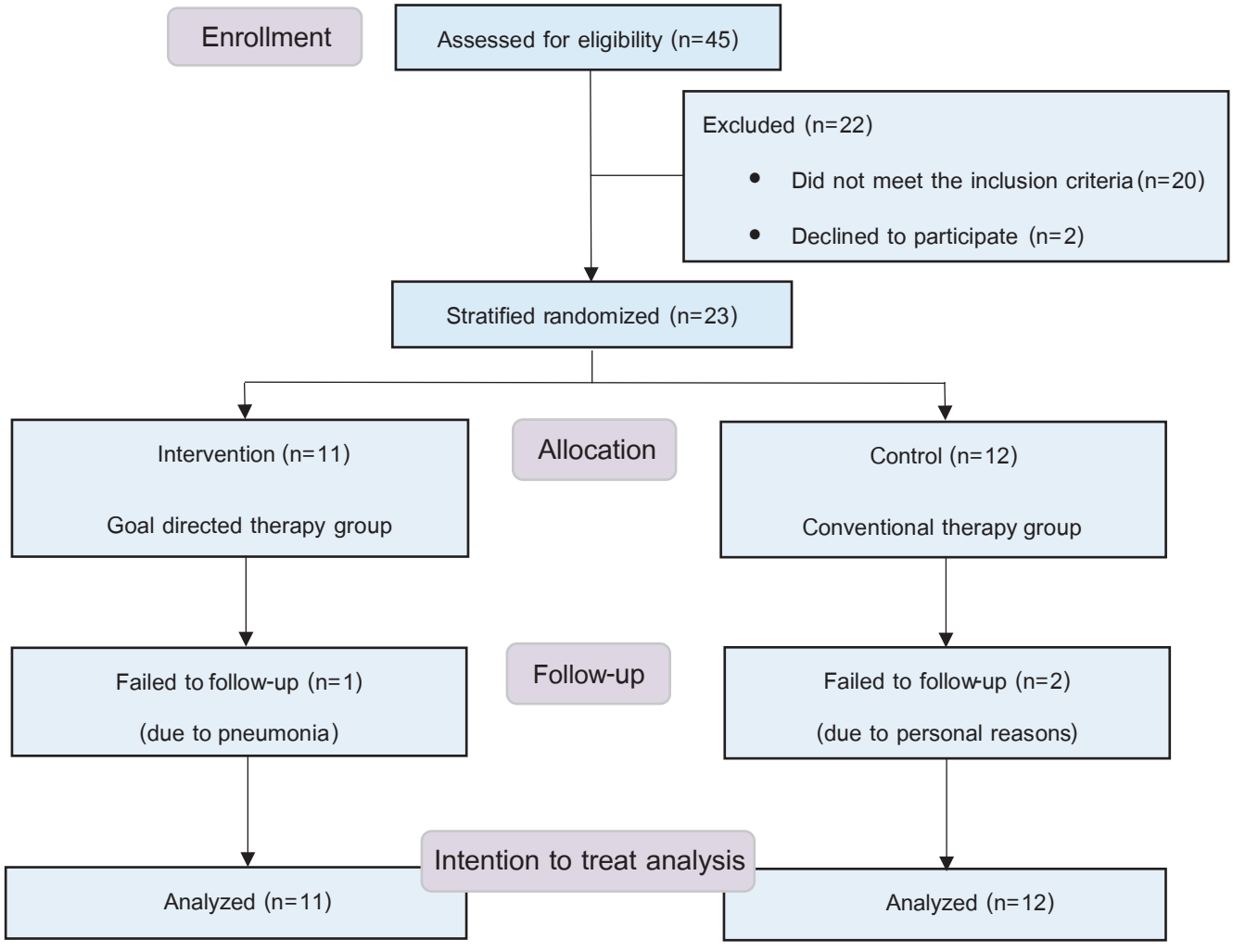

Fig 1. Consolidated Standards of Reporting Trails (CONSORT) diagram showing the flow of participants in the study

prior to the study, known unstable cardiovascular or pulmonary diseases, and any contraindication for receiving physiotherapy (PT), or declined to participate.

The sample size calculation was based on a previous study.[13] The nQuery, with a type-I error at 0.05 and 80% power of test, indicated that at least 10 participants per group would be required to detect a statistically significant difference in gross motor function improvement between the two groups. The recruitment process was on a first-come-first-served basis until the target number of 23 children had been reached, to compensate for potential loss to follow-up about 10%.

Interventions

Attending physiatrists evaluated all participants and prescribed individualized PT programs, such as hydrotherapy, bicycling, strengthening and stretching exercises, balance and mobility training, modalities, and tools applications. Both groups received 50 minutes of PT for 12 sessions. The therapists assigned daily home exercise programs suitable for each patient. Caregivers were responsible for the home programs and for recording their compliance in a logbook (Fig 2).

For GDT group, an additional process was included, which was a team conference involving the physiatrist, physical therapist, child, and caregivers, where an individualized goal was agreed for each patient. The goal-development process included an assessment of the child's performance and motor capacity, identification of a specific, measurable, achievable, relevant, and timed (SMART) goal, and the conception of the goal-attainment scale (GAS).[14,19] Only one goal was set as the most crucial and relevant to the child and family's needs, and that was possible to achieve based on each child's assessed performance. PT programs were adjusted specifically for goal achievement. The GAS was used to objectively identify and follow progress. For example, if the goal was to sit independently for 30 seconds, the programs emphasized

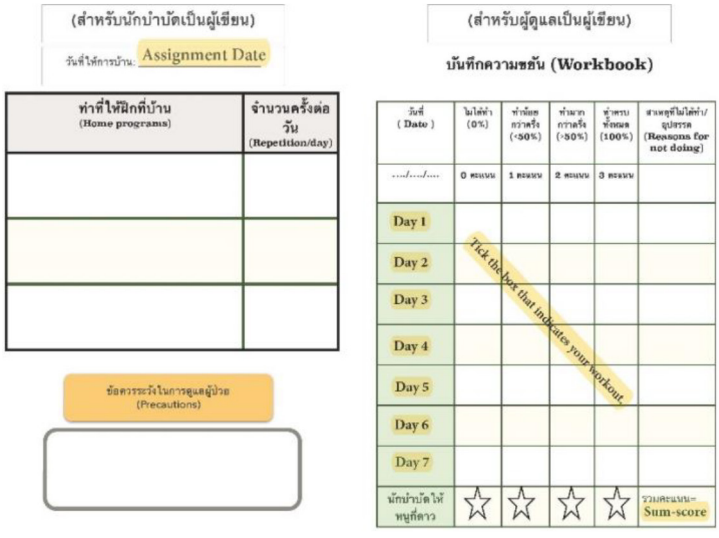

Fig 2. A logbook for recording home program compliance.

exercises to promote core/trunk muscle strengthening and sitting balance. Others programs, such as stretching heel cords or mobilization, were still considered crucial, but were not intensively assigned because these were not related to the set goal. The individualized specific goal was presented in the cover of the logbook for the GDT group to remind all the team and family members of that goal.

Outcome measurements

A study doctor retrieved demographic data, GMFCS level, type of CP, and co-morbidities. The primary outcome of this study was the improvement of gross motor function after the therapy. A blinded physical therapist, who had experience but was not involved in any of the therapy sessions, assessed the participants using the Gross Motor Function Measure (GMFM-66) Thai version[20], a standardized tool for measuring gross motor function in children with CP that contains 66 items for assessing gross motor ability, including: (1) lying and rolling, (2) sitting, (3) crawling and kneeling, (4) standing, and (5) walking. The final GMFM-66 score was calculated with a computer-scoring program: the Gross Motor Ability Estimator. The maximum score is 100, and a higher score means better gross motor function. Here, a change in GMFM-66 score of 1.58 was reported as a clinically meaningful improvement, and a score change of 3.71 could discriminate between great and not great improvement.[21]

Secondary outcomes were the children's QOL, caregiver burden, and home program compliance, assessed by the CP-QOL Questionnaire Parent Proxy Thai version[22], the CP Caregiver Burden Thai version[23], and logbook scoring (Fig 2), respectively. Those questionnaires and a survey for the caregivers' demographic data were self-administered by the caregivers, with the primary caregiver, as the main person the child spends most time with, the preferred candidate to answer the questionnaires, or the other caregivers if the primary caregiver was not available. Outcome measurements were recorded at baseline and immediately after the 12th session of therapy.

In the GDT group, goal achievement was evaluated after completion of all the therapy sessions using the GAS, a tool for identifying specific goals and measuring progress. This assessment was done by the physical therapist who provided the GDT. The GAS consists of 6 grades: -3, for worse than at the start (i.e., a deterioration); -2, for equal to at the start; -1, for less than expected; 0, for expected goal; +1, for somewhat more than expected; and +2, for much more than expected.[24]

Statistical analysis

The analyses were performed using SPSS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). A p-value <0.05 was regarded as statistically significant. Data are presented as the number and percentage for categorical variables, and the mean ± standard deviation (SD) for continuous variables. The baseline patients' characteristics and the results of both groups were assessed for normality using the Kolmogorov–Smirnov test. Chi-square or Fisher's exact test were used to compare categorical variables. For continuous variables, the Student's t-test was used to compare parametric data, and the Mann–Whitney U test was used to compare nonparametric data. Statistical analysis was finally performed by intention to treat analysis. Missing data were replaced by the last observation value.

RESULTS

The study participants comprised 23 children with CP (mean age, 4 years 4 months old; SD 1y4mo). The mean time interval between baseline and final assessments for all was 93.1 (SD 36.3) days. The participants' baseline characteristics are presented in Table 1. Participants in both groups received co-interventions, such as oral antispastic medications and orthotic use, with no statistically significant difference between the groups.

All participants in the GDT group had a team meeting to set a SMART goal related to gross motor function. All showed an improvement from baseline according to their individualized GAS, except one child that was lost to follow-up due to pneumonia. Overall, 6 from 11 children achieved their goals as expected or more than expected (GAS>0).

Comparison of the outcomes before and after the therapies is demonstrated in Table 2. The GDT group showed substantial improvements in GMFM-66 total score and all aspects of gross motor function except for walking & running. The CT group showed no significant improvement in GMFM-66 total score, but improvements in sitting and crawling & kneeling. CP-QOL was significantly increased only in the GDT group, while caregiver burden was decreased after the treatments in both groups.

Comparison of the improvements between the groups is shown in Table 3. The GDT group had significantly higher improvement in GMFM-66 total score and in walking & running subscale compared with the CT group. The increments of CP-QOL in the GDT group were significantly higher than in the CT group, while the decrements of caregiver burden were not different between groups.

Home program compliance was higher in GDT (69%) than CT (42%) group (p=0.010). All participants in the GDT group complied with performing more than half of the assigned tasks; while in the CT group, only 16% complied. Examples of logbook are shown. A participant in GDT group aimed to walk with a walker and well complied with walking exercise at home (Fig 3), while one in CT group revealed moderate compliance (Fig 4).

DISCUSSION

GDT showed superior outcomes compared with CT in improving gross motor function and the QOL of children with CP, consistent with previous studies[13,14,19], even if the dose of treatment at the hospital in this study was limited to just once or twice a week for 12 sessions. GDT facilitated good compliance with home exercise programs, whereby the children still received a high intensity of therapies from their caregivers. Healthcare professionals acted as a coach and trained the caregivers as therapists, which could be practically applied in patients with socioeconomic issues limiting them visiting hospital frequently.[16] This model may also applied in low- to middle-income countries with limited human resources.

GDT resulted in a higher improvement in gross motor function, when compared with CT. Although the amount of therapies performed in the hospital was similar in both groups, the GDT group managed to achieve a greater dosage of therapeutic exercise at home. It is known that strength and muscle endurance affect gross motor outcomes and depend on the amount of intervention the patient receives, so consistently performing exercises can increase strength and endurance.[25,26] In addition, the PT program can be set to integrate specific activities to promote goal achievement. Therefore, those could be reasons for the greater improvement of gross motor outcomes in this group. Gross motor function improvement after GDT was clinically meaningful: the average change of GMFM-66 of 11.7±6.8 showed great improvement[21], most likely due to the very young age of the participants in this study, which is well-known to offer a higher chance of gross motor improvement.[21]

It is known that the standard practice of rehabilitation is goal oriented. Treatment programs in CT should be also for serving goals, considering that physiatrists prescribed those for individuals. The lower outcomes might be from ineffective communication about the goals. As a result, team members could not realize what programs to focus on and a lack of motivation to comply. The predominant home program compliance in GDT group was possibly due to the team meeting including a number of key elements for enhancing adherence to the prescribed home programs, especially effective goal setting. Here, a SMART goal was set by consensus between the multidisciplinary team and the patients' caregivers, which helped tailoring the goals to the patients' needs. One goal was chosen for each patient that was possible for them to achieve easily to encourage and motivate their adherence to physiotherapy rather than setting more difficult or multiple goals.[27] The use of GAS encouraged communication and collaboration between the team members, and facilitated patient and family involvement.[28] Moreover, the team meeting expedited effective communication, which enhanced reciprocal relationships, ameliorated problems, and facilitated caregiver/parental empowerment by their engagement with the professionals, who were perceived as collaborators instead of authoritative experts. Empowerment, motivation,

TABLE 1. Baseline characteristics of the participants and their caregivers.

	GDT group (n=11)	CT group (n=12)	*p-value*
Participants' data			
Female gender, n (%)	6 (54.5%)	6 (50.0%)	0.837
Age (years)[1]	4.4 ± 1.2	3.6 ± 1.4	0.183
GMFCS level[2], n (%)			0.925
I–II	7 (63.6%)	7 (58.3%)	
III–IV	4 (36.4%)	5 (41.7%)	
Topography classification, n (%)			0.169
Unilateral type	7 (63.6%)	4 (33.3%)	
Bilateral type	4 (36.4%)	8 (66.7%)	
Comorbid disease, n (%)			
Intellectual disability	8 (72.7%)	7 (58.3%)	0.492
Epilepsy	2 (18.1%)	2 (16.6%)	0.928
Duration of treatments (days)[1]	105.0 ± 40.9	82.1 ± 29.0	0.199
GMFM-66 at baseline[1]	68.6 ± 17.1	55.6 ± 21.5	0.140
Caregivers' data			
Age (years)[1]	47.3 ± 12.9	43.2 ± 16.0	0.570
Relation to participants, n (%)			0.314
Primary caregiver	4 (36.4%)	7 (58.3%)	
Other caregiver	7 (63.6%)	5 (41.7%)	
Family income, n (%)			0.543
Not enough to use	6 (54.5%)	4 (33.3%)	
Enough to use	3 (27.3%)	6 (50.0%)	
Retained	2 (18.2%)	2 (16.7%)	
Education level, n (%)			0.708
Undergraduate	9 (81.8%)	9 (75.0%)	
≥ Bachelor's degree	2 (18.2%)	3 (25.0%)	

[1]Mean ± SD, [2]GMFCS = Gross Motor Function Classification System.

TABLE 2. Outcomes comparison within the goal-directed therapy (GDT) and conventional therapy (CT) groups.

Outcomes	GDT (n=11)			CT (n=12)		
	Before	After	*p-value*	Before	After	*p-value*
GMFM-66[1]						
Total score	68.6 ± 17.1	80.3 ± 11.8	0.013*	55.6 ± 21.5	60.3 ± 21.7	0.132
Lying & rolling	90.2 ± 14.3	99.4 ± 1.3	0.006*	80.5 ± 16.3	84.33 ± 15.3	0.157
Sitting	87.0 ± 16.2	96.4 ± 6.7	0.006*	69.2 ± 21.6	75.0 ± 21.3	0.039*
Crawling & kneeling	75.6 ± 12.0	87.4 ± 10.6	0.027*	60.4 ± 16.8	70.6 ± 20.9	0.024*
Standing	54.3 ± 31.2	70.4 ± 27.3	0.021*	38.0 ± 25.4	41.4 ± 26.5	0.202
Walking & running	35.3 ± 22.1	48.3 ± 20.4	0.115	30.42 ± 31.9	30.5 ± 29.9	0.678
CPQOL[1]	313.9 ± 59.3	349.3 ± 52.5	0.046*	301.8± 29.9	310.5± 31.8	0.543
Caregiver burden[1]						
Time usage	43.6 ± 18.5	34.6 ± 15.6	0.037*	50.9 ± 11.0	42.1 ± 12.4	0.041*
Difficulty	42.9 ± 17.0	31.9 ± 13.3	0.043*	46.5 ± 7.2	35.6 ± 9.1	0.047*

[1] Mean ± SD, **p-value* ≤ 0.05.

TABLE 3. Comparison of the improvements after the completion of goal-directed therapy (GDT) and conventional therapy (CT).

Outcomes	Score difference (post – pre-treatment)		*p-value*	Difference (95% CI)
	GDT	CT		
GMFM-66[1]				
Total score	11.7 ± 6.8	4.5 ± 3.9	0.011*	7.2 (2.3-12.0)
Lying & rolling	9.2 ± 13.2	3.7 ± 4.7	0.243	5.5 (-2.9-13.9)
Sitting	9.4 ± 11.2	5.8 ± 7.1	0.399	3.6 (-4.5-11.8)
Crawling & kneeling	11.8 ± 4.4	10.2 ± 12.7	0.683	1.6 (-7.2-10.4)
Standing	16.1 ± 19.2	3.4 ± 8.6	0.078	12.7 (-0.1-25.5)
Walking & running	13 ± 7.4	0.1 ± 3.7	0.000*	12.9 (7.8-18.0)
CPQOL[1]	32.1 ± 25.9	8.6 ± 3.6	0.013*	23.4 (5.9-41.0)
Caregiver burden[1]				
Time usage	-9.0 ± 3.6	-8.7 ± 3.8	0.875	0.25 (-3.5-3.0)
Difficulty	-11.0 ± 4.8	-11.7 ± 3.8	0.684	-0.75 (-3.0-4.5)

[1] Mean ± SD, **p-value* ≤ 0.05.

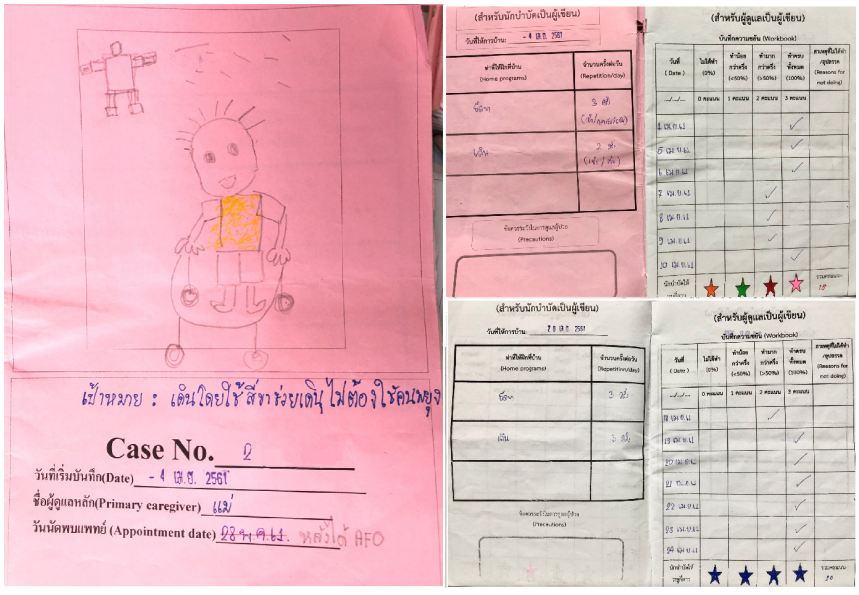

Fig 3. An example of logbook of a participant in the goal-directed therapy (GDT) group

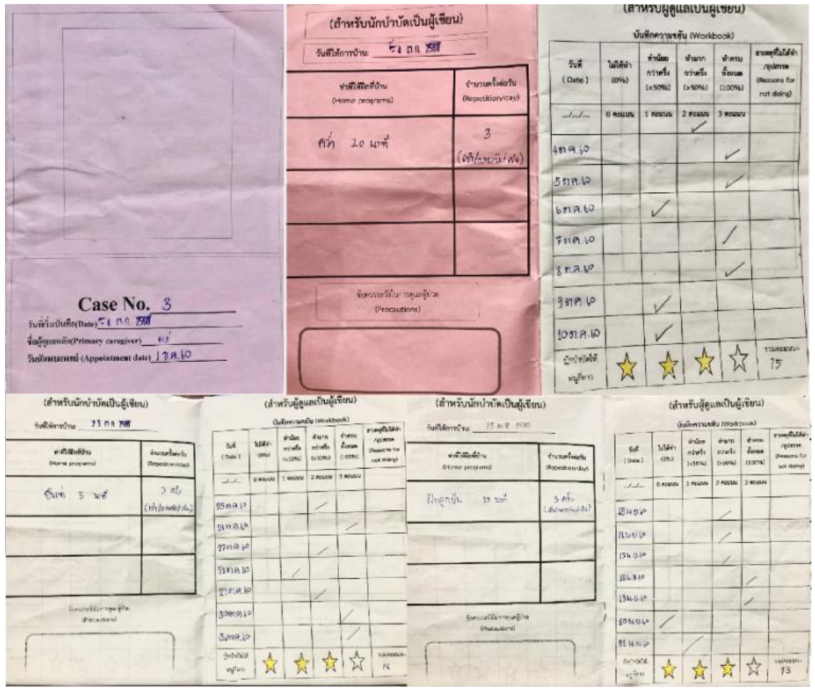

Fig 4. An example of logbook of a participant in the conventional therapy (CT) group

and reciprocal relationships are known determinants of effective parent-delivered therapy in children with CP.[29]

Caregivers' perception of their children's QOL increased after GDT. Previous studies reported gross motor function, cognitive level, complications, pain, and parental stress as factors associated with the QOL of children with CP.[5,30,31] Therefore, the improved QOL in the present study was possibly related to the improvement of gross motor function after treatment. GDT was more effective toward improving gross motor function; consequently, the QOL increment in the GDT group was higher than in the CT. Additionally, effective communication between the multidisciplinary professionals and caregivers via the team meeting in GDT possibly reduced caregivers' stress level, which might have influenced how they reported their child's QOL.[32,33]

Interestingly, caregiver burden was improved in both groups. Considering that the caregivers had to bring their child to the hospital for the PT programs and they were also instructed to spend time doing home exercise programs, in addition to their routine time spent helping the child with daily activities, like eating, personal care, etc., this seemed to be their socio-structural constraints or the objective burdens.[34] Another aspect of caregiver

burden is the subjective burden or emotional distress, which may diminish over time by being able to identify positive aspects of their special parenthood.[34] Family empowerment through home program education was applied to all the caregivers in this study. The results support the findings of a previous study in Thailand, whereby interventions that empowered caregivers' ability to care for their child could reduce caregiver burden.[23]

There are limitations in this study. The physical therapists could not blinded due to the nature of the intervention. Consequently, the therapists who believed in GDT might have put in more effort during the therapy sessions. However, this might be another key to the success of GDT, in that the therapists, as key team members, would do their best for each child to achieve their set goal. Such a potential bias could be reduced by using a blinded assessor and an objective primary assessment tool. Considering baseline parameters, topography, duration of treatment and initial GMFM seemed to favor GDT although those were not statistically significant difference between groups. The nature of children continues to develop over time. Pattern of paralysis and gross motor function at baseline were predictors for good outcome after some interventions.[35,36] Children with a lower initial GMFM achieved higher improvement after selective dorsal rhizotomy.[36] Consequently, assessing a longer period of follow-up and the factors associated with the success of GDT could be very interesting for future studies.

CONCLUSION

GDT demonstrated clear gains for children with CP for improving their gross motor function and QOL. The keys to success are: 1) customized SMART goal setting, 2) focus on integrative therapies to serve meeting that goal, 3) effective communication among multidisciplinary professionals, caregivers, and the child, and 4) family empowerment. In limited resource settings where hospital PT programs could be utilized just once or twice a week, GDT still showed impressive outcomes. Home programs could be a solution to maximize the intensity of interventions. Patients and caregivers are an important part of success, especially once they are motivated and voluntarily make a commitment to comply with home programs to achieve their goals.

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Effectiveness of Ayurved Siriraj Prasa-Nam-Nom Recipe on Breast Milk Volume in Early Postpartum Women: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

Objective: To explore the effectiveness of Ayurved Siriraj Prasa-Nam-Nom (ASPNN) recipe on breast milk production in early postpartum women.

Materials and Methods: Fifty-four normal vaginal term delivery mothers who had inadequate milk volume were enrolled into this randomized, double-blind, placebo-controlled trial. All participants received ASPNN or placebo 1,500 mg three times/day for 3 days in the hospital and 7 days at home. Primary outcomes, including breast milk volume, %creamatocrit, and level of prolactin, were evaluated on day 1 and day 3. Satisfaction scores, adverse effects, and types of breastfeeding were also determined.

Results: On day 3, milk volume was increased in both groups. The median volume of ASPNN group was 19 ml, while that of the placebo group was 30 ml. The median %creamatocrit of ASPNN and placebo group were 7.17% and 6.98%, respectively. Mean serum prolactin levels of ASPNN and placebo group were 321.76 ± 114.23 ng/ml and 323.78 ± 116.68 ng/ml, respectively. Although the effects were not different from the placebo, the reduction of prolactin in ASPNN was lower. Minor adverse effects included skin rash and mild diarrhea. Exclusive breastfeeding rate on day 11 in ASPNN and placebo group were 92.6 % and 88.5%, respectively.

Conclusion: Short term ASPNN supplementation produced no direct effect on breast milk volume, creamatocrit, and serum prolactin. It was safe and might help maintaining serum prolactin. A future trial with more participants and longer period should be conducted to confirm the effect of ASPNN on breast milk quantity and quality.

Keywords: Creamatocrit; galactagogue; prolactin; inadequate breast milk volume; Prasa-nam-nom (Siriraj Med J 2022; 74: 11-18)

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INTRODUCTION

Breastfeeding is standard method for infants feeding. Exclusive breastfeeding for at least 6 months has been globally promoted as a standard infant feeding by the United Nations International Children's Emergency Fund (UNICEF) and the World Health Organization (WHO) through a huge campaign "Ten Steps to Successful Breastfeeding". Multiple lines of evidence support that breastfeeding improves health of infants and mothers. It decreases not only the incidence but also the severity of neonatal infections and reduces postnatal mortality rate.[1] However, WHO reported in 2018 that only about 44% of infants worldwide are exclusively breastfed.[2] Breastfeeding failure is a result of multifactorial determinants, including negative reaction toward breastfeeding at work, negative attitude toward colostrum in some societies, fatigue and intensity from work, short maternity leave (<6 weeks), poor advice on breastfeeding position and latching, as well as insufficient supports.[3] Other factors also include short nipple length[4] and perception of babies' dissatisfaction of breastmilk and inadequate milk volume.[5,6]

Human milk production can be successfully increased by means of psychological support, stress-relief-techniques and medications.[6] Plasma concentration of prolactin (PRL) plays an important role in human milk production. Dopamine is a physiologic inhibitor of PRL release. Therefore, domperidone which acts as a hypothalamic dopaminergic receptor blocker has been widely used as a galactagogue to promote lactogenesis. The drug was reported to enhance PRL.[7]

Thai traditional medicine (TTM) is a holistic approach to take care of human health. There are several TTM methods used for postpartum women, such as hot salt pot compression, massages, hot herbal charcoal seats and herbal medicines.[8] Prasa-Nam-Nom is a Thai herbal galactagogue mixture which has been traditionally used to improve milk production. This herbal formula helps increasing blood circulation and results in enhancing milk secretion and purifying its content. Prasa-Nam-Nom recipe has been listed in TTM text books as a safe herbal medicine and has been used for more than three decades. Various preparations of Prasa-Nam-Nom are widely described and accepted as a galactagogue, including our Ayurved Siriraj Prasa-Nam-Nom (ASPNN) recipe. The ingredients of ASPNN include garden spurge (*Euphobia hirta* Linn.), weeping fig (*Ficus benjamina* Linn.), rauwolfia (*Rauvolfia serpentina* Linn. Benth. ex Kurz.), and wild ginger (*Zingiber zerumbet* (L.) Smith.). Among these, *E.hirta* exhibited galactagogue effects in vivo by enhancing milk production, prolactin secretion, and the development of lobuloalveolar system of mammary glands.[9,10]

Despite its increasing popularity, there is limited evidence to support its uses. Tuntratuang (2017) reported that a Prasa-Nam-Nom used in Nong Bua Lamphu Hospital (no detail on its composition) increased breast milk volume of the mothers after caesarean section better than domperidone at 24, 48, and 72 hours.[11] Jankaew and Narumitmontri (2020) reported that a herbal recipe containing *Anthocephalus chinensis* (Lam.) A. Rich ex Walp. and *Diospyros variegata* Kurz administered five grams thrice a day for one week enhanced breast milk flow at 32 and 40 hours after the delivery better than domperidone.[12]

The objectives of this study were to explore the effectiveness of ASPNN recipe on breast milk production in women with inadequate milk volume during early postpartum period. Furthermore, its safety and impact on the level of serum prolactin hormone and the milk quality were also investigated.

MATERIALS AND METHODS

Study design

This randomized, double-blind, placebo-controlled study was conducted at the postpartum ward, Department of Obstetrics and Gynaecology, Siriraj Hospital, Mahidol University, Bangkok, Thailand between November 2010 and December 2012. The research protocol was reviewed and approved to be ethical by the Siriraj Institutional Review Board No. 743/2554 (EC3). An inform consent was obtained from each participant before the trial began. The study was registered with Thai Clinical Trials Registry with the project number of TCTR20190218004.

Participants

Subjects were enrolled if they were eligible to the inclusion criteria: age of ≥18 years old; first vaginal delivery at term; milk volume at 24-hour after the delivery <49 ml/breast pump.[13] The exclusion criteria included having medical disorders (hypertension, type 2 diabetes, thyrotoxicosis and hypothyroidism); previous allergy to ASPNN or its ingredients; inability to eat vegetables or spicy food; severe breastfeeding problems (abnormal breast anatomy, short nipples, mastitis, breast abscess and severe fetal tongue-tie); taking other medications (domperidone, metoclopramide, antidepressive drugs, caffeine and alcohol). Subjects were withdrawn if they were unable to take 90% of total drugs or had gastrointestinal unwanted effects.

Sample size calculation

According to our previous study, normal milk volume on day three after the delivery was 49 ± 18 ml/ breast pump.[13] In this study, an increase in milk production

up to 15 ml was considered to be clinically significant. By setting the probability of 2-sided type I error and the power of test at 0.05 and 80%, respectively, a minimum of 24 subjects in each group was required. To prevent loss to follow-up cases, 10% more of participants was added which resulted in the total number of 54 participants.

Randomization

For patient allocation, the treatment codes were randomly generated prior to the trial by a computer software nQuery Advisor® version 7 (Statistical Solutions Ltd., Cork, Ireland, United Kingdom). The codes were block randomized into two groups of the ASPNN group and the placebo group and kept strictly confidential in sequence in sealed opaque envelopes by a research nurse who had no responsibility for patient care in this study. The nurse would open each envelope for the treatment assignment without knowing the identity of the drugs administered by either the physicians or the patients.

Intervention

This study was conducted in a double-blind fashion. Each participant received three 500-mg capsules of either ASPNN or placebo thrice a day (4,500 mg/day) 30 minutes before meal for three days. All subjects equally obtained three meals and two snacks with total calories of 2,100 kcal daily. On day three, 2 milliliters of breast milk were collected for fat content evaluation and 6 milliliters of blood were taken for PRL level assessment. The volunteers were requested to answer a questionnaire regarding their satisfaction to the taken medication. Prior to the hospital discharge, participants in each group received more medications to continue their treatment for another seven days based on the traditional prescription of Prasa-nam-nom decoction, which is 7 - 10 days. On day eleven, they were interviewed by phone regarding any unwanted effects and type of infant feeding (Fig 1).

Participants' compliance was assessed by interviewing and counting the remaining capsules. Subjects in both groups received the standard postpartum care in the similar environment and were well-supported for breastfeeding by the same lactation consultant nurse. During the study period, they were encouraged to breastfeed every 2-3 hours in proper latch-on position and correct techniques. They also obtained the same amount of food and water in one day while a food and water chart were daily recorded for monitoring.

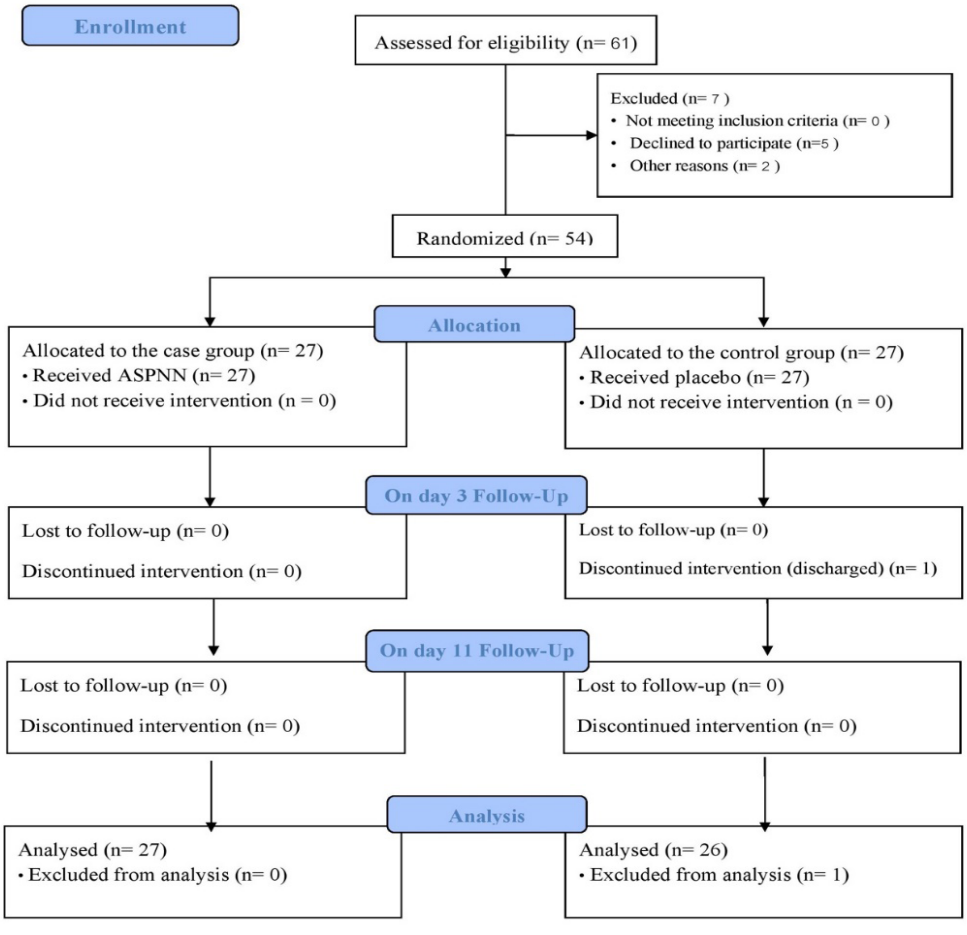

Fig 1. Study flow diagram based on the CONSORT 2010 flow diagram

Drug preparation

The 500-mg of ASPNN and identical-looking placebo capsules were prepared under the good manufacturing practice by Ayurved Siriraj Manufacturing Unit of Herbal Medicine and Products, Center of Applied Thai Traditional Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University (Bangkok, Thailand). All herbal ingredients were selected, washed and oven-dried at 45-55 °C for 6-8 hours. Then, they were chopped, grounded and filtered into brown fine powder. The mixtures were weighed and filled into 500-mg green opaque capsules using a capsule filling machine. Placebo capsules were made with green opaque capsule shells with the same appearance with those used to make ASPNN, but filled with fine granular flour, magnesium stearate and talcum. Both ASPNN and placebo capsules were similarly packed in sealed opaque envelopes. Then the capsules were tested for weight variation and disintegration to ensure that they met with the specification. To ensure the similarity of the given medications, all herbal and placebo capsules were sufficiently produced for the whole study within the same batch. All capsules were stored in a cool dry place at room temperature during the study period.

Breast milk volume collection and measurement

Breast milk was collected on day 1 before the drug administration and on day 3 before discharge using an electrical breast pump (Lactina Electric Plus breastpump by Medela CO. Switzerland) with moderate pumping power for 15 minutes on each side. The milk volume was presented as mL of breastmilk from both sides per pump (Fig 2). The collected breast milk was later brought to the infants.

Creamatocit measurement

Creamatocrit is a method widely used in lactation research. It is used to determine lipid content and energy content in breast milk.[14] The assessment technique requires a hematocrit centrifuge machine, a standard glass capillary tube and a hematocrit reader. 75 µl of collected milk was transferred into a glass capillary tube which was sealed one end by clay and centrifuged for 30 minutes at a speed of 3,000 rpm. The creamatocrit value was evaluated on day 1 before the drug administration and on day 3 before discharge and demonstrated by a percentage of the length of the cream layer to the length of the milk column in the tube (Fig 3).

Fig 2. (a) breast milk volume was measured before the drug administration and on day 3 and (b) an electrical breast pump

Fig 3. Creamatocit measurement (a) a hematocrit centrifuge machine and (b) a hematocrit reader.

Prolactin level assessment

Serum prolactin levels (PRL) were determined on day 1 before the drug administration and on day 3 before discharge using Prolactin II assay (Roche Diagnostic) and analyzed by Modular Analytics E170 module (Roche Diagnostic, Mannheim, Germany). This technique was an electrochemiluminescence immunoassay based on the sandwich principle.

Satisfaction scores, adverse effects, and types of breastfeeding

Satisfaction scores and adverse effects were determined on day 3. After a discharge from the hospital, the participants continued the intervention for another 7 days at home. Then satisfaction scores, adverse events, and types of breastfeeding were assessed on day 11 by a telephone interview. The participants rated their satisfaction with odor and flavor of the intervention on a scale of 1-5, where 1 = very unsatisfied, 2 = unsatisfied, 3 = neutral, 4 = satisfied, and 5 = very satisfied. The participants were asked with an open-ended question for self-report adverse effects. Lastly, they were asked for types of breastfeeding whether they fed the baby with only breast milk (exclusive breastfeeding) or combine with formula supplementation (partial breastfeeding).

Statistical analysis

All statistical analyses were performed using SPSS version 18 for Windows software (SPSS, Inc., Chicago, IL). All data were presented as mean ± SD or median (min, max). Independent t- test or Mann-Whitney U test were used to compare these values between two groups. Paired t-test or Wilcoxon Signed Rank test were used to compared results before - after of each group. Satisfaction scores and adverse effects were presented as case number and percentage. The comparison between two groups was performed using Pearson Chi- square. Breastfeeding types on day 11 were presented as case number and percentage. The comparison was performed with Fisher's exact test. The statistical significance was set at $p < 0.05$.

RESULTS

From the total enrolled 61 women, there were 54 eligible women who were randomly allocated into the placebo group or the ASPNN group. On day three, one case in the placebo group dropped out. Therefore, the data from only 53 cases were used for statistical analysis (Fig 1).

At the beginning, maternal age, body mass index, total gestational weight gain, baby birth weight, pumped milk volume, creamatocrit and serum PRL level in both groups were similar (Table 1).

After three days of the interventions, the median milk volume in both groups remarkably improved from 0.15 to 19 ml in the ASPNN group and 0.3 to 30 ml in the placebo group (Table 2). Maximum milk volume of the ASPNN group was higher than that of the placebo group. Breast milk volume, increased milk volume, creamatocrit and PRL of both groups were not difference. Serum PRL of the ASPNN group on day 3 was not different from day 1 ($p = 0.31$), while that of the placebo group significantly reduced ($p = 0.03$).

ASPNN drug was highly accepted by the women in terms of its odor and flavor (Table 3). Minor adverse effects were reported, including skin rash and mild diarrhea, with spontaneous recovery (Table 4). The percentage of exclusive breastfeeding on day 11 of the ASPNN group was 92.6%, whereas that of the placebo group was 88.5%.

TABLE 1. Demographic data (before the treatment)

Characteristic	ASPNN (n = 27)	Placebo (n = 26)	P-value
Age (years), mean ± SD	23.74 ± 4.95	24.27 ± 5.38	0.36
Body mass index (kg/m^2), mean ± SD	20.57 ± 2.91	20.53 ± 2.52	0.48
Total gestational weight gain (kg), mean ± SD	12.54 ± 3.96	12.89 ± 3.43	0.37
Baby birth weight (g), mean ± SD	3,013.33 ± 288. 42	2,981.15 ± 311.58	0.35
Day-1 breast milk volume (ml), median (min, max)	0.15 (0, 5)	0.3 (0, 4.1)	0.94
Day-1 creamatocrit (%), median (min, max)	7.25 (2.58, 14.23)	6.03 (2.13, 12.34)	0.47
Day-1 prolactin (ng/mL), mean ± SD	339.18 ± 164. 21	386.54 ± 192.61	0.17

TABLE 2. Breast milk volume, creamatocrit and serum prolactin level on day 3 in each group and the changes

Data	ASPNN (n=27)	Placebo (n=26)	p-value
Day-3 breast milk volume (ml), median (min, max)	19 (0, 139)	30 (0.01, 110)	0.53
Breast milk volume changes (ml)#, median (min, max)	18.55 (0, 137.3)	29.75 (0.01, 110)	0.58
Day-3 creamatocrit (%), median (min, max)	7.17 (3.94, 10.71)	6.98 (2.52, 11.52)	0.28
Creamatocrit changes (%)#, median (min, max)	-0.07 (-7.8, 8.13)	0.37 (-4.31, 4.94)	0.49
Day-3 prolactin (ng/mL), mean ± SD	321.76 ± 114.23	323.78 ± 116.68	0.47
Prolactin changes (ng/mL)#, mean ± SD	- 17.42 ± 92.95	- 62.77 ± 169.62	0.12

compared to the baseline

TABLE 3. Satisfaction with odor and flavor of the interventions (day 3)

Topics	ASPNN (n = 27)	Placebo (n = 26)	p-value
Satisfaction			
Excellent	17 (63.0%)	23 (88.5%)	0.031
Delight	10 (37.0%)	3 (11.5%)	
Odor			
Very satisfied	14 (51.9%)	12 (46.2%)	0.408
Satisfied	11 (40.7%)	8 (30.8%)	
neutral	2 (7.4%)	5 (19.2%)	
Unsatisfied	0 (0%)	1 (3.8%)	
Flavor			
Very satisfied	13 (48.2%)	15 (57.7%)	0.557
Satisfied	11 (40.7%)	10 (38.5%)	
neutral	3 (11.1%)	1 (3.8%)	

Value is presented by number of cases (%)

TABLE 4. Self - reported adverse effects

Adverse Effects	ASPNN (n = 27)		Placebo (n = 26)		p-value
	Day 3	Day 11	Day 3	Day 11	Day 3
None	25 (92.6%)	27 (100%)	26 (100%)	26 (100%)	0.368
Skin rash	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	
Mild diarrhea	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	

Value is presented as number of cases (%)
(The is no p-value on day 11 because the adverse effect is a constant)

DISCUSSION

Compromised lactation is originated from several factors, including preglandular, glandular or postglandular problems.[15] Phase II lactogenesis occurs 3 - 7 days after the delivery. In this phase, the quantity of breast milk becomes higher at the end of the first week after the delivery. However, many early-postpartum women perceive that they might have insufficient breastmilk and this leads them to stop breastfeeding. Therefore, it is necessary to initiate a successful breastfeeding as soon as possible to encourage exclusive breastfeeding later.[16] In this study, we focused on the implementation of ASPNN, which has been used to stimulate breast milk production in Thai traditional medicine, in order to help early-postpartum women initiating breastfeeding.

ASPNN administration for 3 days showed no statistically significant effect to breast milk volume compared to the placebo. This might due to the short duration of the intervention, which was limited by the usual hospital stay. Galactagogues, such as domperidone or metoclopramide, are usually prescribed for 5 - 28 days.[17] A study from Nong Bua Lamphu Hospital showed that Prasa-Nam-Nom could increase milk volume in 72 hours.[11] However, this study measured the 24 - hour milk volume, while our study measured milk volume from only one pump in a day.

Lipid is a crucial component of breast milk which provides energy to infants. Creamatocrit measurement is simple, inexpensive, and effective to determine lipid content and calories in breast milk. There was also a strong relationship between the creamatocrit value and the infants' body weight increment.[14] The percentage of fat component in breast milk was improved in both groups (25 - 38%). Interestingly, there was a trend of increased creamatocrit in the ASPNN group compared to the placebo group. According to the TTM knowledge, ASPNN has been prescribed in order to promote milk production and to purify the milk. Therefore, further investigation should be conducted to explore the effect of ASPNN on quality of breast milk.

PRL is among reproductive hormones that are responsible for milk production and secretion and becomes a pharmacological target for galactagogues.[17] Although ASPNN showed no statistically significant effect on PRL concentrations, there was a trend that the herbal medicine could maintain PRL level which is important for a successful breastfeeding. The level of PRL is high in the first two hours after delivery and declines after that. Infant suckling stimulates PRL.[17] In our study, the participants were encouraged to breastfeed their babies every 2-3 hours and this should help maintaining PRL level. However, the placebo group showed a significant reduction in PRL. This suggests that ASPNN might be beneficial for lactogenesis by its impact on PRL.

After discharged, all participants continued their treatment for another week and were interviewed by phone regarding any adverse effects and types of infant feeding. This study shows that short term use of ASPNN is safe for lactating women with only minor and recoverable adverse effects. Its safety for longer period of use is still needed to monitor. The rates of exclusive breastfeeding in both groups were high, which might due to the effectiveness of overall lactation program that could initiate breastfeeding in early postpartum phase.

Traditionally, Prasa-Nam-Nom decoction in conjunction with other treatments, including body massages, hot salt pot, hat charcoal seat, herbal hot spa (Yu-fai) are given to nursing mother for 4 - 6 weeks. This holistic care approach aims to relieve muscle pain, restore the body balance, improve blood circulation, promote perineum wound healing and uterine involution.[8] ASPNN decoction has been used in Ayurved Siriraj Clinic of Applied Thai Traditional Medicine. However, the decoction is inconvenient and difficult to drink. The preparation process is also difficult to control when the participants were at home. Therefore, ASPNN capsule was used in this study instead to ensure that every participant in ASPNN group receive similar intervention. According to the satisfaction survey, the capsule was well-accepted similarly to the placebo.

The advantage of this study was that we designed the experiment to minimize interference to the participants. The breastfeeding was encouraged but voluntarily performed. The milk collection was done only once a day to avoid disturbing baby suckling. Maternal food and water intake were monitored as these affects milk production. Not only the quantity of breast milk, the quality was also assessed. Although the results were not significant, the information can be used in future research.

The limitation of this study was that the main outcomes were determined on day 3 but ASPNN may produce significant benefits later. Although many factors affecting breast milk production and other outcomes were controlled or monitored in the current study (eg. frequency of infant suckling, proper latch-on techniques, monitoring of food and water intake), some uncontrollable factors still exist, such as maternal stress, exact food and water intake, and exact times of milk and blood collection. Future clinical trials should be performed with larger sample size and evaluate outcomes on day 7 afterward. The milk and blood collection times should be similar in all participants. Even though management of maternal

stress was routinely performed in the postpartum ward, it should also be assessed.

CONCLUSION

Short term supplementation of ASPNN produce no direct effect on breast milk volume, creamatocrit, and serum prolactin. It was safe and might help maintaining serum prolactin. A future trial with more participants and longer period should be conducted to confirm the effect of ASPNN on breast milk quantity and quality.

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Abbreviations

ASPNN: Ayurved Siriraj Prasa-Nam-Nom; PRL: Prolactin level; TTM: Thai traditional medicine

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Authors'contributions

PC : analyzed and interpreted the data and contributed in writing the manuscript.
CT : collected the data and performed creamatocit measurement.
AI : collected the data and performed creamatocit measurement.
NL : conducted the production of medicines, analyzed the data and contributed in writing the manuscript.
PA : contributed in planning the study protocol and provided information for data analysis.
TL : contributed in planning and development of this research.
SB : recruited the participants, collected blood and breast milk, and gave the treatment.
NK : recruited the participants, collected blood and breast milk, and gave the treatment.
SP : performed Prolactin level assessment.
AN : contributed in planning and development of this research.
TT : analyzed the data and contributed in planning the study protocol and in writing the manuscript.
All : authors read and approved the final manuscript.

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Clinical Characteristics of Female Patterned Hair Loss in Patients Attending Hair Clinic in Thailand

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ABSTRACT

Objective: To study the clinical features and associated factors of female pattern hair loss (FPHL) in premenopausal and menopausal women patients.

Materials and Methods: This is a retrospective chart review of FPHL patients visited hair clinic, Siriraj Hospital from June 2012 to May 2015. Demographic data, family history and history of hair loss were evaluated. Factors associated with FPHL were analysed.

Results: There were 267 patients (180 premenopausal women and 87 menopausal women) in this study. The mean age of onset of patients was 35.5±12 years (premenopausal FPHL) and 60.5±7 years (menopausal FPHL). Positive family history of androgenetic alopecia (AGA) was 48.3%, mainly in first-degree relatives. The data showed an increased incidence of FPHL with advancing age. The most common presentation is Ludwig grade I. The study showed that patients also have dyslipidemia (16.9%), hypertension (16.5%), diabetes mellitus (10.9%), hypothyroidism (4.9%), anemia (3.7%), and hyperthyroidism (2.9%). In multivariate analysis, significant associations were found between low ferritin level <70 µg/L and premenopausal FPHL (OR 5.51, 95% CI 2.26-15.14, P = 0.01).

Conclusion: Maternal family history of AGA seems to have a greater influence on premenopausal FPHL. Low serum ferritin levels <70 µg/L were significantly associated with FPHL in premenopausal women.

Keywords: Female pattern hair loss; androgenetic alopecia; family history; ferritin; premenopause; menopause (Siriraj Med J 2022; 74: 19-26)

Abbreviations
OR : Odds ratio
CI : confidence Interval

INTRODUCTION

Female pattern hair loss (FPHL) is a common cause of non-scarring alopecia, resulted from the miniaturization of hair follicles. This condition presents as a diffuse hair loss and prominent in the frontal, crown and parietal areas. In contrast to other scalp diseases such as tinea capitis and scalp psoriasis, the patients typically present with patchy hair loss and erythematous plagues with silvery scales, respectively.[1,2] Patients with FPHL have a lower self-image and a lower quality of life. The prevalence of FPHL is varied among ethnicities and increases with age.[3] The overall prevalence is lower in the Asian population (5.6% in the Korean study of Paik et al.)[3] compare to Caucasians (19% in the US study of Mansouri et al.).[4] In addition, one study recently conducted in Thailand reported the prevalence of disorders of skin appendages was 1.0% during the five-year period.[5]

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The pathophysiology of this condition is not well established. The role of androgens in FPHL is different from male pattern hair loss. Individuals with 5-α reductase deficiency or androgen insensitivity syndrome still have FPHL.[6] Estrogen may also play a protective role through inhibition of 5-α reductase which explain the higher prevalence in menopausal group.[7] Other findings that associated to this condition such as insulin resistance, metabolic syndrome, coronary artery disease, hypothyroidism, hyperprolactinemia, breast feeding, ultraviolet light exposure were reported.[8-12] The diagnosis can be made mainly by history taking and physical examination. Skin biopsy is not necessary except for differentiating it from other causes of hair loss. The laboratory evaluations for thyroid function, ferritin level, and androgen state are essential in some cases with suspicious history.

There were mostly publications about the prevalence and risk factors related to FPHL in Western and few Asian studies from South Korea, Taiwan, China and India.[3,10,13,14] As prominent differences in the prevalence and factors of female pattern hair loss in difference countries and race are known. This study investigates about these aspects in different backgrounds which might have various impacts on the effects of these factors. For providing more information of FPHL management in the future.

MATERIALS AND METHODS

Study design

A retrospective chart review of female pattern hair loss patients visited hair clinic, Department of Dermatology, Siriraj Hospital, Mahidol University, a tertiary center in Bangkok, Thailand from June 2012 to May 2015 was performed. The study protocol was approved by the Siriraj Institutional Review Boards. The diagnosis of female pattern hair loss was made by a dermatologist based on history taking, physical examination of pattern of hair loss based on Ludwig type and excluded other causes of hair loss. The data obtained from medical records including demographic data, family history of pattern hair loss, medical history, underlying disease, menstruation history, pregnancy and lactation history in past 12 months, significant weight loss in 6 months, features of hyperandrogenism (e.g. seborrhea, severe acne, infertility, hirsutism, overweight), detailed history of hair loss, physical examination by using Ludwig's classification and laboratory investigation (hemoglobin, hematocrit, creatinine, HbA1C, thyroid stimulating hormone, serum ferritin, antinuclear antibody, antithyroid antibody) at diagnosis were noted.

Sample size calculation and statistical analysis

Since no previous study compared clinical characteristics between premenopausal and menopausal FPHL patients, the sample size was calculated based on the family history of AGA in FPHL patients. Previous studies in Korean and China reported 45.2% and 32.4% of FPHL patients had family history of AGA, respectively.[3,15] The estimated family history of AGA in FPHL patients was 40% with 6% error, therefore a total sample size of 257 patients was needed.

Data was analysed using statistical package for social science (SPSS) software version 18 statistical programs. The chi-square test was performed to compare categorical data. *P* values using the traditional cutoff of *P* less than 0.05 were calculated to determine statistical significance. A logistic regression and multivariable regression model were used to evaluate the relationship of each possible risk factor to FPHL.

RESULTS

There were 316 patients with a medical record diagnosis of ICD10 L64.9 Androgenetic alopecia, unspecified in Hair clinic, Department of Dermatology, Siriraj Hospital, Mahidol University from June 2012 to May 2015. The subjects were divided into 2 groups by the first presentation of FPHL, premenopause and menopause. Premenopause is defined as the whole of the reproductive period before the menopause, while menopause is defined as the permanent cessation of menstruation.[16] Of the 316 patients, 49 patients diagnosed with alopecia areata were excluded. 267 eligible patients (180 premenopausal women and 87 menopausal women) met the criteria for this study.

Characteristics of FPHL

Of the 267 patients, the mean age of hair loss onset among all patients was 43.5±15 years. The mean age of onset of premenopausal FPHL patients was 35.5±12 years, while 60.5±7 years was noted in menopausal FPHL patients (Table 1).

Family history

Both male and female relatives with androgenetic alopecia (AGA) counted as family history. A positive family history of AGA was found in 129 of 267 (48.3%) patients and mainly 121 of 267 (45.3%) patients noted in history of first-degree relatives. 66 of 267 (24.7%) patients reported a family history of AGA in paternal relatives, 27 of 267 (10.1%) patients in maternal relatives, 36 of 267 (13.5%) patients in both paternal and maternal relatives and 138 of 267 (51.7%) patients had no family history of AGA (Table 1).

TABLE 1. Characteristics of female pattern hair loss patients: Medical profiles

Patients	Premenopause (n=180)	Menopause (n=87)	Total (n=267)
Age of onset (years), mean±SD	35.5 ± 12	60.5 ± 7	43.5 ± 15
Race, n (%)			
Thai	180 (100)	85 (97.7)	265 (99.3)
Non-Thai	0 (0.0)	2 (0.3)	2 (0.8)
Family history of AGA, n (%)	95 (52.7)	34 (39.1)	129 (48.3)
1st degree	87 (48.3)	34 (39.1)	121 (45.3)
2nd degree	8 (4.4)	0 (0.0)	8 (2.9)
3rd degree	0 (0.0)	0 (0.0)	0 (0.0)
Parental family history of AGA, n (%)			
None	85 (47.2)	53 (60.9)	138 (51.7)
Paternal family history	47 (26.1)	19 (21.8)	66 (24.7)
Maternal family history	23 (12.8)	4 (4.6)	27 (10.1)
Both positive	25 (13.9)	11 (12.6)	36 (13.5)
Postpartum in 1 year, n (%)	2 (1.1)	0 (0.0)	2 (0.8)
Significant weight loss, n (%)	0 (0.0)	1 (0.1)	1 (0.4)
Menstruation, n (%)			
Regular	154 (85.6)	69 (79.3)	223 (83.2)
Irregular	23 (12.8)	11 (12.6)	34 (12.7)
Hyperandrogenism, n (%)	6 (3.3)	0 (0.0)	6 (2.3)
Seborrhea	1 (0.1)	0 (0.0)	1 (0.4)
Severe acne	4 (2.2)	0 (0.0)	4 (1.5)
Infertility	6 (3.3)	0 (0.0)	6 (2.3)
Hirsutism	2 (0.1)	0 (0.0)	2 (0.8)
Overweight	1 (0.1)	0 (0.0)	1 (0.4)
Underlying disease, n (%)	50 (27.8)	63 (72.4)	113 (42.3)
Vitiligo	0 (0.0)	0 (0.0)	0 (0.0)
Hypothyroidism	8 (4.4)	5 (5.8)	13 (4.9)
Hyperthyroidism	5 (2.8)	3 (3.5)	8 (2.9)
Atopy	0 (0.0)	0 (0.0)	0 (0.0)
Dyslipidemia	14 (7.8)	31 (35.6)	45 (16.9)
Anemia	9 (5.0)	1 (1.2)	10 (3.8)
Diabetes mellitus	8 (4.4)	21 (24.1)	29 (10.9)
Hypertension	8 (4.4)	36 (41.4)	44 (16.5)
Previous treatment, n (%)	34 (18.9)	16 (18.4)	50 (18.7)
Topical minoxidil	34 (18.9)	15 (17.2)	49 (18.4)
Oral antiandrogen	0 (0.0)	1 (1.2)	1 (0.4)
History of taking OCP, n (%)	11 (6.1)	3 (3.5)	14 (5.2)
Smoking, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Duration, n (%)			
<3 months	18 (10.0)	9 (10.3)	27 (10.1)
3-6 months	25 (13.9)	13 (14.9)	38 (14.2)
>6-12 months	15 (8.3)	4 (4.6)	19 (7.1)
>1-2 years	38 (21.1)	22 (25.3)	60 (22.5)
>2-5 years	41 (22.8)	18 (20.7)	57 (21.4)
> 5 years	42 (23.3)	22 (25.3)	64 (23.9)
Hair manipulation, n (%)	18 (10.0)	14 (16.1)	32 (11.9)
Coloring	17 (9.5)	6 (6.9)	23 (8.6)
Straightening	7 (3.9)	3 (3.4)	10 (3.8)
Flat iron	0 (0.0)	0 (0.0)	0 (0.0)
Hair dryer	1 (0.1)	0 (0.0)	1 (0.4)
Curling	11 (6.1)	9 (10.3)	20 (7.5)

*Significant at $P = 0.05$, Data expressed as mean±SD or n (%); AGA, androgenetic alopecia; OCP, oral contraceptive pill.

Association between FPHL and possible risk factors

In the univariate analysis, many factors were associated with having early onset FPHL. Statistically significant associations were noted between premenopausal FPHL and these factors: dyslipidemia (OR 0.15, 95% CI 0.07-0.30, $P = 0.01$), diabetes mellitus (OR 0.14, 95% CI 0.06-0.35, $P = 0.01$), hypertension (OR 0.01, 95% CI 0.03-0.15, $P = 0.01$), family history of AGA (OR 1.72, 95% CI 1.02-2.90, $P = 0.04$), maternal family history (OR 3.06, 95% CI 1.02-9.14, $P = 0.03$), low level of thyroid stimulating hormone <0.27 μIU/ml (OR 0.14, 95% CI 0.03-0.77, $P = 0.01$), low ferritin level <70 μg/L (OR 2.81, 95% CI 1.15-6.86, $P = 0.02$) and positive antinuclear antibody (OR 0.16, 95% CI 0.03-1.06, $P = 0.04$) (Table 2).

Independent factors were analysed by multivariable regression model. Significant associations were found between low ferritin level <70 μg/L and premenopausal FPHL (OR 5.51, 95% CI 2.26-15.14, $P = 0.01$) (Table 3).

Past medical history

There were 2 patients (0.8%) with each history of postpartum and lactation within 1 year and only one patient (0.4%) with significant weight loss in 6 months. 14 of 267 (5.2%) patients reported history of taking oral contraceptive pill within 6 months. Regular menstruation was found in 223 of 267 (83.2%) of patients. Only 6 patients (2.3%) had got the features of hyperandrogenism. There were common medical problems among patients included dyslipidemia (16.9%), hypertension (16.4%), diabetes mellitus (10.9%), hypothyroidism (4.9%), anemia (3.7%) and hyperthyroidism (2.9%) in respectively (Table 1).

Previous treatment history

50 of 267 (18.7%) patients reported history of previous treatment for FPHL. Topical minoxidil was used in majority of patients (18.4%) and one patient (0.4%) had been treated with oral antiandrogen (Table 1).

History of hair loss

For most patients, the time from hair loss onset to seeing a hair specialist was longer than one year. There was previous history of hair manipulation in 32 of 267 (11.9%) patients including hair coloring (8.6%), curling (7.5%), straightening (3.8%) and hair dryer (0.4%) (Table 1).

Overall, the age-specific prevalence of female pattern hair loss (FPHL) increased with advancing age and peaked in age group 50-59 years (24.7%). Most patients (61.0%) were Ludwig grade I, followed by grade II (29.2%) and grade III (9.7%). The age-specific prevalence of severe hair loss (Ludwig grade ≥II) was relatively increasing; however, it was slightly lower in those aged 40-49 years. There were mostly recorded Ludwig grade I in patients with age group 0-19 years and Ludwig grade ≥ II in age group 50-59 years (Fig 1).

DISCUSSION

As prominent differences in the prevalence and factors of female pattern hair loss in difference countries and race are known. No study regarding the clinical characteristics and factors associated with FPHL in Thailand has been reported. This study investigated in difference background which might have various impacts on the effects of these factors. In this study demonstrated the trend of increased incidence of FPHL with advancing age and Ludwig grade I was the most common type, similar to previous studies in other population.[3,15,17-22] Ludwig grade ≥ II were more common in patients aged ≥50 years. This may be due to the fact that less cosmetically concern about hair loss in older adults and higher Ludwig grade with advancing age may be influenced by the duration of disease progression. Although most patients in this study were premenopausal cases, the trend of increased incidence of FPHL with advancing age was observed.

FPHL can occur in any time of life. The mode of inheritance in AGA has not been characterised. There were previous reports of higher prevalence of AGA in patients with paternal rather than maternal inheritance, similarly to this study.[20,23] Moreover, female patients were more likely to have positive maternal family history.[23,24] Specifically, this study demonstrated that positive maternal family history was statistically significantly associated with premenopausal FPHL. This may be due to some inherited genes from maternal side. Further studies are needed to determine the association of early onset FPHL and family history of AGA in FPHL patients. The youngest patient with a positive maternal family history of AGA was 16 years old. These results suggested that paternal family history of AGA has more effect on FPHL than maternal family history of AGA. However, maternal family history of AGA was seem to be greater influence in premenopausal FPHL.

Hypertension, insulin resistance and increased cardiovascular risk have been described associated with early onset FPHL compared with healthy subjects.[9] Possible explanations were higher aldosterone, C-protein, D-dimer and insulin levels in women with FPHL.[9] This study demonstrated that menopausal women with FPHL had higher rate of hypertension, dyslipidemia, diabetes mellitus. There may be explained by more commonly

TABLE 2. Univariate analyses of risk factors associated with early onset female pattern hair loss

Patients, n (%)	Premenopause n=180	Menopause n=87	Total n=267	OR	95% CI	*P* Value
Irregular menstruation	23 (12.8)	11 (12.6)	34 (12.7)	1.06	0.49-2.31	0.87
History of OCP	11 (6.1)	3 (3.5)	14 (5.2)	1.82	0.49-6.71	0.36
Hyperandrogenism	6 (3.3)	0 (0.0)	6 (2.3)	0.66	0.61-0.73	0.08
Underlying disease						
Hypothyroidism	8 (4.4)	5 (5.8)	13 (4.9)	0.76	0.24-2.42	0.64
Hyperthyroidism	5 (2.8)	3 (3.5)	8 (2.9)	0.80	0.19-3.43	0.76
Dyslipidemia	14 (7.8)	31 (35.6)	45 (16.9)	0.15	0.07-0.30	0.01*
Anemia	9 (5.0)	1 (1.2)	10 (3.7)	4.52	0.56-36.30	0.12
Diabetes mellitus	8 (4.4)	21 (24.1)	29 (10.9)	0.14	0.06-0.35	0.01*
Hypertension	8 (4.4)	36 (41.4)	44 (16.4)	0.01	0.03-0.15	0.01*
Family history of AGA	95 (52.7)	34 (39.1)	129 (48.3)	1.72	1.02-2.90	0.04*
1st degree	87 (48.3)	34 (39.1)	121 (45.3)	1.47	0.87-2.48	0.14
2nd degree	8 (4.4)	0 (0.0)	8 (2.9)	0.66	0.61-0.72	0.04*
3rd degree	0 (0.0)	0 (0.0)	0 (0.0)	-	-	-
Parental family history of AGA						
None	85 (47.2)	53 (60.9)	138 (51.7)	-	-	-
Paternal family history	47 (26.1)	19 (21.8)	66 (24.7)	1.35	0.74-2.47	0.33
Maternal family history	23 (12.8)	4 (4.6)	27 (10.1)	3.06	1.02-9.14	0.03*
Both paternal and maternal	25 (13.9)	11 (12.6)	36 (13.5)	1.12	0.52-2.40	0.77
Hemoglobin <12 g/dl	23/123 (18.7)	8/73 (11.0)	31/176	1.87	0.78-4.43	0.15
Hematocrit <36 %	22/123 (17.9)	15/73 (20.5)	37/196	0.84	0.41-1.75	0.65
Abnormal TSH						
TSH <0.27 µIU/mL	2/66 (3.0)	6/34 (17.6)	8/100	0.14	0.03-0.77	0.01*
TSH >4.20 µIU/mL	6/66 (9.1)	3/34 (8.8)	9/100	1.03	0.24-4.41	0.96
Low ferritin level						
Ferritin <40 µg/L	27/86 (31.4)	6/29 (20.7)	33/115	1.75	0.64-4.80	0.27
Ferritin <70 µg/L	48/86 (55.8)	9/29 (31.0)	57/115	2.81	1.15-6.86	0.02*
Positive ANA	7/24 (29.2)**	5/7 (71.4)***	12/31	0.16	0.03-1.06	0.04*

*Significant at $P = 0.05$, Data expressed as mean±SD or n (%); *OR,* odds ratio; *CI*, confidence interval

**Positive ANA pattern in premenopausal FPHL including fine speckle with titer of 1:640, fine speckle with titer of 1:160, fine speckle with titer of 1:100, fine speckle with borderline titer, borderline ANA, titer of 1:2560 with no data of ANA pattern and no data of ANA pattern and titer

***Positive ANA pattern in menopausal FPHL including fine speckle with titer of 1:640, fine speckle with titer of 1:320, nucleolar with titer of 1:320, fine speckle with borderline titer and borderline ANA

AGA, androgenetic alopecia; OCP, oral contraceptive pill; TSH, thyroid stimulating hormone; ANA, antinuclear antibody

TABLE 3. Multivariate analyses of risk factors associated with early onset female pattern hair loss

Patients	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Age	0.77	0.72-0.83	0.008*	0.99	0.98-1.01	0.58
Family history of AGA	1.72	1.02-2.90	0.04*	1.21	0.07-21.44	0.84
Maternal family history	3.05	1.02-9.14	0.03*	2.84	0.24-32.96	0.40
Dyslipidemia	0.15	0.07-0.30	0.01*	0.18	0.06-1.66	0.51
Diabetes mellitus	0.14	0.06-0.35	0.01*	0.64	0.08-5.57	0.36
Hypertension	0.01	0.03-0.15	0.01*	0.54	0.09-3.96	0.48
Ferritin <70 µg/L	2.81	1.15-6.86	0.02*	5.51	2.26-15.14	0.01*

*Significant at $P = 0.05$, Data expressed as mean±SD or n (%); *OR*, odds ratio; *CI*, confidence interval
AGA, androgenetic alopecia; OCP, oral contraceptive pill

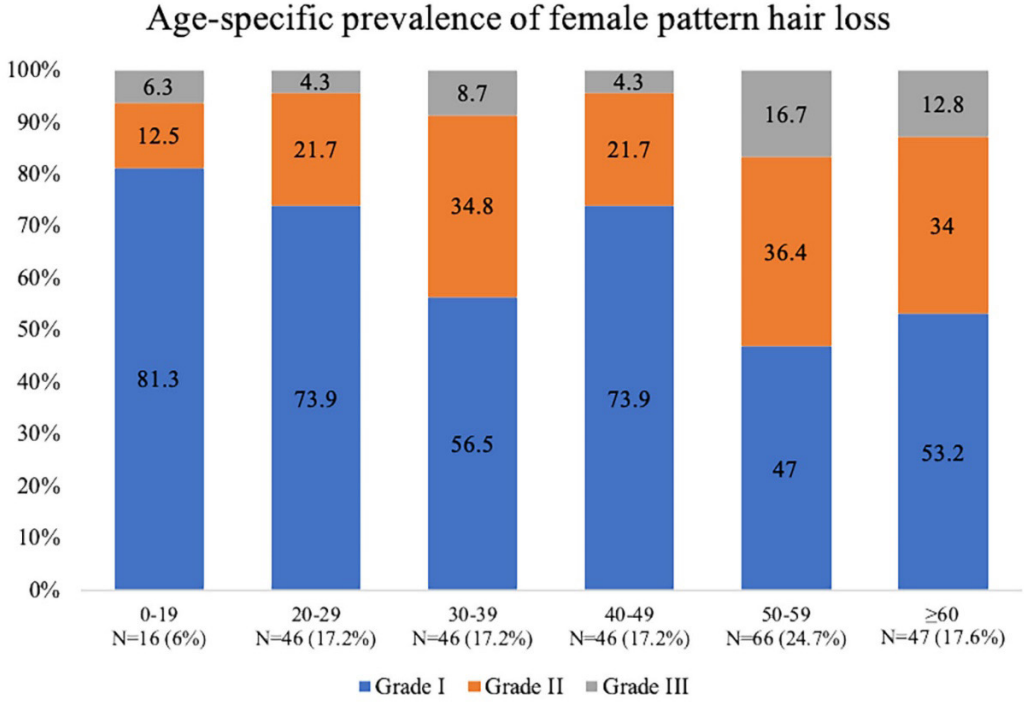

Fig 1. Age-specific prevalence of female pattern hair loss (FPHL)

increasing frequency of those in elderly patients. However, further comparative study will be needed to evaluate the association between hypertension, insulin resistance and increased cardiovascular risk to FPHL.

Previous studies have noted the relationship of iron deficiency and FPHL. Whether defined iron deficiency by using serum ferritin level, the definition has ranged from serum ferritin level less than or equal to 15 µg/L, less than or equal to 40 µg/L. The sensitivity of using those cut-off level of serum ferritin level was 59%, 98% in respectively, with about 99%, 98% specificity based on comparison with complete absence of iron staining on bone marrow aspiration.[25-27] Coenen et al.[25] noted that all patients with serum ferritin less than 70 µg/L had a lack of macrophage and/or sideroblast iron stains on bone-marrow aspiration and would be considered to have iron deficiency anemia. Rushton et al.[28] reported that there was increased scalp hair shedding and decreased

hair volume in women with serum ferritin less than of equal to 70 µg/L. In 2003, Kantor et al.[29] found a lower mean serum ferritin level in FPHL patients who was under 40 years old (mean = 37.3 µg/L; n = 52) than control subjects (mean = 59.5 µg/L; n = 11). In this study, mean level of ferritin in FPHL patients was higher (mean = 212.15±206 µg/L) compared to previous studies.[29,30] There did seem to be lower mean of ferritin level in menopausal (mean = 84.94±100.9 µg/L; n = 87) than premenopausal (mean = 201.67±248.5 µg/L; n = 180) FPHL patients. The results could be related to differences in the diet, genetic, ethnic variations of the study populations and lack of data pertaining to FPHL in our country. Furthermore, this study demonstrated that serum ferritin levels <70 µg/L were significantly associated with FPHL in premenopausal women which was similar to previous studies.[28,31] However, some studies did not support the association between FPHL and iron deficiency.[32-34] No established guideline for routine investigations and role of iron supplement therapy in women with FPHL. Due to various cut-off levels of ferritin, we commonly prescribed iron supplement to FPHL patients with serum ferritin levels <70 µg/L based on previous studies[25,28,34] and several reasons. First, cut-off level of ferritin >70 could cover more patients with iron deficiency anemia. Moreover, we also found that giving iron supplement in FPHL patients with serum ferritin level <70 µg/L helped their hair regrowth and decreased hair shedding. However, additional comparative study with larger sample sizes of the correlation between FPHL and iron deficiency status or responsiveness of oral iron supplement are necessary to answer the efficacy of iron supplement therapy in FPHL patient.

The limitation of the present study are some missing data due to retrospective approach. Furthermore, the study is from a single center and may not accurately represent the general population. Further case control design or population based descriptive study helps to identify the exact prevalence and risk factors of FPHL patients.

CONCLUSION

In conclusion, this study described clinical characteristics of FPHL and the factors associated with FPHL in premenopausal and menopausal female. Maternal family history of AGA was seem to be greater influence in premenopausal FPHL. Moreover, low serum ferritin levels <70 µg/L were significantly associated with FPHL in premenopausal women.

Conflicts of Interest: None declared

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Aflibercept as Adjunctive Treatment for Filtration Surgery in Neovascular Glaucoma

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ABSTRACT

Objective: To investigate intravitreal aflibercept (IVA) injection as an adjunctive treatment to trabeculectomy with mitomycin C (TMC) and panretinal photocoagulation (PRP) for neovascular glaucoma (NVG).

Materials and Methods: PRP and IVA (2 mg/0.05 ml) injection were given, and TMC was performed within 2 weeks after IVA. Additional PRP, laser suture lysis, subconjunctival 5-fluorouracil injection, and bleb needling were performed after TMC if indicated. Best corrected visual acuity (BCVA), intraocular pressure (IOP), surgical complications, and number of anti-glaucoma medications were collected.

Results: Five eyes from 5 consecutive patients were included. Two eyes had proliferative diabetic retinopathy (PDR), 2 central retinal vein occlusion, and 1 ocular ischemic syndrome (OIS) (mean initial IOP: 46.8±6.8 mmHg). NVI regression occurred in one eye after PRP alone, and in one eye after PRP and IVA resulting in a good IOP control with topical medical therapy. The other 3 underwent TMC. The preoperative IOP was 34 (OIS), 54 (PDR), and 50 (PDR) mmHg. The 3-month postoperative IOP decreased to 8, 8, and 4 mmHg, respectively, and to 21, 10, and 6 mmHg, respectively, at the last visit. Only the one OIS eye required postoperative topical IOP-lowering medications. Final BCVA was improved, unchanged, and decreased in 2, 2, and 1 eye, respectively. No intraoperative/postoperative complications or NVI recurrence were observed (mean follow-up: 10.7 months).

Conclusion: Intravitreal aflibercept was shown to be a potentially effective additional treatment to PRP and TMC in patients with NVG.

Keywords: Aflibercept; adjunctive treatment; filtration surgery; neovascular glaucoma (Siriraj Med J 2022; 74: 27-33)

INTRODUCTION

Neovascular glaucoma (NVG) is a devastating secondary glaucoma characterized by the development of neovascularization of the iris (NVI) and anterior chamber angle (NVA), which causes obstruction of aqueous outflow and increased intraocular pressure. It is estimated that 97% of NVG patients develop neovascularization as a result of posterior segment ischemia.[1,2] The common causes of this condition include proliferative diabetic retinopathy (PDR), central retinal vein occlusion (CRVO), and ocular ischemic syndrome (OIS).[1,3] Ocular ischemia leads to the production of pro-angiogenic factors that diffuse into the anterior segment and cause NVI, NVA, and fibrovascular membranes.[4] Vascular endothelial growth factor (VEGF) is one of the pro-angiogenic factors found in the ocular fluids of both PDR patients and NVG patients.[4] Panretinal photocoagulation (PRP) is a standard treatment for retinal ischemic conditions that is combined with treatment of the underlying disease.[5] However, PRP causes the death of healthy retinal cells, permanently diminishes visual fields, and gradually regresses the neovascularization. The use of

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anti-VEGF, such as bevazicumab and ranibizumab, has been reported to rapidly reduce the ischemic process, reverse neovascularization, and limit ocular tissue damage.[6,7] Treatment of NVG with combination PRP, intravitreal anti-VEGF, and trabeculectomy with mitomycin C (TMC) was reported to reduce intraoperative bleeding and improve surgical outcomes.[8-11] In 2011, the US Food and Drug Administration approved aflibercept (Eylea®; Regeneron, New York / Bayer, Berlin, Germany) for the treatment of neovascular age-related macular degeneration (AMD).[12] Aflibercept is a novel recombinant fusion protein that is made up of portions of vascular endothelial growth factor receptor 1 (VEGFR 1) and VEGFR 2 fused to the Fc portion of human immunoglobulin G1 and VEGF-receptor ligand binding elements. Aflibercept exhibits higher affinity for VEGF-A/-B compared to previously-known anti-VEGF molecules, and binds to all of the VEGF isoforms (VEGF-B and -C, placental growth factor-1/-2).[13] The aim of this study was to investigate the effects of intravitreal aflibercept (IVA) injection as an adjunctive treatment to TMC and PRP in patients with NVG.

MATERIALS AND METHODS

This prospective study included NVG patients who consecutively presented at the Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during November 2018 to October 2019. The study protocol was reviewed and approved by the Siriraj Institutional Review Board (SIRB) (certificate of approval number Si726/2018). Written informed consent was obtained from all study patients prior to participation. All patients received PRP and maximum tolerated IOP reduction, including systemic acetazolamide. Aflibercept was injected intravitreally (2 mg in 0.05 ml) through the pars plana in the operating theater within 1 week after the first session of PRP if NVI still persisted. TMC was carried out within 2 weeks after IVA in eyes with intraocular pressure (IOP) greater than 21 mmHg with maximal anti-glaucoma medications.

Surgical technique

All surgeries were performed by NK. The operated eye was draped and then a lid speculum was placed. Xylocaine hydrochloride 2% was injected subconjunctivally at the superonasal quadrant. A fornix-based conjunctival flap was created by disinsertion of the conjunctiva and Tenon's capsule at the 2-3 o'clock position. Oblique relaxing incisions at one side facilitated adequate scleral exposure. Diathermy was applied to control the hemostasis of the sclera. A no.15 surgical blade was used to create a partial thickness triangular scleral flap 3.5 (base) x 3.5 (height) mm. The cellulose sponge soaked with mitomycin C (MMC) 0.4 mg/ml was placed over the sclera under the Tenon's capsule and the conjunctiva for 2-3 minutes. The duration of MMC application was based upon the preoperative evaluation of potential risks for surgical failure, including the status of the conjunctiva over the operating area and the patient's age. Generally, a thicker Tenon's capsule and/or a younger age underwent a longer application of MMC. The MMC at the surgical area was then extensively washed out with balance saline solution (BSS).

A 20-gauge needle was used to performed paracentesis at the nasal or temporal clear cornea. The sclerotomy was done using a 15-degree blade. A Kelly Descemet's punch may be needed to widen the sclerotomy for an adequate size. Peripheral iridectomy was performed then the scleral flap was sutured with 10-0 nylon sutures at its apex and sides. The number and the location of sutures were customized for each patient to facilitate optimal aqueous outflow. The fornix-based conjunctival flap was repositioned to the limbus, tightly anchored with round needle 10-0 nylon sutures, and the relaxing incisions were closed with a continuous suture. The surgical area was tested for leaks before the injection of subconjunctival dexamethasone.

Postoperative procedure

A combination of topical 1% prednisolone and antibiotics were administered 4-6 times a day for 7 days. A topical eye drop containing a combination of antibiotics and dexamethasone was applied 4 times a day for the following 1 month, and then reduced to twice a day thereafter. Best corrected visual acuity (BCVA), IOP, number of anti-glaucoma medications, and the present of NVI were compared between pre- and post-IVA, and before and after TMC. The intraoperative and postoperative complications were also noted. The patients were scheduled for follow-up at 1 day, 1 week, and every 4-8 weeks. Each postoperative visit involved a full eye examination and Seidel test for the presence of bleb leak. Postoperative interventions such as laser suture lysis, 5-fluorouracil (5-FU) injection, bleb needling, or any other procedures were performed under surgeon (NK) consideration.

RESULTS

Five eyes from 5 patients with a mean age of 70.8 years were enrolled in this study during the November 2018 to August, 2019 study period. One patient was male and 4 patients were female. The underlying causes of NVG

included 2 eyes with PDR, 2 eyes with CRVO, and 1 eye with OIS. The eye with OIS underwent several sessions of PRP before participation. The partial regression of NVI had been observed. The mean IOP at presentation was 46.8±6.8 mmHg. After the first session of PRP in eyes without prior PRP, regression of NVI observed in one eye with CRVO, and the IOP was controlled with topical anti-glaucoma medications. The remaining 4 eyes received IVA due to the minimal regression and the persistence of NVI. The mean IOP among those 4 eyes was 37.9 mmHg before IVA, and 36.1 at 1 week after IVA. There was no observed complication related to IVA. Iris neovascularization was absolutely regressed within 1 week in all 4 eyes after IVA. Intraocular pressure was controlled in 1 eye with CRVO after PRP and IVA with maximal topical therapy. The remaining 3 eyes (2 PDR, 1 OIS) underwent TMC. The preoperative IOP of each patient was 34 (OIS), 54 (PDR), and 50 (PDR) mmHg (mean: 46 mmHg). Postoperatively, the IOPs decreased to 8, 8, and 4 mmHg (mean: 6.7 mmHg), respectively, at 3 months, and to 21, 10, and 6 mmHg (mean: 12.3 mmHg), respectively, at the last visit. Only the eye with OIS required postoperative medications. The mean follow-up among all five operated eyes was 10.7 months. No intraoperative or postoperative complication was noted. The BCVA was improved in 2 eyes, unchanged in 2 eyes, and decreased in 1 eye. There was no recurrence of NVI or significant symptoms in any of the 5 study eyes. Pre- and post-procedural characteristics compared among the 5 study eyes/patients are shown in (Table 1). Change in IOP over time from baseline compared among the 3 patients who underwent TMC is shown in (Fig 1).

DISCUSSION

The primary goal of management of NVG associated with ischemic retinopathy is to control IOP and the ischemic conditions of the retina. The treatments for NVG consist mainly of PRP, endolaser, or retinal cryotherapy to decrease oxygen consumption by ischemic retina.[4] PRP is a major procedure that involves ablating the retina to control retinal ischemia, which results in regression of neovascularization of both the anterior and posterior segments.[5] However, the regression of ocular neovascularization may not be rapid enough to limit further ocular tissue damage, such as the peripheral anterior synechiae, and NVG. Among the 5 cases in our series, regression of NVI occurred after PRP alone in one eye, and after combination PRP and IVA in one eye. IOP could be controlled in these 2 eyes with topical IOP lowering medications alone. This may be explained by the fact that prompt treatment in the early stage of the disease to eliminate the progression of ocular neovascularization can prevent further ocular complications. The effects of intraocular administration of anti-VEGFs in NVG included rapid regression of neovascularization, IOP reduction, and improved surgical outcome.[6,8-11] In several previous reports, the regression of NVI usually observed within 1 week after administration of anti-VEGF.[14,15] This effect may persist for several weeks (4 to 28 weeks). According to the half-life of anti-VEGFs and their transitory effects, multiple injections may be needed. The transient effect of anti-VEGF underscores the demand for a simultaneous treatment to eradicate the source of angiogenic factors, such as retinal photocoagulation. The combined treatment of intravitreal anti-VEGF and retinal ablation to control the retinal ischemic condition is theoretically more effective.[16] Anti-VEGF rapidly, but transiently reduces neovascularization in the early treatment stage, whereas PRP prolongedly controls ischemia of the retina over the long-term. In addition, intravitreal anti-VEGFs have demonstrated their benefit as an alternative treatment for NVG patients in whom retinal ablation could not be applied due to inadequate visualization. We considered administering anti-VEGF as soon as possible, especially in eyes in which the status of the ocular media contraindicates retinal treatment. Our group previously reported that application of PRP and anti-VEGF may be considered simultaneously during the same visit to expedite the regression of neovascularization.[11] Bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA, USA) is a recombinant humanized monoclonal antibody to VEGF. It is approved as an anti-angiogenic agent for the treatment of metastatic colorectal cancer in combination with chemotherapy.[17,18] Off-label use of intraocular injection of bevacizumab has been reported as a treatment for macular degeneration, PDR, retinal vascular occlusive disorder, and NVG.[6,7,19] Ranibizumab (Lucentis®; Genentech) is a recombinant humanized antibody antigen-binding fragment (Fab) that was approved for the treatment of neovascular AMD by the US Food and Drug Administration due to its ability to neutralize all active forms of VEGF-A.[20,21] Intravitreal bevacizumab (IVB) and intravitreal ranibizumab (IVR) have been reported as adjunctive treatment in NVG not only to reduce rubeosis iridis, but also to improve the surgical outcome of glaucoma surgeries.[8-11] Aflibercept is the most recently introduced anti-VEGF for the treatment of neovascular AMD.[22,23] According to our review of the literature, aflibercept has not yet been widely studied as a treatment for NVG. The additional effect of this anti-VEGF compared to the previous anti-VEGFs is that it has a longer half-life, and a higher binding affinity for

TABLE 1. Pre- and post-procedural characteristics compared among the 5 study eyes/patients.

Case number	1	2	3	4	5
Age (years)	80	73	78	54	69
Gender	Female	Female	Female	Male	Female
Lateralization	RE	LE	RE	LE	LE
Underlying condition	CRVO	CRVO	OIS	PDR	PDR
Initial BCVA (logMar)	1.0	1.6	1.0	2.0	2.0
Initial IOP (mmHg)	42	52	36	54	50
No. of medications	4	3	4	4	4
Lens	IOL	NS2+	IOL	IOL	IOL
Interventions	PRP	PRP/IVA	PRP/IVA/TMC	PRP/IVA/TMC	PRP/IVA/TMC
Days between PRP and IVA	-	1	272	4	Same day
BCVA at last visit (logMar)	1.0	1.2	1.0	0.3	3.0
IOP at last visit (mmHg)	13	12	10	8	1
Numbers of medications at last visit	4	3	3	0	0
Follow-up (months)	6.0	1.1	31.6	20.2	17.0

Abbreviations : RE, right eye; LE, left eye; CRVO, central retinal vein occlusion; OIS, ocular ischemic syndrome; PDR, proliferative diabetic retinopathy; BCVA, best-collected visual acuity; logMar, logarithm of the minimum angle of resolution; IOP, intraocular pressure; IOL, intraocular lens; NS2+, mild nuclear sclerosis cataract; PRP, panretinal photocoagulation; IVA, intravitreal aflibercept; TMC, trabeculectomy with mitomycin C

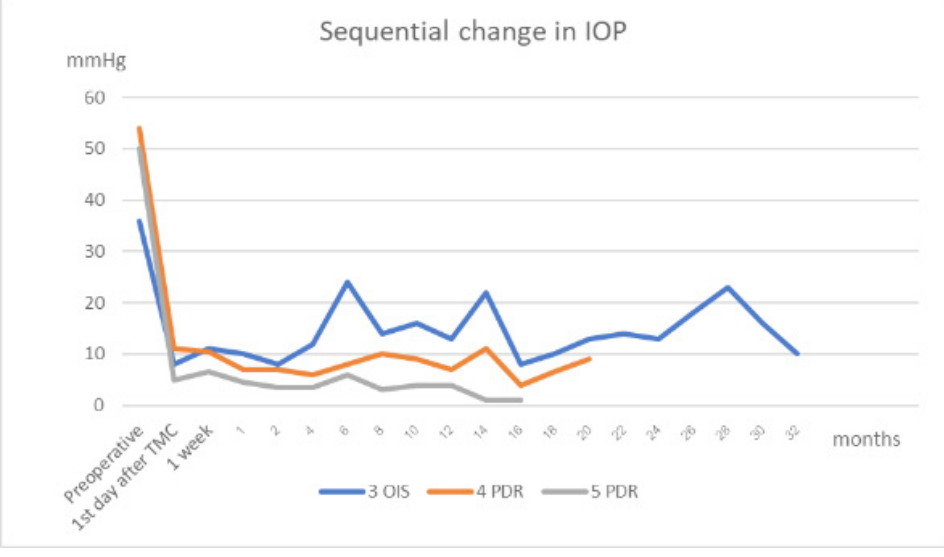

Fig 1. Change in intraocular pressure (IOP) over time from baseline compared among patient 3 (ocular ischemic syndrome [OIS], patient 4 (proliferative diabetic retinopathy [PDR], and patient 5 (PDR) (Abbreviations: mmHg, millimeters of mercury; TMC, trabeculectomy with mitomycin C).

VEGF-A. The use of aflibercept can, therefore, decrease the frequency of injections.[24] Often times, IOP may not be adequately controlled with anti-glaucoma medications alone in recalcitrant NVG despite the regression of ocular neovascularization. This may due to permanent damage to the aqueous drainage channel. When this happens, surgical intervention is required. Three eyes in our series subsequently underwent TMC due to uncontrolled IOP after PRP and IVA. Our study demonstrated that aflibercept injected intravitreally before TMC resulted in prompt regression of NVI, which resulted in reduced intraoperative bleeding and good postoperative IOP control. Moreover, there was no recurrence of NVI during the mean follow-up of 10.7 months, and no complication related to IVA and TMC was observed. The eye with OIS had localized filtration bleb resulted in occasional IOP spike compared to the eyes with PDR. The OIS eye underwent bleb needling with 5-FU injection 3 times. This difference in IOP control and the filtration bleb morphology may due to the nature of underlying disease. Trabeculectomy has long been the gold standard surgical treatment for glaucoma.[25,26] In NVG, the success of filtration surgery needs both the neovascularization control and wound healing modulation at the filtering site. Several studies have reported frequent intraoperative complications, subsequently poor surgical success of TMC in NVG, especially in eyes with persistent ocular neovascularization.[2,27] The management of NVG with a combination treatment of PRP, intravitreal anti-VEGF injection, and TMC has been reported as an alternative option.[8-11] In addition to its anti-VEGF property, anti-VEGF may provide an additional effect on the wound-healing modulation at the filtering area. Anti-VEGF influenced an inhibitory effect on fibroblast activity and wound healing response that may improve the surgical success of trabeculectomy in NVG.[28,29] Our findings have shown the potential of IVA as an adjunctive treatment in filtration surgery for NVG. In 3 patients who underwent TMC, IVA induced preoperative regression of NVI, which minimized intraoperative bleeding and postoperative inflammation, and this resulted in the enhanced success of TMC. There were no significant local or systemic side effects associated with IVA in our series. That said, the side effects and complications from the use of anti-VEGF include central retinal arterial occlusion, endophthalmitis, retinal detachment, increased intraocular pressure, conjunctival hemorrhage, and cataract.[30-33] Several studies reported short-term or long-term IOP elevation after intravitreal anti-VEGF injection.[34,35] There was no clinically significant change in IOP observed in our study. The surgical result was encouraging in terms of IOP control and visual outcome. At last visit, all eyes had IOP under 21 mmHg with or without topical IOP lowering medications, and had improved or preserved visual acuity. Moreover, recurrence of neovascularization was not detected during the follow-up period (range: 16-62 weeks). We hypothesized that in addition to reduced VEGF production from PRP and anti-VEGF injection, VEGF had a new drainage channel via trabeculectomy. The new VEGF drainage channel may explain the non-recurrence of neovascularization, the favorable surgical outcome, and the need for repeat anti-VEGF injection. However, owing to the short-term follow-up period in this study, the possibility of long-term recurrent neovascularization still exists. All eyes in our study demonstrated benefits from IVA and filtration at the last follow-up, including being symptom-free. Most of the eyes had retained visual acuity and had good IOP control.

Preoperative IVA combined with TMC appears to be a safe and effective management for IOP controlling in NVG. Although its effect is temporary, aflibercept may provide an adjunctive effect to PRP because of its swift and dramatic biologic effect. The application of IVA may also be considered before performing the glaucoma drainage device surgery. The long-term outcome of this technique is still undetermined. The repeated application of IVA may be needed to achieve a long-term effect.

Limitations and strengths

Despite the small sample size, the short-term study period, and the lack of a control group, this is the first study to report a series of NVG eyes who underwent TMC after IVA. Further study with a larger sample size, a longer follow-up period with a control group should be conducted to evaluate the safety profile and the long-term efficacy of this technique.

CONCLUSIONS

Preoperative intravitreal administration of aflibercept was shown to be a potentially effective additional treatment to PRP and TMC in patients with NVG. Moreover, IVA was found to be safe and effective for controlling IOP and preserving visual acuity. The rapid regression of NVI minimized intraoperative complications during trabeculectomy, and may have improved the short-term surgical result after TMC. All eyes in this series remained symptom-free, preserved their visual acuity, and had good IOP control. A further study with a long-term follow-up in a larger study population needs to be conducted to evaluate the long-term outcomes.

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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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A Study of Early Parenteral Nutritional Support and Factors Associated with Clinical Outcomes in Major Pediatric Burn Patients

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ABSTRACT

Objectives: to study parenteral nutrition within 7 days post admission (early PN) and factors affecting clinical outcomes in major pediatric burn patients.

Materials and Methods: A retrospective study was conducted regarding pediatric burn patients who had over 15% of their total body surface area (TBSA) with second- or third-degree burns. All the patients were classified as requiring early PN support or non-early PN support.

Results: 124 major pediatric burns were reviewed. Eighty-six patients (65.2%) were male, and their median age was three years (0.3-15 years). Early PN showed no association with LOS (p=0.480) or a 30-day mortality (p=0.529). The children's age, wound infections, and abdominal distension were the independent associated factors of LOS (p=0.025, 0.001, and 0.003 respectively). Pneumonia and urinary tract infection were independent factors associated with 30-day mortality (p=0.025 and N/A, respectively).

Conclusions: Early PN in acute pediatric burns was not associated with LOS or 30-day mortality. It can be considered as options of nutritional support in acute, major pediatric burns. Effective management of wound infections and abdominal distension may reduce LOS.

Keywords: Early PN; EN; major pediatric burn; nutritional support; treatment outcomes (Siriraj Med J 2022; 74: 34-39)

INTRODUCTION

Optimal nutritional support is considered one of the key treatments of major pediatric burns, since malnutrition and inadequate nutrient delivery have been associated with bad clinical outcomes.[1,2] Moreover, children have limited macronutrient stores and relatively higher energy requirements than adults who are admitted into the intensive care unit; these factors can lead to substantial caloric and macronutrient deficits.[3,4] Feeding is thought to attenuate the metabolic stress response, prevent oxidative cellular injury, and modulate immune responses. This has led to a shift from nutritional support as adjunctive care to an actual therapy in cases of major pediatric burns. The enteral route is preferred for providing nutrition.[5] However, major pediatric burn patients are often too ill to be fed normally by mouth, and nasogastric or nasoduodenal tube-feeding is often not well- tolerated due to gastric dysmotility or ileus from acute, severe

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stress. Interruption of enteral feeding also frequently occurs for various reasons, such as medical or surgical contraindications, or radiologic, bedside, or surgical procedures.[6] Severe burn injury causes a persistent and prolonged hypermetabolic state and increased catabolism, which result in increased muscle-wasting and cachexia. The metabolic rates of burn patients can surpass twice the norm, and failure to supply these energy requirements causes impaired wound healing, organ dysfunction, and susceptibility to infection. Accurate assessment and provision of nutritional needs is essential for these patients.[7] Therefore, parenteral nutrition (PN) is often initiated to supplement the insufficient enteral intake. Published guidelines on the timing and thresholds of initiation, as well as the composition and doses of supplemental PN, vary widely.[8-10] Moreover, concerns about overfeeding have led to even more uncertainty. A recent survey showed significant differences in nutritional practices in PICUs worldwide, in terms of macronutrient goals, estimation of energy requirements, timing of nutrient delivery, and thresholds for starting supplemental PN. The latest guideline suggests late initiation of PN support (after 7 days) in PICU due to the result of better treatment outcome and lower PN related complication which studied mainly in non-burn population.[11,12]

Objective

To study parenteral nutrition within 7 days post admission (early PN) and any factors with clinical outcomes in major pediatric burn patients

MATERIALS AND METHODS

Study design and population

This was a retrospective study involving the review of pediatric burns charts for patients admitted to the Burn Unit at Siriraj Hospital in Bangkok from January, 1999 to September, 2019. This study was conducted under the Siriraj Institutional Review Board's (SIRB) approval (COA no. Si 834/2019). Patients age ≤ 15 years who were injured ≥ 15% of their total body surface area (TBSA) with second- or third-degree burns were enrolled. All patients were given enteral nutrition (EN) as soon as they had a stable hemodynamic within 24 hours after admission with calculated goal of daily caloric requirement by using Curreri junior formula. The patients received all types of parenteral nutrition within seven days after admission to achieve calculated goal of daily caloric requirement; those children were classified as being in the early PN group. The others were in the non-early PN group. Patients with an incomplete medical record were excluded from analysis.

Statistic analysis

The data was analyzed by SPSS, version 18.0. The demographic data was presented in frequency, percentage, and median. The length of hospital stay (LOS) and 30-day mortality between the early PN and non-early PN groups were determined using the odds ratio (OR). The independent factors associated with the LOS were analyzed by using the linear regression coefficient, and adjusted factors were analyzed using the backward multiple linear regression model. Fisher's exact test and the Mann-Whitney U test statistical analysis were performed with a univariable analysis of factors associated with 30-day mortality. The independent factors associated with 30-day mortality were reported in OR using binary logistic regression analysis, and the adjusted factors by using the backward multiple linear regression model. A P value of 0.05 was considered statistically significant.

RESULTS

124 patients' data were analyzed. Twenty-two patients (17.7%) were identified in the early PN group, and 102 patients (82.3%) were in the non-early PN group. The medium age was three years old. The degree of burns were as follows: second- degree burns: 120 (96.8%) patients; third-degree burns: 1 (0.8%) patient; and mixed second- and third-degree burns: 3 (2.4%) patients. The median percentage of those with whose total body surface area was burned (%TBSA) was 30 (15, 90). The type of burns consisted of scald burns (87, or 70.2%); flame burns (36, or 29.0%); and other (1, or 0.8%). (Table 1)

The comparisons of 30-day mortality and the LOS of patients who were early PN (n=22) vs. non-early PN (n=102) are reported in Table 2. There was no statistically significant difference in the 30-day mortality [1 (4.5%) vs 4 (3.9%), P=1.000] and median LOS [42 (12, 70) vs. 49 (2, 143), P=0.086] between the groups. The early PN group had a slightly shorter LOS.

A multivariate logistic regression with forward selection revealed that early PN [-3.564 (-13.516, 6.388) (p=0.480)] had no statistically significant effect on the LOS, while wound infection [13.567 (5.734, 21.399) (p=0.001)], abdominal distension [13.460 (4.528, - 22.391) (p=0.003)], and age <1 year [0.817 (0.104, 1.530) (p=0.025)] significantly affected the LOS. (Table 3)

The overall 30-day mortality of pediatric burns in our center was 17.7 %. A univariate analysis with Fisher's exact test or the Mann-Whitney U test forward selection showed that the early PN group had no statistically significant effect on 30-day hospital mortality, in contrast to the non-early PN group [1 (20.0%) vs. 21 (17.6%) (p=1.000)]. However, a few factors did have a statistically

TABLE 1. Characteristic of major pediatric burn patients.

Characteristics	N = 124
Sex, male, n (%)	82 (66.1%)
Median age (min, max) (years)	3 (0.3, 15)
Groups, n (%)	
Early PN	22 (17.7%)
Non-early PN	102 (82.3%)
Degree of burn, n (%)	
Second-degree	120 (96.8%)
Third-degree	1 (0.8%)
Second- & third-degree	3 (2.4%)
Median % TBSA (min, max)	30 (15, 90)
Burn type, n (%)	
Scald burn	87 (70.2%)
Flame burn	36 (29.0%)
Other	1 (0.8%)

Abbreviations: PN = parenteral nutrition, % TBSA = % Total body surface area

TABLE 2. Length of hospital stay (LOS) and 30-day mortality in the early PN vs. non-early PN groups of major pediatric burn patients.

Outcome	Groups		*p*-value
	Early PN (n=22)	Non-early PN (n=102)	
LOS (days)			
Median (min, max)	42 (12, 70)	49 (2, 143)	0.086
30-day mortality, n (%)	1 (4.5%)	4 (3.9%)	1.000

Abbreviations: PN = parenteral nutrition

TABLE 3. Independent factors associated with Length of hospital stay (LOS).

Factors	Unadjusted coefficient (95%CI)	*p*-value	Adjusted coefficient (95%CI)	*p*-value
Early PN	-8.778(-19.077, 1.521)	0.094	-3.564 (-13.516, 6.388)	0.480
Age < 1 year	1.248(0.525, 1.971)	0.001	0.817 (0.104, 1.530)	0.025
Wound infection	16.652(8.887, 24.417)	<0.001	13.567 (5.734, 21.399)	0.001
Abdominal distension	16.208(6.571, 25.846)	0.001	13.460(4.528, - 22.391)	0.003

Abbreviations: PN = parenteral nutrition

significant effect on 30-day mortality: the duration of ventilator use [8 (1, 34) vs. 0 (0, 55) (p<0.001)]; pneumonia [5 (100%) vs. 12 (10.1%) (p<0.001)]; sepsis [5 (100%) vs. 16 (13.4%) (p<0.001)]; urinary tract infection [4 (80.0%) vs. 8 (6.7%) (p<0.001)]; and percentage of burn area [70 (43,90) vs. 28 (15,80) (p=0.002)]. (Table 4)

A multivariate logistic regression with forward selection revealed that the early PN group [0.356 (0.014, 8.901) (p=0.529)] had no statistically significant effect on 30-day mortality, but urinary tract infection (UTI) [34.750 (1.641-735.686) (p=0.023)] and pneumonia did. (Table 5) Pneumonia had a statistically significant effect on 30-day mortality, but the number calculator reported this as non-shown (N/A) because the program could not identify this data.

DISCUSSION

Major pediatric burns in our center found more common in preschool age. They got injury from hot water and liquid more than flame. These findings are reported the same around the world except in our center male victims are more common.[13,14] They may cause from normal pattern of preschool children development to explore new thing with touching and gripping anything without fear of danger due to lack of experience. Most of small children love to play with water, so kettle, pot and pan are thought to be one of the favorite toys for them. This awareness should be raised in parents for injury prevention purpose.

Feeding intolerance is one of the common problems in critically ill trauma patients. Instability of hemodynamic,

TABLE 4. Univariable analysis of factors associated with 30-day mortality in major pediatric burn patients.

Factor	30-day mortality		*p*-value[a]
	Yes (n=5)	No (n=119)	
Early PN, n (%)	1 (20.0%)	21 (17.6%)	1.000
Duration of ventilator use			
Median (min, max)	8 (1, 34)	0 (0, 55)	<0.001
Pneumonia	5 (100%)	12 (10.1%)	<0.001
Sepsis	5 (100%)	16 (13.4%)	<0.001
UTI	4 (80.0%)	8 (6.7%)	<0.001
% TBSA Burn			
Median (min, max)	70 (43,90)	28 (15,80)	0.002

[a]Fisher's exact test or the Mann-Whitney U test
Abbreviations: PN = parenteral nutrition, %TBSA = % Total body surface area

TABLE 5. Independent factors associated with 30-day mortality.

Factors	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*- value
Early PN	1.167(0.124, 10.974)	0.893	0.356(0.014, 8.901)	0.529
UTI	55.500(5.532, 556.775)	0.001	34.750(1.641-735.686)	0.023
Pneumonia	N/A	N/A	N/A	N/A

Abbreviations: PN = parenteral nutrition, UTI = urinary tract infection

ileus from massive fluid and electrolytes disturbance from post resuscitation aftermath of exsanguinating bleeding combining with post injury response by inflammatory cytokines flush worsen intestinal continuity and mobility. Major burn is considered as severe injury that create systematic change in all system of body. Abrupt change of extravascular electrostatic pressure and intravascular oncotic pressure result in rapid decrease of intravascular volume which partly pathophysiology of burn shock. Adequate fluid resuscitation lessen outcome of end organ failure like kidney injury and dead.[15] Intestinal dysfunction of acute major burns can cause from non-burn tissue edema effect from post burn systemic response, electrolytes imbalance and hypoalbuminemia. Major burns standard nutrition recommendation of very early enteral nutrition initiation and caloric goal achievement in a week[16-18] might not success in every case.[19]

PN is one of the options to supply calories to reach daily goal in enteral intolerance and offer some specific nutrients support. For energy providing purpose, it can be used as partial caloric supplement or main supply source of daily energy requirement. In adult critically ill patient including adult major burns, late initiation of PN after first 7 days is recommended in low nutritional risk patient that did not achieve caloric goal via enteral route or contraindicated of enteral nutrition (EN). Early PN is encouraged only when EN is not feasible in high nutritional risk or severely malnourished patients.[12,16,17] Standard nutritional guideline for critically ill children patient also recommend the same, but specialized population like major pediatric burns cannot apply into this guideline.[5] This exception cause from the lack of evidence in the treatment outcome of supplement PN support in this group of patient. The threshold of PN initiation in pediatric is lower (1-3 days in infants and 4-5 days in older children) than adult due to low energy reserve and growth and development effect.[20] The current recommendation based on the evidence that most studies reported no significant improving outcome, but trend to increase complication from PN such as infection like catheter related blood steam infection (CRBSI).[21-23]

PN role in major adult burn is limited that was reported increased mortality without modulate inflammatory response.[24,25] The outcomes of comparing the early and late initiation of PN in major pediatric burn patients are lack of evidence biggest trial like a multicenter PEPaNIC RCT (Early versus Late Parenteral Nutrition in the Pediatric ICU) conducted in 2016 enrolled only 10 cases of burn. The study conclusion from PEPaNIC RCT is not good to apply in major pediatric burn population. No previous studies in Thailand had reported the compared outcomes of early PN support in major pediatric burn patients. Our study found no statistically significant difference in LOS and 30-day mortality between the groups of major pediatric burn patients with and without early PN support. Our findings support the less restricted use of early PN in major pediatric burn patients who, according to the recent guidelines, qualify as critically ill pediatric patients were preserved and recommended PN only for high nutrition risk patients and their trend in late initiation more than early.[11,26]

We found that the main factors associated with LOS are age less than one year, wound infection, and abdominal distension. Burn injury in infants is more complicated to treat than the injury in the same severity in older child patient. Abdominal distension can lead to feeding problem that can prolong disease natural history and LOS. The duration of ventilator use, and/or the presence of pneumonia, sepsis, and/or urinary tract infection, and the percentage of burned skin affects 30-day mortality. Overall, the 30-day mortality rate in our center was 17.7%, compared with 21.3%[27] and 31.3%[28] from other studies.

There are a few limitations in this study. First, as this is a retrospective, observational, single-center study, our findings may not be generalizable to other institutions or ethnic populations. The investigators relied on available documentation to determine the number of days of poor nutrient intake prior to patients' PN initiation. Also, a prior power-analysis calculation was not conducted, and the small number of in-hospital deaths may have affected the outcomes analysis.

CONCLUSION

Early parenteral nutritional support for major pediatric burn patients had no effect on their length of hospital stay or 30-day mortality rate. The patients' age, wound infection, and abdominal distension were independent factors associated with their length of hospital stay. The duration of ventilator use, along with pneumonia, sepsis, urinary tract infection, and the percentage of burned skin were independent factors associated with 30-day mortality.

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Conflicts of interest: It is hereby declared that there are no conflicts of interest.

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Factors Predicting Survival in Ruptured Hepatocellular Carcinoma Treated with Surgical Resection

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ABSTRACT

Objective: Today, ruptured hepatocellular carcinoma (HCC) is a less frequently encountered problem globally due to availability of cancer surveillance protocols for the high-risk population. However, in Thailand, a number of patients do not enroll in screening programs, leading to high rates of ruptured complications. In fit-for-surgery and clinically stable patients, hepatectomy means long-term survival. This study aimed to identify predictive factors of survival in resected patients.

Materials and Methods: A retrospective review of patients with ruptured HCC who underwent liver resection between January 2013 and December 2019 at Siriraj Hospital was performed. The clinical data and outcomes of patients was analyzed.

Results: A total of forty-two patients with ruptured HCC underwent resection or 9.8% of all operable HCC cases. There were 6 patients (14.3%) who suffered from postoperative liver failure and one patient (2.4%) died within 30 days. Overall survival (OS) and recurrence-free survival (RFS) were 90%, 64%, 52% and 42.5%, 24%, 16% at 1, 3, and 5 years, respectively. The factors affecting OS were tumor size ≥10 cm, vascular invasion, and positive resection margin.

Conclusion: Surgical resection in ruptured HCC provides long-term survival. Predicting factors affecting overall survival were large tumor size, vascular invasion, and positive resection margin. Patient selection is a key for better patient's outcomes.

Keywords: Ruptured hepatocellular carcinoma; surgical resection; risk factors; survival (Siriraj Med J 2022; 74: 40-47)

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common malignancy of the liver and the sixth most common worldwide.[1] In Thailand, HCC is the most common malignancy in males and second-most malignancy in females.[1] Although most HCC patients are asymptomatic, some patients suffer with intra-abdominal bleeding due to a ruptured tumor. The incidence rate of ruptured HCC varies among countries, however, it is more common in Asia than in the West where the rate fluctuates between 3% to 26%.[2] In Thailand, the incidence rate is approximately 12.4%.[3] The outcomes of patients with ruptured HCC

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varies widely depending on the treatment and other factors such as performance status, vital signs on arrival, amount of intraperitoneal hemorrhage, comorbidities and background liver parenchyma disease. Moreover, outcomes of liver resection are significantly better than other treatments with a mortality rate of 4.4% compared to 85-100%, respectively.[4,5] In some reports, patients with ruptured HCC who had surgical resection had similar overall survival (OS) compared to non-ruptured HCC patients.[6-9]

In this study, our aim was to identify predictive factors that affect survival rate in ruptured HCC patients undergoing surgical resection.

MATERIALS AND METHODS

This retrospective study was performed in patients diagnosed with ruptured HCC who had liver resection at Siriraj Hospital between January 2013 and December 2019. The patients were diagnosed with ruptured HCC through preoperative imaging such as ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI), or by the intraoperative finding of hemoperitoneum. The decision to perform a surgery depended on the patient's condition and the attending surgeon. While major hepatectomy was defined as resection of 3 Couinaud's segments or more, minor hepatectomy was defined as resection of less than 3 Couinaud's segments. After a successful liver resection, patients had to follow-up with postoperative imaging using either CT or MRI every 3-6 months to evaluate the surgical outcome and to detect the recurrence of HCC. The definition of HCC recurrence is the appearance of hypervascular patterns in a CT or MRI scan with early enhancement in the arterial phase and rapid washout during the porto-venous phase. The patients who were diagnosed with recurrence were evaluated for the possibility of curative treatments such as resection or ablation. Trans-arterial chemoembolization (TACE), trans-arterial radioembolization (TARE) or other systemic therapies were offered if definitive treatments were not possible.

The outcomes we focused on in this study included overall survival (OS) and disease-free survival (DFS). While OS was defined as the length of time from date of operation to the date of death or last follow-up DFS was defined as the time from the date of operation to the date of confirmed HCC recurrence. An extrahepatic recurrence or peritoneal seeding tumor was confirmed by imaging or pathological reports.

The characteristics of patients with ruptured HCC were analyzed to determine related factors influencing outcomes. This study was approved by the human research committee of Faculty of Medicine Siriraj Hospital, Mahidol University.

Variable data was expressed as mean ± standard deviation (SD) and number (percentage). The OS and DFS values were calculated using the Kaplan-Meier survival analysis. Meanwhile, the univariable and forward stepwise multivariable Cox regression analysis were performed to investigate predictors and all the data was analyzed using SPSS software, version 17.0 [IBM, Illinois].

RESULTS

Between January 2013 and December 2019, a total of 460 patients with HCC underwent surgical resection at Siriraj Hospital. Of the total, 45 patients (9.8%) were categorized as those presenting clinical signs of HCC. However, three patients were excluded from the study due to the incomplete recording of data. Therefore, a total of 42 patients were included in this analysis. Most of patients were males (78.6%) and the average age of patients was 59.5. Fourteen patients (33.3%) showed signs of hypovolemic shock and of this total, 10 (71.4%) required emergency trans-arterial embolization (TAE). Regardless, 6 patients from the non-hypovolemic shock group also underwent TAE due to ongoing bleeding. The average tumor size was 7.9 cm and remarkably, almost all the patients had liver function status defined as Child-Pugh class A (97.6%). The patient demographic data is described in Table 1. Major liver resections were performed in 11 patients (26.2%) and median intraoperative blood loss was 600 mL (range 80 - 5,000 mL). Twenty-six patients (61.9%) had vascular invasion; 7 patients (16.7%) had a positive resection margin. The intraoperative and pathologic outcomes are described in Table 2.

Regarding the postoperative period, one patient (2.4%) died within 30 days due to post-hepatectomy liver failure. The postoperative morbidity rate was 28.6% (12/42) and the median length of stay 6 days (range 4 - 58 days). The median overall survival (OS) was 61.1 months and median follow-up time 22 months. The overall survival was 90%, 64%, 52% at 1, 3, and 5 years respectively (Fig 1A). Meanwhile, recurrence-free survival (RFS) was 42.5%, 24%, 16% at 1, 3, and 5 years respectively (Fig 1B). Twenty-nine patients (69%) had a recurrence of HCC at the median time of 7.5 months. The type of recurrence was intrahepatic recurrence (17/29, 58.6%), intrahepatic and extrahepatic recurrence (10/29, 34.5%), and extrahepatic recurrence (2/42, 6.9%). Seven patients (16.7%) suffered from peritoneal recurrence.

Risk factors that affected OS and RFS were analyzed with a univariable and multivariable Cox regression analysis. At least eight factors (univariable Cox regression analysis) affected OS: the preoperative factors were - tumor size ≥10 cm, macrovascular invasion, hematocrit <30%, time-to-surgery ≤7 days, intraoperative factors - blood

TABLE 1. Demographic data.

Characteristics	n = 42
Patient's characteristics	59.5 ± 13.1
Age (years)	
Gender	
Male	33 (78.6%)
Female	9 (21.4%)
Tumor size (cm)	7.9 ± 3.2
Macrovascular invasion	4 (9.5%)
No. of tumor	
1	37 (88.1%)
2	3 (7.1%)
3	2 (4.8%)
Hypovolemic shock	14 (33.3%)
Preoperative embolization	16 (38.1%)
MELD score	8 (6-14)
Child-Pugh	
grade A	41 (97.6%)
grade B	1 (2.4%)
Time to surgery	
≤7 days	12 (28.6%)
>7 days	30 (71.4%)
Preoperative Laboratory results:	
Total bilirubin (mg/dL)	0.70 ± 0.37
Albumin (mg/dL)	3.88 ± 0.50
Prothrombin time	12.9 ± 1.0
Creatinine	0.93 ± 0.20
Hematocrit (%)	35.6 ± 7.1
AFP	6,522 ± 20,855

Data was presented as mean ± SD, median (range) or number (percentage)

TABLE 2. Intraoperative and pathologic result.

Outcomes	n = 42
Intra-operative outcome	
Type of hepatectomy	
Major	11 (26.2%)
Minor	31 (73.8%)
Operative time (min)	150 (75 – 660)
Intra-operative blood loss (mL)	600 (80 – 5000)
PRC transfusion	18 (42.9%)
Pathological results	
Differentiation	
Moderately	33 (78.6%)
Poor	4 (9.5%)
Extensive necrosis (after embolization)	5 (11.9%)
Vascular invasion	26 (61.9%)
Satellite lesion	9 (21.4%)
Positive margin	7 (16.7%)

Major hepatectomy = liver resection ≥3 Couinaud's segments
Data was presented as mean ± SD, median (range) or number (percentage)

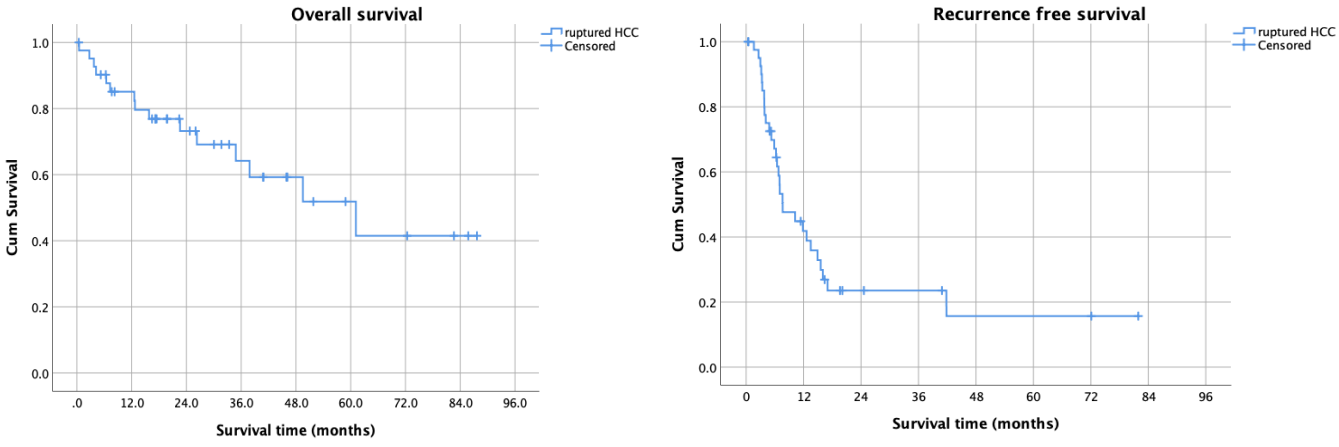

Fig 1. **A**- Kaplan-Meier estimation of overall survival of all patients. 5-year OS was 52%.;
B- Kaplan-Meier estimation of recurrence-free survival of all patients. 5-year DFS was 16%.

loss > 1,000 mL, blood transfusion, and pathological factors - vascular invasion, positive resection margin (Table 3). Using a multivariable analysis, only three factors i.e. tumor size ≥10 cm, vascular invasion, and positive resection margin were documented for OS. There were 3 pathological factors affecting RFS; i.e. vascular invasion, positive resection margin, and satellite lesion (Table 4). Among these factors, tumor size ≥10 cm, time-to-surgery ≤7 days, vascular invasion, positive resection margin were found to be major factors leading to worst OS outcome (Fig 2).

DISCUSSION

Spontaneous rupturing of HCC is a catastrophic complication with a high mortality rate. Today, patients with HCC receive treatment early due to worldwide implementation of surveillance programs in high-risk patients, resulting in less people presenting ruptured HCC. However, the incidence rate of ruptured HCC in Asian countries is higher than in Western countries. In our institution, Siriraj Hospital, the ruptured HCC rate was 9.8% of all resectable HCC cases, which is comparable to reports from other Asian countries such as Hong Kong (9%)[10], Taiwan (26%)[11], and Japan (2.3%).[12]

Ruptured HCC usually shows aggressive tumor biology. According to our data, there was high rate of vascular invasion (61.9%) and 9 patients (21.4%) had a large tumor of over 10 cm. In this study, patients with tumor size ≥10 cm, vascular invasion, and positive tumor margin had significant shorter overall survival rate.

This study provided results of long-term survival with low morbidity in select surgical candidates. However, almost all operable cases had a well-preserved liver function (CTP-A, 97.6%) and most operations were partial hepatectomies (73.8%). The strategy of using non-urgent surgical resections despite tumor bleeding was stopped, either spontaneously or by embolization. The surgery was performed after patient condition was stabilized. Surgery performed within 7 days significantly affected the overall survival. Moreover, post-hepatectomy liver failure was encountered only in 6 patients (14.3%) with only one case of mortality (2.4%). The 5-year overall survival was 52%, which is better than results in several other studies[4,6,7] and comparable with some large series.[8,13] This study strongly demonstrates that surgical resection plays a role in the treatment of ruptured HCC patients including those at the advanced tumor stage.

Peritoneal recurrence is uncommon in non-ruptured HCC when compared to the high rates of ruptured HCC. Peritoneal recurrence was found only in 16.7% of patients in this study and this was comparable to previous studies which reported a rate between 11 - 40% with median time to recurrence being 6 - 11 months.[14-18] Ruptured HCC and time-to-surgery were the two most common deciding factors for peritoneal recurrence, however, there is some supportive and contradictive evidence regarding this.[14,15,19,20]

Most ruptured HCC patients usually presented poor liver function and were excluded from liver resection. Therefore, the number of ruptured HCC patients who could be candidates for liver resection was limited. Nevertheless, a further prospective study with a large number of ruptured HCC patients, including a multicenter study should be carried out to clarify additional predictive factors of ruptured HCC patients treated with surgical resection.

TABLE 3. factors affecting overall survival of ruptured HCC undergoing resection by univariable and multivariable Cox regression analysis.

Factors	Univariable analysis			Multivariable analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Pre-operative factors						
Age: ≥60 years	0.664	0.235 – 1.879	0.44			
Pre-operative hypotension	0.658	0.196 – 2.208	0.50			
Pre-operative embolization	0.675	0.230 – 1.979	0.47			
Tumor size ≥10 cm	**3.987**	**1.234 – 12.88**	**0.02**	**5.487**	**1.387 – 21.72**	**0.02**
Macrovascular invasion	**5.621**	**1.442 – 21.92**	**0.01**			
Multiple tumor	1.856	0.514 – 6.700	0.35			
MELD score >8	1.892	0.662 – 5.406	0.23			
Time-to-surgery: ≤7 days	**4.063**	**1.398 – 11.81**	**0.01**			
Laboratory results:						
Total bilirubin >1.0 mg/dL	1.648	0.548 – 4.960	0.37			
Albumin <3.5 mg/dL	1.800	0.568 – 5.708	0.32			
Hematocrit <30 %	**4.011**	**1.193 – 13.481**	**0.03**			
Prothrombin time >13 second	1.237	0.438 – 3.494	0.67			
AFP >200 IU/mL	1.930	0.644 – 5.778	0.24			
Intra-operative factors						
Major hepatectomy	2.272	0.656 – 7.871	0.20			
Operative time: ≥180 min	1.436	0.464 – 4.450	0.53			
Blood loss >1000 mL	**3.803**	**1.347 – 10.74**	**0.01**			
Blood transfusion	**4.013**	**1.355 – 11.89**	**0.01**			
Pathological factors						
Vascular invasion	**7.068**	**1.581 – 31.60**	**0.01**	**6.165**	**1.195 – 31.80**	**0.03**
Positive resection margin	**4.881**	**1.204 – 19.79**	**0.03**	**9.663**	**1.181 – 79.06**	**0.03**
Satellite lesion	1.297	0.356 – 4.724	0.69			

TABLE 4. factors affecting recurrence of ruptured HCC undergoing resection by univariable and multivariable Cox regression analysis.

Factors	Univariable analysis			Multivariable analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Pre-operative factors						
Age: ≥60 years	0.746	0.357 – 1.558	0.44			
Pre-operative hypotension	0.543	0.218 – 1.349	0.19			
Pre-operative embolization	0.697	0.327 – 1.487	0.35			
Tumor size ≥10 cm	0.927	0.370 – 2.320	0.87			
Macrovascular invasion	1.125	0.337 – 3.759	0.85			
Multiple tumor	1.989	0.672 – 5.884	0.21			
MELD score >8	1.877	0.825 – 4.273	0.13			
Time-to-surgery: ≤7 days	1.642	0.755 – 3.571	0.21			
Laboratory results:						
Total bilirubin >1.0 mg/dL	1.103	0.441 – 2.756	0.83			
Albumin <3.5 mg/dL	1.064	0.397 – 2.851	0.90			
Hematocrit <30 %	1.430	0.541 – 3.784	0.47			
Prothrombin time >13 second	1.061	0.484 – 2.324	0.88			
AFP >200 IU/mL	1.151	0.500 – 2.652	0.74			
Intra-operative factors						
Major hepatectomy	0.876	0.354 – 2.163	0.77			
Operative time: ≥180 min	0.653	0.275 – 1.551	0.33			
Blood loss >1000 mL	0.644	0.271 – 1.532	0.32			
Blood transfusion	0.773	0.361 – 1.653	0.51			
Pathological factors						
Vascular invasion	**2.295**	**1.044 – 5.049**	**0.04**	**2.590**	**1.153 – 5.819**	**0.02**
Positive resection margin	**5.174**	**1.838 – 14.56**	**<0.01**	**5.753**	**1.835 – 18.04**	**<0.01**
Satellite lesion	**3.051**	**1.317 – 7.070**	**<0.01**	**3.052**	**1.273 – 7.318**	**0.01**

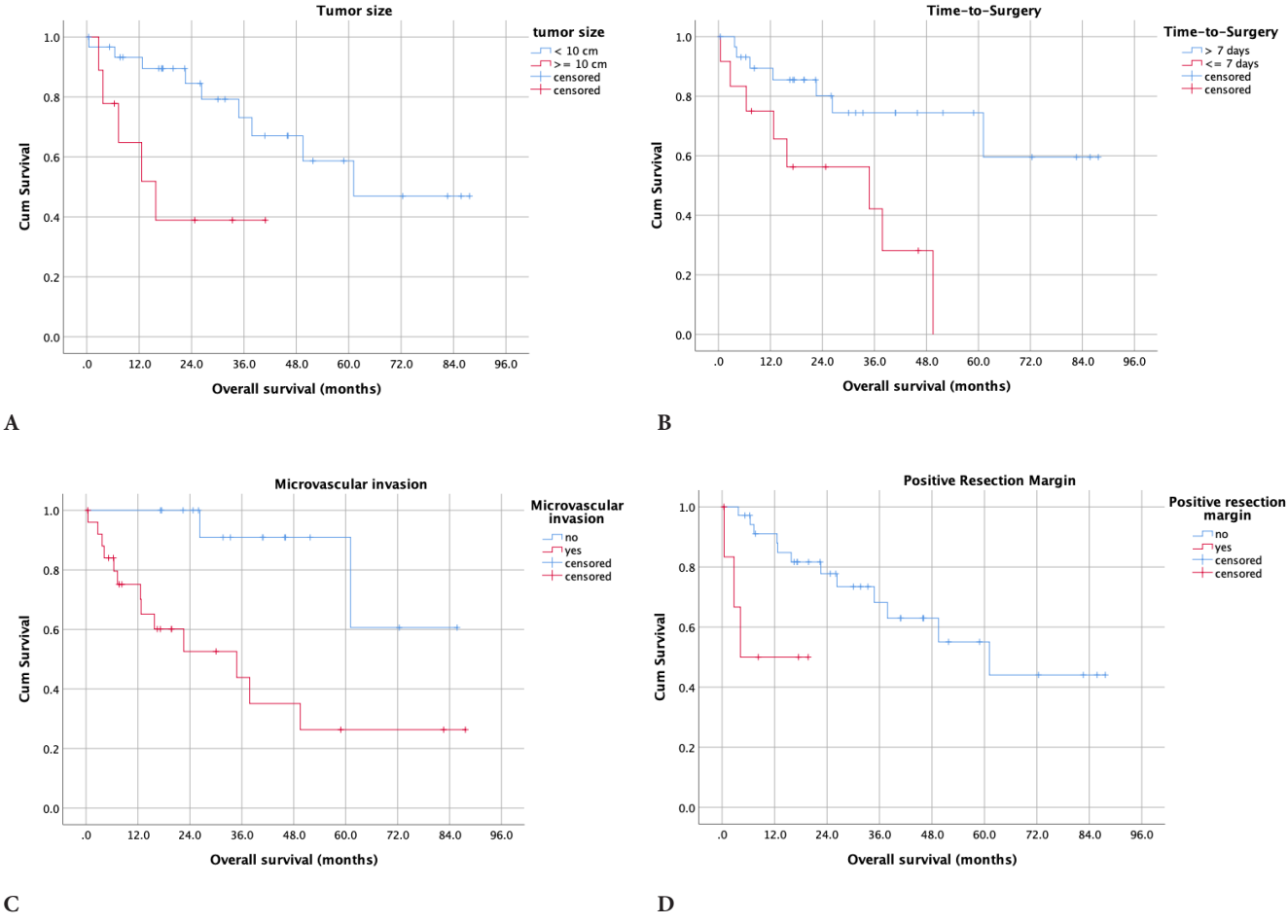

Fig 2. Kaplan-Meier estimation of overall survival of patients. **A:** Patients with Tumor size ≥ 10 cm had significantly poor OS (median OS 61.1 months [size <10 cm] vs 15.8 months [size ≥10 cm], p = 0.01); **B:** Time-to-Surgery ≤7 days had significantly poor OS (median OS 34.8 months [≤7 days] vs Not reach [>7 days], p <0.01); **C:** HCC with microvascular invasion had significantly poor OS (median OS 34.8 months [with vascular invasion] vs not reach [without vascular invasion], p <0.01); **D:** Patients with positive resection margin had significantly poor OS (median OS 61.1 months [free resection margin] vs 4.2 months [positive resection margin], p = 0.01)

CONCLUSIONS

Surgical resection in ruptured HCC provides long-term survival. Predicting factors affecting overall survival were large tumor size, vascular invasion, and positive resection margin. Patient selection is a key for better patient's outcomes.

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Prevalence and Factors Associated with the Loss of PTEN Expression in Patients with Lung Cancer

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ABSTRACT

Objectives: Phosphatase and tensin homolog (PTEN) is a major tumor suppressor gene and is involved in cell survival control. PTEN loss of expression (PTEN-) is associated with a poor outcome. Our study investigated the prevalence of *PTEN*- in terms of its characteristics and disease prognosis for lung cancer patients.

Materials and Methods: In total, 167 tissue blocks from lung cancer patients at Chareonkrung Pracharak Hospital between January 2010 and December 2020 were studied through immunohistochemistry staining (IHC) for PTEN expression. The clinicopathological factors, IHC features, and epidermal growth factor receptor (EGFR) status were analyzed in association with PTEN- in term of prognosis and the overall survival (OS).

Result: Adenocarcinoma was the major subtype (85.6%) and most patients (90.6%) were diagnosed at stage IV of lung cancer. The prevalence of PTEN- was 66.5%. A location at the left lower lobe (LLL) location and the absence of tumor-infiltrating lymphocytes (TILs) were significantly associated with PTEN- (p=0.039, p=0.046), while the smoking was likely correlated but not statistically significant (p=0.09). The median OS for PTEN- was not significantly different from PTEN+ (8.88 vs 7.20 months, p=0.38). However, smoking, Eastern cooperative oncology group (ECOG) status and primary symptoms were significantly associated with poorer OS.

Conclusion: The prevalence of PTEN- was higher in our studies. Absent TILs and a LLL location were independent factors associated with PTEN-. However, a right upper lobe (RUL) location with PTEN- tended to have a poor prognosis. Interestingly, better survival was found in active smokers with PTEN-. Further survival studies in cases with no TILs lesions and active smokers in associations PTEN expression and other immune-related biomarkers, such as programmed death–ligand 1 (PD-L1), are warranted.

Keywords: PTEN; immunohistochemistry; tumor infiltrating lymphocytes; lung cancer (Siriraj Med J 2022; 74: 48-63)

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide, and the second leading cancer in males and females. In 2019, lung cancer was responsible for the death of 1.37 million people in the United States.[1] In Thailand, lung cancer is the third most common cancer in males, accounting for 24.74% of all cancers, and the fourth most common cancer in females, accounting for 7.26% of all cancers, while the most common cancers overall are hepatobiliary cancer in males and breast cancer in females.[2]

Lung cancer can be classified as either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). NSCLC, which is more common (accounting for 85% of lung cancers), can be sub-classified as adenocarcinoma (AC, 40%), squamous cell carcinoma (SC, 25%-30%), and large cell carcinoma (10%-15%).[3,4] Regarding its genetic and molecular basis, the dysregulation of cell growth

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and development may predispose some patients to lung cancer as well as determine its severity.[5] Tumor suppressor genes (TSGs), in particular *PTEN (phosphatase and tensin homolog)*, are considered significant controllers of cell survival and the cell cycle process; thus their dysregulation may lead to the development of several cancers, including lung cancer.[6-10] *PTEN*, located on chromosome 10q23, regulates the cell cycle via the PI3K/AKT pathway. In the development of lung cancer, *PTEN* dysfunction, including its loss of function, protein instability, and somatic mutation, has been noted to be associated with the malignant transformation of lung cells.

Several studies reported that approximately 41.2%-43.7% of lung cancer patients have a loss of *PTEN* expression.[11-13] Moreover, *PTEN* dysregulation was found to be associated with an advanced tumor stage, partly due to the anchorage-independent growth mechanism.[14] In lung cancer patients, the loss of *PTEN* expression *(PTEN-)* made negatively regulates phosphatidylinositol 3 phosphate level in the P13K/AKT pathway[15], and this alteration may be associated with the development of lung cancer.[16] *PTEN* loss of expression *(PTEN-)* was related to characteristics of NSCLC patients, including male, a previous history of smoking, and lung cancer with a poorly differentiated type, increased lymph node involvement, high distant metastasis, and late-stage, and thus it is a factor for a poor prognosis.[17] Further, patients with a combination of the *PTEN-* and *p-AKT+* have a lower 5-year survival rate and median survival time. Though the associations between the loss of *PTEN* expression and SCLC is still unclear. Alterations in the *PTEN* pathway have been regularly reported in SCLC.[18] In the genetic mice model, the *PTEN* inactivation could accelerate the SCLC with more aggressive behavior.[19] *PTEN-* is also an independent poor prognostic factor for NSCLC.[11-13] Furthermore, lung cancer patients with a loss of *PTEN* expression and the positive *epidermal growth factor receptor (EGFR)* mutation may be resistant to chemotherapy and targeted therapy.[20,21]

Consequently, our study aimed to explore the prevalence of *PTEN* loss of expression in Thai lung cancer patients, and also its associated clinical characteristics and possible effects on disease prognosis.

MATERIALS AND METHODS

This retrospective study was approved by the Human Research and Ethics Committees of Bangkok Metropolitan Administration, Bangkok, Thailand. (No. S013h/63_EXP). Informed consent was waived because the study involved anonymous data extraction with no direct patient or public involvement.

Study population

In total, 191 lung cancer patients underwent either transbronchial biopsy (53.9%), transcutaneous biopsy (24.6%), lobectomy (3.6%), or tissue biopsy (lymph node, skin, bone, mass) (18%) at the Charoenkrung Pracharak Hospital between 1 January 2010 and 31 December 2020 and were enrolled in the study. The inclusion criteria were patients aged >15 years old diagnosed with lung cancer by histopathological studies. If there was insufficient tissue for the further analysis of *PTEN* expreesion, the patients were excluded from the study. The patients' demographics and clinical characteristics, including age, gender, smoking, associated symptoms (such as chronic cough, progressive dyspnea, hemoptysis, and chest pain), Eastern cooperative oncology group (ECOG) performance status, tumor size and its primary location, histopathological study, duration of follow-up, and living status, were collected. Ancillary immunohistochemical reports, including *thyroid transcription factor 1 (TTF – 1)* and *epidermal growth factor receptor (EGFR)* mutation, were also collected.

Histopathology and immunohistochemistry (IHC) for PTEN

Formalin-fixed paraffin-embedded (FFPE) blocks were cut 4 μm in thickness by microtomy (Thermo Fisher Scientific HM355S automated microtomy). Slides were stained by hematoxylin and eosin (H&E). All the previous H&E slides were reviewed for confirming the diagnosis and evaluating the tissue adequacy for further *PTEN* immunohistochemical study by two pathologists at pathologists at the Department of Pathology, Charoenkrung Pracharak Hospital, blinded to the patient information and clinical data. The laboratory was certified for Laboratory Academic Standards by The Royal College of Pathologist of Thailand. Histopathological parameters, including histologic subtype and tumor-infiltrating lymphocytes (TILs), were evaluated. Unstained whole-section FFPE slides from 167 FFPE blocks were heated at 70 ^{0}C for 30 min in a hot air oven (Memmert UF55) before running them in a Ventana BenchMark XT automatic sample preparation system (serial number KPXT715667). The IHC staining process included deparaffinization by EZ prep (LOT#G13961) and cell conditioning by CC1 (LOT#G06580) for 56 min and antigen retrieval by a primary peroxidase inhibitor. Rabbit monoclonal primary antibody VENTANA PTEN clone SP218 (LOT#G27111) was used as the primary antibody in this study. Automated incubation was performed for 16 min. OptiView HQ links and OptiView HQ Universal links were mixed to ensure amplified signals, followed

by use of the Ventana OptiView TM Universal DAB Detection Kit. Counterstaining was performed by dyeing with Ventana Hematoxylin II for 12 minutes, followed by bluing reagent for 6 minutes. The oil was removed from each slide by soap and the slide was subsequently dehydrated with 95% ethyl alcohol, absolute alcohol, and xylene, respectively. Finally, a coverslip was placed on the slide and it was then mounted with mounting media.

IHC scoring of PTEN

The IHC score for *PTEN* was modified from the previous literature.[11] The interpretation and scoring of *PTEN* IHC was performed in terms of either the intensity of staining or the average number of positive tumor cells, as independently evaluated by two pathologists. *PTEN* IHC slides were visually scored using a bright field microscope (Olympus BX43) under an objective lens (40x magnification) and eye pieces lens (10x magnification and field number 22). The positive internal control staining included the bronchial epithelial cells and stromal cells. Five 40x high-power fields were selected to includes 200 cell counts. The interpretation of *PTEN* expression involved the cytoplasmic and/or nuclear staining pattern. The average percentage of positive tumor cells is reported as the following: 0 = no tumor cells stained, 1 = 10%-20% of cells stained, 2 = 20%-50% cells stained, and 3 = >50% of cells stained. The intensity of positive cell staining was categorized as follows: 0 = no appreciable staining in the cells, 1 = barely detectable staining as compared with normal stromal cells, 2 = readily appreciable brown staining distinctly marking the cell cytoplasm/or nucleus, and 3 = dark brown staining in the cytoplasm and/or nucleus. *PTEN* IHC scoring was rendered, with regard to the calculated results for the intensity and the average percentage of positive tumor cells, ranging from 0 to 9. A score of 2 or less is defined as negative *PTEN* IHC expression, whereas 3 or greater is defined as positive *PTEN* IHC expression.

Using 200-400x (a 10x eyepieces and a 20-40x objective lens) microscopic magnification, tumor infiltrating lymphocytes (TILs) are the percentages of TILs in the stromal compartment (% stromal TILs), defined as the area of mononuclear cells (including lymphocyte and plasma cell) infiltration, between the cancer cells with no direct contact, in the stromal tissue. The 10% of more stromal TILs is considered positive.[22]

Statistical analysis

Data were analyzed for the prevalence of *PTEN* loss of expression (*PTEN*-) in lung cancer, the associations between *PTEN* loss of expression and the clinicopathological factors, IHC features, and the *EGFR* type status. The parametric statistical analysis was performed using SPSS version 26 software (IBM Corp., Armonk, NY). The patients' demographic and clinical characteristics were expressed as a numbers, percentages, median, and mean and standard deviation (SD). Categorical variables were analyzed by Pearson chi-square test and Fisher's exact test for the normally distributed data. A p-value less than 0.05 was considered statistically significant. Kaplan–Meier analysis and the log-rank test were used to analyze the results from the survival study. Cox proportional hazard regression was applied to determine the *PTEN* loss of expression and the variables affecting the survival status. According to Seol-Bong Yoo *et al.*[13], the prevalence of the *PTEN* loss of expression in NSCLC was 42.4% and was used to calculated the sample size. In this study, there were only 8 patients with SCLC; therefore, only descriptive statistics were applied. Moreover, no prevalence of *PTEN* loss of expression was previously reported.

RESULTS

Among the 191 lung cancer patients enrolled on the study, 24 were excluded because 23 had no available FFPE blocks and one who had inadequate tumor tissue for the IHC study. Subsequently, a total of 167 patients were included in the study for the data analysis. Two pathologists independently interpreted the PTEN IHC staining on each slide. For the first 40 slides, the interpretation kappa values were 0.975 for the inter-observer reliability and 0.95 for the intra-observer reliability, accordingly. For the overall 167 slides, the inter-observer reliability was 0.98. Three discordantly interpreted slide results between the two pathologists were re-evaluated and further discussed until consensus was reached on each slide (positive or negative *PTEN* staining)

The mean age of the patients was 64.8 ± 11.77 years old, with a median age of 65.0 years old (ranging from 32-93 years), with 51.5% having an age ≥65 years old. The majority of patients were male (57.5%). Out of the 167 lung cancers cases, NSCLC was accounted for 159 cases (95.2%) and SCLC 8 cases (4.8%). Lung cancer was the most commonly found in patients who had never smoked group (46.1%), while its incidence in those with a smoking history group, which was active (37.7%), secondhand (6.6%), or former smokers (9.6%), was 53.9%. Clinical symptoms included chronic cough (77.2%), progressive dyspnea (68.3%), hemoptysis (19.8%), and chest pain (24.6%), and the most common ECOG score at the time of diagnosis was 1 (54.5%), respectively. Among

the NSCLC cases, 85.6% of patients had adenocarcinoma subtypes, and 90.6% were diagnosed at an advanced stage (stage IV). Moreover, all 8 SCLC cases were involved extensive disease (ED). Approximately half the patients (57.6%) had a tumor sized 3-7 cm at the greatest diameter. Regarding of the location of the lung lobes, primary lung cancer was mostly located at the right upper lung area (34.1%). Surprisingly, most lung cancer tissues (64.1%) displayed no tumor-infiltrating lymphocytes. The prevalence of PTEN loss of expression was 66.5%. Most lung cancer tissues were stained positive for TTF-1 (77.5%) and Napsin A staining (63.2%). Regarding the EGFR mutation, 46 cases (47.8%) were located on either EXON 19 (68.2%) or EXON 21 (18.2%).

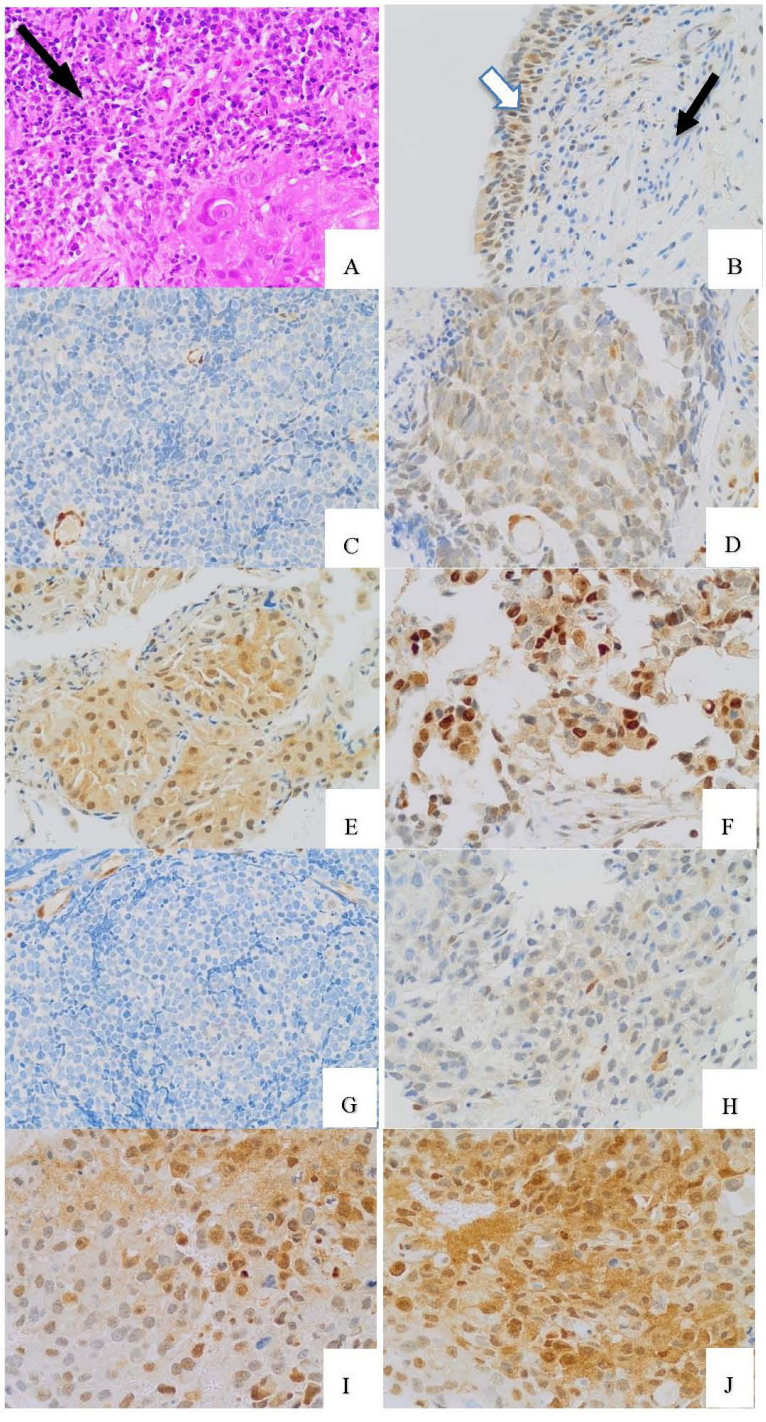

Fig 1. **A:** NSCLC with TILs (dark arrow) (H&E, x40); **B:** Normal bronchial epithelium (white arrow) and stromal cell (dark arrow) with nuclear and cytoplasmic staining of PTEN. PTEN Intensity was categorized as follows: **C:** 0 if no appreciable intensity stain. **D:** 1 if barely stain; **E:** 2 if appreciable brown; **F:** 3 if dark brown stain in the cytoplasm and/or nucleus. The different percentages of PTEN IHC were demonstrated as follows: **G:** no tumor cell staining or 0%; **H:** 10% - 20%, **I:** 20% - 50%; **J:** more than 50% in the NSCLC patients.

TABLE 1. Demographics, clinical characteristics, and histopathological and immunohistochemical studies of the patients with lung cancer.

Characteristic variables	Number	Percentage (%)
Gender (n = 167)		
Male	96	57.5
Female	71	42.5
Age (years) (n = 167)		
≥65	86	51.5
<65	81	48.5
Smoking (n = 167)		
Never smoked	77	46.1
Active smoker	63	37.7
Secondhand smoker	11	6.6
Former smoker	16	9.6
Chronic cough (n = 167)	129	77.2
Hemoptysis (n = 167)	134	80.2
Progressive dyspnea (n = 167)	114	68.3
Weight loss (n = 167)	100	59.9
Chest pain (n = 167)	41	24.6
ECOG status (n = 167)		
0	8	4.8
1	91	54.5
2	36	21.6
3	21	12.6
4	11	6.6
Histological type of lung cancer (n = 167)		
NSCLC	159	95.2
SCLC	8	4.8
NSCLC subtype (n = 159)		
Adenocarcinoma	143	89.9
Squamous cell carcinoma	12	7.5
Large cell neuroendocrine carcinoma	2	1.3
Other (e.g. carcinosarcoma)	2	1.3
Tumor size (n = 167) (cm.)		
<3	15	9.3
3–7	95	58.6
>7	52	32.1
Location of the primary tumor (n = 167)		
RUL	57	34.1
RML	20	12
RLL	31	18.6
LUL	32	19.1
LLL	26	15.6
Center	1	0.6

TABLE 1. Demographics, clinical characteristics, and histopathological and immunohistochemical studies of the patients with lung cancer. (Continue)

Characteristic variables	Number	Percentage (%)
Tumor-infiltrating lymphocytes (TILs) (n = 167)		
Present	60	35.9
Histological pattern (n = 167)		
Acinar	89	53.3
Papillary	8	4.8
Solid	61	36.5
Lepidic	9	5.4
Degree of differentiation (n = 167)		
Moderate	157	94
Poor	10	6
PTEN expression (n = 167)		
Loss of expression	111	66.5
TTF-1 IHC (n = 142)		
Positive staining	110	77.5
EGFR mutation (n = 46)	22	47.8
Exon 19	15	68.2
Exon 20	1	4.5
Exon 21	4	18.2
Double mutation	2	9.1
Treatment (1st line)		
1st line chemotherapy	79	47.3
1st line tyrosine kinase inhibitor (TKI)	12	7.2
1st line surgery	3	1.8
1st line radiotherapy	15	9
no definite treatment/palliative care	58	34.7
Living status		
Alive	23	13.77
Dead	144	86.23

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status, NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer, PTEN = phosphatase and tensin homolog, TTF-1 = thyroid transcription factor 1, EGFR = epidermal growth factor receptor, RUL=Right upper lung, RML=Right middle lung, RLL=Right lower lung, LUL=Left upper lung, and LLL=Left lower lung

Correlation between PTEN expression and survival time

In terms of the correlation between the *PTEN* status and clinical outcome, *PTEN*- was less common in lung cancer that was primarily located at the left lower lobe (LLL), compared to at the right upper lobe (RUL) (p = 0.039, OR = 0.36), and in the absence of TILs (p = 0.045, OR = 1.96). Whereas an age <65 years old and smoking were likely correlated with the *PTEN* status (p = 0.056, OR = 0.22 and p = 0.089, OR = 1.75, respectively).

According to the multivariate analysis, the absence of TILs (p = 0.017, adjusted OR = 2.5), location at the LLL (p = 0.026, adjusted OR = 0.297), and age <65 years old (p = 0.04, adjusted OR = 0.47) were independent factors correlated with the PTEN loss of expression. In addition, SCLC and smoking behavior were also marginally significantly associated with the PTEN loss of expression (p = 0.054 and 0.089, respectively).

The median follow-up time was 8.04 months (range, 0.01–94.80). Most patients (65.3%) had received specific treatments (including 1st line chemotherapy (47.3%), 1st line radiotherapy (13.8%), 1st line tyrosine kinase inhibitor (7.2%), or surgery for primary cancer (1.8%)), while the remaining 34.7% had received no aggressive treatment due to their poor baseline status. The median overall survival (mOS) was 8.88 months, with 2- and 5-year overall survival rates of 19.7% and 7.4%, respectively. Of note, 27 patients (16.17%) had CNS/spine metastasis at the 1st diagnosis.

TABLE 2. Association among clinical status, immunohistochemical study, *EGFR* mutation of primary lung cancer, and PTEN expression.

Clinicopathological, immune-molecular features	Total n (%)	PTEN (+) n (%)	PTEN (-) n (%)	P-value
Gender				
Male	96 (57.5)	33 (58.9)	63 (56.8)	0.79
Female	71 (42.5)	23 (41.1)	48 (43.2)	
Age				
≥65	86 (51.5)	23 (41.1)	63 (56.8)	0.056
<65	81 (48.5)	33 (58.9)	48 (43.2)	
Smoking				
No smoking	77 (46.1)	31 (55.4)	46 (41.4)	0.089
History of smoking	90 (53.9)	25 (44.6)	65 (58.6)	
Chronic cough				
Yes	129 (77.2)	41 (73.2)	88 (79.3)	0.37
No	38 (22.8)	15 (26.8)	23 (20.7)	
Progressive dyspnea				
Yes	114 (68.3)	39 (69.6)	75 (67.6)	0.786
No	53 (31.7)	17 (30.4)	36 (32.4)	
Hemoptysis				
Yes	33 (19.8)	12 (21.4)	21 (18.9)	0.70
No	134 (80.2)	44 (78.6)	90 (81.1)	
Weight loss				
Yes	100 (59.9)	34 (60.7)	66 (59.5)	0.88
No	67 (40.1)	22 (39.3)	45 (40.5)	
Chest pain				
Yes	41 (24.6)	15 (26.8)	26 (23.4)	0.63
No	126 (75.4)	41 (73.2)	85 (76.6)	
Size				
<3 cm	15 (9.3)	2 (3.6)	13 (12.3)	0.12
3–7 cm	95 (58.6)	39 (69.6)	56 (52.8)	
>7 cm	52 (32.1)	15 (26.8)	37 (34.9)	

TABLE 2. Association among clinical status, immunohistochemical study, *EGFR* mutation of primary lung cancer, and PTEN expression. (Continue)

Clinicopathological, immune-molecular features	Total n (%)	PTEN (+) n (%)	PTEN (-) n (%)	P-value
Primary site				
RUL (0)	57 (34.1)	17 (30.4)	40 (36)	0.31
RML (1)	20 (12)	5 (8.9)	15 (13.5)	
RLL (2)	31 (18.6)	8 (14.3)	23 (20.7)	
LUL (3)	32 (19.2)	12 (21.4)	20 (18)	
LLL (4)	26 (15.6)	14 (25)	12 (10.8)	
Central (5)	1 (0.6)	-	1 (0.9)	
ECOG status				
0	8 (4.8)	1 (1.8)	7 (6.3)	0.49
1	91 (54.5)	30 (53.6)	61 (55)	
2	36 (21.6)	14 (25)	22 (19.8)	
3	21 (12.6)	7 (12.5)	14 (12.6)	
4	11 (6.6)	4 (7.1)	7 (6.3)	
Type				
NSCLC	159 (95.2)	56 (100)	103 (92.8)	0.05
SCLC	8 (4.8)	-	8 (7.2)	
Stage (NSCLC)				
I	4 (2.5)	1 (1.8)	3 (2.9)	0.21
II	4 (2.5)	1 (1.8)	3 (2.9)	
III	7 (4.4)	4 (7.3)	3 (2.9)	
IV	144 (90.6)	49 (89.1)	95 (91.3)	
Stage (small cell)				
Extensive disease	8 (100)	0 (0)	8 (100)	-
NSCLC				
Adenocarcinoma	143 (89.9)	47 (83.9)	96 (93.2)	0.12
Squamous cell CA	12 (7.5)	6 (10.7)	6 (5.8)	
Large cell / NE	2 (1.3)	1 (1.8)	1 (1)	
Other	2 (1.3)	2 (3.6)	-	
TILs				
Yes	60 (35.9)	26 (46.4)	34 (30.6)	0.045
No	107 (64.1)	30 (53.6)	77 (69.4)	
TTF-1				
Yes	110 (77.5)	39 (79.6)	71 (76.3)	0.66
No	32 (22.5)	10 (20.4)	22 (23.7)	
EGFR mutation				
Yes	22 (47.8)	11 (55)	11 (42.3)	0.39
No	24 (52.2)	9 (45)	15 (57.7)	
EGFR mutation				
EXON 18	-	-	-	0.79
EXON 19	15 (68.2)	8 (72.7)	7 (63.6)	
EXON 20	1 (4.5)	-	1 (9.1)	
EXON 21	4 (18.2)	2 (18.2)	2 (18.2)	
Double mutation	2 (9.1)	1 (9.1)	1 (9.1)	
Treatment				
1st line chemotherapy	79 (86.8)	27 (87.1)	52 (86.7)	0.003
1st line (TKI)	12 (13.2)	4 (12.9)	8 (13.3)	

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status, NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer, PTEN = phosphatase and tensin homolog, TTF-1 = thyroid transcription factor 1, EGFR = epidermal growth factor receptor, RUL=Right upper lung

Patients who had a history of smoking, chronic cough, progressive dyspnea, no hemoptysis, chest pain, weight loss, larger tumor size, and lower ECOG status had a lower mOS time than in the opposite group (p = 0.003, 0.005, 0.015, 0.008, 0.03, 0.001, 0.001, and 0.001, respectively). Nevertheless, gender, age, a subtype of lung cancer (adenocarcinoma vs squamous cell subtypes) and primary brain/spine metastasis, presence of TILs, TTF-1, and *EGFR* mutation showed no significant difference in the mOS time between the comparative populations (p >0.05).

In all lung cancer patients, the mOS was 8.88 months (ranging from 0.01 to 94.8 months). There was no significant difference in mOS between the *PTEN*+ and *PTEN*– groups in NSCLC (7.20 vs. 8.88 months) (p = 0.38) and also no significant difference in mOS between the *PTEN*+ and *PTEN*– groups in adenocarcinoma (7.20 vs 9.96 months) (p = 0.23) (Fig 2.1 and 2.2).

TABLE 3. Association among the clinicopathologic, immune-molecular features and median overall survival (mOS) and hazard ratio (HR) (n = 167).

Clinicopathological, immune-molecular features	Total n (%)	mOS month	95% CI n (%)	P-value	Hazard ratio	95% CI n (%)	P-value
Gender							
Male	96 (57.5)	7.68	5.54–9.82	0.368	reference	-	-
Female	71 (42.5)	13.63	8.91–18.35		0.86	0.62–1.20	0.369
Age							
≥65	86 (51.5)	8.64	4.86–12.42	0.256	reference	-	-
<65	81 (48.5)	9.96	7.25–12.67		0.823	0.60–1.15	0.258
Smoking							
No smoking	77 (46.1)	14.57	8.83–20.31	0.003	reference	-	-
History of smoking	90 (53.9)	6.36	4.62–8.10		1.64	1.18–2.29	0.004
Chronic cough							
No	38 (22.8)	19.2	13.94–24.46	0.005	1.75	1.17–2.62	-
Yes	129 (77.2)	7.2	5.20–9.2		reference	-	0.006
Progressive dyspnea							
No	53 (31.7)	18.84	12.39–25.29	0.015	reference	-	-
Yes	114 (68.3)	7.2	4.44–9.96		1.55	1.08–2.20	0.016
Hemoptysis							
No	134 (80.2)	7.68	4.89–10.47	0.008	reference	-	-
Yes	33 (19.8)	10.08	0–20.67		0.55	0.35–0.86	0.009
Weight loss							
No	67 (40.1)	18.24	9.70–26.78	0.001	reference	-	-
Yes	100 (59.9)	7.08	4.57–9.59		1.77	1.25–2.49	0.001
Chest pain							
No	126 (75.4)	9.96	5.92–14	0.03	reference	-	-
Yes	41 (24.6)	7.2	2.93–11.47		1.54	1.04–2.28	0.032

TABLE 3. Association among the clinicopathologic, immune-molecular features and median overall survival (mOS) and hazard ratio (HR) (n = 167). (Continue)

Clinicopathological, immune-molecular features	Total n (%)	mOS month	95% CI n (%)	P-value	Hazard ratio	95% CI n (%)	P-value
ECOG status							
0	8 (4.8)	35.16	22.83–47.49	<0.01	reference	-	-
1	91 (54.5)	14.52	10.19–18.85		2.28	0.92–5.66	0.076
2	36 (21.6)	3.79	1.40–6.18		2.36	2.08–13.84	0.001
3	21 (12.6)	5.88	2.63–9.13		5.99	2.21–16.23	<0.001
4	11 (6.6)	4.6	0–9.52		3.92	1.30–11.84	0.016
Primary site							
RUL (0)	57 (34.1)	16.03	9.81–22.26	0.024	reference	-	-
RLL (2)	31 (18.6)	9.24	5.91–12.57		0.96	0.59–1.54	0.86
LUL (3)	32 (19.2)	3.79	2.01–5.57		1.60	1.01–2.54	0.046
LLL (4)	26 (15.6)	5.58	1.19–10.57		1.90	1.16–3.09	0.01
Type							
Adenocarcinoma	143 (92.3)	9.24	6.49–11.99	0.26	reference	-	-
Squamous cell CA	12 (7.7)	11.64	0.03–23.25		0.68	0.34–1.34	0.27
Brain/spine metastasis							
No	140 (83.8)	9.24	6.39–12.09	0.31	reference	-	-
Yes	27 (16.2)	6.72	3.60–9.84		1.254	0.81–1.95	0.32
Size							
<3 cm	15 (9.3)	20.88	3.61–39.15	<0.01	0.45	0.23–0.88	0.02
3–7 cm	95 (58.6)	11.28	7.44–15.12		reference	-	-
>7 cm	52 (32.1)	5.28	3.23–7.33		2.13	1.46–3.10	<0.001
TILs							
Yes	60 (35.9)	7.08	1.36–12.80	0.68	reference	-	-
No	107 (64.1)	8.88	5.9–11.86		1.08	0.76–1.52	0.67
TTF-1							
No	32 (22.5)	10.08	6.75–13.41	0.88	reference	-	-
Yes	110 (77.5)	7.92	4.68–11.16		1.03	0.68–1.56	0.88
PTEN							
No	56 (33.5)	7.2	1.97–12.43	0.38	reference	-	-
Yes	111 (66.5)	8.8	6.62–11.14		0.85	0.60–1.22	0.38
EGFR							
No	24 (52.2)	7.44	4.62–10.26	0.41	reference	-	-
Yes	22 (47.8)	14.52	11.99–17.05		0.76	0.39–1.46	0.41

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status, TILs = tumor-infiltrating lymphocytes, TTF-1 = thyroid transcription factor 1, PTEN = phosphatase and tensin homolog, EGFR = epidermal growth factor receptor.

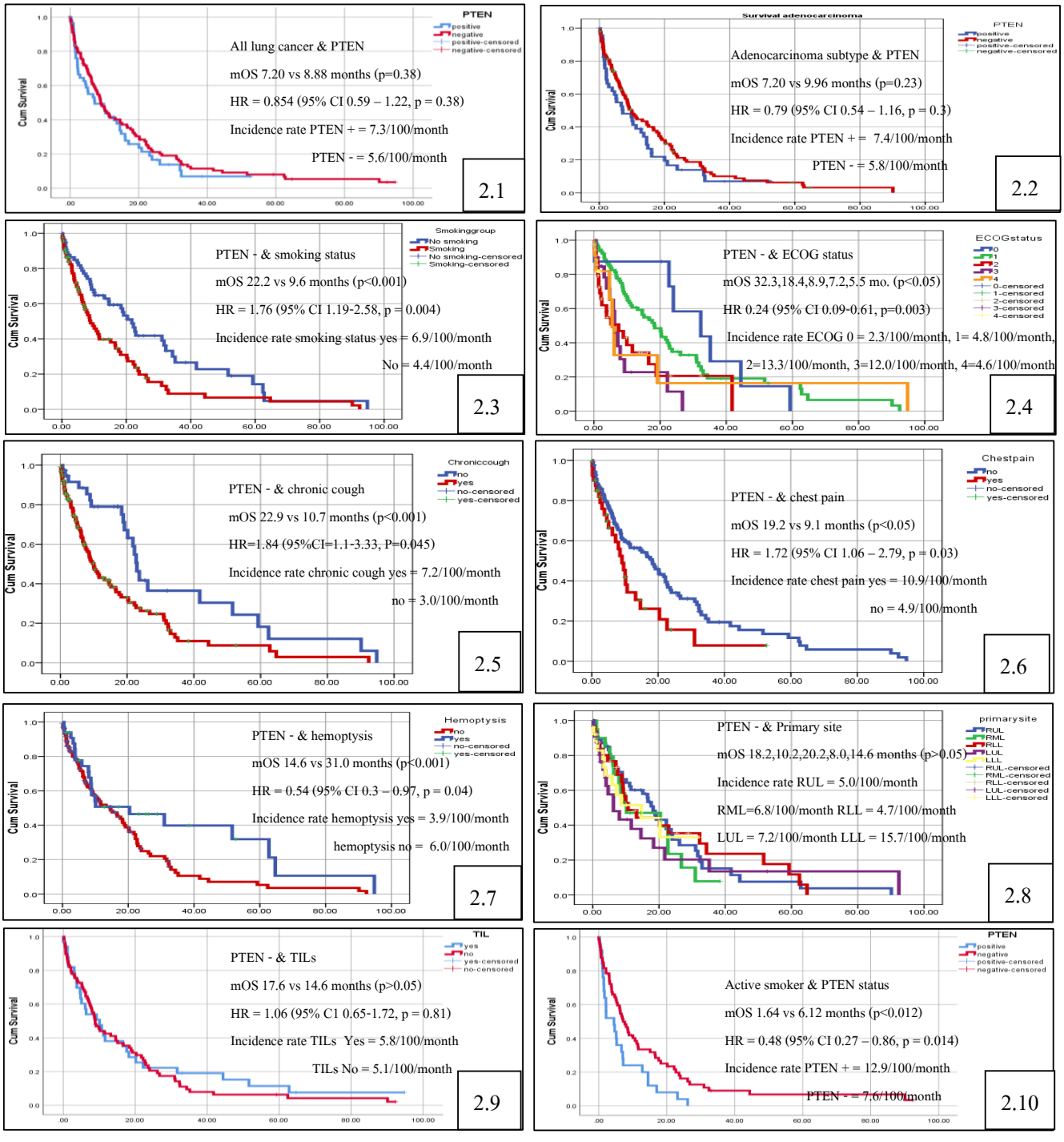

Fig 2. The Kaplan–Meier graph demonstrating the mOS of the patients with lung cancer **(2.1)**, adenocarcinoma subtypes **(2.2)**, and active smoking **(2.10)** in association with the *PTEN* status. **Fig 2.3–2.9** display the mOS of *PTEN*- correlated with the smoking status, EGOG status, present symptom (chronic cough, progressive dyspnea, hemoptysis), primary site, TILs.

In the subgroup analysis of *PTEN*– patients, the mOS was significantly decreased with the presence of smoking (Fig 2.3). high ECOG status (Fig 2.4), chronic cough (Fig 2.5), chest pain (Fig 2.6) and hemoptysis (Fig 2.7). Nevertheless, there was no significant difference in the mOS depending on the primary tumor site (Fig 2.8). Fig 2.9 reveals that the mOS in the no TILs feature was longer than the mOS in the TILs group, but was no statistical difference. Among the active smokers, the mOS was longer in the *PTEN*- group than in the *PTEN*+ group (Fig 2.10). The mOS in either presence or absence of EGFR mutation was not significantly different (7.44 vs 14.52 months) (p = 0.41).

DISCUSSION

As the function of *PTEN* is to inhibit the Akt kinase activity in the kinase/Akt/mTOR pathway, *PTEN* deletion results in high levels of activated Akt, which brings out the G1 cell cycle and possible progress to pathogenesis of the NSCLC and subsequent metastasis.[23] Furthermore, the absence of *PTEN* creates an immunosuppressive microenvironment, thus facilitating tumor growth and metastasis.[24] *PTEN*- creates an immunosuppressive microenvironment.[25] On the contrary, the presence of CD8+ T-lymphocyte infiltration is correlated with a better prognosis in several cancers.[26] The expression of *PTEN* protein could also block the programmed cell death receptor-1 (PD-1). Consequently, several studies have reported the expression of *PD-1* is related with the expression of *PTEN*.[27]

Our study is the first to report the overall prevalence of *PTEN*- in Thai patients with lung cancer (66.5%), with a prevalence of 64.8% in NSCLC patients and 100% in SCLC patients. Previously, the prevalence of *PTEN*- in NSCLC patients was reported to be approximately 24%-59.86%[11,13,28-32], In the literature, adenocarcinoma (AC) is the most common subtype of NSCLC, representing approximately 40% of patients.[33] However, in this study, the prevalence of AC subtypes was very high (84.4% in males and 87.3% in females), compared to the reported prevalence according to a SEER study in 2018[34] and in the 2015 report of the National Cancer Research Institute of Thailand[2], which to be reported a rate of approximately 50%-60%.

Of note, the percentages of AC and *PTEN*- in our study population were relatively high. Possible explanations for this include the higher cut-off score of 3 and higher we employed, similar to the study of Tang *et al.* in NSCLC patients[11], whereas many previous studies utilized lower cut-off scores of 1-2.[11,35-40] Table 4 described the PTEN expression in lung cancer patients from each study and their correlated parameters.

TABLE 4. The studies of the PTEN loss of expression in lung cancer patients and their correlated clinical parameters.

References	Histology	Finding	PTEN score	Number of patients	Related clinical parameters with PTEN-
Soria et al.[28]	NSCLC	Protein loss	Intensity - absence	24% (30/125)	No related parameters
Chang et al.[41]	NSCLC	Protein loss	< 3	59.86% (173/289)	LN metastasis, smoking status, low survival rate
Yoo et al.[42]	NSCLC	Protein loss	No data	42.4% (122/288)	SC, smoking status, low progression free survival
Scrima et al.[43]	NSCLC	Protein loss	0-25%	39% (41/104)	SC subtype
Tang et al.[11]	NSCLC	Protein loss	0-2	46.1% (47/102)	Poor survival of p-Akt^{S473} positive
Goncharak et al.[32]	NSCLC	Protein loss	0-2	41.4% (43/104)	Advanced disease, LN metastasis, low survival rate
Kim et al.[37]	AD, SC	Protein loss	0-2	37.4% (34/91)	High histological grade, pathological T stage, N stage, short survival in AD
Thiva et al., These study	NSCLC	Protein loss	<3	64.8% (103/159)	Absence of TILs, poor location at LLL, age <65 years, smoking

Moreover, we preferred the detection of *PTEN* expression by the IHC method over *PTEN* mRNA study as the method is more sensitive for studying overall survival. Further, in the meta-analysis, NSCLC patients with *PTEN* loss of expression had an unfavorable prognosis, whereas the results could not be demonstrated when the PTEN mRNA method was utilized.[24]

In our study, patients with hemoptysis had a longer mOS than asymptomatic patients (p = 0.008). Thus, hemoptysis might prompt both patients and doctors' concerns, thus allowing early investigation, followed by prompt treatment, eventually extending the overall mOS period. Furthermore, similar to the study of Port *et al*., our study indicated that a smaller tumor size and lower ECOG performance status were correlated with a prolonged mOS.[44] Moreover, no significant difference in mOS was observed between the age groups and genders, corresponding with another study by Franceschini *et al*.[45]

In NSCLC, no significant difference was seen in mOS between *PTEN*– and *PTEN*+ (p = 0.38). Though most studies expected a lower mOS in NSCLC patients with *PTEN* - [31,35-38,40,42,46-50], some studies reported a longer mOS.[51] The differences could be possibly due to different races and ethnicities, techniques to detect *PTEN* loss of expression (including polymerase chain reaction, fluorescence *in situ* hybridization, IHC), and cut-off score in IHC. Notably, in our study, a higher proportion of AC was observed, and this NSCLC subtype is more responsive to treatment.

Interestingly, all the SCLC patients in our study were at an extensive stage with *PTEN*-, compared with those with the NSCLC subtype (p = 0.05). The mOS of SCLC was short, only 5.28 months (range. 0.96-9.61). Regarding previous reports on the prevalence of SCLC, possible SCLC-Y accounted for approximately 10% of all four subtypes. However, only in the SCLC-Y subtype is the oncogenesis related to the mTOR pathway and associated with *PTEN* loss of expression. Presumably, all the SCLC cases in our study were likely to be the SCLC-Y subtype. However, further studies to analyze the subtypes of the SCLC are required to demonstrate the actual prevalence of each subtype in Thai patients.

In this study, there were no significant differences in mOS in patients with smoking history, a more advanced stage, and squamous cell type with *PTEN*- status (p = 0.089, 0.237, and 0.053); though previous studies demonstrated a correlation between smoking status[41,52,53], stage[13], and squamous cell subtype[13,31], with *PTEN*-. In the PTEN- group in our study, patients with a smoking history had a longer mOS than those with a negative smoking history (p = 0.012; 6.12 vs 1.68 months, HR (*PTEN* +/-) = 0.48, 95%CI 0.27-0.86, p = 0.014). Chang *et al*. also reported an unclear association between the *PTEN* status and smoking behavior.[41] Of note, the frequency and intensity of smoking are known risk factors for lung cancer, but these were not extensively included in the analysis due to the limited data availability in the medical records.

Interestingly, our study is the first to report that the location of the primary tumor at the RUL was significantly correlated with *PTEN*-, compared to at the LLL location (p = 0.03). Patients with the primary site at the RUL also had a significantly longer mOS than at the LUL and LLL locations (16.03 months vs. 3.79 months and 5.58 months (p = 0.024) (LUL:HR 1.60, 95%CI 1.01–2.54, p = 0.046 and LLL:HR = 1.90, 95%CI 1.16–3.09, p = 0.01). For the primary location of NSCLC, Lee *et al*. reported that a lower lobe group had a higher mortality rate than non-lower lobe ones (48.6% and 40.3%, p <0.0001) with less frequent *EGFR* mutation.[54] Lung cancer with the primary location at the upper lobe may contain different immunomolecular processes that might interfere with the survival outcomes. These hypotheses probably lead to the need for personalized medicine.

In the *PTEN*+ group, the location of the primary site was significantly associated with the mOS, of which the RUL location had a longer mOS than the LLL location. However, this association was not found in the *PTEN*- group. Nevertheless, in the *PTEN*+ group, the primary location of the tumor at the RUL had a longer mOS than at the LLL location [32.4 months vs 14.8 months (p = 0.0001), HR 3.14, 95%CI 1.52–6.52, p = 0.002]. Therefore, further studies on the *PTEN* expression using the IHC of the patients with the primary location of lung cancer at the upper lobe may reveal its prognostic capability.

For the histopathological studies, the *PTEN*- was found to be associated with the negative findings of TILs (p = 0.045). In our study, there was no significant difference in mOS using the TILs status (p = 0.67). On the contrary, Schalper *et al*. and Gao *et al*. reported better survival outcomes in NSCLC and triple-negative breast cancer with the presence of TILs, respectively.[55,56] In our mOS study in NSCLC, no TILs feature was not a prognostic factor, probably not only the level of activated Akt in the mTOR pathway, influencing cellular survival, and other tumor suppressor functions, such as chromosome integrity and DNA repair[57], take part in the survival process. Other confounding factors, such as a small sample size number, the method used to identify *PTEN*, and scoring of the *PTEN* expression by IHC, were influential in these studies.

CONCLUSION

The prevalence of the PTEN loss of expression in NSCLC in our study population was quite a bit higher than in previous studies. The pathological no TILs feature and RUL location were found to be independent factors associated with the PTEN loss of expression. The small cell subtype and the smoking group were nearly significantly related to negative *PTEN* staining. Smoking status, all symptoms, ECOG status, RUL location, and tumor size >7 cm were found to play unfavorable prognostic roles in the overall survival in NSCLC. In the subgroup study, PTEN loss of expression with RUL location tended to involve a poor prognosis. Interestingly, a better survival outcome was shown in the active smoker group with *PTEN* negative. Further survival study of the no TILs pathological status and active smoker subgroups in terms of PTEN expression and other immune-related biomarkers, such as IHC for programmed death–ligand 1 (PD-L1), with an adequate sample size and proper study design is warranted.

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A Scenario of COVID-19 Disease on Mental Health Among the General Age-groups

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ABSTRACT

The COVID-19 (SARS-CoV-2) virus causes a respiratory disease with physical and mental health effects, ending at general morbidity and fatality from some latest coronavirus strains, at times. During the present pandemics, people stay mainly at home, contributing to some elevated stress levels. World Health Organization (WHO) contemplates that the additional steps like, quarantine and self-isolation have stimulated daily routines of peoples, leading to a rise in agitation, oppression, sleeplessness, alcohol addiction, drug-addictions and suicidal behaviors; consequently, causing increase in cases of domestic violence, even. At this stage, health service providers cannot help the poor, elderly people, children who are susceptible to pre-medical adverse conditions. This work aims to highlight the general scenario of the mental health locally in India during COVID-19 pandemic. Some lifestyles, such as yoga, meditation, *Ayurvedic* medication, avoiding reading on corona too much and watching TV about it, while staying with the own family with the popular healthier lifestyles are recommended to alleviate stress.

Keywords: Mental health; stress; COVID-19 (Siriraj Med J 2022; 74: 64-67)

INTRODUCTION

The COVID-19 (SARS-CoV-2) emerged in Wuhan city China in December 2019.[1,2] The COVID-19 virus started from and Mid-March 2020; while, the local governments declared lockdown of all activities.[2,3] Outbursts of the virus and the consequential moves endured by the governments to limit the spread of the virus have maximized discomfort for an expansive pump-up the mental health ramifications.[4,5] To break chain of virus transmissions, several safety measures have been implemented,viz., quarantine for travelers and avoiding direct contact with people; eventually, closing down of academic institutions, employment and platforms of relaxation were done.[6] On the other hand, these preventive measures have adversely impacted the mental health of individuals allover.[2] The negative impacts of quarantine and self-isolation on mental health are due to the lack of liberty, monotony, and distrust that could produce general declination.[7] Particularly, several questions emerge in each young mind lacking the social outbursts, especially in children staying away from classrooms and schoolmates; eventually, they turn to their parents to get some answer and company. Thus, through the generated frustration and helplessness arising from the lack of social activity in schools, children might have a lasting traumatic effect on their mental health as the loss of coordination and a mindful-home study.

Furthermore, senior citizens might stay away from family members because of inherent clinical reasons and some typical adverse immunological response(s). Eventually, the physical family-distance could have

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significant adversity on the mental health in the pandemic. It could not stop the generated nervousness, unhappiness and initiate devastating circumstances that can induce an erratic behaviorial pattern. Because senior citizens rely on family members for their routine ongoing requirements, covid-related self-isolation could harm the family system. Moreover, health personnel employed as the frontline force for patients could result in the mentally suffering of particular patients in the form of fear of contracting a disease; the long hours of service of health workers may result in insufficiency of ancillary protective equipment. Moreover, there could be a negative mental-health effect from the news of the mortality of fellow patients, apart from the load of the patient and unavailability of effective medicines.

Social concerns affecting mental health

A psychological-interventional medical team was formed for attending to patients affected in the pandemic. The team consisted of psychiatrists, clinical psychologists and psychiatric nurses, who formulated interventions for planning separately for different groups. Indeed, the different stages of flow of people or/and patients are picturised (Fig 1).

Health experts could understand the mental health status of various groups of the society affected by the pandemic. The timely identification of high-risk patient groups, especially those with prior mental health issues, is essential to prevent extreme suicide and other negative impulsive behaviors. Interventions should be based on a comprehensive assessment of risk factors leading to psychological problems, poor mental health before a crisis, and a previous bereavement that might injure one or family members. Moreover, the described life-threatening circumstances, panic, separation from family and low household income may fuel the fire, at least due to the process of lockdown.

Impact on mental health

India registered the first case of COVID-19 in Kerala in January 2020; the numbers have been rising regularly. In India and several nations, the devastating pandemic lead to an alarming state of national lockdown.[8,9] Preventive interventions such as social isolation and quarantine suggested by WHO affect mental health.[2,10] Psychological depression is caused by abrupt changes in everyday life, leading to lockouts and extended isolation.[8] Fatigue, nausea, exhaustion, insomnia, and social rejection are the principal mental health conditions associated with the COVID-19 pandemic.[5] Particularly, exhaustion, anxiety, and stress go hand in glove with the pandemic.[12,13] Thus, COVID-19 can contribute to an increased suicide risk; strict lockout rules, social distancing, travel limits could lead to a state of fear and anxiety, often leading to depression, which is not yet explicitly studied. However, studies into the prevalence of psychiatric morbidities by general individuals in the pandemic, health care staff and COVID-19 patients estimated that half of the population experienced psychological impacts related to pandemics.[14]

The most frequently recorded conditions were poor quality of sleep, depression and psychological distress. It is found that some groups of the population, such as women, infants and the elderly, are at greater risk of complications when a stressful accident occurs.[15] Social encouragement and cold reactions often influence increased mental health conditions. Health personnels, older adults, and pregnant women may feel more frustrated.[16] As it is,

Fig 1. The different stages of flow of people or/and patients.

it can be challenging for children and teenagers to stay at home permanently without personal meetings with peers that make them feel agitated and bored.[17] Sudden travel bans triggered during severe pandemics have initiated panic among internal migrant workers in India. They were expected to migrate several thousand miles to reach their respective indigenous locations under this condition and be back to work. The condition for most citizens causing evident psychological trauma has been worsened by significant work reductions, unemployment, pay reduction, and the increasing economic downturn.[18] Lockdown has facilitated the excessive use of social media and multimedia channels to disrupt sleep habits and negatively affect mental wellbeing from the tendency of having updated news. Notably, a 20% rise in mental illness cases since the Indian Psychiatry Society was reported in the pandemic in Lancet 2020.[19] Patients with pre-existing psychological disorders such as, depression or anxiety or a history of abuse may be more at risk of discomfort during these severe times.[20]

Quarantine is increasingly taking a toll on people as a stringent attempt to contain the transmission of the infection; eventually, life under quarantine has an acute impact on mental health for disrupted of everyday life from the quarantine. This affected individuals to lose control of their lives, in general. Another leading factor to depression is social self-rejection from fearful persons engaging with cured or suspicious patients. Thus, acute fear and depression for a comparatively longer-term are correlated with the failure to activate social networks.

Strategies undertaken

Indeed, the frontline staff is credited for the suitable attempts to minimize the number of COVID-19 incidents.[21] Hydroxychloroquine could be deliberated under strict medical monitoring for individuals with high-risk characteristics for other severe diseases.[22] Another strategy of treatment practiced in India is plasma therapy. The App in smartphones, 'Arogya Setu', helps to inform people in their vicinity about the COVID-19 condition, and self-assessments are available to individual personal health.[16] So far, the Indian Government has approved emergency usage for the *Covishield* of Serum Institute of India and *Covaxin* of Bharat Biotech. There are many ways of alleviating boredom by remaining busy with the mobile, keeping oneself occupied with daily activities and re-discovering hobbies and interests. Often the beneficial pursuits are healthy behaviors, dietary habits and suitable hobbies. Furthermore, common in Indian households, usages of ayurvedic herbs and herbal drugs such as, *Ayurvedic* preparations of cinnamon, *Tulsi*, turmeric, daily 'yoga and meditation' simultaneously boost immunity, which can help ease the generated stress and anxiety.[23,24] Throughout these challenging days, stress control, counselling and communication are vital for public life.

CONCLUSION

The present COVID-19 war is a challenging in testing ones emotional ability, patience and stamina. Mainly, Indians are accustomed to struggles and sacrifices from a very early age. The public must be aware of the common psychological effects of a pandemic through special TV programmes, as it can take months for the impact of COVID-19 on the slow-creeping mental wellbeing to become completely evident. To control its effects, it needs collaborative contributions from psychiatrists and the health care system, as well. India is a land of spirituality and yoga. It has a great legacy of traditional mindfulness of meditative practices and traditional medicines in the banner of *Ayrvedic* products; nonetheless, each country has own developments of age-old traditional medicines. Such practices have been known to reduce psychiatric disorders; those will prove too beneficial significantly and support integrating meditation for improved mental and physical wellbeing into our everyday regimen. Maintaining a constructive mindset, coping methods and recognizing the issue-point would help solve the mental health challenges in this hour of crisis.

Additionally, the stress due could ramify other related problems, family responsibilities, personal health concens therefrom, health problems affecting other family members and the overall family economy. The struggle when becomes related to economy in most countries gets involved with males, indeed.

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Cardiovascular Presentation in Pheochromocytoma: What We Should be Aware

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ABSTRACT

Pheochromocytoma is a catecholamine-producing tumor that although being a rare disease, it poses diagnostic problems because its clinical presentation often mimics certain diseases, including cardiovascular disorders. The effects of excessive catecholamine secretion cause a variety of cardiovascular presentations ranging from hypertension to life-threatening cases such as hypertensive emergency, shock, supraventricular or ventricular arrhythmias, pulmonary edema, and acute coronary syndromes. The principal medical treatment for pheochromocytoma is a blockade of adrenergic receptors. However, surgical or tumor resection often provides complete resolution of abnormal myocardial dysfunction or arrhythmias, so this approach remains the mainstay of treatment that should be performed as soon as the diagnosis of pheochromocytoma is established. As clinicians, we must be aware of the characteristics of the cardiovascular manifestations of pheochromocytoma to make an earlier diagnosis and more appropriate management.

Keywords: Pheochromocytoma; cardiovascular manifestations; endocrine heart disease (Siriraj Med J 2022; 74: 68-74)

INTRODUCTION

Pheochromocytoma is derived from the Greek "Phios" which means black, "Chroma" which means colour, and "Cytoma" which means tumor. Pheochromocytoma refers to the blackish-brown colour of tumor cells when stained with chromium salts.[1] These tumors usually originate from the adrenal glands, with a triad of clinical symptoms; headache, palpitations, and diaphoresis accompanied by paroxysmal hypertension.[2] Pheochromocytoma is a rare disease, with an estimated prevalence is 0.1-0.6%. The incidence of new cases is 2-8 cases per 1 million people. These tumors are often benign, with a malignancy prevalence of only 10% of all patients with pheochromocytoma.[3] The median age at diagnosis is 40 years.[4]

Pheochromocytoma is a functional tumor derived from chromaffin cells of the adrenal medulla and paragangliomas. Chromaffin cells secrete catecholamines, such as adrenaline (epinephrine), norepinephrine, and dopamine. Most pheochromocytomas secrete mainly norepinephrine, and only about 15% secrete epinephrine.[1] In 20% of cases, chromaffin cells grow outside their normal locations in the adrenal glands, such as in the organs of Zuckerkandl (75%), thorax, mediastinum, abdomen, and pelvis. 70% of pheochromocytomas that grow outside the adrenal glands and 5% inside the adrenal glands are malignant. The dopamine beta-hydroxylase enzyme, which converts dopamine to norepinephrine, is absent in immature tumors. So, this is why dopamine-secreting tumors have

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a higher probability of malignancy.[1] Many other factors can cause pheochromocytoma. In most cases, genetic and environmental factors play a significant role. 25-33% of pheochromocytomas are due to familial factors. Mutations in the VHL, RET, NF1, SDHB, and SDHD genes are all known to cause familial pheochromocytoma or extra-adrenal paraganglioma.[5]

MATERIALS AND METHODS

We conducted an explanatory review to summarize evidence from literatures focusing on cardiovascular presentation and principal management of pheochromocytoma. We included peer-reviewed articles published from 2004 to 2020 on PubMed, EMBASE, The Cochrane Library, and Google Scholar.

General clinical presentation and principal management

The pheochromocytoma clinical presentation is varied, so it is called "The Great Masquerader". The typical classic triad of symptoms are palpitations, headache, and diaphoresis. The presence of these three clinical manifestations accompanied by hypertension often leads to the diagnosis of pheochromocytoma. However, pheochromocytoma can be asymptomatic for many years.[1] In addition to the classic triad, other symptoms that patients with pheochromocytoma often complain of is anxiety, tightness, chest pain, abdominal or low back pain, nausea and vomiting, tremors, flushing, dizziness, blurred eyes, and paresthesias. A catecholamine crisis can also lead to heart failure, pulmonary edema, arrhythmias, and intracranial haemorrhage.[6] When we suspect pheochromocytoma, additional examination such as urine or plasma metanephrine levels and imaging studies such as computed tomography (CT) scan and magnetic resonance imaging (MRI) are needed.

The mainstay of treatment for pheochromocytoma is definitive surgery such as total or partial adrenalectomy. However, specific therapy for each cardiovascular manifestation, especially emergencies, is essential for life-saving efforts while waiting for definitive surgery preparation. Preoperative preparation is crucial, including blood pressure maintained below 160/90 mmHg before surgery. Classically, blood pressure is controlled with alpha-blockers (phenoxybenzamine 0.5-4 mg/kg body weight (BW)). Prazosin, terazosin, and doxazosin can be used as alternatives to short-acting alpha-blockers. Cardio-selective beta-blockers such as metoprolol and atenolol can be used once adequate alpha-blocker effects have been achieved. Antihypertensives such as calcium channel blockers or angiotensin-converting enzyme (ACE) inhibitors can also be used effectively.[1] Minimal invasive techniques (laparoscopy or retroperitoneoscopy) have become the standard approach to pheochromocytoma surgery due to fewer complications and faster healing than open surgery.[6] Compared to open surgery, minimal invasive techniques only need a small incision, which is related to better cosmetic results.[6]

Cardiovascular presentation of pheochromocytoma

Cardiovascular manifestations in pheochromocytoma are range from mild to life-threatening emergencies due to the effects of catecholamine excess[7] (Fig 1).

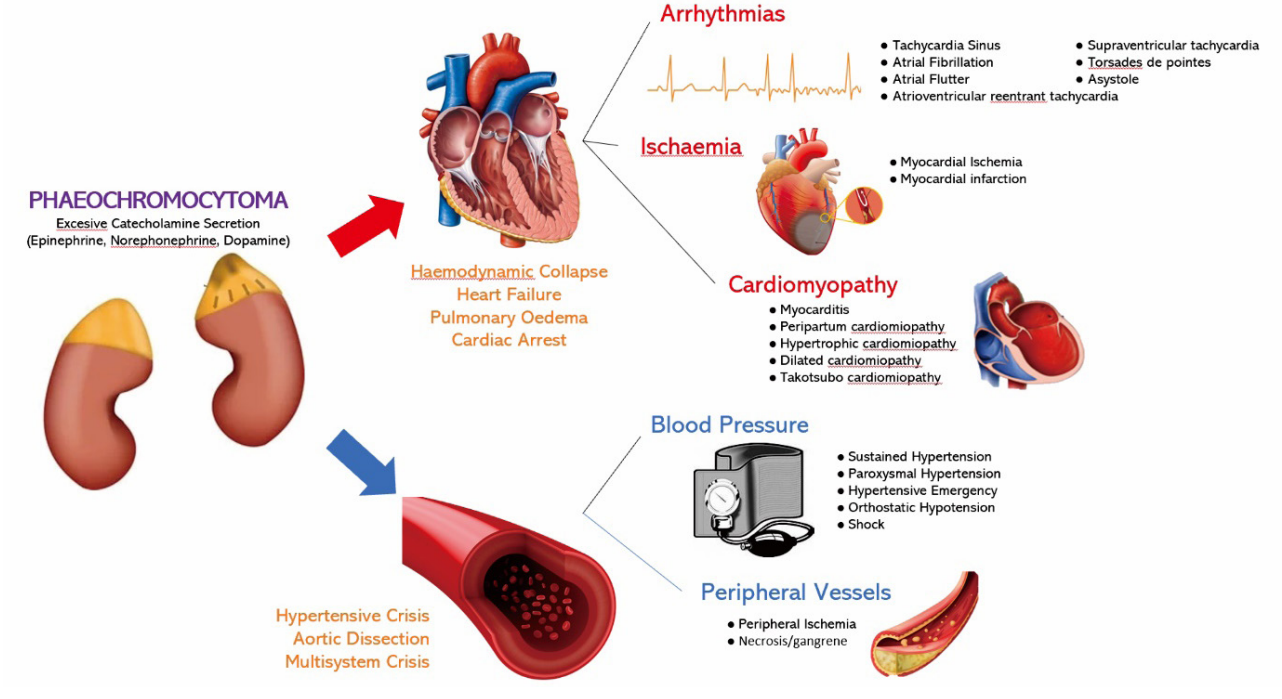

Fig 1. Cardiovascular Presentations of Phaeochromocytoma.

Hypertension in pheochromocytoma

Approximately 90% of patients with pheochromocytoma have paroxysmal hypertension or sustained hypertension. Hypertension in pheochromocytoma is usually characterized by high peripheral resistance and a low cardiac index. Noradrenaline increases peripheral vascular resistance, which increases systolic blood pressure (SBP) and diastolic blood pressure (DBP). While adrenaline increases cardiac output and SBP, it does not affect DBP due to its stimulatory effect on two adrenergic receptors causing peripheral vasodilation. In adrenaline-secreting pheochromocytoma, episodic symptoms often appear as palpitations, headache, syncope, anxiety, and hyperglycemia due to over-stimulation of receptors. Meanwhile, noradrenaline-producing tumors are often characterized by sustained hypertension due to the effects of excess catecholamines that stimulate alpha receptors. Tumors that predominantly secrete dopamine often result in normotension or even hypotension.[7]

Sustained hypertension is usually associated with structural abnormalities of the arteries, but it is still debated whether structural changes in blood vessels cause hypertension or vice versa. An increase in the media-lumen ratio results from vascular remodelling. Endothelial function abnormalities are also found in patients with pheochromocytoma, although this is also present in other causes of secondary hypertension.[7]

Angiotensin receptor blockers, calcium channel blockers, β-blockers, and α blockers have all been used for blood pressure management, even though there is no consensus on preferred medicines. For patients planning to have tumor resection surgery, initial treatment with α-blockers 10-14 days before surgery can be given. After achieving adequate α-blockade, the patient can be treated with β-blockers to achieve heart rate control. Phenoxybenzamine is a preferred α-blocker. It is started with an initial dose of 10mg twice daily and increased by 10-20mg every third day.[8]

Orthostatic hypotension in pheochromocytoma

Orthostatic hypotension often accompanies persistent hypertension, occurring in 70% of patients with pheochromocytoma.[9] Orthostatic hypotension in untreated hypertension individuals should be regarded as a pheochromocytoma diagnostic indication. Most are asymptomatic and rarely lead to syncope. Although hypotension usually occurs only in patients with a dominant adrenaline or dopamine secretion pheochromocytoma, but it can also occur in noradrenaline secretion.[10] Impaired vasoconstrictor response to catecholamines, hypovolemia, desensitization of alpha-adrenergic receptors, and feedback inhibition of sympathetic noradrenaline released by sympathetic inhibition or stimulation of presynaptic alpha-2 adrenergic receptors are all possibilities for orthostatic hypotension.[7]

Management of orthostatic hypotension in patients with pheochromocytoma should focus on efforts to restore volume. Steroid administration is often associated with fatal side effects, so there is no indication for mineralocorticoid administration in patients with orthostatic hypotension.[10] Before surgical therapy, the restoration of blood volume must be maintained so that there is no decrease in blood volume leading to hypotension and shock. Pheochromocytoma should be suspected when the unexplained vascular collapse is associated with significant abdominal pain, pulmonary edema, leukocytosis, mydriasis unresponsive to light stimulation, cyanosis, diaphoresis, and hyperglycemia. Hypotension can occur in patients with an acute myocardial infarction or acute cardiomyopathy. Shock in patients with pheochromocytoma can also be triggered by certain drugs such as steroid hormones, dopamine receptor antagonists, and tricyclics.[10]

Arrhythmia in pheochromocytoma

In patients with pheochromocytoma, excessive amounts of catecholamines can stimulate beta-adrenergic receptors and trigger mild or severe arrhythmias. However, many factors will trigger this condition. In humans and mice with pheochromocytoma, prolonged catecholamine stimulation decreased receptor density and induced cardiovascular adrenergic receptor desensitization. Reflex bradycardia and nodal escape rhythms have been reported during hypertensive emergencies and episodes of increased vagal tone.[7] Palpitations are reported in 50-70% of pheochromocytoma patients. Pheochromocytoma can cause various arrhythmias, including supraventricular tachycardia, atrial fibrillation or flutter, ventricular fibrillation, atrioventricular reentrant tachycardia, torsades de pointes, and sinus arrest.[9] Patients with paroxysmal or recurrent arrhythmias accompanied by diaphoresis, hypertension, anxiety or pallor, should be suspected of having a diagnosis of pheochromocytoma.[10]

Intravenous esmolol, a short-acting cardio-selective beta-blocker, can be used to control the rate of atrial fibrillation or atrial flutter at a dose of 0.5 mg/kg intravenously over 1 minute followed by an intravenous infusion of 0.1-0.3 mg/kg per minute. However, it is necessary to give an alpha-blocking agent before using a beta-blocker so that there is no excessive stimulation of alpha receptors resulting in a hypertensive emergency.[7]

Myocardial ischemia and myocardial infarction in pheochromocytoma

Only a minority of patients with pheochromocytoma have symptoms related to myocardial ischemia or myocardial infarction. Recent clinical studies have found an association between pheochromocytoma and myocardial infarction. In some cases, myocardial damage is documented on electrocardiogram (ECG), echocardiography, and angiography. In cases for which angiographic data were obtained, less than half of patients had significant coronary atherosclerosis and classic coronary risk factors. In these patients, the excess amount of catecholamines secreted by the tumor exacerbates the course of myocardial infarction. Meanwhile, in cases without significant coronary atherosclerosis, myocardial infarction is caused by catecholamines' direct toxic effects, which cause necrosis and fibrosis of myocardial cells. High catecholamine levels cause ischemia by increasing myocardial oxygen consumption, disrupting the hemodynamic balance of myocardial supply and demand, and rising afterload.[5]

Patients can present with chest pain, tachycardia, diaphoresis, anxiety accompanied by an ECG appearance of T waves, hyperacute T, diffuse low voltage, and other nonspecific ECG changes. One essential ECG change in pheochromocytoma is repolarization ECG changes associated with QT interval prolongation.[11] It happened due to the effects of catecholamines that stimulate coronary artery vasoconstriction and simultaneously increase myocardial oxygen demand through stimulation of heart rate and cardiac contractility, resulting in ECG abnormalities without coronary atherosclerosis. However, the clinician could find cardiac marker elevation.[9,12]

Patients with symptoms and indications of an acute myocardial infarction will often be treated with β-adrenergic blockade. However, suppression of β-adrenergic mediated vasodilation in skeletal muscle causes paradoxically higher blood pressure owing to unopposed α-adrenergic receptor activation, exacerbating the patient's condition.[9] Combination of these two drugs is recommended

Cardiomyopathy and myocardial hypertrophy in pheochromocytoma

Phaeochromocytoma can cause cardiomyopathies, such as hypertrophic, dilated, takotsubo, and peripartum cardiomyopathy. Several mechanisms may explain myocardial damage associated with catecholamines. Catecholamines can directly affect the myocardium by increasing sarcolemmal permeability, lipid mobility, free radical production, or calcium overload. Myocardial damage can occur secondary to a persistent increase in cardiac oxygen demand along with decreased oxygen availability due to the effects of catecholamines that trigger coronary vasoconstriction and platelet aggregation.[7]

High levels of catecholamines in the blood released by tumors can trigger myocarditis and cardiomyopathy. Myocarditis has previously been reported in patients with pheochromocytoma. In a case report of 26 patients at the Mayo Clinic who died of pheochromocytoma complications, 58% were diagnosed with active myocarditis. The pathological changes of active myocarditis are similar to the lesions found in the myocardium of animals treated with catecholamine injections in several laboratories.[9]

High levels of catecholamines in the blood can cause dilated or hypertrophic cardiomyopathy. Patients with pheochromocytoma and heart failure have atypical symptoms that are difficult to distinguish from idiopathic dilated cardiomyopathy. Patients with advanced dilated cardiomyopathy who will undergo heart transplantation should be excluded from causes of reversible cardiomyopathy, including pheochromocytoma. Several patients who had undergone successful heart transplantation then later diagnosed with pheochromocytoma have indistinguishable clinical symptoms compared to dilated cardiomyopathy.[13]

Takotsubo cardiomyopathy is a condition of patients with signs and symptoms of acute myocardial infarction without coronary artery stenosis or spasm with the appearance of the heart in the form of a Japanese octopus fishing pot, called "Takotsubo". The rounded apex of the heart indicates the effect of local toxic concentrations of catecholamines in the absence of coronary artery disease. High concentrations of noradrenaline stimulate basal hyperkinesis, increase mechanical stress on the apex wall and end-diastolic pressure. Another patient with pheochromocytoma-induced cardiomyopathy has severe left ventricular dysfunction, basal segment akinetic, midventricular, and apical hyperkinetic. This percentage is called "Inverted Takotsubo Cardiomyopathy". Therefore, there is no definite pattern of ventricular dysfunction in patients with pheochromocytoma-induced Takotsubo cardiomyopathy.[7] Treatment of pheochromocytoma-induced-cardiomyopathy is primarily supportive care until definitive surgical therapy can be performed.[9]

Peripheral ischemia in pheochromocytoma

Although very rare, pheochromocytoma can result in peripheral ischemia and vasculopathy that results in necrosis or gangrene. This complication occurs as a result of severe vasoconstriction or diffuse arterial vasospasm induced by hypercatecholaminemia. Ischemia can also occur due to arterial occlusion of cardiac thrombus embolism in patients with catecholamine-induced arrhythmias,

but this is extremely rare.[6] The patient can have a similar presentation with peripheral artery disease, complaining about claudication or limb pallor.[9]

Early detection of these situations is critical to prevent vasoconstrictive medicine, which can exacerbate distal ischemia in both the upper and lower limbs. In the case of pheochromocytoma induced acute limb ischemia (ALI), treatment of choice includes an open surgical or using catheter direct thrombectomy (CDT). Early trials show successful CDT in 70% of cases.[14]

Life-threatening cardiac manifestations

In some cases of pheochromocytoma, the cardiac presentation that appears can be life-threatening and cause death. The most common life-threatening cardiac manifestation of pheochromocytoma is a hypertensive emergency due to the rapid and excessive release of catecholamines from the tumor. Malignant arrhythmias, shock, aortic dissection, and acute heart failure due to myocardial dysfunction are less common.[15]

Hypertensive emergency in pheochromocytoma

An increase in systolic blood pressure in patients with pheochromocytoma can reach a very high and dangerous value above 200 mmHg. This situation becomes a life-threatening hypertensive emergency when accompanied by acute target organ damage. Hypertensive emergencies can also result from using certain drugs in patients with pheochromocytoma, such as beta-blockers that are not accompanied by adequate use of alpha-blockers. Hypertensive crises can happen in 75% of patients with pheochromocytoma as often as once weekly. Symptoms of a hypertensive crisis may vary, such as headache, confusion, visual disturbances, or tachycardia. This condition can cause organ damage, such as acute myocardial infarction, congestive heart failure, or cerebrovascular disorders.[9] Intravenous phentolamine is the treatment choice for a hypertensive emergency in patients with pheochromocytoma. The drug is usually given in an intravenous bolus dose of 2.5-5 mg at a 1 mg/min rate. This dose should be repeated every 3-5 minutes until hypertension is controlled because of the short half-life of phentolamine. Phentolamine can also be given by continuous infusion. However, due to the availability of other drugs with safer pharmacokinetic profiles, phentolamine is no longer used as a hypertensive emergency treatment during surgical anaesthesia. Intravenous vasodilators such as nicardipine, sodium nitroprusside, nitroglycerin, and fenoldopam provide more effective short-term control in intraoperative hypertension. It is easier to titrate, has a shorter duration of action, and can be used alone or with other vasodilators. Another alternative therapy is urapidil, a selective alpha1 adrenergic receptor antagonist that can be given as an intravenous bolus dose (25-50 mg) or accompanied by an infusion (50-100 mg/kg per minute).[7]

Malignant arrhythmia in pheochromocytoma

Although rare, pheochromocytoma can result in QT prolongation, even malignant arrhythmias such as ventricular tachycardia.[16] Several recent case reports have described cases of ventricular tachycardia accompanying a patient with suspected acute myocardial infarction but no coronary artery stenosis on angiography. The more common arrhythmias in pheochromocytoma are sinus tachycardia, atrial fibrillation, and atrial flutter.[7]

Bradycardia has been reported in 10% of patients with pheochromocytoma due to noradrenaline secretion. Episodes of nodal rhythm, altering sinus intervals, giving rise to ectopic heartbeats, both atrial and ventricular. Other mechanisms, such as vagal increase by the baroreceptor reflex due to a sudden increase in arterial pressure and desensitization of adrenergic receptors, can also cause bradycardia, mainly found in adrenaline-secreting pheochromocytoma.[12]

Sinus arrest due to catecholamine release, often accompanied by nodal escape rhythms, has been reported in several case reports. On the other hand, atrioventricular dissociation associated with pheochromocytoma only occurred in a few cases.[17] Unfortunately, the diagnosis of pheochromocytoma leading to sinus arrest, AV dissociation, supraventricular and ventricular arrhythmias are often delayed. The patient has had a pacemaker inserted and even ablated his bundle. Tumor resection therapy can solve all cases without the need for a pacemaker or ablation.[12]

Several cases of pheochromocytoma have complications of malignant arrhythmias, namely life-threatening ventricular tachycardia, such as a young woman from Beijing who complained of the classic triad of pheochromocytoma with recurrent ventricular tachycardia in the course of the disease.[18] Paulin et al., suggested that the sudden release of catecholamines from pheochromocytoma is related to ventricular tachycardia (VT) mechanism.[19] Excess catecholamines cause abnormal electrical activity of the myocardium, such as the excessive opening of ion channels and the increased function of the ion exchange pump, which causes large amounts of sodium, potassium, and calcium to enter through the membrane in automatic, intensive myocardial conductivity. In addition, excess catecholamines can reduce the threshold for ventricular fibrillation, resulting in sudden death or

abnormal repolarization that underlies various types of rapid arrhythmias.[12]

Sustained ventricular tachycardia in pheochromocytoma can be treated by ablation. However, such therapy cannot stop the systemic arrhythmogenic mechanisms due to the effects of catecholamines, so tachyarrhythmias will often recur. A Pheochromocytoma should be considered a diagnosis in a hypertensive patient with a typical clinical presentation of palpitations and headaches with an ECG showing VT or other tachyarrhythmias. Biochemical and imaging tests will guide the diagnosis, and symptoms often have a complete resolution after tumor surgery.[20] Preoperative use of phentolamine may be considered. Beta-blockers, such as propranolol, can be used in catecholamine-induced tachyarrhythmias but should be started after adequate doses of alpha-blockers administration to prevent hypertensive emergencies.[12]

Shock in pheochromocytoma

Sudden cessation of catecholamine secretion in patients with inadequate circulating volume, and desensitization of adrenoreceptors due to prolonged exposure to catecholamine-induced hypertension, are the main mechanisms that explain shock in patients pheochromocytoma.[10] Hemorrhagic necrosis of catecholamine-producing tumors are also associated with severe shock. Another less well-known mechanism is shock due to the negative inotropic effect of hypocalcemia. Shock in patients with pheochromocytoma is often responsive to volume addition.[7]

CONCLUSION

Cardiovascular presentation of pheochromocytoma varies widely, ranging from mild to life-threatening abnormalities requiring immediate action, such as hypertensive emergencies, malignant arrhythmias including VT and ventricular fibrillation, shock, and acute heart failure or pulmonary edema. Therefore, it is necessary to consider pheochromocytoma diagnosis in patients with cardiovascular presentations accompanied by unstable blood pressure and/or the classic triad of symptoms, namely headache, palpitations, and diaphoresis.

Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Authors' contributions

RA has given substantial contributions to the conception of the design of the manuscript. RA and IPD were major contributors in writing the manuscript. IPD and LFKW are editing the manuscript for publications. All authors have participated in drafting the manuscript, BSP revised it critically. All authors read and approved the final version of the manuscript.

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