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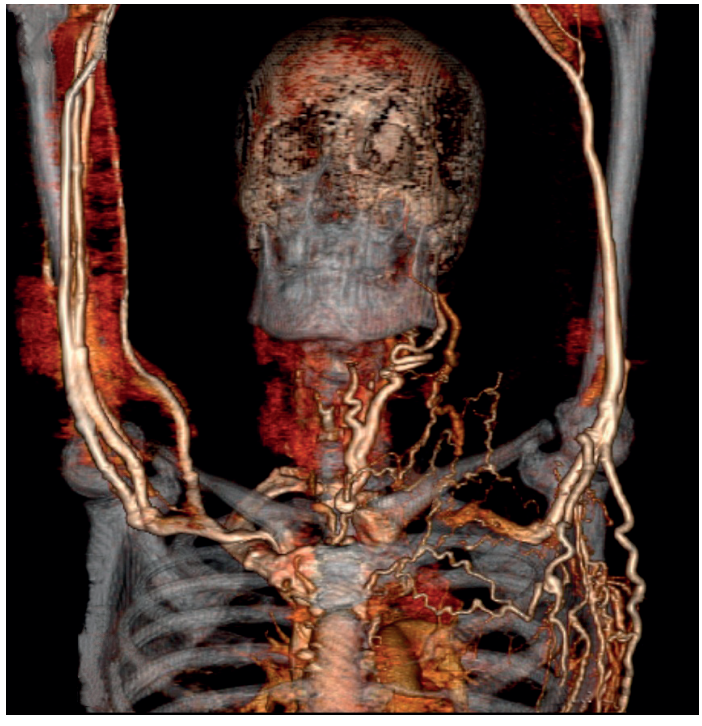

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Prevalence and Pattern of Diseases of The Skin and Subcutaneous Tissue in A Primary Care Area in Thailand

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ABSTRACT

Objective: Information on the population-based prevalence study of skin diseases is still lacking. The study explores the prevalence and pattern of diagnosed skin diseases of the population in a primary care area of a university hospital in Thailand.

Materials and Methods: Skin disease patients were identified using the International Statistical Classification of Diseases and Related Health Problems 10th Revision codes (L00-L99). Retrospective data were obtained from the hospital electronic medical record between 2015-2019. The number of clinic visits and the number of skin disease diagnoses were counted. The five-year period prevalence was calculated by dividing the number of cases by the population in the primary care area.

Results: During the five-year period, in a population of 29,969, we found 3,770 patients (12.6% of 29,969 population) who made 7,433 outpatient visits with the diagnoses of skin diseases. Infections of the skin and subcutaneous tissues were the most common (37.3%), followed by dermatitis (29.7%), urticaria and erythema (13.9%), other disorders of the skin and subcutaneous tissue (8.6%), and papulosquamous disorders (1.7%). The five-year period prevalence of skin diseases per 100,000 persons was as following: cellulitis (2,296), urticaria (1,682), psoriasis (177), atopic dermatitis (420), seborrheic dermatitis (227), alopecia areata (50), vitiligo (23), and pemphigus (10).

Conclusion: Infection and dermatitis were the two most common skin diseases in the primary care area population. Atopic dermatitis, psoriasis, seborrheic dermatitis, and decubitus ulcer were less commonly found. Our prevalence data should be the "at least" prevalence of skin diseases due to possible underreporting.

Keywords: Prevalence; skin disease; Thailand (Siriraj Med J 2021; 73: 357-362)

INTRODUCTION

Skin diseases are one of the most common presentations clinicians encountered in the daily practice. They are accounted for approximately 10.0% of a general practitioner's workload and 6.0% of outpatient referrals.[1] Skin conditions contributed 1.8% to the global burden of disease measured in Disability-Adjusted Life Year from 306 diseases and injuries in 2013. Excluding mortality, skin diseases were the fourth leading cause of disability worldwide.[2]

There is still a lack of information on the population-based prevalence study of skin diseases. Most studies on the prevalence of skin disease report the percentage of various skin diseases and use the medical record data from mostly the tertiary care hospital which may not reflect the population they serve, therefore the prevalence

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of skin disease in the population could not be accurately calculated.

Thai national health insurance was overseen by three different schemes: (i) the civil servants' medical benefit scheme (ii) the social security scheme and (iii) the universal coverage health scheme (72.0% of the population).[3] Naresuan university hospital provided health care for patients from all health scheme and also had their primary care area covering five subdistricts surrounding the hospital. The population in these five subdistricts in year 2017 was 46,474. Among 46,474, there were 29,969 who were registered under tax-funded universal coverage health scheme. This study aimed to explore the prevalence of skin disease in the population of 29,969 in the primary care area of the hospital whom were registered under the universal coverage health scheme.

MATERIALS AND METHODS

We conducted a retrospective study using the hospital electronic medical record database. All visits of skin diseases diagnosed with the International Statistical Classification of Diseases and Related Health Problems 10th Revision code for Diseases of the skin and subcutaneous tissue (L00-L99) from January 2015 to December 2019 were collected. The given diagnosis, sex, age, date of diagnosis, address, and health scheme were recorded for each patient visit. At the date of the first recorded diagnosis, individuals were considered as prevalent cases from that date onward. A patient who was seen for the same diagnosis during the five-year period was counted as one case, regardless of the number of visits. On the other hand, patients who received more than one skin disease diagnosis were considered and counted as separate cases for each of the diagnosis. The five-year period prevalence per 100,000 persons of various skin diseases was calculated by dividing the accumulated number of diagnosis cases from 2015 to 2019 with the 2017 year's population (29,969 persons) which is in the median of the five years. Visits and cases of patients from other health schemes were also analyzed and compared with the universal coverage health scheme patients in the primary care area. Statistical analysis was performed using Stata, version 9.0 IC (Stata Corp LP, College Station, TX, USA). This study was approved by the institutional ethics review board with an IRB no. P3-007/2020.

RESULTS

During the five-year period (2015-2019), out of 29,969 population living within the service areas and registering with the universal coverage health scheme, there were 3,770 cases and 7,433 visits with the diagnosis of diseases of the skin and subcutaneous tissue (L00-L99). The five-year period prevalence of skin disease (L00-L99) was 12,579 per 100,000 persons (3,770 x 100,000/29,969) or 12.6 percent for these population. Forty-six percent of the patients were male. The mean, median, and interquartile range of age were 33 years, 30 years, and 7-58 years, respectively.

The frequency of skin disease diagnosis cases was shown in Table 1. Infections of the skin and subcutaneous tissues were the most common skin diseases (36.2%), followed by dermatitis and eczema (31.3%), urticaria and erythema (13.5%), other disorders of the skin and subcutaneous tissue (8.6%), disorders of skin appendages (8.3%) and papulosquamous disorders (1.7%). Papulosquamous disorder patients and bullous disorder patients had a high number of visits per case with 6 visits per case and 9 visits per case, respectively.

A list of the prevalence of some selected skin disorders is presented in Table 2. Dermatitis diagnosis was the most common (19.6% of all skin diseases), followed by cellulitis (18.2%) and urticaria (13.4%). Alopecia areata, vitiligo, lupus erythematosus, lichen planus, and pemphigus were more commonly seen in female than male patients.

Fig 1 shows the distribution of age of patients with various skin diseases. Fig 2 shows the seasonal variation of skin disease with cellulitis becoming more common during the rainy season.

The data of skin disease patients from other health schemes who visited the hospital during the same period was also analyzed. There were 14,233 cases (28,977 visits) which consist of dermatitis (35.8%), infections of the skin and subcutaneous tissues (21.0%), urticaria and erythema (16.2%), disorders of skin appendages (10.0%), papulosquamous disorders (2.9%), bullous disorder (0.7%), radiation-related disorder (0.7%), and other disorder (12.6%).

DISCUSSION

In our study, the five-year period prevalence of diseases of the skin and subcutaneous tissue skin diseases (ICD10 L00-L99) in the primary care area population was 12,579 per 100,000 persons which translated into approximately twelve percent of the population affected by any skin diseases during the five-year period. Infections of the skin and subcutaneous tissues (36.2%), dermatitis (31.3%), and urticaria (13.5%) were among the most common skin diseases. A study from a hospital in Iran, infections of the skin and subcutaneous tissues were found to be the most common skin diseases (32.1%),

TABLE 1. Frequency of diagnosed skin disease groups of population in the primary care area.

ICD-10 code	ICD-10 description	Visits	%	Cases	%	Prevalence*
L00-L08	Infections of the skin	2,382	32.4	1,366	36.2	4,560
L10-L14	Bullous disorder	89	1.2	10	0.3	30
L20-L30	Dermatitis	2,070	27.8	1,180	31.3	3,940
L40-L45	Papulosquamous	395	5.3	63	1.7	210
L50-L54	Urticaria and erythema	905	12.2	508	13.5	1,700
L55-L59	Radiation-related disorders	9	0.1	7	0.2	20
L60-L78	Disorders of skin appendages	543	7.3	312	8.3	1,040
L80-L99	Other disorders**	1,040	14.0	324	8.6	1,080
Total		7,433		3,770		12,579

*five-year period prevalence per 100,000 persons calculated by (number of case / population of 29,969) x 100,000
**L80-L99 including such as vitiligo, seborrheic keratosis, lupus erythematosus, vasculitis, etc. ICD-10= International Statistical Classification of Diseases and Related Health Problems 10th Revision

TABLE 2. Five-year period prevalence of selected skin diseases among universal coverage scheme population in the primary care area.

ICD-10 code	Disease description	Age Mean Median (IQR)	Sex % male	Cases Number	Percent*	Prevalence per 100,000 persons**
L30	Dermatitis	26, 13 (3-51)	44.5	738	19.6	2463
L03	Cellulitis	46, 53(25-65)	48.7	688	18.2	2296
L50	Urticaria	25, 18 {5-42)	42.4	504	13.4	1682
L23-L25	Contact dermatitis	30, 26 (7-49)	43.0	167	4.4	557
L20	Atopic dermatitis	17, 5 (0.6-25)	57.1	126	3.3	420
L40	Psoriasis	48, 52 (37-61)	49.1	53	1.4	177
L89	Decubitus ulcer	69, 73 (60-80)	38.9	49	1.3	164
L21-L22	Seborrheic dermatitis	20, 5 (0.2-38)	51.5	44	1.1	227
L63	Alopecia areata	29, 21 (15-47)	20.0	15	0.4	50
L80	Vitiligo	35, 18 (14-61)	28.6	7	0.2	23
L93	Lupus erythematosus	53, 55 {47-59)	33.3	6	0.2	20
L43	Lichen planus	61, 59 (56-65)	25.0	4	0.1	13
L10	Pemphigus	70, 66 (64-79)	0.0	3	0.1	10

*percent of all skin diseases (ICD-10 L00-L99) cases in primary care area ** five-year period prevalence per 100,000 persons calculated by (number of case/population of 29,969) x 100,000 IQR=interquartile range

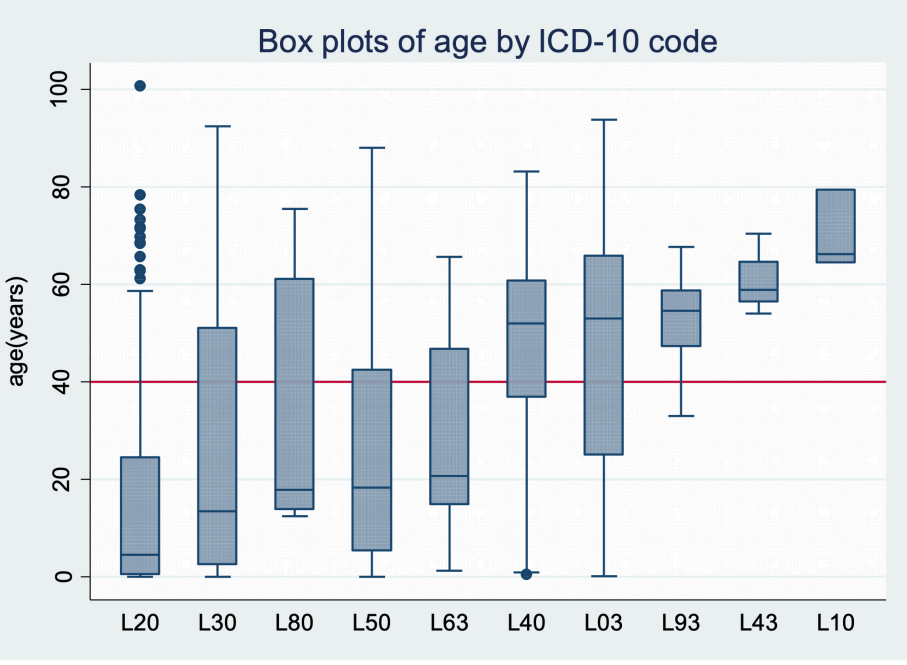

Fig 1. Age at the first visit of skin disease diagnosis (ICD-10 code) in five-year period sorted by the median of age. L20=atopic dermatitis L30=unspecified dermatitis L80=vitiligo L50=urticaria L63=alopecia areata L40=psoriasis L03=Cellulitis L93=lupus erythematosus L43=lichen planus L10=pemphigus

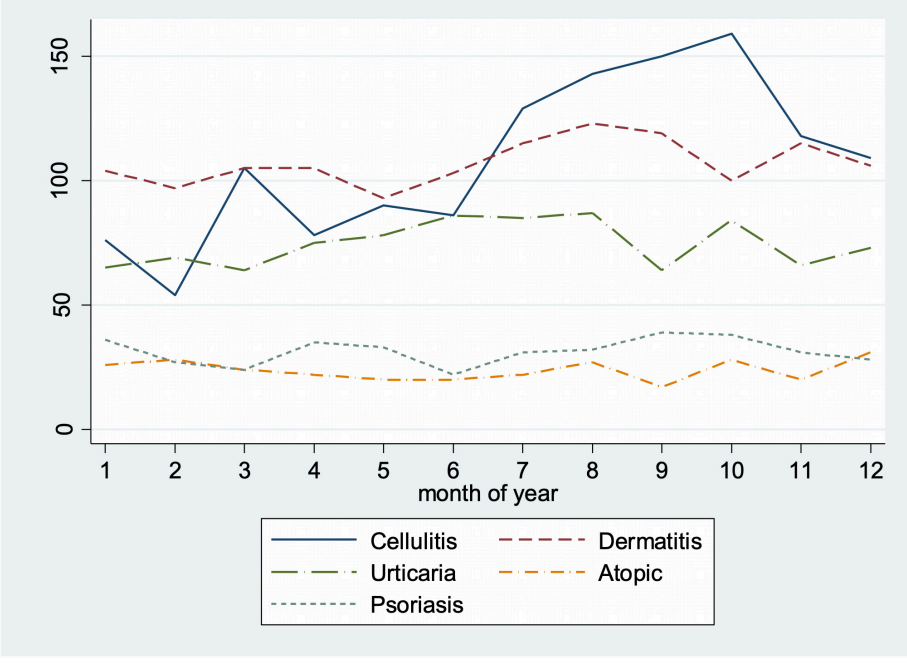

Fig 2. Skin disease visits by the month of the year

followed by dermatitis (24.5%), papulosquamous disorders (3.8%), urticaria and erythema (2.8%).[4] A large hospital network database study from Mexico, a study in a pediatric dermatologic clinic in Thailand, a study in Thai elderly patients showed infections as 32.1%, 21.9, and 21.9% of all skin diseases, respectively.[5,6,7]

The most common skin disease in a questionnaire survey were warts (41.3%), followed by acne (19.2%) and contact dermatitis (15.0%).[8] The global burden of disease study showed 0.4% of burden for dermatitis (atopic, contact, and seborrheic dermatitis), 0.3% for acne vulgaris, 0.2% for psoriasis, 0.2% for urticaria, 0.1% for pyoderma and cellulitis.[2]

In our study, the proportion of patients with bullous disorder and papulosquamous disorder in other health schemes was higher than universal coverage scheme in the primary care area, 2.9% versus 1.7 %, and 0.7% versus 0.3%, respectively. A referral bias might explain the difference between two groups as patients with more complex and serious diseases were more likely to require a referral to our tertiary care hospitals.

Pemphigus, lichen planus, lupus erythematosus, cellulitis, and psoriasis, tend to be the disease of adult and elderly population as the median ages were above 40 years. Where as the median ages in patients with the diagnosis of atopic dermatitis, unspecified dermatitis,

vitiligo, urticaria, and alopecia areata were below 40 years. A study from the United States found that the incidence rates of many skin diseases were affected by age of the population.[9]

In our study, dermatitis was the most common skin problem; however, the terminology is often nonspecific, and may reflect a variety of dermatitis conditions. The prevalence of dermatitis is 2,463 per 100,000 persons. A postal community survey from the United Kingdom found dermatitis in 6,100 per 100,000 persons[10], which is higher than our study which used data from the electronic medical record

The five-year period prevalence of cellulitis was 2,296 per 100,000 persons. The number of cases were higher during the rainy season, which could be related to agricultural activities during the season. The incidence rate of cellulitis in the US was 2,460 per 100, 000 person-years, with a higher incidence among males and individuals aged 45-64 years.[11]

Urticaria prevalence in our study was 1,682 per 100,000 persons. In the studies from South Korea, and Taiwan, the prevalence per 100,000 persons of chronic spontaneous urticaria were 160-450, 690-790, respectively.[12,13] In our study, atopic dermatitis prevalence of all age groups was 420 per 100,000 persons. The School survey study found that the global prevalence per 100,000 persons of atopic dermatitis in children aged 6-7 years was 7900.[14]

The five-year period prevalence per 100,000 persons of psoriasis and seborrheic dermatitis was 177, and 227, respectively. A community study in Turkey showed the prevalence per 100,000 persons of psoriasis in people older than 20 years of age was 1200, and the prevalence of seborrheic dermatitis was 5,200.[15] In the estimation by the Global Psoriasis Atlas website, psoriasis prevalence in Thailand was extrapolated at 0.3% (confidence interval 0.06-1.65) or 300 per 100,000 persons.[16] Whereas seborrheic dermatitis prevalence varies between 2.0% and 12.0% throughout the world.[15]

The five-year period prevalence of alopecia areata is 50 per 100,000 persons. The prevalence of alopecia areata in South Korea was 155 per 100,000 persons.[17] The five-year period prevalence of vitiligo is 20 per 100,000 persons. Previous studies found that the prevalence of vitiligo ranges from 60 to 2,280.[18] The five-year period prevalence of pemphigus is 10 per 100,000 persons. The previous study found standardized point prevalence pemphigus of 5.2 cases per 100,000 adults.[19]

The prevalence of diseases depends on the study design (hospital medical database analysis, cross-sectional survey with medical exam, questionnaire survey), types of measure (point, period, or lifetime prevalence), and case definition (self-reported, physician's, or dermatologist's diagnosis). We chose to report five-year period prevalence which allowed more time to detect more patients, so more accurate data with chronic skin diseases but might mix incident and prevalent case altogether.

The main limitation of our current study is by using the data from the medical record instead of a cross-sectional medical exam survey could lead to an underestimation of the true prevalence. Most five-year prevalence of skin diseases in our study was still lower than prevalence in other previous studies. The prevalence in our study could be underreported from many reasons such as patients with mild diseases might not have sought clinical treatment, some might have tried over-the-counter treatment from local pharmacies, or some may have chosen to go to other health facilities. Our prevalence data should be the "at least" prevalence of skin diseases.

The population included in this study was also relatively small. Our studied population was the universal coverage health scheme which tend to have lower socioeconomic status and educational levels. Skin disease patients were identified using ICD-10 code, L00-L99 (Diseases of the skin, and subcutaneous tissue), which did not include skin tumors, vascular disease such as a venous ulcer, fungal, viral, and parasitic infection.

The strength of the study is the setting of definite population in primary care area of a university tertiary hospital, in which dermatology consultation was highly accessible under the universal health coverage scheme.

CONCLUSION

In conclusion, during a five-year period, 12.6 % of the population in primary care was affected by skin diseases. Among common skin diseases with a five-year period prevalence greater than 1.0% were dermatitis, cellulitis, and urticaria. Atopic dermatitis, psoriasis, seborrheic dermatitis, and decubitus ulcer were less commonly found (prevalence less than 1.0% but more than 0.1%). Vitiligo, alopecia areata, lupus erythematosus, and pemphigus were the least common, less than 0.1%. The finding would help to understand the burden of skin disease for proper healthcare management, proper medical training and disease prevention

Conflict of interest: There are no potential conflicts of interest to declare.

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Job-Seeking Anxiety Resilience and Family Influence on Career Decision-Making among Senior Undergraduate Students in Southern Thailand during the COVID -19 Pandemic

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ABSTRACT

Objective: A study of job-seeking anxiety, resilience quotient and family influence on career decision-making is apparently lacking; especially in regards to the impact of the financial crisis during the COVID-19 pandemic, and other factors, such as the political climate currently in Thailand. This study aimed to determine job-seeking anxiety, resilience quotient and family influence on career decision-making and related factors among senior, undergraduate university students.

Materials and Methods: A cross-sectional descriptive study was conducted in a university in Southern Thailand; from May to October, 2020. Two hundred and seventy-one participants completed questionnaires regarding personal and parental demographic characteristics, resilience, family influence scale, job-seeking self-perception and job-seeking anxiety.

Results: The prevalence of job-seeking anxiety among participants was low (40%), moderate (50%) and high (10%). In univariate analysis, factors significantly associated with job-seeking anxiety were: female gender (p=0.03), effects of the COVID-19 pandemic, economics and the current, political situation Thailand (p=0.03), resilience quotient (p<0.001) and job-seeking self-perception (p<0.001). The predictors of job-seeking anxiety, from multivariate analysis, were: resilience quotient (β = -1.169, P<0.001) and job-seeking self-perception (β = 2.232, P<0.001).

Conclusion: Our study provided evidences that a significant proportion of senior, undergraduate students had experienced job-seeking anxiety, with the protective factor of job-seeking anxiety being individual resilience quotient. This information could be used as an effective management strategy for providing mental health promotion, and prevention for senior, undergraduate university students.

Keywords: COVID-19; family influence; job-seeking anxiety; resilience quotient (Siriraj Med J 2021; 73: 363-372)

INTRODUCTION

Anxiety is a normal, individual response when experiences trigger or insecure situations occur, unless it is persistent, overwhelming and unmanageable. Anxiety disorders can cause distress as well as have debilitating impacts on daily activities and mental health.[1] According to a report, issued in 2016 by department of mental health ministry of Public Health of Thailand, it was found that an estimated 140,000 people; above 18 years old, were diagnosed with anxiety disorders.[2]

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The last year of study in university appears to be the most difficult time when students face pressures from both study and career planing. The discribtion of a career is the job or profession that someone does for a long period of their life. Therefore, the concerns about career's and job-seeking can cause some stress and anxiety for senior students[3], in accordance with establishing personal goals and self-identity. Besides job-seeking and self-perception, previous studies have shown that influences by family[4] (i.e., family status, education and parents' expectations) and socio-demographic factors (i.e., gender and academic major) were associated with job-seeking stress, depression and suicide ideation.[5]

Moreover, in 2019 the youth unemployment rates increased rapidly from 3.7 to 3.9 percent[6], in particular because of the impact of the financial crisis caused by the COVID-19 pandemic. The COVID-19 pandemic also can be stress factors for public health actions; such as social distancing, which can increase increase stress and anxiety, due to the impact on temporality closures of some business.

One of the important factors in coping with both stress and hardship is resilience. Resilience quotient (RQ) is defined as: the individual process of adapting using emotional stability, will power and coping skills to recover from trauma, tragedy, threats and stressful circumstances.[7]

Although, the issue of job-seeking has become the most important concern and cause for anxiety for university students, few studies in Thailand about job-seeking anxiety related resilience quotient and family influence on career decision-making have been published; especially during the COVID-19 pandemic. Therefore, the purposes of this research were to examine job-seeking anxiety and to determine the associations between job-seeking anxiety, resilience quotient and family influence on career decision-making among senior, undergraduate students, in Prince of Songkla University, during the COVID-19 pandemic in order to provide mental health promotion and prevention in the future.

MATERIALS AND METHODS

A cross-sectional descriptive study was conducted, using online questionnaires, at the Prince of Songkla University, HatYai Campus, Thailand; from May to October, 2020. The study protocol was approved by the Office of Human Research Ethics Committee (HREC) Prince of Songkla University (REC.63-236-9-2). Action, informed consent was obtained from participants prior to enrollment into the study.

The Student Affairs personnel of Prince of Songkla University, who were instrumental in distributing the online survey using convenience sampling to 584 senior, undergraduate students in Hatyai campus (Fig 1) within 13 faculties; classified into 4 groups 1.) Pure Science; including, the Faculty of Science 2.) Applied Science and Technology; including, the Faculty of Engineering, Faculty of Natural Resources, Faculty of Agro-Industry and Faculty of Environmental Management 3.) Health Sciences; including, Medical Technology, Faculty of Traditional Thai Medicine and the Faculty of Veterinary Science 4.) Social Science and Arts; including, Faculty of Law, Faculty of Economics, Faculty of Liberal Arts, Faculty of Management Sciences, and International college. The inclusion criteria were senior students who were willing to participate in our study, aged above 18, and had enrolled in the Faculty of Veterinery Science, in the academic year of 2015, and in other Faculties in the academic year of 2017; and were able to read and understand Thai. We excluded 313 students, who were unintened for job seeking, suspended, planned for further study (n=233), had job commitments to scholarship (n=29) and informed unwilling to answer (n=51). The total of 271 participants were enrolled into the study.

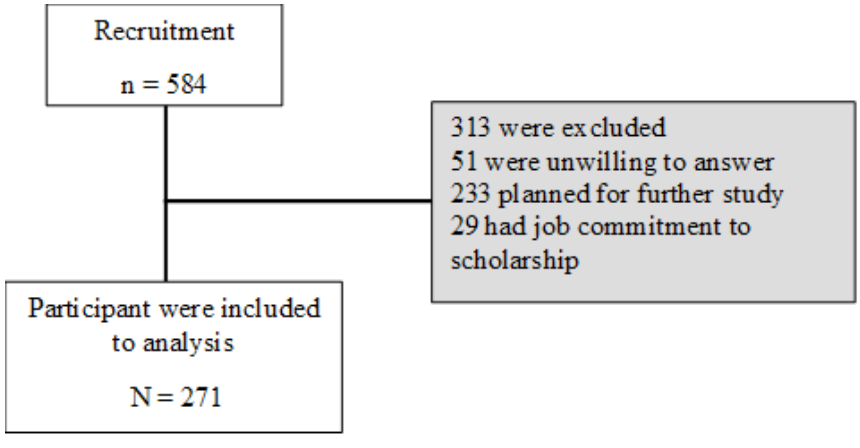

Fig 1. Flow diagram of participant enrolment.

Measurement

All participants completed the seven parts of the online questionnaires: personal demographic data, parental demographic data, the effect of the COVID-19 pandemic, economics and the current political situation in Southern Thailand on job-seeking, Thai resilience questionnaires (Thai-RQ), Family Influence Scale (FIS) for evaluation of familial influence to job-seeking, Job-seeking self-perception questionnaires and Job-seeking anxiety questionnaires, by themselves. All questionnaires were sent online to senior, undergraduate students by the Student Affairs department.

The effect of the COVID-19 pandemic, economics and current political situation in Thailand on job-seeking was used to evaluate the opinion of current conflicts and situations toward job seeking. Which was developed by the research and measured by a question scoring of likert scale; 1 to 5. The scores of 1 or 2 were interpreted as low, 3 as moderate, and 4 or 5 as a high effect. The Item Objective Congruence (IOC) Index for content validity assessment was 1.00.

The 20-item, Thai-RQ[8]., which was developed by the Department of Mental Health, Ministry of Public Health, Thailand, was used to assess personal resiliency consisting of 3 domains: emotional stability, will power and coping skills. Each answer was scored on a scale of 1 to 4. The total scores ranged from 20 to 80. The scores 20-54, 55-69, 70-80 indicated low, normal, and high resilience quotient, respectively. The reliability of the Thai-RQ, assessed by Cronbach's alpha, was 0.75.

Measurement of family influence to job-seeking by Fouad[9] was conducted by the Thai version of the Family influence scale (FIS). This consists of 22 questions; including, 8-items of information support, 5-items on financial support, 4-items of family expectations, and 5-items on values and beliefs. Each answer was scored on a scale of 1 to 5. The family influence level was classified as: low (<27), moderate (27-34), and high (>34) by the total sum. The Cronbach's alpha of FIS was 0.798.[10]

Job-seeking, self-perception questionnaires that were modified from factors associated with anxiety of job application questionnaire composed of 15 items. The reliability of this questionnaire assessed by Cronbach's alpha was 0.886.[3] The questions included 6-items of personal perceptions, 4-items of experience and abilities perceptions, and 5-items on job perceptions. Each answer was scored on a scale of 1 to 5. The scores 15-35, 36-55, 56-75 indicated low, moderate, and high levels, respectively. The Job-seeking anxiety questionnaire, an instrument for measuring severity of job-seeking anxiety, consisted of 25 items; which represented: mood, thoughts, and physical symptom responses. Each answer was scored on a scale of 1 to 5. The scores 25-58, 59-91, 92-125 indicated normal to low, moderate, and high levels of job seeking anxiety, respectively. The Cronbach's alpha of this test was 0.9[3]

Statistical analysis

R software version 3.5.1 was used to perform analysis.[11] Descriptive statistics were preformed using mean and standard deviation (SD). Univariate analyses for associations of independent variables with job-seeking anxiety were analyzed using an independent sample *t* test, one-way analysis of variance (one-way ANOVA), Kruskal-Wallis test, Pearson's correlation and Spearman correlation. Cohen's conventions criteria[12] were used to evaluate the degree of association, a correlation coefficient of 0.10 (represente a weak or small association), 0.30 (represent a moderate correlation and 0.50 or higher (represent a strong or large correlation). Significant factors obtained from univariate analysis were analyzed using multiple linear regressions, to examine factors predicting job seeking anxiety. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Of the 584, senior undergraduate students in Prince of Songkla University; who were recruited into our study, two hundred and seventy-one participants met the inclusion criterias. Table 1 shows resilience quotient, family influence scale, job-seeking self-perceptions and job seeking anxiety status. The majority of participants had a moderate level of resilience quotient, family influence scale, job-seeking self-perceptions and job seeking anxiety status.

The mean age (SD) of participants was 21.78 (0.76) years; range 20-25 years. Most of them were female, lived in other provinces outside of Songkhla, studied in the applied Science department; mean GPAX (SD) = 2.86 (0.45), and had no underlying diseases. Their field of study coupled with job interest was principally matched. Their families' status was mostly co-habiting partner, and income was approximately between 10,000-39,999 Baht per month. The effect of economics and the current political situation, including COVID-19, on their job-seeking anxiety was almost, entirely high. Baseline data regarding socioeconomic status is presented in Table 2.

Table 2 presents a comparison of the demographic data, and current political situation as well as effects of COVID-19, in relation to job-seeking-anxiety. (moods, cognition and physical symptom responses). Female gender was significantly associated with higher job-

TABLE 1. Resilience quotient, family influence scale, job-seeking self-perceptions and job seeking anxiety status of participants.

	Resilience quotient (RQ)		Family influence scale (FIS)		Job-seeking self-perceptions		Job seeking anxiety	
	n	%	n	%	n	%	n	%
Low	71	26	8	3	45	17	108	40
Moderate	181	67	195	72	174	64	136	50
High	19	7	68	25	52	19	27	10
Mean (S.D.)	58.7 (7.84)		73.54 (11.74)		46.79 (10.64)		65.02 (18.63)	
Min - Max	31-80		44-110		19 - 71		30-115	

seeking anxiety and physical symptom responses. Past history of mental illness was only associated with greater mood responses. Paternal career and family income were significantly associated with cognition effects. Economics and current political situations (as well as those relating to COVID-19) were significantly associated with higher job-seeking anxiety, moods and cognition. By contrast, age, faculty, hometown, GPAX, field of study and job interest match, family status, parental education and maternal career were not associated with job seeking anxiety ($P > 0.05$).

Table 3 demonstrates correlation between resilience quotient, family influence scale, job-seeking self-perception and job-seeking anxiety. Resilience quotient were significantly associated with job-seeking anxiety with high degree of negative correlation. ($r>0.5$). Although, significantly factors positively correlated with job-seeking anxiety with low degree of association were the family's information support, values and beliefs ($r<0.30$). Family expectations was only associated with cognition with low degree of association ($r<0.30$). Furthermore, the family's financial support and summary of family influence scale were not associated with job-seeking anxiety. Personal, experience and abilities and job perceptions, which represented job-seeking self-perception, were significantly, positively correlated with job-seeking anxiety and its consequences.

Table 4 shows predictors for job-seeking anxiety. Gender, economics and the current political situation (Including effects from COVID-19), resilience quotient and job-seeking self-perception were entered into multiple linear regression to analyze with job-seeking anxiety.

The final model presents the associations of resilience quotient and job-seeking self-perception on job-seeking anxiety. These factors explained 41.7% of the variance of the job-seeking anxiety score. Job-seeking self-perception was the strongest predictor for job-seeking anxiety ($\beta=2.232$, $P<0.001$).

DISCUSSION

This study aimed to explore job-seeking anxiety in senior, undergraduate student, and identify each sociodemographic factor related to job-seeking anxiety. Anxiety levels can be classified using class interval scales as low, moderate and high. The prevalence of job-seeking anxiety was similar to the study by Ua-ariyapanichkul et al[3], which also studied senior university students. We found that most participants had moderate to high level of job-seeking anxiety (60%). This finding showed that job-seeking anxiety was a problem after graduation, for university students.

In regards to sociodemographic factors, females were considered a significant variable to job-seeking anxiety. This finding was similar to previous study performed by Fengsu Hou et al[13] that females were experiencing more severe stress and anxiety symptoms than male gender especially in Covid-19 situation. Moreover, resulting from gender differences in regards to a comparison of lay-off rates in Thailand (Q2 of 2019 and 2020) were 2.3% in women and 1.5% in men[14] that might result in an anxious with job seeking.

Particularly during the COVID-19 pandemic, the financial situation has become a crisis. In consequence, unemployment rates sharply increased in double-time in Q2 of 2019 and 2020[15], causing anxiety in senior, university students. In this study, the perspectives on COVID effects were mostly in the high level (82.29%).

TABLE 2. Comparison of the demographics data and current conflict situation (COVID-19) in relation to job-seeking-anxiety and its effects (moods, cognition and physical symptoms responses).

	Total (N=271)	%	Job-seeking anxiety											
			Mean (SD)	Overall Statistic (df)	*P**	Mean (SD)	Moods Statistic	*P**	Mean (SD)	Cognition Statistic (df)	*P**	Mean (SD)	Physical symptoms Statistic (df)	*P**
Gender				*t* (269)	0.03		*t* (269)	0.18		*t* (269)	0.11		*t* (269)	0.013
Male	75	27.68	61.0 (19.10)			19.0 (6.28)			17.8 (6.26)			24.2 (8.99)		
Female	196	72.32	66.5 (18.30)			20.2 (5.81)			19.2 (6.12)			27.2 (8.80)		
Age				*t* (269)	0.63		*t* (269)	0.72		*t* (269)	0.69		*t* (269)	0.63
<22 years old	101	37.27	65.7 (19.20)			20.0 (5.88)			19.0 (6.53)			26.7 (8.75)		
>22 years old	170	62.73	64.6 (18.30)			19.7 (6.01)			18.7 (5.98)			26.2 (9.07)		
Group of Faculty				*F* (4,266)	0.42		*F* (4,266)	0.6		*F* (4,266)	0.27		*F* (4,266)	0.4
Pure Science	10	3.69	65.7 (19.40)			20.2 (6.71)			19.4 (7.43)			26.1 (3.17)		
Applied Science	146	53.87	63.3 (18.50)			19.5 (5.84)			18.2 (5.94)			25.7 (0.75)		
Health Science	12	4.43	66.6 (23.40)			21.4 (7.24)			20.4 (7.48)			24.8 (3.42)		
Social Science and Arts	103	38.01	67.2 (18.10)			20.2 (5.91)			19.5 (6.20)			27.5 (0.82)		
Hometown				*t* (269)	0.77		*t* (269)	0.37		*t* (269)	0.64		*t* (269)	0.76
Songkhla	104	38.38	64.6 (16.90)			19.4 (5.91)			18.6 (5.99)			26.6 (7.98)		
Other	167	61.62	65.3 (19.70)			20.1 (5.98)			19.0 (6.31)			26.2 (9.51)		
GPAX				*F* (4,266)	0.70		*F* (4,266)	0.71		*F* (4,266)	0.68		*F* (4,266)	0.65
<2.5	63	23.25	64.8 (18.30)			19.6 (5.58)			19.0 (6.26)			26.3 (8.75)		
2.5-2.99	104	38.38	65.3 (17.60)			19.6 (5.60)			19.0 (5.73)			26.7 (8.71)		
3-3.49	78	28.78	63.6 (18.70)			20.0 (6.23)			18.2 (6.15)			25.4 (8.66)		
>3.5	26	9.59	68.7 (23.20)			21.0 (7.41)			19.9 (7.81)			27.8 (11.14)		
Underlying disease				*t* (269)	0.35			0.71			0.26			0.16
No underlying disease	238	87.82	64.6 (18.90)			19.9 (6.03)			18.7 (6.29)			26.1 (9.01)		
Underlying disease	33	12.18	67.8 (16.60)			19.5 (5.39)			20.0 (5.21)			28.4 (8.27)		

TABLE 2. Comparison of the demographics data and current conflict situation (COVID-19) in relation to job-seeking-anxiety and its effects (moods, cognition and physical symptoms responses). (Continue)

				Job-seeking anxiety									
	Total			Overall			Moods			Cognition		Physical symptoms		
	(N=271)	%	Mean (SD)	Statistic (df)	*P**	Mean (SD)	Statistic	*P**	Mean (SD)	Statistic (df)	*P**	Mean (SD)	Statistic (df)	*P**
Field of study and Job interest match				*M*	0.97			0.22			0.71			0.52
Yes	169	62.36	65.0 (18.70)			20.4 (6.04)			18.6 (6.01)			25.9 (9.07)		
No	102	37.64	65.1 (18.70)			19.5 (5.89)			18.9 (6.29)			26.6 (8.89)		
Family status				*t* (269)	0.56			0.7			0.77			0.46
Cohabiting partner	192	70.85	64.6 (18.30)			19.8 (5.96)			18.8 (6.16)			26.1 (8.83)		
Other	79	29.15	66.0 (19.50)			20.1 (5.97)			19.0 (6.26)			27.0 (9.23)		
Father's education				*F* (6,264)	0.53			1.00			0.31			0.32
Uneducated	5	1.85	71.2 (12.10)			20.4 (5.03)			21.6 (3.71)			29.2 (5.67)		
Primary school	61	22.51	61.3 (17.00)			19.5 (5.88)			17.3 (5.35)			24.5 (8.4)		
Secondary school	71	26.20	66.0 (19.20)			19.6 (5.63)			19.3 (6.03)			27.1 (9.09)		
Vocational education	54	19.93	64.3 (19.60)			20.0 (6.08)			18.9 (6.57)			25.44(9.02)		
Bachelor's degree	73	26.94	67.0 (19.20)			20.1 (6.40)			19.5 (6.82)			27.4 (9.19)		
Master's degree and higher education	7	2.58	67.3 (14.10)			20.4 (6.27)			17.6 (4.58)			29.3 (9.67)		
Father's career				*F* (5,265)	0.20			0.59			0.048			0.39
Employee/laborer	52	19.19	60.7 (15.30)			19.2 (5.52)			17.2 (5.11)			24.3 (7.33)		
Government and state enterprise	64	23.62	66.7 (18.50)			20.2(6.01)			19.5 (6.75)			27.0 (8.90)		
Agriculturist	57	21.03	66.7 (20.00)			20.4 (6.30)			19.3 (6.31)			27.1 (9.43)		
Business owner	64	23.62	63.3 (19.30)			19.1 (5.84)			18.0 (5.96)			26.1 (9.43)		
Other/ unemployment	34	12.55	69.0 (19.20)			20.6 (6.20)			20.9 (6.22)			27.6 (9.42)		

TABLE 2. Comparison of the demographics data and current conflict situation (COVID-19) in relation to job-seeking-anxiety and its effects (moods, cognition and physical symptoms responses). (Continue)

	Total (N=271)	%	Mean (SD)	Job-seeking anxiety Overall Statistic (df)	*P**	Moods Mean (SD)	Moods Statistic	*P**	Cognition Mean (SD)	Cognition Statistic (df)	*P**	Physical symptoms Mean (SD)	Physical symptoms Statistic (df)	*P**
Mother's education				*F* (6,264)	0.09			0.19			0.06			0.26
Uneducated	4	1.48	65.5 (17.20)			20.8 (5.85)			17.8 (6.4)			27.0 (7.53)		
Primary school	65	23.99	59.7 (18.40)			18.5 (5.93)			17.1 (5.61)			24.1 (9.06)		
Secondary school	70	25.83	66.7 (20.40)			20.3 (6.00)			19.4 (6.27)			27.1 (9.99)		
Vocational education	44	16.24	67.7 (15.30)			20.6 (5.52)			19.4 (5.78)			27.8 (6.42)		
Bachelor's degree	79	29.15	65.2 (18.00)			19.7 (6.13)			19.1 (6.32)			26.5 (8.74)		
Master's degree or higher	9	3.32	75.3 (21.40)			23.3 (5.34)			22.9 (7.93)			29.1 (11.25)		
Mother's career				*F* (5,265)	0.15			0.26			0.08			0.41
Employee/laborer	57	21.03	60.8 (16.40)			18.6 (18.61)			17.5 (5.83)			24.7 (8.12)		
Government and state enterprise	50	18.45	70.1 (17.10)			21.1 (21.14)			20.8 (6.39)			28.1 (8.47)		
Agriculturist	42	15.50	65.5 (19.10)			20.2 (20.19)			18.8 (5.76)			26.5 (9.71)		
Business owner	68	25.09	64.9 (20.80)			20.0 (20.03)			18.5 (6.57)			26.4 (9.76)		
Other/ unemployment	54	19.93	64.6 (18.40)			19.4 (19.43)			18.8 (5.90)			26.4 (8.47)		
Family income (baht)				*F* (4,266)	0.15			0.2			0.03			0.53
<10,000	41	15.13	68.0 (21.30)			20.8 (6.77)			19.7 (6.64)			27.4 (9.83)		
10,000-39,999	159	58.67	63.3 (18.50)			19.5 (5.76)			18.1 (5.96)			25.7 (8.95)		
40,000-69,999	39	14.39	70.1 (18.60)			21.2 (5.85)			21.2 (6.49)			27.7 (9.15)		
70,000	32	11.81	63.4 (14.60)			18.7 (5.76)			18.2 (5.64)			26.5 (7.40)		
Economic and current situation (COVID-19)				*F* (3,267)	0.03			0.02			0.02			0.15
Low (1-2)	10	3.69	51.8 (17.10)			14.8 (5.47)			15.5 (5.17)			21.5 (7.60)		
Moderate (3)	38	14.02	61.6 (15.10)			19.2 (4.65)			17.0 (4.60)			25.5 (8.61)		
High (4-5)	223	82.29	66.2 (19.00)			20.2 (6.08)			19.3 (6.36)			26.7 (9.01)		

df = Degree of freedom. * *P* was based on an independent sample *t* test (*t*), one-way ANOVA (*F*), or Mann-Whitney U test (*M*) as appropriate.

TABLE 3. Mean, standard deviation and bivariate correlation of study variables.

		Resilience quotient (RQ)		Family influence scale (FIS)				Job-seeking self-perceptions				Job-seeking anxiety			
	Mean (SD)	RQ total score	Information support	Financial support	Family expectation	Values and Beliefs	FIS total Score	Personal perceptions	Experience and abilities perceptions	Job perception	Job-seeking perceptions total score	Moods	Cognition	Physical symptoms	Job-seeking anxiety total score
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
(1)	58.70 (7.84)	-													
(2)	28.48 (5.34)	**r = 0.265 *P* < 0.001**	-												
(3)	17.67 (2.77)	r = 0.152 *P* = 0.12	**r = 0.504 *P* < 0.001**	-											
(4)	13.89 (3.20)	r = -0.059 *P* = 0.332	**r = 0.250 *P* < 0.001**	**r = 0.384 *P* < 0.001**	-										
(5)	13.50 (5.31)	**r = -0.129 *P* = 0.033**	**r = 0.198 *P* = 0.001**	**r = 0.191 *P* =0.002**	**r = 0.473 *P* < 0.001**	-									
(6)	73.54 (11.74)	r = 0.082 *P* = 0.181	**r = 0.731 *P* < 0.001**	**r = 0.656 *P* < 0.001**	**r = 0.691 *P* < 0.001**	**r = 0.717 *P* < 0.001**	-								
(7)	16.27 (4.26)	**r = -0.261 *P* < 0.001**	**r = -0.157 *P* = 0.010**	r = -0.086 *P* = 0.158	r = 0.067 *P* = 0.272	r = 0.046 *P* = 0.449	r = -0.053 *P* = 0.388	-							
(8)	13.34 (3.52)	**r = -0.271 *P* < 0.001**	**r = -0.143 *P* = 0.018**	**r = -.0120 *P* = 0.048**	**r = 0.128 *P* = 0.036**	r = 0.025 *P* = 0.681	r = -0.047 *P* = 0.438	**r = 0.629 *P* < 0.001**	-						
(9)	14.00 (4.26)	**r = -0.321 *P* < 0.001**	**r = -0.231 *P* < 0.001**	r = -0.113 *P* = 0.064	**r = 0.140 *P* = 0.021**	**r = 0.205 *P* = 0.001**	r = -0.001 *P* = 0.991	**r = 0.505 *P* < 0.001**	**r = 0.572 *P* < 0.001**	-					
(10)	46.79 (10.64)	**ρ = -0.318 *P* < 0.001**	**ρ = -0.200 *P* = 0.001**	**ρ = -0.120 *P* = 0.048**	**ρ = 0.131 *P* = 0.032**	ρ = 0.108 *P* = 0.077	ρ = 0.035 *P* = 0.569	**ρ = 0.860 *P* < 0.001**	**ρ = 0.851 *P* < 0.001**	**ρ = 0.808 *P* < 0.001**	-				
(11)	19.84 (5.95)	**r = -0.511 *P* < 0.001**	**r = -0.197 *P* = 0.001**	r = -0.084 *P* = 0.167	r = 0.061 *P* = 0.318	**r = 0.153 *P* = 0.012**	r = -0.024 *P* = 0.699	**r = 0.398 *P* < 0.001**	**r = 0.475 *P* < 0.001**	**r = 0.539 *P* < 0.001**	**ρ = 0.545 *P* < 0.001**	-			
(12)	18.82 (6.18)	**r = -0.556 *P* < 0.001**	**r = -0.181 *P* = 0.003**	r = -0.048 *P* = 0.436	**r = 0.146 *P* = 0.016**	**r = 0.173 *P* = 0.004**	r = 0.024 *P* = 0.688	**r = 0.331 *P* < 0.001**	**r = 0.295 *P* < 0.001**	**r = 0.468 *P* < 0.001**	**ρ = 0.421 *P* < 0.001**	**r = 0.704 *P* < 0.001**	-		
(13)	26.36 (8.94)	**r = -0.467 *P* < 0.001**	**r = -0.177 *P* = 0.003**	r = -0.116 *P* = 0.057	r = 0.051 *P* = 0.404	**r = 0.125 *P* = 0.039**	r = -0.037 *P* = 0.542	**r = 0.273 *P* < 0.001**	**r = 0.225 *P* < 0.001**	**r = 0.292 *P* < 0.001**	**ρ = 0.305 *P* < 0.001**	**r = 0.564 *P* < 0.001**	**r = 0.740 *P* < 0.001**	-	
(14)	65.02 (18.63)	**r = -0.576 *P* < 0.001**	**r = -0.208 *P* = 0.001**	r = -0.098 *P* = 0.107	r = 0.092 *P* = 0.130	**r = 0.166 *P* = 0.006**	r = -0.017 *P* = 0.777	**r = 0.368 *P* < 0.001**	**r = 0.358 *P* < 0.001**	**r = 0.467 *P* < 0.001**	**ρ = 0.460 *P* < 0.001**	**r = 0.824 *P* < 0.001**	**r = 0.912 *P* < 0.001**	**r = 0.905 *P* < 0.001**	-

Note: solid numbers, in bold, are statistically significant (P<0.05

TABLE 4. Predictors of job-seeking anxiety.

Predictors*	Job-seeking anxiety				
	r^2	β	Standard error	p-value	95%CI
Resilience quotient (RQ)	0.332	-1.169	0.12	<0.001	-1.39, -0.94
Job-seeking self-perceptions	0.417	2.232	0.35	<0.001	1.55, 2.92
Constant		103.008	9.28	<0.001	84.74, 127.25

* This model was adjusted for gender, economics and current the political situation (Including COVID-19).

Likewise, the COVID-19 pandemic represented as a positive variable on job-seeking anxiety.

Next, overall grade point average representing the average value of the accumulated final grades earned in courses over time at university. This factor showed to be non relevant in job-seeking anxiety, which was also indicated in a previous study; explaining that not all the subjects were concordant to career options. Furthermore, academic major's and matching between fields of study and job interest was considered insignificant, due to more career options being available nowadays. This can makes it unnecessary to apply for a job related to a particular field of study.[3]

Participations who had history of mental illness was found to be marginal significant to job seeking anxiety. The relatively, small amount of participants in this study might lead to the difficulty in identifying these predictors. Otherwise, the results were possibly associated to job-seeking anxiety. Similarly to a previous study, psychological abnormalities appeared to be a clearly positive factor on job-seeking anxiety, and depression in university students.[16]

When considering family influence: we found that family's information support, values and beliefs were associated to an increase in job-seeking anxiety. Surprisingly, parental expectations did not have an influence on child anxiety, likewise to a previous study[3,17], in believing expectations decreased by child's age. Moreover, the ages of last years undergraduate students were mature enough and reasonable for self-judgment. In the same way, no differences were found on indices of family status in these ages.

A significant effect of family income on job-seeking anxiety was found. Meanwhile, the cognition part of job-seeking anxiety seemed to be related with senior university students whose fathers were unemploymed was especially found. Eventually, University students who suffered from financial stress were also pressured to apply for a job.

The results of this study showed a certainity in the negative correlation between anxiety and each part of Resilience Quotient. Previous studies revealed that the score of Resilience Quotient was strongly, inversely related to scores of anxiety[18] using emotional stability, will power and coping skills.[7]

Self-perceptions in the meaning of understanding their personal, job, and experiences and abilities in job-seeking positively correlated with anxiety states. In other words, anxious participants were more conservative in their estimations of their action capabilities, which was concordant in a previous study.[19]

Meanwhile, gender, Job-seeking self-perceptions, Resilience quotient (RQ), Economics and current situations (COVID-19) were associated with job-seeking anxiety. However, the only effect of the Job-seeking self-perceptions and Resilience quotient (RQ) could predict job-seeking anxiety. These findings were similar to the study of Sood S, et al.[20] that resilience and psychological well-being associated with perceived distress of university Students During COVID-19. Self-esteem and anxiety of job applications by Tiensirirerk P[21], that showed the similarity between self-estreem and resilience quotient as protective factors for anxiety.

There were some limitations of this study. First, response rates using online-survey were slightly low as they were indicated in the research by Fincham J[22,23], in that response rates for an online survey were approximately 25-30%. The response rates varied depending on types and time of surveys. Besides, this data was collected during the semester and during the COVID-19 situation. Another difficulty was being unable to encourage and identify non-response groups for reanswering.

In addition, small sample sizes were also limited by the large amount of the excluded group, who planned for further study. We suggest examining their anxiety level and impact of job-seeking anxiety on this decision-making. All variables were collected using self-report questionnaires, without clinical interviews, and use of a cross-sectional study, which might not be able to detect changes of anxiety over time. Therefore, a longitudinal study is also suggested to clarify this.

In conclusion, we recommend that the Student Affairs Department create an effective management strategy, for providing mental health promotion and prevention for university students. As roles of universities on resilience and mental health promotion were remarkably mentioned in previous studies, elimination of any discrimination towards gender, religion, race and other identities should be done. Not only settings in the university, but also the quality of teaching and mentoring should be emphasized, so as to promote a mentally healthy environment afterwards.

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Direct CT Venography in ESRD Patients: Technical Experience and Findings

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ABSTRACT

Objective: The aims of this study were to describe direct computed tomography venography (CTV) for upper limb venous system evaluation and to report on findings in end-stage renal disease (ESRD) patients.

Materials and Methods: Direct CTV was performed using a 64-multidetector computed tomography (MDCT) scanner with simultaneous injection of diluted iodinated contrast (IC); 1:4 at both elbows and 2-phase scanning namely, the direct venous, and the arterial phases. The findings in ESRD patients evaluated between November 2013 and March 2019 were retrospectively reviewed.

Results: Forty CTV examinations (600 venous segments) were performed and the volume of IC used per patient was 38 mL. Number of lesions found in a patient ranged from 1 to 6 and the majority had 1 to 3 lesions (30/38 patients). Stenosis and thrombosis were the two most common findings (112/600) and were equally prevalent. The three most common sites of steno-occlusive complications were the brachiocephalic vein (29 lesions), the internal jugular vein (25 lesions), and the subclavian vein (16 lesions). The most common site of stenosis was the brachiocephalic vein (18 lesions), whereas the most common site of thrombosis was the internal jugular vein (20 lesions). No venous aneurysms or ruptures were found. IC extravasation at the site of injection occurred in one arm in one patient.

Conclusion: Direct CTV has the advantage of requiring lower IC volume while maintaining direct visualization of the venous system similar to conventional venography.

Keywords: Computed tomography venography; contrast material; upper limb,vascular access (Siriraj Med J 2021; 73: 373-379)

INTRODUCTION

Venous steno-occlusive disease after establishment of vascular access is a common complication in end stage renal disease (ESRD) patients. Evaluation of the venous system is important for acquiring information on the lesion, which then facilitates treatment planning. Various non-invasive and invasive imaging techniques can be used to assess the venous system. The color Doppler ultrasound is a non-invasive technique that is easy to perform but its use is limited in some venous segments, especially the central veins.[1] Contrast-enhanced magnetic resonance (MR) imaging is also non-invasive, and does not require iodinated contrast (IC); however, it is not easily accessible and is expensive.[2] Conventional venography (CV) is the gold standard for evaluating the venous system; nevertheless, it is invasive and it requires injection of a

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considerable amount of IC. Multi-detector computed tomography venography (MDCT-V) is fast, easy to perform, provides high spatial resolution, and has high accuracy.[1,3-5] Additionally, three-dimensional images can be acquired by CTV, whereas only two-dimensional images are obtained with CV.

The two types of CTV are direct and indirect CTV, and the indirect CTV technique is considered to be the standard. It is performed by injecting IC at a dose of 2 mL/kg (total 100-200 mL) in a single extremity, usually the unaffected side, and scanning during the arterial phase (second passage) using either a test bolus or bolus tracking.[1,4-5] However, some fresh thrombi may show identical density as contrast-enhanced blood and, thereby, yield false negative results.[6] Moreover, inflow phenomena can mimic an intra-luminal thrombus (Fig 1). Direct CTV of a lower extremity was first reported in 1994 during which diluted IC was injected in a superficial vein and scanning was performed before the IC entered the right atrium (first passage).[6] In contrast to indirect CTV, the advantages of direct CTV are lower IC injection volume and the fact that scanning time is independent of the patient's blood flow velocity.[7] There are a few reports of direct CTV in upper extremities and the techniques used are variable; nevertheless, the direct CTV technique may be considered as a replacement for indirect CTV for evaluating the upper limb venous system.

Therefore, here, we describe our technique and experience in performing direct CTV of the upper extremity, discuss venous complications after vascular access, and investigate findings of venous complications in ESRD patients.

Fig 1. "Inflow phenomenon" Non-opacified blood (arrow) from left brachiocephalic vein draining into opacified blood in superior cava, causing partial hypodensity mimicking thrombus

MATERIALS AND METHODS

We retrospectively reviewed findings from ESRD patients who had undergone CTV of both upper limbs between 1st November 2013 and 31st March 2019. We identified 38 patients (18 males and 20 females) with an average age of 65 years (range 29-85 y). We excluded one patient who underwent upper extremity CTV with only single arm injection. In the cohort, one patient underwent three studies while another required two studies, all at different times. Data from a total of 40 exams were included, and information on the patient's presenting symptoms, and types of vascular accesses were collected. The presence, sites, and number of stenosis and/or occlusion were determined. The clinical data were accessed from medical records while CTV findings were accessed from the PACS database.

The study was approved by the university review board, approval No.282/2019. Informed consent was waived due to the retrospective nature of the study.

CTV technique

Images were obtained with patients in the supine position with their heads directed toward the gantry. Hands were placed in the head-holder, superior to the head, and immobilized with tape to limit motion. A tourniquet was not used, as recommended by Mavili et al.[8]

CTV was performed using a 64-MDCT scanner (Toshiba Aquillion one, Tokyo, Japan) with tube potential set at 100 kV, and current at 300 mA. The parameters for CTV were beam collimation 0.5 mm, pitch 0.641, slice thickness 1.0 mm, and reconstruction interval 0.8 mm.

The scanning protocol progressed in the cranio-caudal direction from the elbow to the superior vena cava. To explore image quality and its usefulness, the scanning phases were initially set, as follows-non-contrast scan and three post-contrast phases. The first post-contrast phase was scanned after a delay of 7 seconds from contrast injection (first passage). The second phase was repeated at the same scan length immediately after the first phase for arterial opacification (second passage), and the third phase was repeated immediately after the second phase at the same scan length. We observed that no additional information was acquired from the third phase. Moreover, if the patient experienced an arterio-venous fistula development, the opacified blood was rapidly washed out from vascular system and no vascular enhancement was observed during the third phase. Therefore, the third post-contrast phase was

omitted. However, the second phase was retained because the internal jugular veins were better opacified in the second phase than in the first phase. This is because, in the absence of central vein obstruction, the internal jugular veins are usually not opacified in the first phase.

IC fluids, with concentrations of 320, 350 and 370 mgI/mL (Visipaque, Omnipaque, and Ultravist, respectively) were simultaneously administered through a 20-22 gauge IV catheter into the subcutaneous superficial veins of both forearms just below the elbows using an automated injector. Initially, the rate of injection was 2 mL/s for each extremity but based on our observations, it was increased to 3 mL/s. Next, IC concentrations were optimized as follows. Initially, IC was diluted at a ratio of 1:2 in saline which led to very high IC concentration in the veins and artifacts. Therefore, we titrated IC dilution to 1:4. The volume of diluted IC injected was 75 mL for each extremity, and thus, a total of 38 mL IC was used for each patient.

Image analysis and interpretation

Acquired data were transferred to a workstation (Vitrea 5.2.512.6014), which allowed processing of multiplanar reconstructions, including maximum intensity projection (MIP), multiplanar reformations (MPR), and volume rendering (VR). The images were then sent to a PACS (Infinite Healthcare, Seoul, South Korea). All CTV examinations were arranged such that they were performed before the patients' hemodialysis session.

Conventional transverse images, reformatted sagittal, and coronal reconstructions, MIP, and VR 3D images were used for interpretation. All images were analyzed by radiologists on duty. A total of 8 general radiologists with variable experience, ranging from 1 to 26 years, interpreted the images.

Statistical analyses

Data were analyzed using R software, version 3.5.1 and SPSS statistical software ver. 22 (SPSS, Chicago, Illinois). Residuals were examined for assumption of normality and heteroscedasticity. Continuous variables are expressed as mean ± standard deviation. Categorized variables are presented as numbers with percentages. Association between patient's gender and occurrence of either stenosis or thrombosis was analyzed using the Chi-squared test of Independence. Lastly, the effect of a patient's age on occurrence of both stenosis and thrombosis was analyzed using binary logistic regression. P values less than 0.05 were considered as significantly different.

RESULTS

Of the 40 patients in the study, two had normal findings; thus the incidence rate was 95%.

Table 1 illustrates patient demographics. Almost all patients had prior vascular access either temporary or permanent, and only one patient had no prior vascular access. About 50% of patients had indwelling catheters while undergoing CTV. Patients' presenting symptoms are listed in Table 2. Most of the patients (27/40; 67.5%) presented with upper limb swelling.

Superficial and deep veins in both arms, and the central veins and internal jugular veins were evaluated and categorized into segments; as basilic vein, cephalic vein, brachial vein, axillary vein, subclavian vein, brachiocephalic vein, internal jugular vein, and SVC, representing a total of 15 segments per study. This study analyzed data from 600 venous segments. The number of lesions found in one patient ranged from 1-6, but a majority of patients had 1-3 lesions (30/38; 78.9%) (Fig 2). Data on sites and types of lesions are provided in Table 3. Stenosis (Fig 3) and thrombosis (Fig 4) were the two most common findings, were equally prevalent, and were seen in 112 of the 600 segments (18.7%). The three most common sites of steno-occlusive complications were the brachiocephalic vein (29 lesions), the internal jugular vein (25 lesions), and the subclavian vein (16 lesions). The most common site of stenosis was the brachiocephalic vein (18 lesions) whereas the most common site of thrombosis was the internal jugular vein (20 lesions). We did not encounter any venous aneurysms or ruptures. Extravasation of IC at the injection site occurred only once and in one arm of one patient.

Patient gender was not significantly related to the occurrence of either stenosis (χ^2 (1) = 0.003, p = 1.00) or thrombosis (χ^2 (1) = 2.308, p = 0.170). Further, patient age did not significantly predict the likelihood of stenosis (Binary logistic regression, χ^2 (27) = 15.984, p = 0.9533) or thrombosis (Binary logistic regression, χ^2 (27) = 32.763, p = 0.205)

DISCUSSION

CTV is a highly accurate, minimally invasive method for diagnosing steno-occlusive disease of the upper extremity veins.[3,10] However, as false positives for occlusion can occur upon external compression of the patient's body, the patient's position on the table is critical during the scan.[4] Additionally, indirect or direct CTV technique, and single site or double site injections have been used. The indirect CT technique uses 100-150 mL of undiluted IC injected at the unaffected arm at a flow rate of

TABLE 1. Patient demographics.

	No. (%)
Gender	
Female	20 (52.6)
Male	18 (47.4)
Types of prior vascular access	
Arteriovenous fistula	17 (42.5)
Arteriovenous graft	13 (32.5)
Temporary vascular access	9 (22.5)
None	1 (2.5)
Presence of indwelling catheter while undergoing CT scan	21 (52.5)

TABLE 2. Patients' presenting symptoms.

Presenting symptoms	No. (%)
Upper limb swelling	27 (67.5)
Failed cannulation	5 (12.5)
High venous pressure*	3 (7.5)
Clot derived from catheter	2 (5)
Decreased fistula flow**	1 (2.5)
Asymptomatic: follow up	2 (5)

* High venous pressure = A venous segment static pressure greater than 0.5 in grafts or fistulae[9]

* Decreased fistula flow = An access flow < 600mL/min in grafts and < 500 mL/min in fistulae[9]

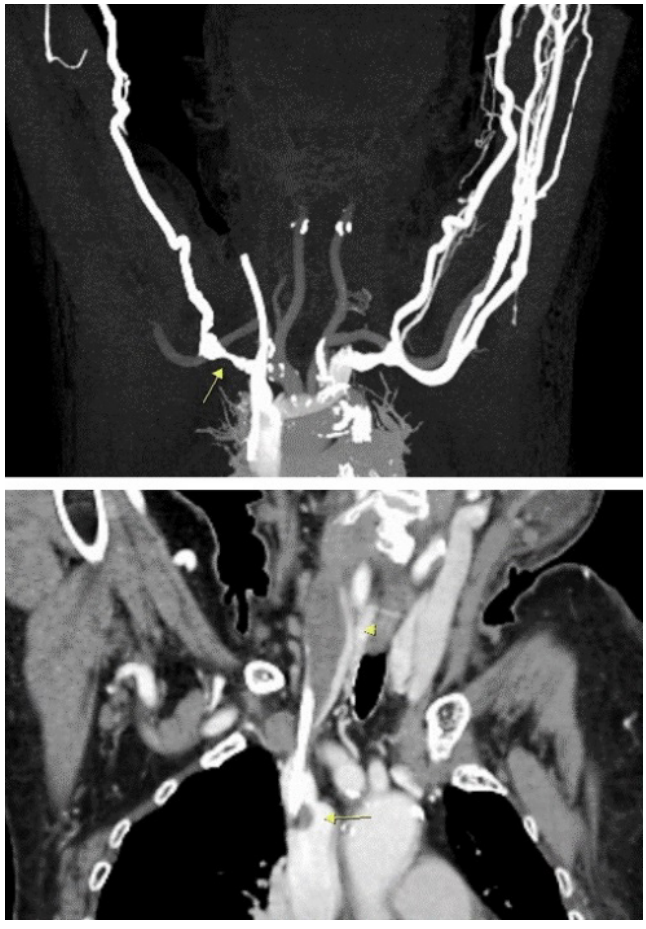

Fig 2. A 85 year-old female with failed left radiocephalic arteriovenous fistula, and indwelling catheter at right internal jugular vein, presents with right upper limb and face swelling. Direct computed tomography venography with maximum intensity projection reconstruction, coronal view reveals stenosis at right subclavian vein (top-arrow), and thrombosis in right internal jugular vein (bottom-arrowhead), and thrombosis in superior vena cava (bottom-arrow)

TABLE 3. Sites and types of lesions.

Site	Stenosis (No.)	Thrombosis (No.)	Total
Superior vena cava	3	4	7
Internal jugular vein	5	20	25
Brachiocephalic vein	18	11	29
Subclavian vein	9	7	16
Axillary vein	5	4	9
Brachial vein	3	2	5
Cephalic vein	10	5	15
Basilic vein	3	3	6
Total	56	56	112

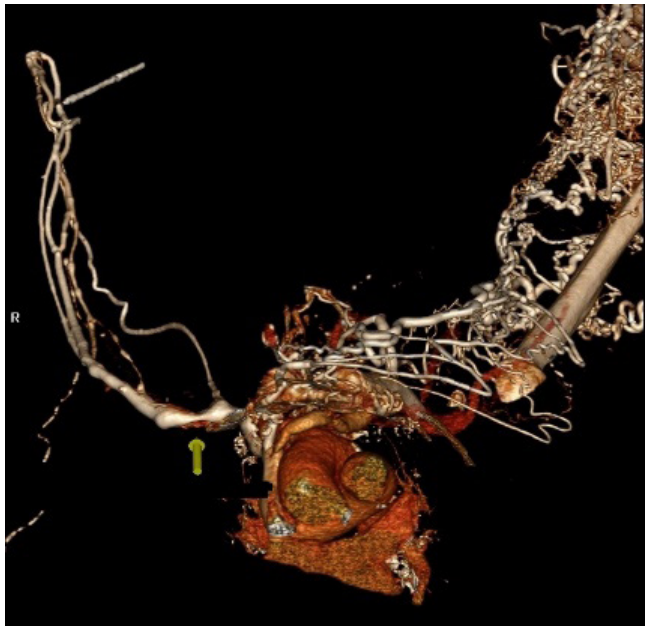

Fig 3. Direct computed tomography 3D volume rendered image of a 81 year-old female shows right axillary vein stenosis (arrow), measured 1.9 mm. in diameter, compared with 9.9 mm. of normal diameter, about 1.0 cm. long. Much collateral veins are noted at left upper limb.

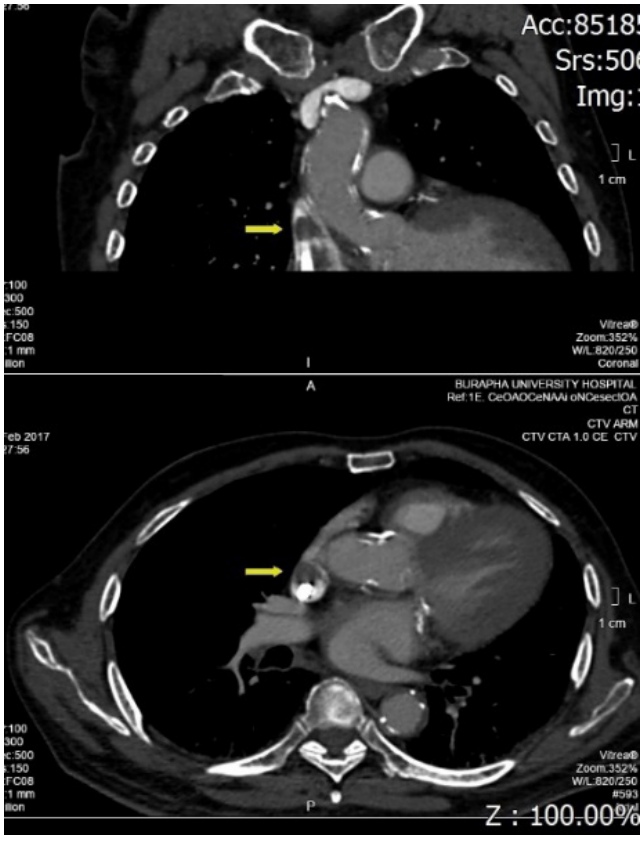

Fig 4. A 82 year-old male presents with failed cannulation at right internal jugular vein. Direct computed tomography venography with multiplanar reconstruction longitudinal view (top) and axial view (bottom) demonstrate thrombus in the superior vena cava (arrows).

3-5 mL/min, along with scanning in the arterial phase using a timing bolus test or bolus tracking.[4-5] There have been few reports of direct CTV for upper extremities and the techniques used are variable. A prospective study of 22 patients used a 1:2 dilution of IC that was injected simultaneously in both arms, along with scanning using bolus tracking.[10] The total volume of IC used per patient in that study was 75 mL. Svensson and colleagues used 45 mL of 1:10 diluted IC, and scanning at 12 seconds; thus, less than 5 mL of IC was used in their study.[11] We used 1:4 diluted IC, which is similar to that used by Kuo et al. who injected IC into both femoral veins.[12] Total volume of of IC used in our patients was 38 mL, and we simultaneously injected IC into both arms to avoid inflow phenomenon at the superior vena cava (SVC), which is a common problem with single arm injection. Our protocol yielded good quality images of bilateral upper extremity veins that were also suitable for mapping. In cases of chronic occlusion, collateral veins were also easily visible (Fig 5). Tanju et al. have used direct contrast-enhanced 3D magnetic resonance venography (MRV) with bilateral injections, and have reported 100% sensitivity and specificity. However, there were only 19 patients in that study.[2]

Multiple contrast agents can be used for venography other than IC such as carbon monoxide[11,13] or gadoterate meglumine[12,14] which provide high quality images but are not feasible for use because of their availability. Non-contrast enhanced MRV has been proposed in patients who want cannot endure risk from either the contrast agent or radiation[13,15], even though contrast-enhanced MRV has a higher diagnostic odds ratio than non-contrast techniques.[14,16]

The incidence of steno-occlusive complications in our cohort was 95%, which is higher than that reported by previous studies.[15-19] This may be because almost all our patients were symptomatic and had undergone prior subclavian catheterization or placement of peripherally inserted long-term catheters for hemodialysis before fistula formation. Prior catheter use is associated with primary patency loss in patients with a fistula or a graft, and this is the strongest independent predictor of upper extremity venous thrombosis.[18-21] Moreover, most of our patients had an indwelling catheter, which is also the strongest independent predictor of upper extremity deep venous thrombosis.[20,22] Additionally, average patient age in the current study was more than 65 years, and available literature suggests that age more than 50 years is a significant risk factor for AVF thrombosis.[21,23]

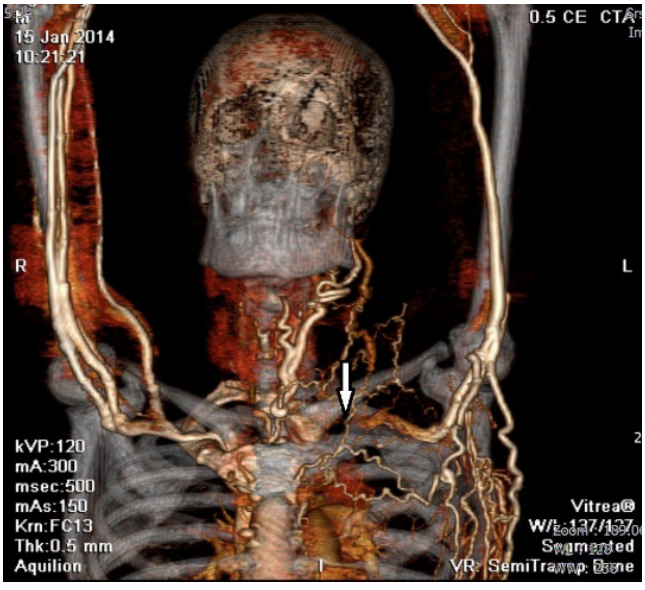

Fig 5. Direct computed tomography venography 3D Volume Rendered image of a 52 year-old male demonstrates left brachiocephalic vein thrombosis (arrow). Much collateral veins are noted at left hemithorax.

Limitations

The limitations of this study are a selection bias due to the lack of a control group, because it is not possible to perform CTV in a healthy individual. Therefore, all participants were symptomatic, which led to a high incidence rate. Further, information on several vascular access characteristics such as anatomic location, and time-to complication development was not available. Another limitation was the potential for variability in image interpretation because of the variation in radiologists' experience. Lastly, the study did not investigate the effects of radiation dose which is a topic of concern. However, as the conversion factor in this region is small, radiation exposure is expected to be minimal[3,24], and radiation hazard in this cohort would be less of a concern given the higher mean age of the patients.[25]

CONCLUSION

We describe a direct CTV technique used in our facility, which involved simultaneous injection of diluted contrast medium in both upper extremities. This technique not only has the benefit of requiring a lower volume of contrast medium while maintaining direct visualization of the venous system similar to conventional venography, but also avoids inflow phenomenon at the SVC. Additionally, mapping for venous reconstruction of both arms was possible based on data acquired. The notable findings in this study are beneficial for future study. Further comparative cross-sectional study or RCT in a larger population to demonstrate the benefit of this novel technique over the conventional method is required.

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Comparison of Radial Echoendoscopy and Predictive Factors in the Evaluation of Patients with Suspected Choledocholithiasis

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ABSTRACT

Objective: The aim of this study was to compare predictive factors and endoscopic ultrasound (EUS) in the diagnosis of choledocholithiasis.

Materials and Methods: Patients with suspected choledocholithiasis were recruited from April 2011 to January 2018. All patient characteristics, EUS findings and ERCP findings were recorded and analyzed.

Results: Eighty patients were enrolled in this study. Clinical symptoms, blood chemistry and liver function tests were similar in patients with and without choledocholithiasis. Using the findings of ERCP as the gold standard, radial EUS had a sensitivity and specificity for the detection of choledocholithiasis of 90.2% and 97.4%, and for choledocholithiasis and/or common bile duct sludge 92.7% and 100%, respectively. For patients with intermediate likelihood and high likelihood of having choledocholithiasis, as calculated from their predictive factors (33 and 45), radial EUS was positive for choledocholithiasis in 51.5% (17/33) and 46.7% (21/45), and ERCP was positive for choledocholithiasis in 54.5% (18/33) and 48.9% (22/45), respectively.

Conclusion: Predictive factors, for both the intermediate and high likelihood groups, were not accurate to diagnose these patients. EUS is a good diagnostic tool and should be performed in both groups of patients to avoid unnecessary ERCP.

Keywords: Echoendoscopy; choledocholithiasis (Siriraj Med J 2021; 73: 380-385)

INTRODUCTION

Cholelithiasis is a common problem and occurs 6-9% in the population.[1] Most patients are asymptomatic but some can develop biliary colic and other complications. Choledocholithiasis is one of the common complications and can occur in about 20% of these patients.[2] Once they have choledocholithiasis, cholangitis and acute biliary pancreatitis can occur. The diagnosis of patients with suspected choledocholithiasis can be made by clinical symptoms, physical examination, changes of liver function test and transabdominal ultrasonography. From previous studies, certain factors were found to improve the diagnostic accuracy of choledocholithiasis by up to 70%. Such factors included clinical ascending cholangitis, the common bile duct being larger than 6 mm with the gall bladder in situ on transabdominal

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ultrasonography, and a bilirubin level higher than 1.8 mg/dL.[3] Some imaging methods also have a role in the diagnosis of this condition, such as Endoscopic Ultrasound (EUS), Computerized Tomography (CT), Magnetic Resonance Imaging and Magnetic Resonance Cholangiopancreatography (MRI and MRCP), but the choice of which method to use depends on the local accessibility and the accuracy of each method.

An Endoscopic Ultrasound is an endoscope with an ultrasound probe at the tip which allows the endosonographer to examine the gastrointestinal tract, hepatobiliary tract and pancreas closely by ultrasound. This method is safe and has a low rate of complications, such as bowel perforation and bleeding (0.12%).[4] The accuracy of hepatobiliary and pancreatic examinations is reported to be as good as MRI and MRCP, at about 91-93% from a previous study.[5] For CT, MRI and MRCP, patients receive radiation or magnetic resonance as well as intravenous contrast media which may deteriorate renal function. Patients with renal insufficiency may have some limitation for both studies. The gold standard for the detection of choledocholithiasis is Endoscopic Retrograde Cholangiopancreatography (ERCP). ERCP can be both diagnostic and therapeutic at the same time, but as it is more invasive, complications can occur at a higher rate than for EUS, at about 4%, and include acute pancreatitis, perforation, bleeding and infection.[6]

EUS and ERCP are both highly accurate for detecting choledocholithiasis. EUS has a sensitivity of 89-94% and a specificity of 94-95% when ERCP findings are used as the gold standard. But EUS has lower complications (Relative risk 0.35, 95% CI 0.2-0.62; p<0.001) and a lower incidence of pancreatitis (Relative risk 0.21, 95% CI 0.06-0.83; p 0.03).[7]

The American Society of Gastrointestinal Endoscopy (ASGE) guideline[3] categorize patients with suspected choledocholithiasis into low, intermediate and high likelihood using predictive factors. Additional tests (MRCP or EUS) are recommended to confirm choledocholithiasis in the intermediate likelihood group before doing ERCP.

This study was designed to compare the sensitivity and specificity of the patients' predictive factors with the EUS findings in the detection of choledocholithiasis, in patients who underwent ERCP.

MATERIALS AND METHODS

This prospective, descriptive study was approved by Ramathibodi Ethical Committee. The study was performed in Ramathibodi Endoscopic unit from April 2011 to January 2018. We recruited patients 18 to 80 years old who were suspected of having choledocholithiasis and transabdominal ultrasonography was negative for choledocholithiasis. Patients had at least one of the following: clinical symptoms of cholangitis (fever, abdominal pain and jaundice), alkaline phosphatase > 300 unit/L, direct bilirubin > 1.8 mg/dL, clinical symptoms of gall stone pancreatitis, common bile duct (CBD) dilatation to at least 6 mm in patients with the gall bladder in-situ or common bile duct dilatation to at least 10 mm in patients after cholecystectomy.[3,8] We excluded patients who were unable to undergo esophagogastroduodenoscopy such as those with esophageal stricture, pyloric stricture and patients who refused to participate in the study. All patients were informed about risks and benefits of both procedures, radial EUS and ERCP. Patients signed the consent forms before starting all procedures.

EUS was performed in these patients as soon as possible by two endosonographers and an Olympus GF-UE160-AL5 Radial Array Ultrasound Gastrovideoscope was used. With the Radial EUS, the CBD was carefully evaluated for choledocholithiasis or common bile duct sludge. We defined choledocholithiasis as a hyperechoic lesion with posterior acoustic shadow or a hypoechoic lesion which was movable in the CBD and common bile duct sludge as hyperechoic foci or content in common bile duct which included microlithiasis and viscous bile fluid. Microlithiasis and viscous bile fluid are known to cause intermittent common bile duct obstruction and pancreatitis.[9-11] ERCP was done either at the same session or within 6 weeks for definite diagnosis and treatment. At ERCP, if the cholangiogram looked suspicious for choledocholithiasis or common bile duct sludge, endoscopic sphincterotomy was done and a balloon or basket extraction was applied to clear common bile duct. The diagnosis was confirmed by two experienced endoscopists.

All predictive factors, findings of radial EUS and findings of ERCP were compared by statistical analysis. All patients were classified by their predictors according to the ASGE guideline 2010 into low, intermediate and high likelihood groups to check the accuracy of this guideline (Fig 1).[3] Descriptive data was presented as mean with standard deviation (SD) or median with range. Factors associated with choledocholithiaisis were analyzed by Chi-square test or T-test. Statistical significance was defined as p-value < 0.05. The sensitivity and specificity values for radial EUS for the detection of choledocholithiasis and choledocholithiasis and/ or common bile duct sludge were calculated, using the ERCP findings as the gold standard. All statistical analyses were performed using STATA version 15.

Very strong	
Common bile duct stone on transabdominal ultrasonography	
Clinical ascending cholangitis	
Bilirubin >4 mg/dL	
Strong	
Dilated common bile duct on transabdominal ultrasonography (>6 mm with gall bladder in situ)	
Bilirubin level 1.8-4 mg/dL	
Moderate	
Abnormal liver biochemical test other than bilirubin	
Age older than 55 year	
Clinical gall stone pancreatitis	
Assigning a likelihood of choledocholithiasis based on clinical predictors	
Presence of any very strong predictor	high
Presence of both strong predictor	high
No predictor present	low
All other patients	intermediate

Fig 1. Predictors of choledocholithiasis[3]

RESULTS

Eighty patients were recruited into this study from a total of 722 patients who underwent ERCP for choledocholithiasis in the same time period. For the 80 patients with suspected choledocholithiasis and negative choledocholithiasis on transabdominal ultrasonography, the mean (±SD) of age was 65.2 (±14.9) years old and 46 patients were female. Patients presented with abdominal pain 87.5% (70/80), fever 58.8% (47/80) and jaundice 71.3% (57/80). The median (range) for alkaline phosphatase was 183 u/L (62-1309), serum glutamic-oxaloacetic transaminase level 112 (16-4289) U/L, serum glutamic-pyruvic transaminase level 142 (13-1782) U/L, total bilirubin 2.5 (0.2-16) mg/dL and direct bilirubin 1.7 (0.1-12) mg/dL. In 69 patients, radial EUS and ERCP were done on the same day/ same setting. For nine patients these two procedures were done with an interval of one day, and in two patients, the interval was 35 days. No complication was noted in all recruited patients.

Radial EUS showed choledocholithiasis in 37 patients and ERCP confirmed choledocholithiasis in 41 patients. The sensitivity and specificity of radial EUS for detecting choledocholithiasis were 90.2% (37/41) and 97.4% (38/39), respectively (Table 1). Baseline characteristics for patients with and without choledocholithiasis, including symptoms, blood chemistry and liver function tests, were similar (Table 2). Only the mean age of patients was higher in patients with choledocholithiasis, mean±SD: 68.66 (14.58) vs 61.54 (14.49), p=0.032.

When we included patients with choledocholithiasis and/ or common bile duct sludge, radial EUS was positive in 63 patients and ERCP was positive in 68 patients. The sensitivity and specificity of radial EUS for detecting choledocholithiasis and / or sludge in common bile duct were 92.7% (63/68) and 100% (12/12), respectively (Table 1). When we analyzed the performance of radial EUS in detecting only common bile duct sludge, the sensitivity was 72.5% (29/40) and the specificity was 95% (38/40), (Table 1).

When the predictors for choledocholithiasis were used to classify the patients according to the ASGE guideline 2010, 2 of our patients were in the low likelihood group, 33 in the intermediate likelihood group and 45 in the high likelihood group. The guideline recommended further investigations for the intermediate group. In this group, radial EUS was positive for choledocholithiasis in 17 patients (51.5%, 17/33) and ERCP was positive for choledocholithiasis in 18 patients (54.5%, 18/33). When we included patients with choledocholithiasis and/ or common bile duct sludge, Radial EUS was positive

TABLE 1. Performance of EUS (ERCP findings as gold standard)

Performance of EUS	Sensitivity EUS/ERCP, n (%)	Specificity EUS/ERCP, n (%)	ROC area, (95% CI)
For choledocholithiasis	37/41 (90.2%)	38/39 (97.4%)	0.94 (0.89-0.99)
For CBD sludge	29/40 (72.5%)	38/40 (95%)	0.86 (0.79-0.93)
For choledocholithiasis and/or CBD sludge	63/68 (92.7%)	12/12 (100%)	0.96 (0.93-0.99)
Categorized by ASGE guideline:			
Intermediate likelihood group			
For choledocholithiasis	17/18 (94.4%)	15/15 (100%)	0.97 (0.92-1)
For choledocholithiasis and/or CBD sludge	25/28 (89.3%)	5/5 (100%)	0.95 (0.89-1)
High likelihood group			
For choledocholithiasis	21/22 (95.4%)	23/23 (100%)	0.97 (0.93-1)
For choledocholithiasis and/or CBD sludge	36.38 (94.7%)	7/7 (100%)	0.97 (0.94-1)

Abbreviations: CBD: common bile duct, ROC: Receiver operating characteristic

in 25 patients (75.8%, 25/33) and ERCP was positive in 28 patients (84.8%, 28/33). For the high likelihood group, radial EUS was positive for choledocholithiasis in 21 patients (46.7%, 21/45) and ERCP was positive for choledocholithiasis in 22 patients (48.9%, 22/45). When we included patients who were positive for choledocholithiasis and/ or sludge in the analysis, radial EUS was positive in 36 patients (80%, 36/45) and ERCP was positive in 38 patients (84.4%, 38/45). The accuracy of EUS in the diagnosis of choledocholithiasis, and choledocholithiasis and/ or common bile duct sludge was high in both groups of patients and is shown in Table 1.

DISCUSSION

Endoscopic Ultrasound is a low risk endoscopic procedure which can evaluate the common bile duct in patients with suspected choledocholithiasis. Previous studies have suggested that a strategy of EUS-directed ERCP for choledocholithiasis allowed 50-70% of the patients to avoid a diagnostic ERCP and reduced complications by 4-7%. At 1-year follow-up, there was no difference in outcomes between patients in both groups.[12-14]

In this study, we recruited patients with suspected choledocholithiasis from clinical symptoms and blood chemistry, without visible choledocholithiasis on transabdominal ultrasonography. The reason that our study recruited this group of patients was that patients who were positive for choledocholithiasis on transabdominal ultrasonography would go straight for ERCP. They would not need to have another non-invasive imaging technique such as EUS to confirm choledocholithiasis. Most of the patients in this study (69/80) underwent radial EUS and ERCP back-to-back, and 9/80 patients had both procedures with an interval of only one day. As a result, the results of both procedures, and the difference between them would unlikely be confounded by the passage of stones in between the procedures. A long waiting time between the two procedures would allow the passing of choledocholithiasis and give a false negative ERCP result. Conversely, cholelithiasis could also be passed into the common bile duct during the waiting time. This would produce a positive ERCP result, and decrease the sensitivity of EUS.

TABLE 2. Comparison baseline characteristic between positive and negative choledocholithiasis (CBD stone) from ERCP.

Characteristic	ERCP_CBD=negative (n=39)	ERCP_CBD=positive (n=41)	P-value
Age, mean (SD)	61.54 (14.49)	68.66 (14.58)	0.032
Sex, n (%)			
male	14 (35.9)	20 (48.78)	0.244
female	25 (64.1)	21 (51.22)	
Abdominal pain, n (%)			
no	5 (12.82)	5 (12.2)	1.000
present	34 (87.18)	36 (87.8)	
Fever, n (%)			
no	18 (46.15)	15 (36.59)	0.385
present	21 (53.85)	26 (63.41)	
Jaundice, n (%)			
no	8 (20.51)	15 (36.59)	0.112
present	31 (79.49)	26 (63.41)	
ALP, median (range)	174 (64, 1309)	196 (62, 503)	0.434
SGOT median (range)	118 (24, 4289)	99 (16, 1065)	0.713
SGPT median (range)	143 (23, 1782)	137 (13, 782)	0.906
GGT median (range)	353 (9, 2344)	462 (31, 1396)	0.201
TB median (range)	2.40 (0.210, 10.80)	2.70 (0.20, 16)	0.721
DB median (range)	1.70 (0.10, 8.60)	1.50 (0.10, 12)	0.743
Categorized by ASGE guideline			
Low likelihood, n (%)	1 (2.56)	1 (2.43)	
Intermediate likelihood, n (%)	15 (38.46)	18 (43.9)	
High likelihood, n (%)	23 (58.97)	22 (53.65)	

Abbreviations: ALP: alkaline phosphatase, SGOT: serum glutamic-oxaloacetic transaminase, SGPT: serum glutamic-pyruvic transaminase, GGT: gamma glutamyl transferase, TB: total bilirubin, DB: direct bilirubin

Common bile duct sludge is known to cause similar complications to choledocholithiasis such as cholangitis, common bile duct obstruction and gall stone pancreatitis. So we also analyzed choledocholithiasis and/ or common bile duct sludge in the study. Radial EUS was found to have high sensitivity and specificity for the detection of choledocholithiasis, at 90.2% and 97.4% respectively, and also for choledocholithiasis and/or common bile duct sludge, at 92.7% and 100%, respectively. Radial EUS had a lower sensitivity, 72.5%, but still a high specificity, 95%, for detecting only common bile duct sludge. The amount of sludge in the common bile duct varied for each patient and may have affected the performance of the radial EUS.

There was no significant difference in the baseline characteristics between patients with choledocholithiasis

and without choledocholithiasis, particularly in terms of clinical symptoms (abdominal pain, fever and jaundice) and blood chemistry including the liver function test. It seems that the predictors were not so useful in this study.

For patients with intermediate likelihood and high likelihood of choledocholithiasis according to the ASGE guideline 2010[3] classification, ERCP was positive for choledocholithiasis 54.5% (18/33) and 48.9% (22/45), respectively. The accuracy of the predictors from this guideline was also low for our patients in both groups. EUS findings were more accurate than the predictors.

Although this study was prospective in design, it only had a small number of patients, making it difficult to suggest the applicability of the findings with any strength. A further large study may confirm the findings of this study.

CONCLUSION

Endoscopic Ultrasound with a radial echoendoscope was highly accurate and a safe endoscopic procedure for the detection of choledocholithiasis when performed by experienced endosonographers. For patients in both the intermediate likelihood and the high likelihood groups, EUS showed benefit and allowed patients to avoid unnecessary Endoscopic Retrograde cholangiopancreatography and its related complications.

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Transepidermal Water Loss after Water Immersion

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ABSTRACT

Objective: To observe changes in transepidermal water loss (TEWL) at different times after water immersion.

Materials and Methods: TEWL values were measured before water immersion and at 3, 5, 10, 15, 20, and 30 minutes after immersion of the skin in water for 5 minutes.

Results: Forty-one healthy volunteers were enrolled with an average age of 30.4±5.5 years. Twenty-five subjects were female and sixteen were male. The TEWL value before water immersion ($TEWL_{baseline}$) was 13.16±7.27 g/m^2/h and TEWL values at 3, 5, 10, 15, 20 and 30 minutes after immersion were 23.21±7.67, 16.12±3.42, 14.76±6.36, 14.45±6.67, 13.53±4.67 and 12.96±5.18 g/m^2/h, respectively. After immersion, TEWL values at 3 and 5 minutes statistically increased compared to $TEWL_{baseline}$ (p<0.001). TEWL values between 10 to 30 minutes gradually dropped with no statistically significant difference compared to the previous period and $TEWL_{baseline}$. Although total water loss from the skin occurred within 30 minutes, 56.9% of it occurred within 10 minutes after immersion. There was no significant difference between $TEWL_{baseline}$ in males and females but the TEWL values at 3, 5 and 15 minutes after immersion in males was higher than in females (p<0.05).

Conclusion: TEWL statistically increased after water immersion for only 5 minutes. The cumulative percentage of TEWL was high within 10 minutes. Gender did not affect TEWL values before immersion; however, males experienced more water loss from the skin than females after immersion. Therefore, moisturizer should be applied immediately before TEWL occurs.

Keywords: Immersion; TEWL; transepidermal water loss (Siriraj Med J 2021; 73: 386-390)

INTRODUCTION

The skin has multiple defensive and regulatory functions. However, its most important function is to act as barrier against external stresses and the percutaneous penetration of chemicals, allergens, and organisms. This barrier function is almost entirely present in the epidermis and in particular the stratum corneum (SC). The skin also maintains water and electrolyte homeostasis and thermoregulation.[1,2]

The functional state of the skin can be investigated by assessing non-invasive biophysical parameters which are influenced by several factors.[2-5] For example, in vivo evaluation by measurement for TEWL has proven to be a reliable indicator of the function of the skin barrier.[3-9] A defective skin barrier leads to increased TEWL along with dry skin.[9,10] Therefore, TEWL values are directly related to the clinical severity of lesions in various skin diseases with altered barrier function such as atopic dermatitis, ichthyosis, contact dermatitis, and psoriasis.[1] To manage dry skin, bathing and application of moisturizer is necessary. How the skin changes at different time periods after bathing can provide useful information for skin care recommendations.

The purpose of this study was to characterize the function of skin barrier as evaluated by TEWL values at different time points after immersion.

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MATERIALS AND METHODS

A prospective study was conducted at the Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University. The study protocol was approved by the Siriraj Ethical Review Board (Si 702/2015) and the written informed consent was obtained from all participants.

Healthy participants with no pre-existing dermatoses or any systemic disorders that could alter the biophysical parameters were enrolled. The participants were asked not to apply any skin product for at least 12 hours prior to testing and to avoid washing the area to be tested on evaluation day. Before any measurements were made, participants were asked to rest for 15 minutes in a room with controlled environmental conditions to help them acclimatize. All measurements were made in highly comparable temperatures and humidity-controlled conditions in accordance with the guidelines for standardized skin parameter measurements in the European Group on Efficacy Measurement and Evaluation of Cosmetics and Other Products (EEMCO) guidelines and by the Standardization Group of the European Society of Contact Dermatitis.[11] The TEWL value was measured using a Tewameter™ TM 300 (Courage and Khazaka electronic, Köln, Germany) and expressed in g/m^2/h. Participants were asked to immerse the test area in water rather than bathe. The volar region of the forearm, which is often selected as the test site in dermatological research, was selected as the test area. After baseline TEWL readings were recorded, the volunteer's forearms were immersed in the water bathtub for five minutes followed by pat drying with towels and then measuring TEWL again at 3, 5, 10, 15, 20, and 30 minutes later. To avoid inaccuracies, each measurement was repeated three times at the nearby areas and the mean value was used for analysis. The patients were monitored for side effects after the measurements were completed.

The data was analyzed using SPSS Statistical software, version 20 (IBM, Chicago, IL, USA). A normal distribution of the data was examined by Kolmogrov-Smirnov test. The demographic data was presented through descriptive statistics with an average ± standard deviation (SD) for normally distributed values and median and range for non-normally distributed data. For normally distributed data, an independent t-test was used to compare the age between genders (Mann-Whitney U test for non-normal distribution). To compare TEWL values at each time point and between genders, an analysis of variance (ANOVA) with Bonferroni post-hoc test was used (Friedman's test with Bonferroni post-hoc test for non-normal distribution). A *p*-value of less than 0.05 was regarded as being statistically significant.

RESULTS

This study contained forty-one healthy volunteers (61% female and 39% male) with an average age of 30.4±5.5 years. There was no statistical difference in age between both genders (p=0.190). The average room temperature was 23.20±0.89°C, and the relative humidity was 47.00±7.38%.

The TEWL value before water immersion ($TEWL_{baseline}$) was 13.16±7.27 g/m^2/h. After immersion, the TEWL value statistically increased at 3 minutes, and 5 minutes, (p<0.001) before rapidly decreasing during 10 to 30 minutes after immersion when it showed no statistical difference compared to the baseline (Table 1).

Cumulative TEWL after immersion, which reflects total water loss from the skin, was 23.2, 39.3, 54.1, 68.5, 82.1, 95.0 g/m^2/h at 3 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 30 minutes, respectively (Fig 1). Regarding the percentage of cumulative TEWL at different time points, 24.4%, 41.4% and 56.9% of TEWL occurred within 3 minutes, 5 minutes, and 10 minutes after immersion, respectively.

The baseline TEWL value in males was the same as in females (p=0.121). The TEWL values in both male and female volunteers increased after immersion in water (p<0.001), however, it became equal to before immersion at 10 minutes (p>0.05). The TEWL values in males were statistically higher than in females at 3 minutes, 5 minutes, and 15 minutes after immersion (Table 2). No side effects were reported from the testing.

DISCUSSION

Non-invasive biophysical skin parameters such as TEWL are widely accepted as a reliable tool for assessment of the skin barrier function. Dry skin is a common condition in many skin diseases. Regular bathing and application of moisturizer to hydrate the skin and prevent water loss is necessary. This study demonstrated a significant increase in TEWL post water immersion compared to the baseline, however, the value returned to the baseline levels after 10 minutes. The cumulative TEWL value within 10 minutes of testing was 56.9% of all water loss in 30 minutes. The baseline TEWL values showed no statistical difference between male and female, but TEWL values in males was higher than females until at least 15 minutes after immersion.

Our data confirmed results from previous studies which showed that TEWL increased after water application.[10,12-14] Although an increase in TEWL values usually relate to impairment of the skin barrier,[7-9,15] the increased in TEWL after immersion was not only due to an impaired skin barrier function. It is assumed that the skin absorbs

TABLE 1. Comparison of TEWL values at different time points.

Time	TEWL (mean±SD) g/m²/h	*p*-value compare to baseline	*p*-value compare to previous measurement
Before immersion (baseline)	13.16±7.27	-	-
3 min after immersion	23.21±7.67	<0.001*	<0.001*
5 min after immersion	16.12±3.42	<0.001*	<0.001*
10 min after immersion	14.76±6.36	1.000	0.166
15 min after immersion	14.45±6.67	1.000	0.656
20 min after immersion	13.53±4.67	1.000	1.000
30 min after immersion	12.96±5.18	1.000	1.000

*p-value <0.05 = statistical significance

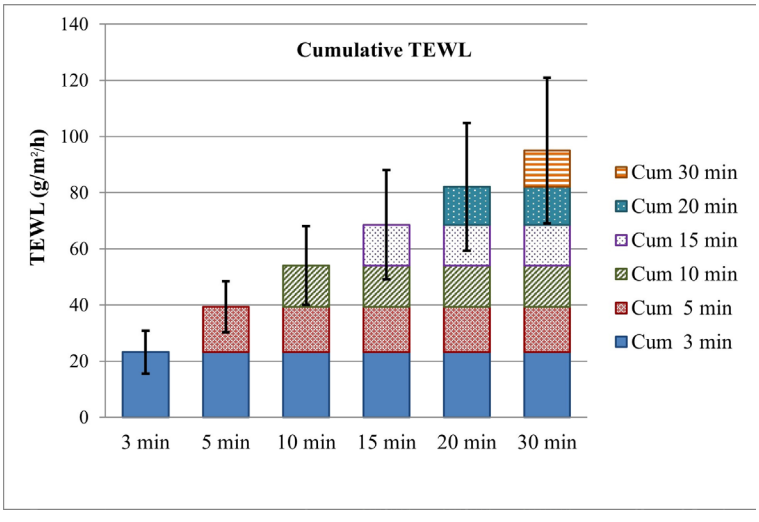

Fig 1. Cumulative TEWL at every time point after immersion.

TABLE 2. Comparison of TEWL values between genders at different time points.

Time	TEWL (mean±SD) g/m²/h		*p*-value compare between genders
	Male	Female	
Before immersion (baseline)	14.77±9.09	12.05±5.55	0.121
3 min after immersion	25.93±10.34	21.34±4.32	0.012*
5 min after immersion	16.84±3.34	15.61±1.79	0.048*
10 min after immersion	16.14±8.25	13.80±4.50	0.130
15 min after immersion	16.78±9.14	12.84±3.53	0.013*
20 min after immersion	14.32±4.84	12.98±4.53	0.237
30 min after immersion	12.94±2.52	12.97±5.18	0.979

*p-value <0.05 = statistical significance

water instantaneously after being immersed; however, the water-holding capacity of the SC is maintained only for a short period of time after which the remaining excess water evaporates from the skin leading to a gradual return to baseline levels.[10,14,16,17]

According to results obtained, immersion increased TEWL values and its accumulation correlates to an absolute total amount of water loss from the skin was high (56.9%) within 10 minutes after immersion.[15] If the TEWL continues, dry skin will get worse. Apply occlusive moisturizer as soon as possible after bathing to maintain SC water content should be recommended as part of the skin care regimen.[1,10,18] Patients with dry skin will benefit from occlusive moisturizer application when skin hydration is still retained.[10,18,19]

Although a precise definition of normal TEWL value does not exist,[8,20] variations in TEWL values have been well-documented.[2,5-7,20] A wide ranges of TEWL values are influenced by several endogenous, exogenous, and environmental factors.[8,20] There is insufficient evidence to conclude that gender affects TEWL.[20] While some studies did not observe much of a difference in TEWL values between genders,[4,15,21] others noticed higher TEWL values in males.[2,4,5,9,21] The baseline TEWL value in our study was not different between genders but the TEWL value after immersion was significantly higher in males who experienced more water loss from the skin than females, possibly due to different hormonal effects, skin conditions, barrier functions, and outdoor working habits and activities.[21]

This study has limitations because the duration of water exposure was only five minutes, which may be too short to reveal profound effects of skin barrier function structurally and functionally. Furthermore, this study measured only TEWL values, other biophysical parameters such as skin capacitance and pH might add more information about dynamic changes in the skin after water immersion. Hence, future studies with longer immersion duration, more frequent measurements, and a range of biophysical skin parameters should provide more helpful information.

CONCLUSION

In conclusion, this study evaluated the effects of the routine practice of water exposure through water immersion of the skin. Since the TEWL value increased significantly for a short period, it is reasonable to encourage the application of moisturizer immediately after bathing to prevent water loss from SC

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Spontaneously Ruptured Hepatocellular Carcinoma Treated by Transarterial Embolization Compared with Conservative Treatment: Survival Outcome and Prognostic Factors

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ABSTRACT

Objective: To report the survival outcome and prognostic factors in the patients with spontaneously ruptured hepatocellular carcinoma treated by transarterial embolization compared with conservative treatment.

Materials and Methods: A retrospective review of 89 patients who had spontaneous rupture of hepatocellular carcinoma (HCC) at Siriraj Hospital between January 2011 and February 2017 were enrolled. Ruptured HCC patients are diagnosed by clinical presentations of abdominal pain/distension, anemia/shock with dynamic liver computed tomography findings as: hemoperitoneum, focal discontinuity or tumor protrusion of the hepatic surface and/or active contrast material extravasation. We compared the survival outcome and prognostic factors of the ruptured HCC patients who received two treatment methods; conservative treatment and transarterial embolization (TAE).

Results: The cumulative median survival time of the ruptured HCC patients was significantly higher in the TAE group (81 days) than in the conservative treatment group (29 days) with p-value = 0.006. There were two significant predictors for post-treatment mortality. First, treatment modality in the TAE group showed a significantly lower mortality rate than in the conservative treatment group with a hazard ratio (HR) 0.454 (p-value = 0.003). Second, a pre-treatment high hematocrit level was a significant predictive factor for lower mortality than a low hematocrit level with a hazard ratio (HR) 0.946 (p-value = 0.016).

Conclusion: TAE results in a good clinical outcome and increased survival rate in the patients with ruptured HCC. A pre-treatment high hematocrit level was a good prognostic factor for the survival in ruptured HCC patients.

Keywords: Prognostic factors; survival outcome; ruptured hepatocellular carcinoma; transarterial embolization (Siriraj Med J 2021; 73: 391-398)

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignant hepatic tumor and the second most common cause of cancer-related death in the world.[1] In Thailand, it occurs as one of the most common cancers in men and the third most common cancer in women.[2] Previous Thai studies have reported that spontaneous tumor rupture is the most common complication found in 16% of cases[2,3] and approximately 10% of patients with HCC die from this severe complication.[4]

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The mechanisms of ruptured HCC have not yet been fully elucidated. Some authors believe that it depends on multifactorial factors[5], including disruption of the feeding artery or a tear at the tumor surface. Others believe that bleeding is likely due to a laceration of a tumor located superficially resulted from a minor trauma. Some hypothesize that increased pressure in the tumor from a sudden occlusion of hepatic vessels causes venous congestion, in conjunction with central necrosis within the tumor and coagulopathy, leading to bleeding or rupture.

In this study, the diagnosis of HCC was based on the *Thailand Guideline for Management Hepatocellular Carcinoma 2019*[6] by the Thai Association for the Study of the Liver (THASL). The most common clinical presentation of ruptured HCC patients is acute abdominal pain with or without shock. However, a definite diagnosis of the ruptured HCC needs to be confirmed by dynamic contrast-enhanced computed tomography (CT).[7,8] The advantages of CT imaging is its ability to demonstrate the tumor location, size, number, degree of hemoperitoneum, portal vein tumor thrombus, and extrahepatic lesions. From a literature review, we found that almost all previous Thai studies reported the treatment outcome in patients with unruptured HCC.[9] There are only two Thai studies concerning the treatment of ruptured HCC patients.[10,11] Also, a study by Kerdsuknirun et al.[12] stated about the overall survival of ruptured HCC patients compared to non-ruptured cases, but the report did not mention the specific treatment.

Consequently, the objective of this study aimed to report the survival outcome and prognostic factors of patients with spontaneously ruptured HCC treated by TAE compared with conservative treatment in Thailand.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of Siriraj Hospital, protocol number 844/2016 (EC3). The research involved a retrospective study of the ruptured HCC patients treated at the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand from January 2011 to February 2017. Inclusion criteria was ruptured HCC patients who were 15 years old or older and treated with TAE or conservative treatment whose CT imaging and clinical data were available. Exclusion criteria was patients who were missing data.

A CT scan of the abdomen (120 kVp; 400 mA; slice thickness, 1.25 mm) was performed for diagnosis in all patients using a 64-slice CT scanner, General Electric (GE) Light speed volumetric CT (VCT), and GE Discovery CT 750HD instruments with an intravenous non-ionic iodinate contrast media (350 mg I/ml) at a dose of 2 ml/kg. We defined ruptured HCC according to dynamic contrast enhanced CT[7,8] demonstrating an arterial enhancing tumor with delayed phase washout associated with hemoperitoneum, focal disruption of the liver capsule, protruding tumor from the hepatic surface area, and/or active contrast extravasation.

A total of 89 patients who met the inclusion criteria of ruptured HCC were reviewed. Demographic data included age, gender, date of diagnosis, and date of death. The laboratory tests: complete blood count (CBC: hematocrit and platelet count), coagulogram (prothrombin time or PT, international normalized ratio or INR), liver function test (LFT: total bilirubin, direct bilirubin, albumin), and types of viral hepatitis were collected. We did not obtain the clinical encephalopathy because it was not recorded in all patients.

For the CT findings, we evaluated the size of the ruptured tumor (measured as the longest diameter in one dimension), number of tumors, location of the ruptured tumor (capsular region or protrusion from the hepatic capsule), ascites, surrounding perihepatic hematoma, portal vein thrombosis, extrahepatic metastasis, and contrast extravasation from the CT scan or angiogram.

After ruptured HCC was diagnosed, the patient underwent immediate resuscitation, including intravenous fluid and blood transfusion with or without abdominal paracentesis. Of the 89 patients, 45 (50.6%) patients received conservative management and 44 (49.4%) patients underwent TAE for emergency hemostatic treatment.

TAE group: In hemodynamic instability patients or patients with continuous bleeding, TAE was chose if the patient's liver function was preserved. The procedures were performed by four experienced interventional radiologists. The tumor location, neovascularization, and active bleeding area were determined by angiogram. Selective arterial embolization was performed in all patients using a 5Fr angiographic catheter (Radiofocus®, Terumo®, Tokyo, Japan) followed by super-selective catheterization using a 2.7Fr microcatheter (Progreat®, Terumo®, Tokyo, Japan). For embolic materials, our center typically uses a temporary occlusive particle, Gelfoam® (Spongostan™ Absorbable Gelatin Sponge, Denmark), which is cut into a small cube approximately 1 mm in size. Seven patients received additional Ethiodized Oil (Lipiodol® UltraFluide, Guerbet, France), an oil-based radio-opaque contrast agent which has a specific characteristic for transient embolization at the hepatic sinusoid level. One patient received additional polyvinyl alcohol, PVA® (Contour®, Boston Scientific, Ireland), which is a permanent embolic particle. Successful control of the bleeding was defined

as stabilization of the hemodynamic and hematocrit level without further pack red cell (PRC) transfusion.

Conservative group: The patients who had hemodynamic stability received the best conservative treatment, such as replacement of the blood component, albumin, diuretic, and/or analgesic drugs with the correction of coagulopathy.

Statistical analysis

The patients' baseline characteristics are shown as medians (ranges) and frequencies. We used the Student's t test and Mann-Whitney U test for the differences between categories or continuous variables. The post-treatment cumulative mortality rate between the two groups (conservative and TAE groups) was analyzed using the chi-square test or Fisher's exact test. Survival time was defined from the diagnosis of ruptured HCC to the patient's date of death. For the multivariate analysis, a COX regression hazard model to demonstrated the predictive factors of post-treatment mortality was performed. A two-tailed p-value of less than 0.05 was considered statistically significant in all the analyses with the SPSS Statistics 23.

RESULTS

In total, 89 patients with ruptured HCC were categorized into two groups according to the treatment modality: group 1, consisting of 45 patients who received conservative treatment, and group 2, consisting of 44 patients who received TAE treatment.

The baseline characteristics of all the patients as well as the clinical variables of the two treatment groups, including the unit of PRC transfusion, laboratory results, and CT findings, are shown in Table 1. Most of the patients with ruptured HCC had evidence of anemia which were evaluated by low hematocrit level and the number of PRC transfusion. The univariate analysis showed that the hematocrit level and total bilirubin were significantly higher in the conservative group compared with in the TAE group (p-value = 0.035 and 0.027, respectively). Portal vein thrombosis and surrounding hematoma were significantly more evidence in the conservative group than in the TAE group (p-value < 0.001 and 0.04, respectively). The other clinical parameters showed no significant difference between the conservative treatment group and the TAE group.

The data of the ruptured HCC patients who received TAE treatment showed contrast extravasation from angiogram in 6 patients (13.6%) and tumoral neovascularization in 44 patients (100%). The embolic material used for TAE were Gelfoam® in 37 patients (84.1%), Gelfoam® with PVA® in 1 patient (2.3%), and Gelfoam® with Lipiodol® in 6 patients (13.6%).

The cumulative median survival time of the ruptured HCC patients was significantly higher in the TAE group (81 days) compared to the conservative treatment group (29 days) (p-value = 0.006). The cumulative survival rates at 1, 3, and 6 months were 46.7%, 28.9%, and 17.8% in the conservative treatment group, and 70.5%, 50%, and 38.6% in the TAE group, respectively (Fig 1).

The results from the multivariate analysis of the significant predictive factors for post-treatment mortality in the patients with ruptured HCC are summarized in Table 2. There were only two significant predictors: the treatment modality in the TAE group, which showed a significantly lower mortality rate than for the conservative treatment group with a hazard ratio (HR) of 0.454 (p-value = 0.003), and the pre-treatment high hematocrit level, which was a significant predictive factor for lower mortality than a low hematocrit level with a hazard ratio (HR) of 0.946 (p-value = 0.016).

DISCUSSION

Spontaneous rupture of hepatocellular carcinoma (HCC) is a serious complication of HCC. It occurs in approximately 3-15% of cases, resulting a high mortality rate between 25%-75%.[13-14] The previous study reported that TAE was an effective treatment modality for ruptured HCC patients, which showed a better survival rate than those in the supportive treatment group.[15-16] Moreover, TAE is less invasive than surgical treatment. Therefore, currently, TAE is the first-line hemostasis in cases of ruptured HCC whereas conservative treatment can be considered in the patients who have stable hemodynamics and no demonstrated active contrast extravasation from CT imaging.

One study from Thailand (2012)[10] involving 94 patients with spontaneous rupture of HCC during 1997-2011, found the median survival of embolized and non-embolized patients were 34 vs. 9 days (p-value = 0.005). Our study showed that the cumulative overall survival rates at 1, 3, and 6 months of the patients with ruptured HCC were higher in the group treated with TAE than those in the conservative treatment group. However, there might be significant factors in two groups that may lead to the different overall survival, as the patients in TAE group had better LFT and the patients in conservative group had more portal vein thrombosis. The median survival rate of the ruptured HCC patients was 29 days in the conservative group and 81 days in the TAE group, with p-value = 0.006, which was longer than in that previous report. It was also probably be due to the development of new technique and better equipment used in the interventional radiology field.

TABLE 1. Baseline clinical data of the 89 patients enrolled in the study and according to two treatment modalities: a conservative treatment group and a transarterial embolization (TAE) group.

Variable	Conservative group (n = 45)	TAE group (n = 44)	p-value
Age (year)	60 (41- 88)	62 (37- 88)	0.51
Gender (male)	38 (84.4%)	35 (79.5%)	0.55
PRC transfusion (unit)	3.4	4.4	0.58
Hct, g/dL	27	24	0.035
Platelet, 1,000/mm^3	204	193	0.65
Prothrombin time	20	18	0.68
Albumin, mg/dL	2.8	3.0	0.198
Total bilirubin, mg/dL	4.7	2.5	0.027
Direct bilirubin, mg/dL	3.5	1.7	0.062
Multiple tumors	42 (93.3%)	40 (81.9%)	0.240
Ruptured tumor size, cm	11.8	10.7	0.28
Viral hepatitis (A/B/C/None)	0 (0%) / 22 (48.9%) / 10 (22.2%) / 13 (28.9%)	1 (2.3%) / 23 (52.3%) / 8 (18.2%) / 12 (27.3%)	0.73 0.181
Ascites (mild/moderate/severe)	7 (15.6%) / 21 (46.7%) / 17 (37.8%)	5 (13.5%) / 29 (56.2%) / 10 (22.7%)	
Protrusion from hepatic capsule	44 (97.8%)	44 (100.0%)	1.000
Capsular region of ruptured tumor	45 (100.0%)	44 (100.0%)	NA
Disruption of hepatic capsule	45 (100.0%)	44 (100.0%)	NA
Portal vein thrombosis	38 (84.0%)	21 (47.7)	<0.001
Extrahepatic metastasis	26 (58.8%)	12 (28.3)	0.068
Hemoperitoneum	45 (100.0%)	44 (100.0%)	NA
Surrounding hematoma	32 (71.1%)	39 (88.6%)	0.040
Active contrast extravasation from CT scan	8 (17.8%)	11 (25.0%)	0.406

Abbreviations: NA = Not applicable, PRC = pack red cell, Hct = hematocrit, CT = computed tomography

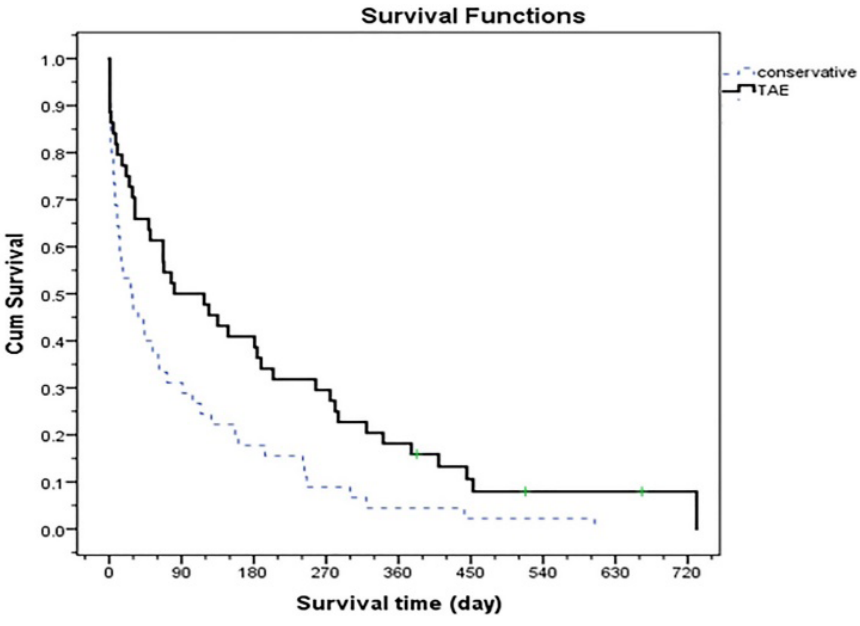

Fig 1. Cumulative median survival rate in the patients with ruptured HCC, showing that the survival rate in the transarterial embolization (TAE) group was significantly higher than in the conservative group (p-value = 0.006).

TABLE 2. Multivariate analysis of the significant predictive factors for post-treatment mortality in ruptured hepatocellular carcinoma patients.

	Multivariate analysis 95.0% CI for Exp (B)			
Variable	**HR**	**Lower**	**Upper**	**p-value**
1. Treatment modality (conservative or TAE)	0.454	0.271	0.761	0.003
2. Portal vein thrombosis	1.518	0.912	2.525	0.108
3. Surrounding hematoma	1.561	0.853	2.857	0.148
4. Extrahepatic metastasis	0.789	0.480	1.296	0.349
5. Hct	0.946	0.904	0.989	0.016
6. TB	0.889	0.609	1.296	0.540
7. DB	1.161	0.756	1.782	0.494

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio, TAE = transarterial embolization, Hct = hematocrit, TB = total bilirubin, DB = direct bilirubin

A recent study from India[17] reported the outcome of conventional transarterial chemoembolization (cTACE) in 16 patients with spontaneously ruptured HCC. They found that the overall cumulative survival rates at 30 days, 180 days, and at 1 year were 87.5%, 72.2%, and 54.1%, respectively. However, in our hospital, we usually perform TAE without additional chemo-infusion in the ruptured HCC cases because these patients mostly have unstable hemodynamics. The primary treatment end point is to achieve hemostasis and save life first. Then, we may schedule for TACE in the next session both via hepatic or extrahepatic collateral arteries supplying HCC.[18]

Another recent study reported that TAE was effective for initial treatment in patients with spontaneously ruptured HCC.[19] They found that large sized tumors, a

poor Child-Pugh classification, and shock were significantly associated with poorer prognosis. Our study did not evaluate the Child-Pugh score because we had incomplete data of clinical encephalopathy to calculate the score in all patients and some patients presenting with shock which could not evaluate the encephalopathy. The multivariate analysis of this study showed two significant predictive factors for lower post-treatment mortality; TAE treatment and pre-treatment high hematocrit level. This meant that the patients who had TAE and pre-treatment high hematocrit would have less mortality than the patients who had conservative treatment and low hematocrit level. However, the amount of PRC transfusion was not a significant variable for indicating the bleeding severity in our study, which may be due to unavailable information regarding blood transfusion unit the patients received before transferring to our hospital.

In the conservative treatment group, 8 (17.8%) of 45 patients showed evidence of active contrast extravasation on their CT findings but the clinician still chose conservative management instead of TAE. This might imply that these patients were not suitable for TAE.

In the TAE group, the CT findings showed active extravasation in 11 (25 %) of 44 patients (Fig 2A, B), but showed no significant impact factor for post-treatment mortality. However, the angiographic findings showed active contrast extravasation in only 6 (13.6%) of 44 patients (Fig 2D), which may explain by spontaneous hemostasis of the bleeding from the time of the CT scan to the angiogram.

The CT appearances in all 89 cases of ruptured HCC were hemoperitoneum (Fig 3A), tumor located at the capsular region and disruption of the hepatic capsule (Fig 3B, C). Protrusion of the tumor from the hepatic capsule was demonstrated in nearly almost patients. Most of the ruptured tumors' size was large, with a mean size of 11.7 cm in the conservative group and 10 cm in the TAE group. This indicated that tumors with large size tend to experience spontaneous rupture more than small size.

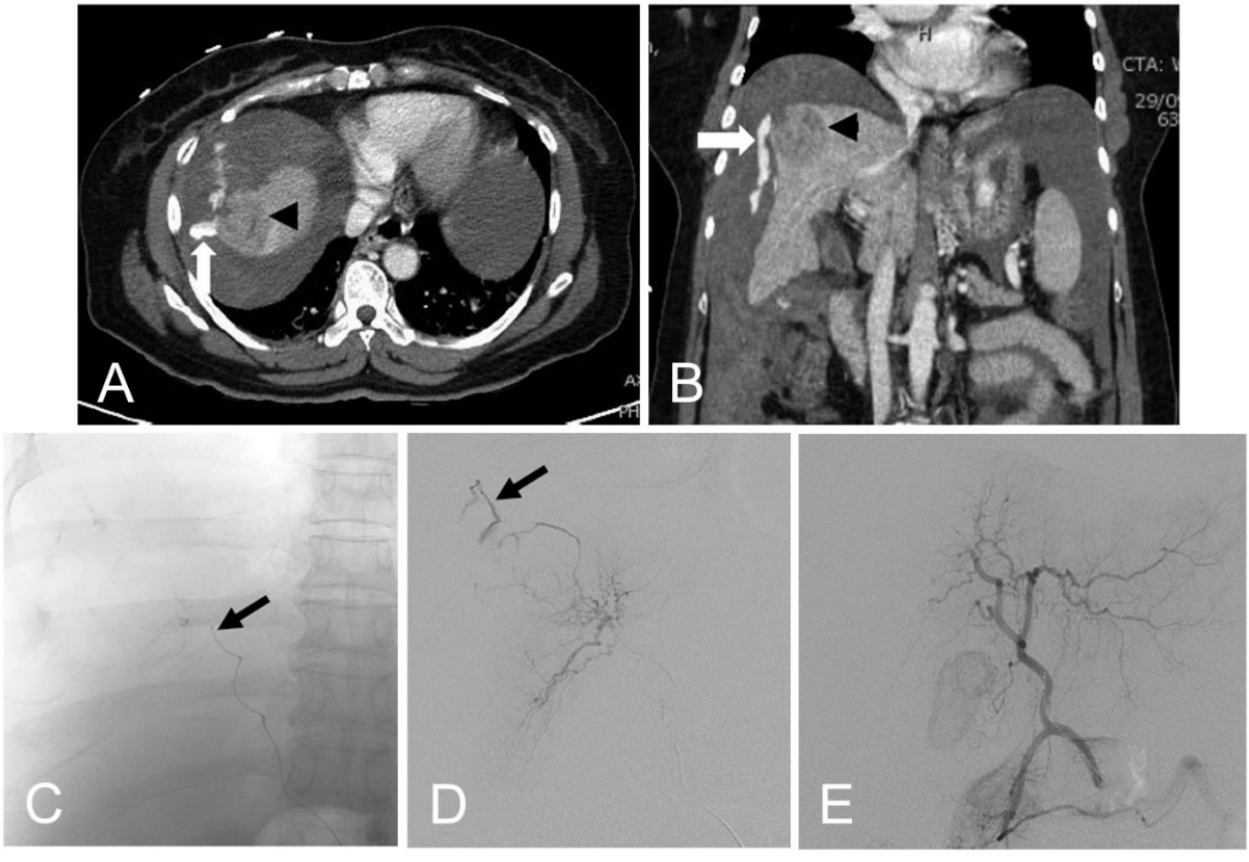

Fig 2. A 55-year-old female with hepatitis B cirrhosis and HCC presented with syncope and hypotension. A. Axial and B. Coronal contrast enhanced portal phase CT scan shows a well encapsulated mass with portal washout at segment 8 of the right hepatic lobe (arrowheads) with ascites and active contrast extravasation (white arrows). C. Scout film angiogram showed a microcatheter placing at superior right hepatic lobe (arrow) D. Selective right hepatic angiography shows a hypervascular lesion with active contrast extravasation from the A8 branch of the right hepatic artery (arrow). E. Post-embolization angiography shows disappearance of the contrast extravasation.

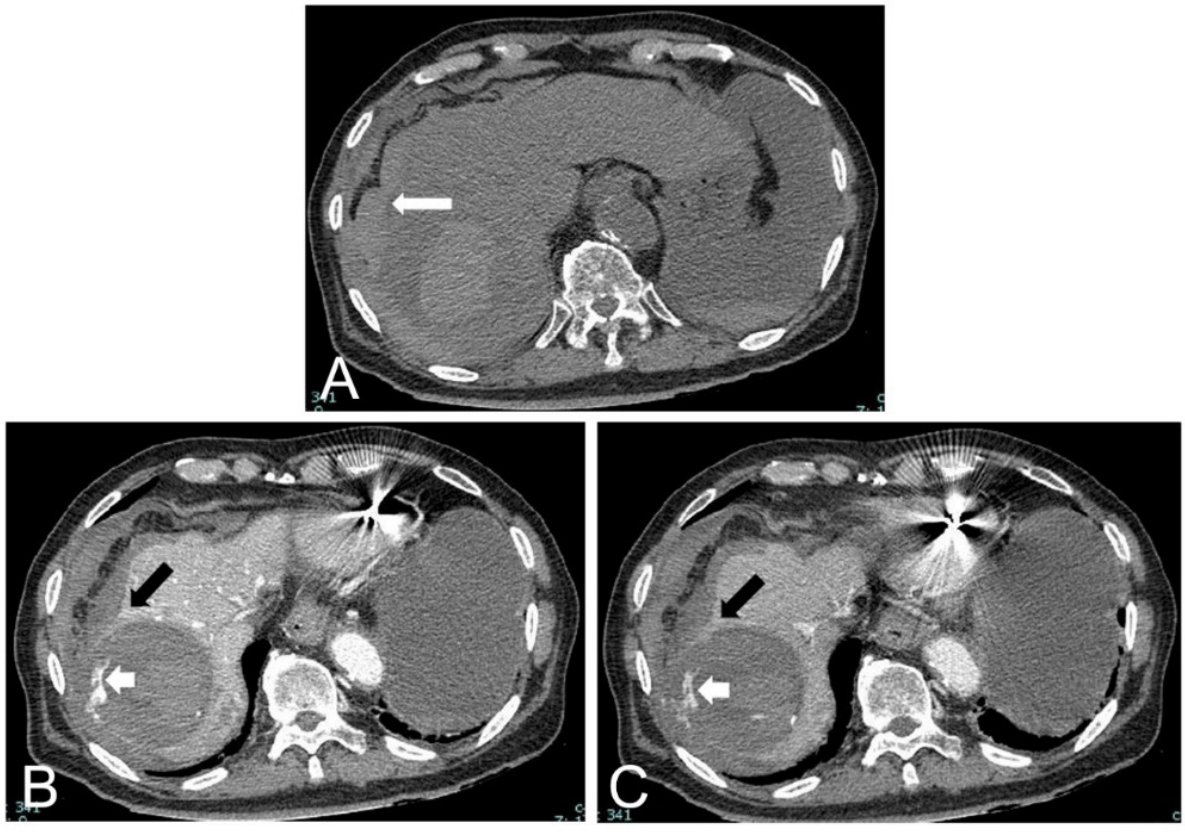

Fig 3. A 81-year-old male with anemia (Hct 22.9%), epigastric distension and tenderness. A. Non-contrast phase CT scan shows hyperattenuating hematoma at the lateral aspect of the right hepatic lobe (long arrow). B. Arterial and C. Delayed phase CT scans show a well-defined arterial enhancing mass at hepatic segment 7, which demonstrated hepatic capsular disruption of the HCC (dark arrows) with active contrast extravasation (short arrows).

There are some limitations in this study. Firstly, this is a small sample size, retrospective study which the treatment modality was decided by the patient's condition and their physicians which can lead to selection bias. Secondly, the previous data of blood transfusion unit from the first referring hospitals was mostly unavailable, so we could not evaluate this significant predictive factor. Lastly, there are some significant different of baseline characteristic associated with outcome and mortality such as LFT and portal vein thrombosis between two groups. Future study with propensity matching such as exclude all the patients who had contraindication for TAE in the conservative treatment group would make the analysis result more reliable in the ruptured HCC patients.

CONCLUSION

TAE results in a good clinical outcome and increased survival rate in the patients with ruptured HCC. A pre-treatment high hematocrit level was a good prognostic factor for the survival in ruptured HCC patients.

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Early Removal of the Etonogestrel Contraceptive Implant and Associated Factors Among Users at the Urban Family Planning Clinic in Siriraj Hospital, Bangkok, Thailand

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ABSTRACT

Objective: To study the rate and reasons for the early removal of etonogestrel contraceptive implants and associated factors at the family planning clinic in Siriraj Hospital.

Materials and Methods: This retrospective cohort study was conducted between May 2015 and December 2019 and contained 1,030 women who received the etonogestrel contraceptive implant. The medical records of demographic characteristics and clinical factors i.e., implant insertion date, implant removal date, reason for implant removal, contraceptive use before implant insertion and after implant removal, documented bleeding pattern and acceptability, were identified.

Results: The mean age of participants was 28.6 ± 6.9 years. About 21% of women (218/1030) prematurely discontinued their etonogestrel implant. A desire to become pregnant was the most common reason for early removal of the etonogestrel implant (32%). Meanwhile, the most common side-effect contributing to early removal was unscheduled bleeding. The associated variables of early etonogestrel implant removal were low BMI (p-value = 0.021) and unacceptability of bleeding pattern at one year (p-value < 0.001) and two years (p-value < 0.001) after insertion.

Conclusion: Early etonogestrel implant discontinuation rate was remarkable and the main reasons for it include a desire to become pregnant and bleeding side effects. Moreover, a lower BMI and unacceptability of bleeding problems also increased the likelihood of early removal of this contraceptive method.

Keywords: Etonogestrel implant; contraceptive method; one rod contraceptive implant; implant removal; implant discontinuation (Siriraj Med J 2021; 73: 399-405)

INTRODUCTION

Family planning plays an essential role in women's health by reducing the mortality rate of unsafe abortions and undesired pregnancies. Today, various methods of modern contraception focus on techniques that have proven to be effective and are widely used.[1] For example, long-acting reversible contraceptives (LARCs) are birth control methods that provide effective contraception long-term without requiring user action. Contraceptive methods that fall under LARC include intrauterine devices (IUDs) and subdermal contraceptive implants.[2] The etonogestrel contraceptive implant (Implanon NXT®) is a single-rod progestin-only device containing 68 mg of etonogestrel preloaded in a 4-cm soft plastic stick.[3] It

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provides protection for three years and its mechanism is based on ovulation inhibition and an increase in the viscosity of the cervical mucus.[4]

Although the three-year etonogestrel contraceptive implant is effective and popular among users, common side effects include unscheduled bleeding, weight gain, acne, headache and loss of libido. These side effects are all possible reasons for the early discontinuation of this contraceptive method.[5] In developing countries, removal of the etonogestrel implant in the first year (13%-28% of all cases depending on the area of study) is common even though these women are still in need of contraception.[6-9] Various studies have noted that women may insist on the early removal of the etonogestrel implant for a variety of reasons. For example, some studies have noted that unscheduled bleeding and other side effects are common reasons for early removal.[10-12] In fact, unscheduled bleeding was the most frequently cited reason for early removal of implants in a large multicenter trial in seven countries.[13] To solve this problem, counseling women on expected bleeding patterns might improve the longevity of implantable progesterone contraceptives. Furthermore, a low body mass index was also associated with early discontinuation of implants in a prior study,[9,14] however, these studies failed to identify sociodemographic predictors or associated factors of early discontinuation.[14-16] Moreover, most studies only had a follow-up period of one year.[6-9] with only a few studies maintaining a three-year follow-up period in a small population group.[17]

Although there are several studies about long-acting reversible contraceptives in Thailand[18-20], published data on the discontinuation rate of etonogestrel implants and associated factors are insufficient. Moreover, Siriraj Hospital provides family planning services and reproductive healthcare for many women. This study aimed to investigate the rate and reasons for early removal of etonogestrel implants and associated factors in large sample size and long-term period. Accordingly, this study will be expedient to determine the current practice of etonogestrel implant discontinuation and findings from this study will also be helpful in improving the reproductive healthcare system.

MATERIALS AND METHODS

This retrospective cohort study was conducted after the Ethics Committee of SIRB (Si 406/2020(IRB2)) approved the study. This study enclosed women who received an etonogestrel contraceptive implant removal at the family planning clinic, Siriraj Hospital between May 2015 and December 2019. In Siriraj Hospital, it is common platform at our institution to provide contraceptive counselling and appropriate instruction regarding the chosen method. The etonogestrel implant available in Thailand during the study period was Implanon NXT®, a single-rod progestin-only device containing 68 mg of etonogestrel preloaded in a disposable applicator and approved by the US FDA for a duration of three years of use.

First, medical records of women who had an etonogestrel implant inserted during the study period were identified. The investigators did not exclude women based on the indication of implant use. Regarding preciseness, two investigators reviewed each medical chart and discrepancies noted were normalized through discussion amongst all investigators. The authors recorded the following parameters from each medical record: sociodemographic characteristics, reproductive and obstetric history, utilization past contraceptive history, implant insertion & removal date, reason for requesting removal, documented bleeding pattern and acceptability. Bleeding patterns, which were recorded at the one year, two year, and three year visit was assorted as either regular bleeding or unscheduled bleeding. Documented bleeding complaints were ascertained by reviewing the charts and classified as acceptable or unacceptable.

The primary outcome was an early removal rate of the etonogestrel implant. "Early removal" was defined as removal of etonogestrel implant within 36 months of insertion. Furthermore, a medical record documented implant removal as early discontinuation either at our institution or any other outside clinic within 36 months after implant insertion. Last but not least, secondary outcomes and associated factors for early removal were also documented.

The data was analyzed using the SPSS software package (SPSS version 18.0; IBM). The demographic data and descriptive statistics were presented as percentage and Mean ± SD while the association between variables and implant discontinuation status was identified by a Chi-square test and Fischer's exact test. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 1,030 women with records of etonogestrel implant insertion during the study period were assembled. The mean age of the participants was 28.6 ± 6.9 years and the majority of women in this study were married and/or parous women. The mean BMI was 23.2 ± 4.7 kg/m^2. and the most recently used method of contraceptives was oral contraceptive pills (35.3%). Also, at least 15% of the women had never used any contraceptive method (Table 1).

TABLE 1. Sociodemographic characteristics of participants.

Characteristics	n (%) (N=1030)
Gravidity	
Nulligravid	113 (11)
Gravidity = 1	558 (54.2)
Gravidity ≥ 2	359 (34.8)
Parity	
Nulliparous	155 (15)
Parity = 1	617 (60)
Parity ≥ 2	258 (25)
BMI	
< 25 kg/m^2	693 (67.3)
≥ 25 kg/m^2	337 (32.7)
Marital status	
Married	933 (90.6)
Single	69 (6.7)
Divorced	28 (2.7)
Recent contraceptive method	
Hormonal method	
Oral contraceptive pills	364 (35.3)
DMPA	168(16.3)
3-year Implant	70 (6.8)
5-year Implant	42 (4.1)
Emergency contraceptive pills	28 (2.7)
Progestin only pills	6 (0.6)
Non-hormonal method	
Male condom	141 (13.7)
Cu-IUD	24 (2.3)
Withdrawal	17 (1.6)
Others	16 (1.6)
Never use	154 (15.0)

About 21% of women (218/1,030) recorded early etonogestrel implant discontinuation (within three years following implant insertion (Table 2)). Among them who had early implant removal, the most commonly cited reason was an intention of becoming pregnant (32.1%), bleeding disturbances (22.5%) and weight gain (12.8%) (Table 3). The "others" parameter in Table 3 includes loss of libido, mood changes, desire for male contraception and a desire for tubal sterilization. For most women in early removal group, the contraceptive method post etonogestrel implant removal was oral contraceptive pills. Moreover, 44% of the women who had used etonogestrel implants until the end desired to have it reinserted after removal.

The authors conducted contemplation to explore the factors associated with etonogestrel implant removal before three years. The associated variables of early etonogestrel implant removal were BMI (p-value = 0.021) and acceptability of bleeding patterns at one year (p-value < 0.001) and two years (p-value < 0.001) after insertion (Table 4). Gravidity, parity and marital status had no relation or impact on the decision to have the etonogestrel implant removed. As mentioned previously about BMI, the authors noticed that a low BMI was associated with early removal of etonogestrel implants (OR = 1.71; p-value < 0.05).

TABLE 2. Duration of etonogestrel implant use at the time of removal.

Duration	n (%) (N=1030)
Early removal	218 (21.2)
< 6 months	17 (1.7)
6 months to 1 year	54 (5.2)
> 1 year to < 2 years	69 (6.7)
≥ 2 years to < 3 years	78 (7.6)
No early removal (≥ 3 years)	812 (78.8)

TABLE 3. Reasons for early removal of etonogestrel implant.

Reason	n (%) (N=218)
Wish to become pregnant	70 (32.1)
Bleeding disturbance	49 (22.5)
Weight gain	28 (12.8)
Acne	23 (10.6)
Separation	23 (10.6)
Dizziness	16 (7.3)
Headache	14 (6.4)
Insertion site problem	5 (2.3)
Others	26 (11.9)

DISCUSSION

Our study shows important data and useful insights about the patterns of etonogestrel implant use and reasons for early removal among users in Thailand. The study also provides the trends in usage as information from all users requesting removal was collected and not just from those who requested early removal of the device. The population in this study was on average around twenty-eight years of age and had a normal BMI which is representative of the Thai population.

In our study, 21% of etonogestrel implant users had their implant removed within three years of insertion. The result was lower than studies in other developing countries.[21-22] This discrepancy might be due to sample size, timing of the study, socio-cultural differences, and the government's role in minimizing early removal of etonogestrel implant. The desire to become pregnant was the most common reason for early removal of implants in this study (32%), which infers that counseling services given to patients about the contraceptive method and its duration during inception is very essential. Moreover, side-effects were cited as the most common reasons for early removals, especially unscheduled bleeding. This has also been found in other studies.[10-13] In comparison to Thai population-based study, Assavapokee N et al. found that the discontinuation rate of etonogestrel implant within 3 years was 16.9% and unscheduled bleeding was the main reason for early implant removal.[23]

Of all the demographic characteristics and clinical factors that we studied; one-year and two-year acceptability of bleeding problems and BMI were the most cited factors associated with early removal of the etonogestrel

TABLE 4. Bivariate comparison of characteristics by etonogestrel implant discontinuation status.

Characteristics	Early removal (N = 218)	No early removal (N = 812)	p-value
Gravidity			0.255
Nulligravid	30 (13.8)	83 (10.2)	
Gravidity = 1	110 (50.4)	448 (55.2)	
Gravidity ≥ 2	78 (35.8)	281 (34.6)	
Parity			0.669
Nulliparous	37 (17.0)	118 (14.5)	
Parity = 1	128 (58.7)	489 (60.2)	
Parity ≥ 2	53 (24.3)	205 (25.3)	
BMI			0.021
< 18.5 kg/m^2	41 (18.8)	97 (12.0)	
18.5-24.9 kg/m^2	105 (48.2)	450 (55.4)	
≥ 25 kg/m^2	72 (33.0)	265 (32.6)	
Marital status			0.065
Married	189 (86.7)	744 (91.6)	
Single	22 (10.1)	47 (5.8)	
Divorced	7 (3.2)	21 (2.6)	
Bleeding problem			
1-year after insertion			
Bleeding pattern			0.539
Regular bleeding	18 (12.1)	84 (10.3)	
Unscheduled bleeding	131 (87.9)	728 (89.7)	
Acceptability			<0.001
Acceptable	135 (90.6)	797 (98.2)	
Not acceptable	14 (9.4)	15 (1.8)	
2-year after insertion			
Bleeding pattern			0.196
Regular bleeding	6 (10.3)	109 (16.9)	
Unscheduled bleeding	52 (89.7)	536 (83.1)	
Acceptability			<0.001
Acceptable	53 (91.4)	635 (98.4)	
Not acceptable	5 (8.6)	10 (1.6)	
3-year after insertion			
Bleeding pattern			1.000
Regular bleeding	0 (0)	185 (23.9)	
Unscheduled bleeding	0 (0)	590 (76.1)	
Acceptability			1.000
Acceptable	0 (0)	743 (95.9)	
Not acceptable	0 (0)	32 (4.1)	

implant. First-year unscheduled bleeding was found in 89% of women in this study, which emphasizes the importance of counseling patients about expected side-effects and appropriate management before offering the contraceptive choice. Acceptance of potential side-effects before choosing the contraceptive method may decrease the early discontinuation rate. Only a few women reported acne, weight increase, dizziness, and headache as reasons for early removal of the etonogestrel implant. The side-effects found in our study are corresponding to the potential side-effects of the etonogestrel implant. In this study, the author observed that Low BMI was associated with early removal of etonogestrel implants. There are studies support that the lower basal BMI may account for the higher percentage of irregular bleeding.[24] Moreover, obese women were 2.6 times less likely to have implant removal for bleeding as compared with normal weight women. It could be hypothesized that the effect of higher endogenous estrogen levels in women with higher BMI stabilizes the endometrium.[14]

Moreover, we also noticed in this study that almost half of the women who had used etonogestrel implants until the end desired to have it reinserted after removal, demonstrating acceptability and satisfaction of this method. As we know that etonogestrel implants offer the benefits of long action of use and reversibility. In this study, we figured out that the most common reasons for intending to use an etonogestrel implant was the desire for a long-acting contraceptive method that did not require frequent follow-ups.

CONCLUSION

In conclusion, the study revealed that early etonogestrel implant discontinuation rate is significant and that the main reasons for early removal was a wish to become pregnant and/or bleeding side effects. The study also suggests that a lower BMI and unacceptability of bleeding problems increases the likelihood of early removal of this contraceptive method.

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Conflict of interests: The authors declare that they have no conflict of interests.

Ethical issues: This study was conducted after obtaining necessary permissions from the Ethics Committee of SIRB (Siriraj Institutional Review Board: 406/2563(IRB2)). All ethics were respected in this study.

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G-control Charts for Contamination Rates of Blood Cultures in a University Hospital

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ABSTRACT

Objective: To determine blood culture contamination rates, and display with a *g*-chart.

Materials and Methods: A retrospective cohort study was conducted. The medical records of patients, from whom blood cultures were obtained in a university hospital, during January and December, 2019 were retrieved and reviewed for contamination. The Centers for Disease Control and Prevention (CDC) criteria were used to classify the blood culture results. The contamination rates were illustrated with a *g*-chart.

Results: We identified 331 false-positive blood cultures, among 32,961 cultured specimens; yielding a contamination rate of 1.0% (95% CI = 0.9% – 1.1%). The highest contamination events occurred in the emergency department (49.2%), pediatric ICU (5.2%) and neonatal ICU (4.8%), respectively. The most common contaminated commensal bacterial genus were coagulase -negative *Staphylococci* (67.1%), *Bacillus* spp. (10.2%) and *Corynebacterium* spp. (7.6%). The *g*-charts could identify 14 abnormal variations, in 41 locations.

Conclusion: The contamination rates found were within ranges of other reports. *G*-charts are simple to construct, easy to interpret and sensitive for detection of real time epidemics.

Keywords: Hemoculture; blood culture; contamination; rate; geometric; SPC chart (Siriraj Med J 2021; 73: 406-412)

INTRODUCTION

Blood cultures play an important role in the management of bloodstream infections, due to it is a critical tool detecting the dangerous presence of living organisms in the blood stream. However, the merits of blood culture results are jeopardized by false positives, resulting from contamination during the taking or processing of blood specimens. Blood culture contamination represents an ongoing source of frustration for clinicians and microbiologists alike. Ambiguous culture results often lead to diagnostic uncertainty in clinical management and are associated with increased health care costs due to unnecessary treatment and testing.[1] There are several steps in the process of taking blood cultures that may influence the contamination rate. Blood culture contamination has been attributed to the transference of organisms from the patient's skin, the immediate environment of the patients, supplies used to obtain or transfer the blood samples or from the hands of the health care worker performing the procedure.[2-3]

In this era of strains on the resources and rising cost of healthcare, it becomes increasingly apparent that decisions must be made on facts, not just opinions. Consequently, data must be gathered and analyzed. This is where statistical process control (SPC) comes in. For over decades, the healthcare setting has benefited from the tools of SPC that have helped guide the decision-making process.[4-5]

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Control chart is the main tool in SPC and usually used for monitoring and improving the ongoing process. Geometric SPC chart (*g*-chart) is based on the geometric distribution and was designed to monitor rare events.

Primary baseline data is an essential part of any quality improvement project. Hence, the primary intention of this study was to determine blood culture contamination rates, and display with a *g*-chart. To document the rates, and variations of blood culture contaminations needed for a blood culture quality improvement project.

MATERIALS AND METHODS

Setting

The study was conducted in Songklanagarind Hospital, a tertiary care, medical school, and training hospital in Southern Thailand. In our hospital, clinical blood culture samples are usually collected at the bedside, from two separated specimens; taken from different venipuncture sites.

Studied samples

Blood culture specimens taken from patients admitted to the hospital, from the 1st of January to the 31st of December, 2019.

Blood sample collection

Prior to venipuncture, the skin was disinfected with a combination of 2% chlorhexidine gluconate and 70% alcohol, for 30 seconds, then allowed to dry, except that taken from infants <2 months, in which 70% alcohol would be used instead. After antisepsis, the veins would not be touch, without use of sterile gloves. Then the vein is pierced with a needle, and drawn into a syringe. Samples were subsequently inoculated into blood culture bottles without change of needles. Blood culture collection kits are not used in this process.

Specimen Processing

Blood samples were obtained in media bottles, and kept at room temperature before being transferred, as soon as possible, to the microbiology laboratory for processing (within 2 hours). Blood culture specimens were incubated in automated instruments for 5 days, or until the automate alarm for positive blood culture.

The automated blood culture system used in the hospital is BD BACTEC FX (BACTEC) by Becton Dickson & Co., sparks, MD. It is used to process blood cultures with isolates identified using MALDI-TOF and biochemical methods, according to standard practices.

Microbiology lab identification

Once blood cultures become positive for growth, either by manual subculture techniques (blood agar, chocolate agar, and MacConkey agar) or signaling from automated systems, a Gram stain is performed. A positive Gram stain result is regarded as a critical value, and the ordering clinician, or another responsible member of the healthcare team providing care to the patient is immediately informed. At this point, subcultures are performed and these allow identification and, if indicated, susceptibility testing is then performed; typically over the next 24-48 hrs. Complete organism identification and organism-specific susceptibility testing is performed on all positive blood culture specimens.

Definitions of blood cultures

1. Positive Blood cultures: Any blood cultures which microorganisms are found.
2. Blood Stream Infection: Positive Blood cultures which the microorganisms are not included in CDC common commensal lists[6] or two blood specimens found the same microorganisms.
3. Secondary Blood Stream Infection: One or more positive blood cultures which the microorganisms are included in CDC common commensal lists6 and also found the same microorganisms at another site of the body.
4. Contaminated Blood cultures: One positive blood culture which the microorganisms are included in CDC common commensal lists[6] with no more than one matching organism identified in 2 separated blood specimens and No primary infection source of the organism identified Contaminated blood culture

Studied variables

The variables in this study include blood sample collecting date, age and gender of patients, wards that request blood culture and results of the blood cultures.

Statistical analysis

Continuous data were described in terms of arithmetic or geometric mean, according to the types of data distribution. Discrete data were presented as percentage. Contamination rates were calculated by dividing the number of contaminated specimens with the total number of cultured specimens. Contamination rates were reported in terms of percentage. The dispersion of data was represented by variance or 95% confidence interval (95% CI). The 95% CI of proportion were estimated based on exact binomial statistics.

Construction of the *g*-charts were done by line graph plotting the numbers of non-contaminated specimens between pairs of contaminated specimens (NBP), in axis *y* against the consecutive contaminated specimen numbers (CSN) in axis *x*. The *y*-axis is displayed in log scale base 2. The chart then starts with the second CSN, and the NBP between the first and the second CSN.[7-8]

We used median of total NBP to define the center line (CL), and used confidence intervals to define control limits of the chart. The confidence intervals were calculated using equations proposed by Yang Z et al.[9] The equation for lower limit is $ln(1 - \alpha/2)/ln(q)$ and the equation for upper limit is $ln(\alpha/2)/ln(q)-1$,where α is the cumulative probability and q is the probability of a non-contaminated specimens.[7]

Definitions of the *g*-chart; lower and upper control limits with calculation formula

Chart limits

1. Lower control limit (LCL): Lower bound of 95%CI and formula is $ln(0.975)/ln(q)$

2. Lower warning limit (LWL): Lower bound of 80%CI and formula is $ln(0.9)/ln(q)$

3. Upper warning limit (UWL): Lower bound of 80%CI and formula is $ln(0.1)/ln(q)-1$

4. Upper control limit (UCL): Lower bound of 95%CI and formula is $ln(0.025)/ln(q)-1$

ln = Natural logarithm or $\log_e$

q = Probability of non-contaminated specimen

The outbreak of blood culture contamination can be diagnosed by any of the following rules; 1) there is one point of the graph that fell under LCL, 2) there are two successive points falling under LWL 3) there are five successive points under CL, and 4) there are six successive points decreasing.

Ethics in research

The study protocol was approved by the Ethics Committees of the Faculty of Medicine, Prince of Songkla University (EC: 62-451-9-1). Because of the observational nature of the study, written informed consent was not required.

RESULTS

Characteristics of studied samples

The study included 32,961 blood culture specimens, from 8,841 hospital patients. The characteristics of the patients are shown in Table 1.

Blood culture results

Using the Center of Disease Control and Prevention (CDC) criteria[6], we could identify 331 (1.0%) contaminated blood specimens among 32,961 of the total blood specimens requested (Fig 1). The Pareto diagram of the number of contamination is illustrated in Fig 2. The contaminated micro-organisms are listed in the appendix.

TABLE 1. Demographic data of the patient, for whom blood culture specimens were taken.

Characteristics		95%CI
Age (year)	Mean = 50.53	49.95 - 51.11
Gender (%)		
Male	51.75	50.71 - 52.79
Female	48.25	47.21 - 49.29
Service (%)		
Emergency	31.22	30.72 - 31.72
Medicine	25.93	25.45 - 26.40
Surgery	18.05	17.64 - 18.47
Pediatric	13.58	13.21 - 13.95
Obstetric & Gynecology	4.04	3.83 - 4.26
Others	7.18	6.90 - 7.45

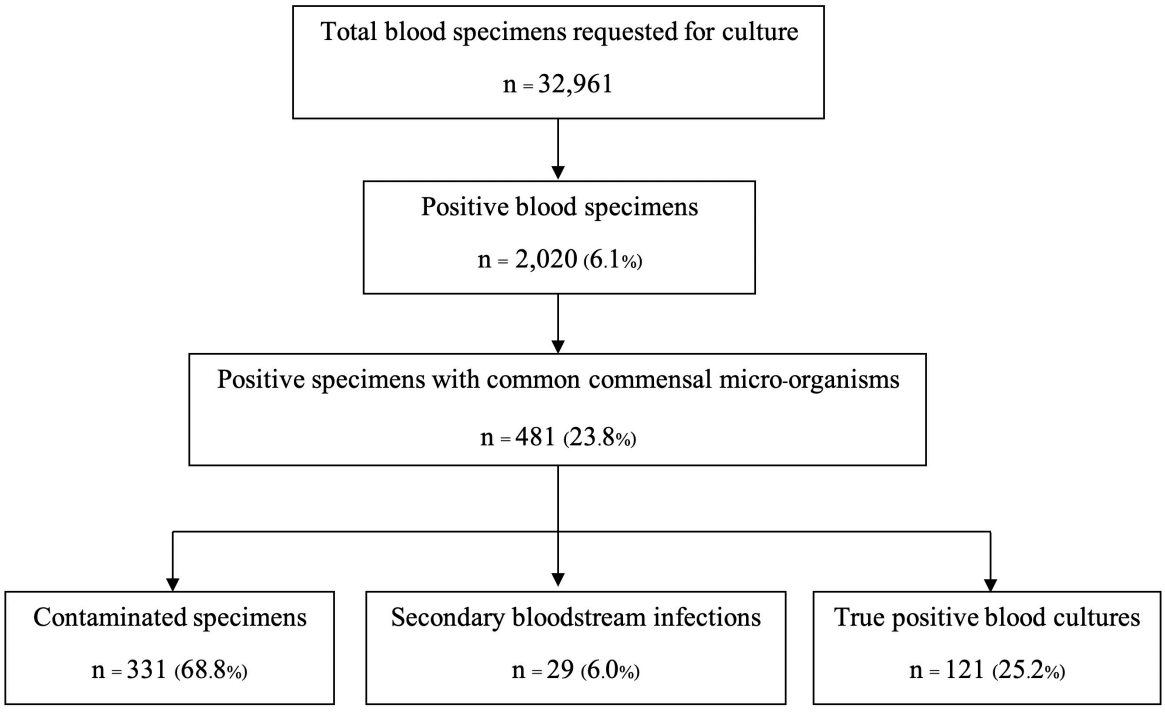

Fig 1. Blood culture results for the year 2019.

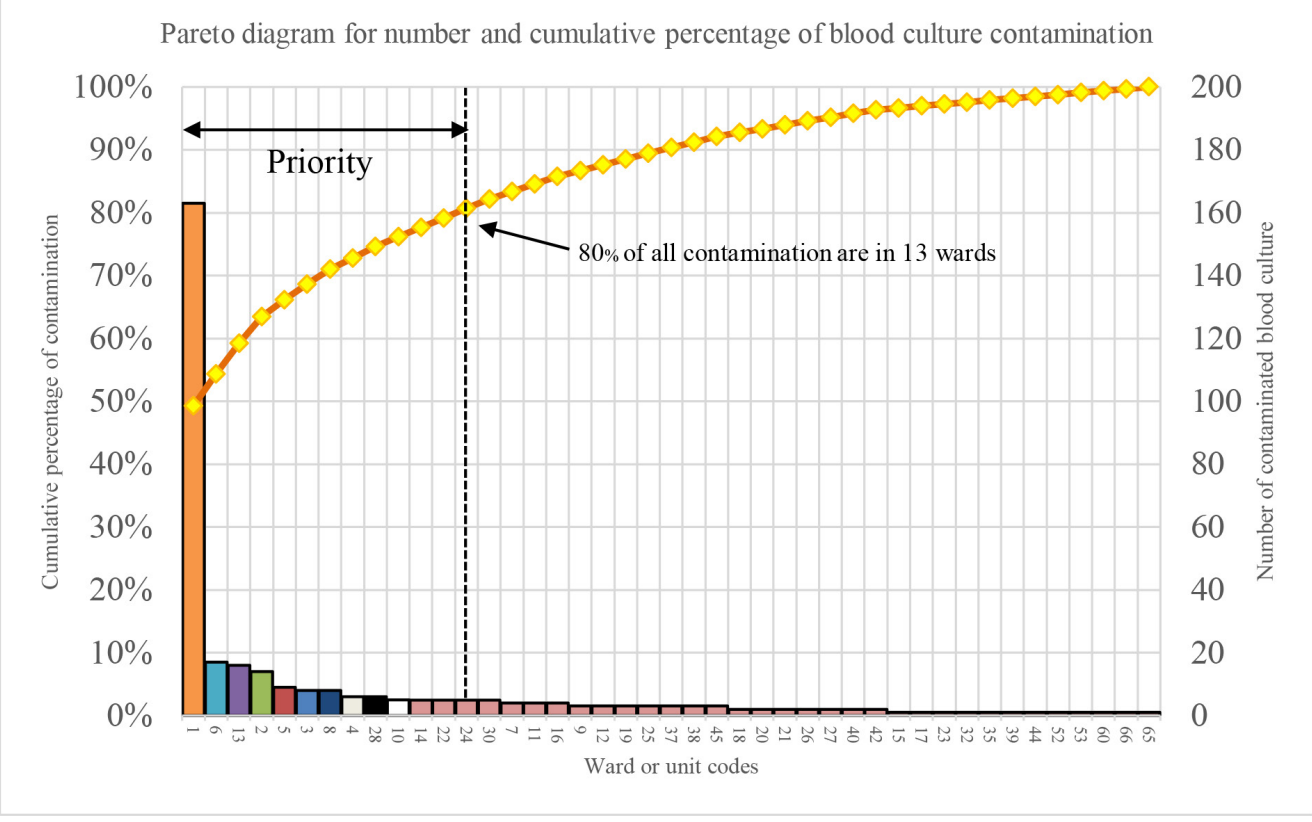

Fig 2. Pareto diagram for the number of contaminated blood culture specimens (presented with diagram) and corresponding cumulative percentage of contamination (presented with line diagram)

Descriptive data of number between contamination

The average as well as variance of numbers between contaminated blood specimens was 98 and 9,127, respectively. The median was 71. The histogram is demonstrated in Fig 3.

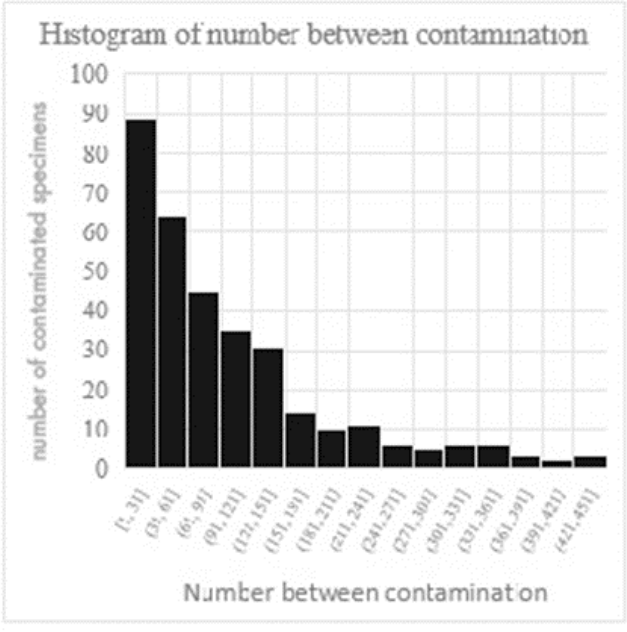

Fig 3. Histogram of the number between contaminations.

g-Control chart

The *g*-Chart of blood culture contamination, in PSU hospital for the year 2019, is illustrated in Fig 4.

Outbreak of blood culture contamination

Outbreak of blood culture contamination in Songklanagarind Hospital is shown in Table 2. We could identify 14 outbreaks in the year 2019. The average run length (average of number between outbreaks) was 19.

DISCUSSION

The study design of this research was a cross-sectional descriptive analysis, which can only study a point in time, and lacks the ability to identify the cause-effect relationship. Therefore, the results can only represent the magnitude of the problem.

Some microorganisms such as Burkholderia pickettii[10] are not enrolled in the common commensal organism's list of the CDC[6]; nonetheless, microorganisms can be causative agents for blood culture contamination. Therefore, this may be the reason for the occurrence of false negative, in the other words, the contamination rate may be possibly lower than the actual result.

Although, Songklanagarind Hospital has no phlebotomy team available the blood sample collection method is practiced via standard protocol.

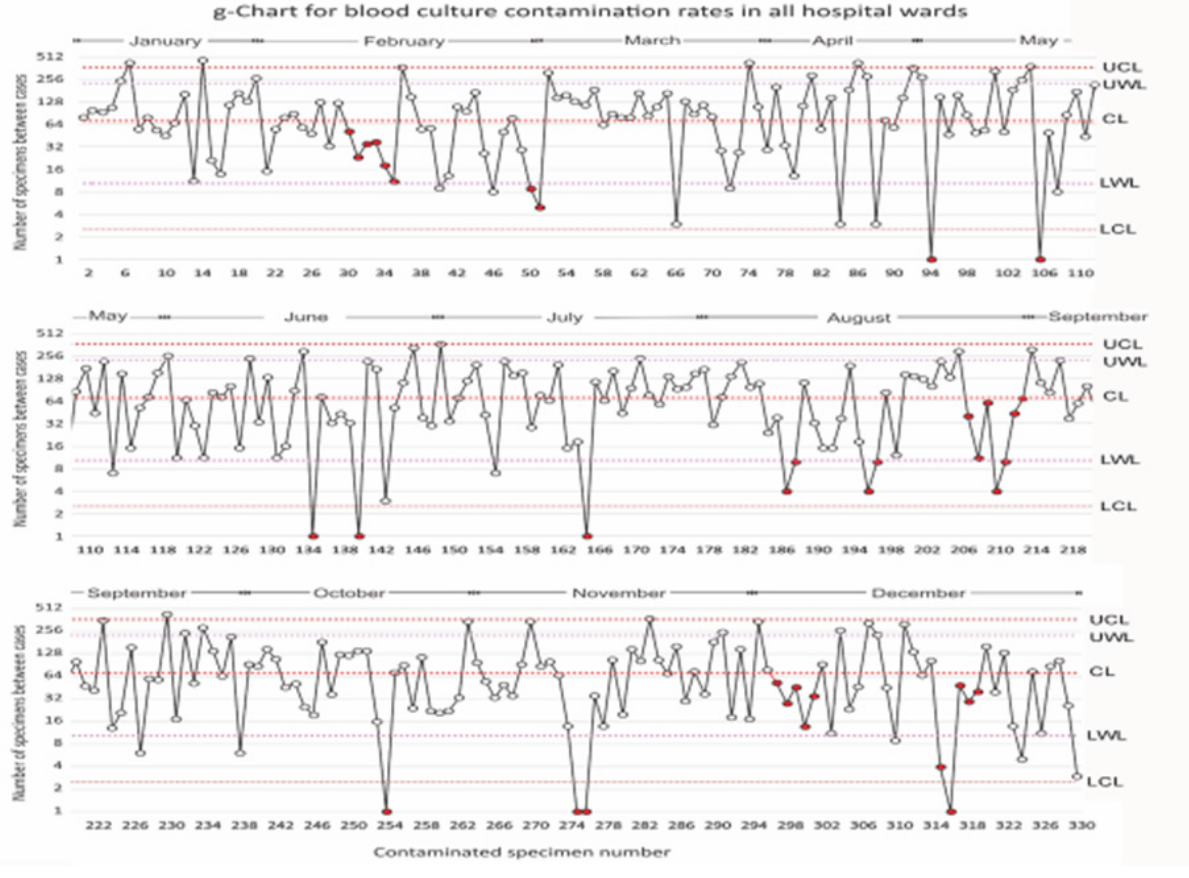

Fig 4. *g*-Chart of blood culture contamination in Songklanagarind Hospital for the year 2019.

TABLE 2. Outbreak of blood culture contamination in Songklanagarind Hospital.

Ward	Number	Criteria	Date of outbreak	
			Month	Day
All wards	1	Five points under	February	8-9
	2	Two points under		23-24
	3	One point under	May	1
	4	One point under		20
	5	One point under	June	16
	6	One point under		18
	7	One point under	July	17
	8	Two points under	August	10
	9	Two points under		15
	10	Five points under		28-30
	11	One point under	October	17
	12	Five points under	November	5-7
	13	Five points under	December	2-3
	14	Five points under		22-23
	Average run length = 19 (95%CI = 13-25)			

The results show that there is a huge difference between contamination in the Emergency Department and other services. It has been suggested that urgent care, lack of ongoing training, workload and nature of present patients may contribute to this. From the literature reviews show that Zahra Hashemizadeh had the highest contamination rate (8.47%) in Neonatal Care Units in Shiraz, Southwest-Central Iran.[11] In contrast, Chang CJ, et al. and Washer LL had the lowest contamination rate (0.2%) in discharged patients from Emergency Department, National Cheng Kung University Hospital, Taiwan, and patients using povidone-iodine and iodine as antiseptics in University of Michigan Health System respectively.[12-13] The contamination rate in a single Emergency Department at a university-affiliated, tertiary care adult hospital in the United States was maintained below 3% during each biweekly interval throughout the intervention period in the study of Self HW et al.[14] They developed the sterile blood culture intervention to convert blood culture collection from a clean to a sterile procedure.

More than 50 % of contaminated microorganism are coagulase-negative staphylococci including *Stahylococcus epidermidis* (37.18%), *Staphylococcus hominis* (8.93%), *Staphylococcus capitis* (7.49%).

The Pareto chart is one of the seven basic tools of quality control. It is a type of chart that contains both bars and a line graph, where individual values are represented in descending order by bars, and the cumulative total is represented by the line. The left vertical axis is the frequency of occurrence and the right vertical axis is the cumulative percentage of the total number of occurrences. The purpose of the Pareto chart is to highlight the most common sources of defects. We used general 80/20 rule to identify the 20% of wards that created 80% of overall contamination

Statistic process control (SPC) techniques have played an effective part in monitoring hospital performance. The Geometric SPC chart (*g*-chart) is appropriately used in this study, because the contamination data has an over-dispersion problem, which is shown in histogram of

number between contaminations (Fig 3). *G*-chart analysis is based on inverse sampling to either detect process changes, or verify improvements faster. Prospective *g*-chart analysis is able to trigger specific awareness when relevant increases or decreases of rare events are detected. Such alarms enable timely root cause analysis, so as to secure early clinical process.[15] Also *g*-Chart is appropriate for very low incident event for its take less effort to collect data and can provide real time outbreak detection".

Previously we actually had no formal blood culture monitoring system. This study provides information needed to priority setting, and establishing baseline data for the hospital's quality improvement, which has never been done before. Quality improvement of blood cultures can reduce additional costs, overuse of antibiotics and drug-resistant bacteria in the hospital.

CONCLUSION

We identified 331 false-positive blood cultures, among 32,961 cultured specimens; yielding a contamination rate of 1.0% (95%CI = 0.9 - 1.1). This blood culture contamination rate is very low when compared to other reports. The *g*-control chart is a very effective tool that can detect 14 abnormal variations in 41 locations, by a 3 outbreak criteria comprising of: 1 point under LCL, 2 points under LWL and 5 points under CL.

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Conflict of interest: All authors of this article certified that there were no financial nor non-financial conflicts of interest.

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Protective Roles of *N-trans*-feruloyltyramine Against Scopolamine-Induced Cholinergic Dysfunction on Cortex and Hippocampus of Rat Brains

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ABSTRACT

Objective: To study the protective effects of *N-trans*-feruloyltyramine (NTF) on scopolamine-induced cholinergic dysfunction, apoptosis, and inflammation in rat brains.

Materials and Methods: Treatments were administered intraperitoneally (i.p.). Wistar rats (8-week-old) were allocated into 4 groups (n = 3) as follows: scopolamine-only, NTF-only, NTF + scopolamine and control. Spatial cognition was evaluated by Morris water maze. ROS assay and Western blot analyses were conducted in 3 brain regions: the frontal cortex, hippocampus, and temporal cortex.

Results: NTF treatment inhibited scopolamine-induced memory impairment and significantly attenuated scopolamine-induced changes in the three brain regions. Investigated scopolamine-associated changes were as follows: increases in ROS production and BACE1 level, decrease in ChAT level, increases in inflammatory and apoptotic markers, and activation of signaling pathway kinases related to inflammation and apoptosis.

Conclusion: With its *in vivo* antioxidant, cholinergic-promoting, anti-apoptosis, and anti-inflammatory biological activities, NTF is a promising candidate to be further investigated as a potential treatment for Alzheimer's-associated neurodegeneration.

Keywords: Acetylcholine; Alzheimer's disease; antioxidant; Morris water maze; *N-trans*-feruloyltyramine (Siriraj Med J 2021; 73: 413-422)

INTRODUCTION

Alzheimer's disease (AD) causes progressive and irreversible deterioration of cognitive functions especially memory.[1] The major pathological characteristics of the human AD brain are extracellular aggregates of amyloid-β (Aβ) and intracellular aggregates of hyperphosphorylated tau, namely the senile plaques and the neurofibrillary tangles respectively.[2] The underlying mechanism of sporadic AD involves cholinergic dysfunction including degeneration of basal forebrain cholinergic neurons and loss of hippocampal cholinergic fibers.[3] Impaired cholinergic transmission affects learning and memory, cortical and hippocampal information processing, and ultimately behaviors.[6] Scopolamine, an antagonist of a muscarinic acetylcholine receptor,[4] induces cholinergic dysfunction and cognitive impairment through oxidative

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stress and neuroinflammation in animal models.[5] Intraperitoneal injection of scopolamine blocks cholinergic neurotransmission, dysregulates cholinergic system, and consequently impairs cognition in rodents.

N-trans-feruloyltyramine (NTF) was purified from *Polyalthia suberosa*, a shrubby tree found throughout southeast Asia and south China whose parts are used in Thai traditional medicine.[6] NTF has potent radical-scavenging antioxidant property, especially from phenolic hydroxyls. NTF and the other purified compound studied by our group, *N*-benzylcinnamide (PT-3), have been demonstrated to be effective in protecting rat cultured cortical neurons against Aβ-induced cytotoxicity by inhibiting ROS production, suppressing apoptotic caspase-3 and Bax, and in turn elevating anti-apoptotic Bcl-2.[7-8] As PT-3 that has elicited protective effects on primary cortical neurons also showed promising outcome in aged rat brain *in vivo*,[9] neuroprotective effects of NTF in vivo had become our next primary study target.

In this research study, the animals were used in the experiment, and they were calculated for the sample size in accordance with the ethical guideline to protect the unnecessary wastage of resources. Consistently, there are several studies that have demonstrated the statistically significant results with a small sample size (n = 3).[9-11] Meanwhile, our preliminary results showed the protective effects of NTF treatment against scopolamine-induced cholinergic dysfunction, including, ROS production, apoptosis, inflammation, and associated signaling pathways in several rat brain regions, specifically the frontal cortex, hippocampus and temporal cortex.

The frontal cortex integrity is correlated with the higher reservation of cognitive performance in aging populations.[12] The hippocampus and especially its associated cholinergic signaling pathway play a crucial role in memory formation.[13] The medial temporal lobe cortex or, in short, the temporal cortex is reciprocally interconnected to the hippocampus and it greatly involved in the hippocampus-associated cognitive processes and cognitive decline such as AD in humans[14] and ischemia-associated dementia in rats.[15] Furthermore, ChAT activity in the temporal cortex is positive correlated with cognitive preservation, but it is negatively correlated with AD pathologies.[16] Importantly, the frontal cortex, hippocampus, and temporal cortex are three of the regions heavily affected by scopolamine[17,18] and aging[9] in the rat brain.

Herein, we studied the neuroprotective effects of NTF treatment on scopolamine-associated cholinergic dysfunction, namely, ROS production, apoptosis, inflammation, and relevant signaling pathways in the rat frontal and temporal cortices and hippocampus.

MATERIALS AND METHODS

Reagents

Scopolamine (Sigma-Aldrich) and NTF were dissolved in a vehicle solution of (v/v) 40% dimethylsulfoxide, 59% phosphate-buffered saline (PBS), and 1% ethanol and diluted in PBS for animal administration. NTF was isolated from the acetone extract of *Polyalthia suberosa* stems as previously described.[6] The test drug NTF was in its form of pure compound. From 997.2 mg of the semi-solid fraction, 88.1 mg (8.83 %) of NTF could be purified by preparative layer chromatography.[6]

Animal experiments

All experimental procedures were approved by the Institute of Molecular Biosciences Animal Care and Use Committee (MB-ACUC) (COA.NO.MB-ACUC 2016/002).

Twelve 8-week male Wistar rats (250-300 g) from the National Laboratory Animal Center, Mahidol University, were individually cared for in cages under 12 h light/dark cycle, 22±1 °C temperature, 45-55 % humidity, and *ad libitum* water and diet. The 12 rats were separated into 4 groups, each with 3 animals, namely, scopolamine treatment only, NTF treatment only, scopolamine plus NTF treatment, and control. Following habituation for 5 days, animals were once per day injected intraperitoneally (i.p.) for 14 days.[19] In the control group, animals were injected with vehicle 1 h before the water maze test for 14 days. In the scopolamine-only group, animals were first injected for 7 days with vehicle followed by scopolamine treatment on day 8 to day 14 (3.0 mg/kg BW) 1 h before the water maze test. In the NTF treatment only group, animals were injected with 1 ml aliquot of NTF (1.5 mg/kg BW) for 14 days 1 h before the water maze test. In the scopolamine plus NTF treatment group, animals were administered with NTF as described above for 7 days and then together with n day 8 to day 14 (3.0 mg/kg BW) 1 h before the water maze test.

Rats were treated with NTF for 7 days prior to MWM training because we would like to investigate the neuroprotective effects of NTF against scopolamine. The NTF effects as a memory enhancer can still remain during the MWM training.[19] The dose of NTF was selected in accordance with our previous findings on the protective roles of N-benzylcinnamide (PT-3).[9] In animal studies the model of scopolamine has been performed using doses between 0.5,1 or 3 mg/kg IP). The effect of scopolamine to induce impairment of learning and memory is dose dependent.[19-20] The highest dose of scopolamine exhibits the greater severity of memory dysfunction. Scopolamine was administered via the injection 1 hr before MWM

training because the half-life of scopolamine is 2.4±1.4 h for its elimination.[21] Central pharmacodynamic effects of scopolamine peak between 1 and 3 h and disappear after 5–6 h.[22]

Morris water maze test

Water maze tests were conducted to evaluate the effects of NTF and scopolamine on the acquisition and retention of spatial memory.[18] Visible platform trial was performed on the day before the training trial. Animals have to associate the relative location of the visible platform with the provided visual cues. On the following training trials, the platform was submerged and invisible. The training trials were conducted over a period of 7 days as follows: day 1, visible platform test; days 2-6, hidden platform tests; and on the same day 6 after hidden platform, probe trial test. These consecutive 6 days correspond to the days 8-13 of the treatment period. Rats in each group were put in the water facing different quadrants, which were altered for each experiment, and the escape latency defined as time spent to find the submerged platform was recorded. In the probe trial, time spent in the target quadrant with the platform was recorded.

The experimental schedule was designed to investigate the protective effect of NTF as a memory enhancer in rats. Rats underwent habituation for 5 days. After that, NTF (1 ml, 1.5 mg/kg. i.p.) or vehicle was administrated once a day for 7 days. Then, 1 h prior to the first trial session, rats were administrated of veh, NTF, scopolamine (3.0 mg/kg) or NTF+Scop (i.p) every MWM training for 6 days, in accordance with the previously described protocol.[23]

ROS production assay

After animals were decapitated without prior anesthesia, the 3 regions of the rat brains – the frontal cortex, hippocampus, temporal cortex – were dissected and frozen at -80°C. For the frontal cortex, the frontal part of the brain was cut straight down coronally and attached subcortical brain regions were removed. For the hippocampus, the bilateral hippocampal tissues with all subsectors were collected. For the temporal cortex, all cortical regions encompassing the hippocampus or the medial temporal lobe were dissected. Reactive oxygen species (ROS) production was measured according to the protocol of our previous work.[24] In brief, 2.4-3.0 mg of brain tissue from each dissected region were homogenized in 80-100 µl of ice-cold Locke's buffer, then 10 µl of homogenate was adjusted to a concentration of 3 mg/ml tissue, incubated with 10 µM 2',7'-dichlorofluorescin diacetate (DCFH-DA) (stock solution in 100% ethanol) for 45 min at 37°C. Fluorescence of the oxidized product 2',7'-dichlorofluorescein (DCF) (485 nm excitation and 535 nm emission) was measured every 10 minutes for 4 times in total at 37°C in a DTX 880 multimode plate reader.

Western blot analysis

Rat brains were dissected into three regions: frontal cortex, hippocampus and temporal cortex).[25] The brain protein contents were extracted with the lysis buffer, centrifuged at 11,000 ×g for 15 min, and collected as a supernatant. The protein samples were loaded onto polyacrylamide gel. The proteins were separated by gel electrophoresis and transferred onto (PVDF) membranes where they were reacted to specific antibodies. On the PVDF membrane, proteins of interest were identified using a detection reagent. The protein bands were quantified and analyzed with the same loading sample groups of its own gel. PVDF membrane was incubated overnight at 4°C with primary rabbit anti-Bax, -Bcl-2, -activated caspase-3, -BACE1, -phospho-p38, -p38, -phospho-JNK, -JNK, -phospho-ERK1/2, -ERK1/2, -phospho-Akt, -Akt, -phospho-NF-κB p65 (Ser536), -NF-κB antibodies (1:1000 dilution) (Cell signaling), -IL-1β (1:1000) (Santa Cruz); and primary mouse anti-ChAT (1:1000) (Millipore) and -IL-6 (1:1000) (Santa Cruz); and rabbit anti-actin (1:2500) (Cell Signaling) for normalization of protein loading. Immunoreactive proteins were visualized by incubating PVDF membranes with secondary horseradish peroxidase-conjugated antibodies (1:1000) (Cell Signaling) at room temperature for 1 h and subsequently with enhanced chemical luminescence (ECL) reagents (Bio-Rad) before signal exposure to Hyperfilm™ (GE Healthcare). Intensities of the visualized protein bands were measured by scanning densitometry (Image J software). The optical density (OD) of protein bands on Hyperfilm was determined and normalized to the OD of β-actin and total phospho proteins. The results were expressed as the percentage of OD values by using the ImageJ software.

Each gel contains four sample groups (Control, Sco, NTF+sco, NTF). Each protein sample of three rats on different brain regions, including frontal cortex (3), hippocampus (3), and temporal cortex (3) was loaded separately on different polyacrylamide gel. Four sample groups of each region in each rat were underwent Western blotting. Each blot was repeated for at least 3 times from different batches of protein samples. The representative blots were shown in figures.

Statistical analysis

Statistical analyses were performed using Prism 6.0 (GraphPad). Differences in the escape latency from the training/hidden platform trials were analyzed using two-way analysis of variance (ANOVA). Differences between means of the 4 groups from all other experiments were determined using one-way ANOVA with pair-wise *p*-values corrected by Tukey's *posthoc* test. Results are deemed significant at $p < 0.05$. In all Western blot analyses, non-phospho proteins (ChAT, Bcl-2, Bax, Activated caspase-3, IL-6, and IL-1β) were quantified and normalized with β-Actin. Meanwhile, phosphoproteins (p-NFκB, p-ERK, p-JNK, p-p38, p-Akt) were quantified and normalized with the total protein counterparts.

RESULTS

Learning and memory in a Morris water maze test of rats administered scopolamine with and without NTF

Control rats became proficient at locating the submerged platform by day 5 of training compared to day 1 ($p < 0.05$) in a hidden platform test (Fig 1A). On day 5 of training scopolamine-treated rats perform significantly less well than controls ($p < 0.01$), but, interestingly, rats treated with scopolamine together NTF have proficiencies superior to those of controls, whereas NTF treatment alone was no more effective than non-treated control. In the probe trial tests, as expected, compared to control rats, those in the scopolamine plus NTF treatment group stay significantly longer in the platform quadrant. Those in the NTF only treatment group spent the same time. Lastly as expected, those in the scopolamine-only group stayed for a significantly shorter duration (Fig 1B).

ROS production in brain tissues of rats administered scopolamine with and without NTF

Scopolamine treatment for 7 days elevated ROS level 2 folds in all three types of brain tissues (frontal cortex, hippocampus, and temporal cortex) over control levels (Fig 2). ROS levels were restored to control levels by NTF administration (7 days prior and during the scopolamine treatment period), while NTF alone (for 7 days) had no effect on ROS levels in all three types of brain tissues.

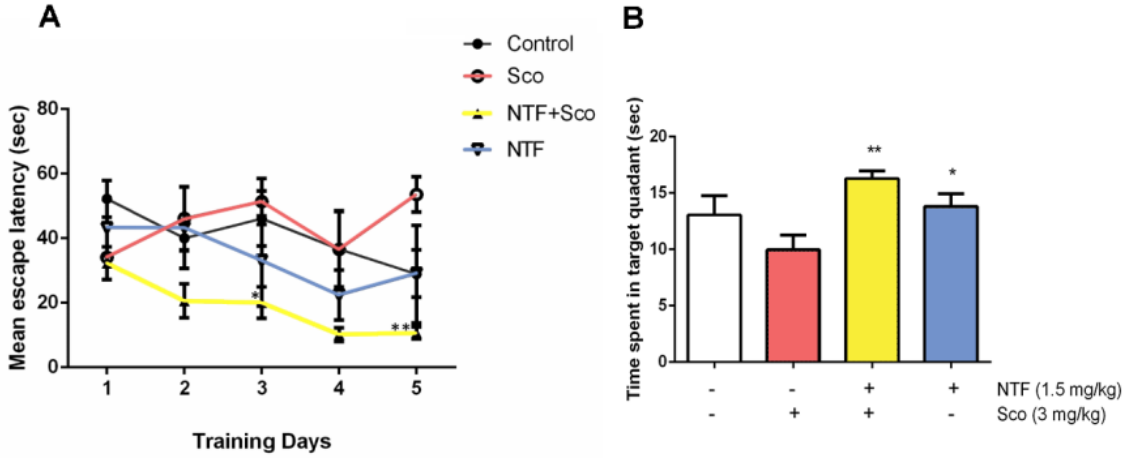

Fig 1. NTF (1.5 mg/kg) was injected i.p. daily to rats 7 days before and together with scopolamine (3.0 mg/kg BW i.p.) 1 h before the 7-day Morris water maze test; probe trial was conducted on day 7. Each data point (n=3) is expressed as mean ± SEM. A NTF prevented scopolamine from elevating escape latency. *p < 0.05 scopolamine-treated with NTF versus (vs) the scopolamine group on day 3, **p < 0.01 scopolamine-treated with NTF vs the scopolamine group on day 5. B NTF restored scopolamine-induced decrease in retention measured as time spent in the target quadrant. *p < 0.05 scopolamine vs NTF, **p < 0.01 scopolamine vs scopolamine with NTF treated group.

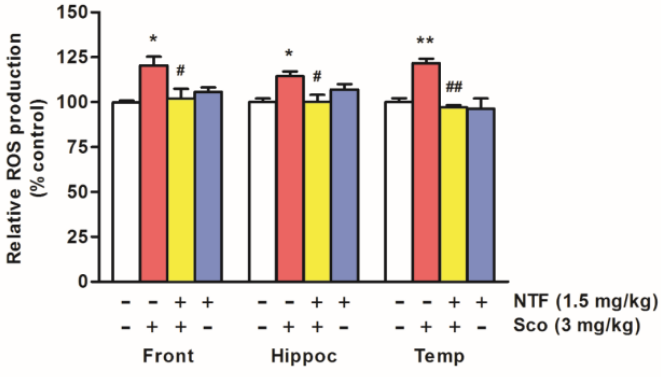

Fig 2. Effects of NTF treatment in scopolamine-treated rats on ROS. Production of ROS in brain homogenates were measured by fluorometric agent DCFH-DA. Each data bar (n=3) is expressed as mean ± SEM. *p < 0.05, **p < 0.01 vs control; #p < 0.05, ##p < 0.01 vs scopolamine-treated group.

Levels of cholinergic, apoptosis, and inflammatory proteins in brain tissues of rats administered scopolamine with and without NTF

Administration of scopolamine to rats significantly decreases ChAT and increases BACE1 levels in the 3 brain regions compared to control tissues (Fig 3A-C), and NTF cotreatment was able to ameliorate these alterations. However, NTF treatment alone had no effect on basal levels of the two proteins.

Exposure to scopolamine causes a significant increase in apoptotic proteins, Bax and activated caspase-3, and a corresponding decrease in anti-apoptotic Bcl-2 in the three types of brain tissues compared to controls with NTF treatment reversing these changes (Fig 4A-C). However, these relative changes in apoptosis-related proteins levels in the brain tissues of rats treated with NTF alone varied with tissue types and thus impacting the ability of NTF to restore the normal levels of apoptosis-related proteins in the scopolamine-treated animals.

Scopolamine significantly increased levels of cytokines – interleukin (IL)-6 and IL-1β – and of activated transcription factor – phosphorylated NF-κB – which were reversed by NTF. The extent of these phenomena relative to control levels also depended on brain tissue types (Fig 5A-C).

Activated cell signaling pathways in brain tissues of rats administered scopolamine with and without NTF

We investigated the activation indicated by the proportion of phosphorylated proteins of JNK, p38, ERK1/2, and Akt. In the frontal cortex (Fig 6A), scopolamine increased p-JNK and p-p38, decreased p-ERK1/2, and increased p-Akt. NTF downregulated p-ERK1/2 but upregulated p-Akt without affecting p-JNK or p-p38. A combination of scopolamine and NTF could only restore p-p38 to control level. In the hippocampus (Fig 6B), scopolamine elevated levels of p-JNK and p-p38, depressed p-ERK1/2 and had no effect on p-Akt. NTF had no effect on levels of p-JNK, increased p-p38, decreased p-ERK1/2, and had no effect on p-Akt. A combination of scopolamine and NTF could only restore p-ERK1/2 to control level and had no effect on the p-Akt level. In the temporal cortex (Fig 6C), scopolamine raised levels p-JNK and p-p38, lowered p-ERK1/2, and had no effect on p-Akt. NTF had no effect on levels of p-JNK, p-p38, or p-ERK1/2 but decreased p-Akt. A combination of scopolamine and NTF restored levels of p-JNK, p-p38 and p-ERK1/2, and had no effect on p-Akt. Table 1 shows the quantitative protein values as the percentage of change in expression relative to control groups in Western blot analysis.

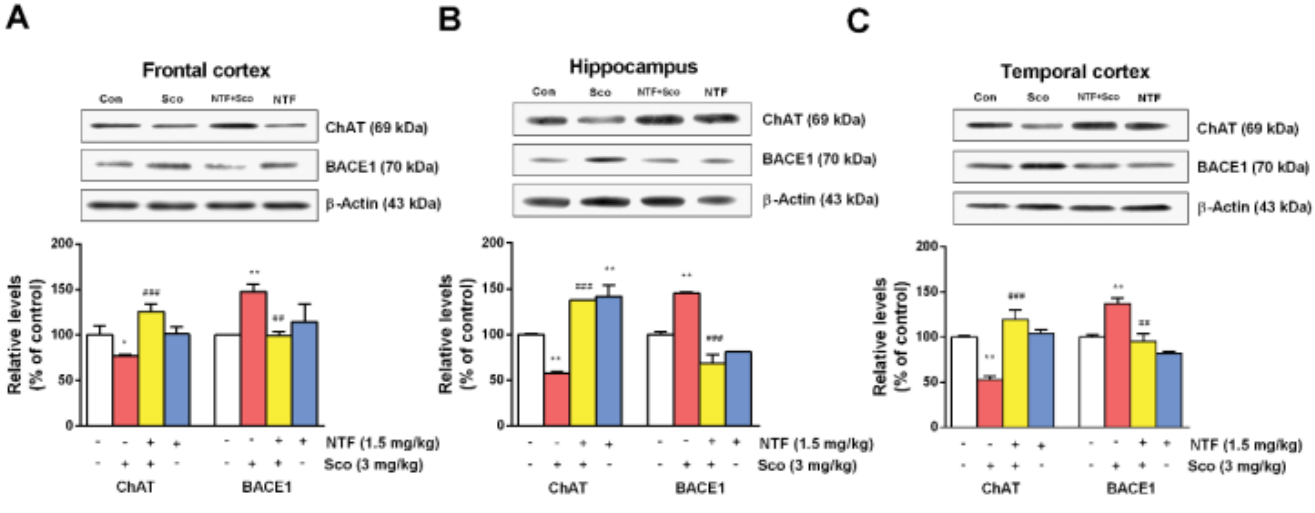

Fig 3. Effects of NTF on scopolamine-induced cholinergic dysfunction in rats on the levels of ChAT and BACE1 in: A frontal cortex, B hippocampus, and C temporal cortex. Each data bar (n=3) is expressed as mean ± SEM. *p < 0.05, **p < 0.01 vs control group; ##p < 0.01, ###p < 0.001 vs scopolamine-treated group

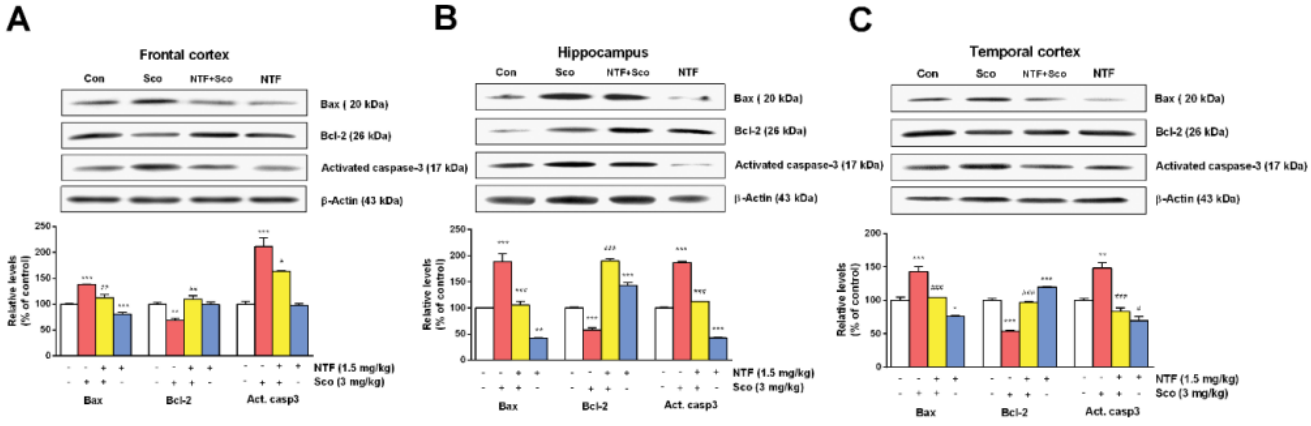

Fig 4. Effects of NTF on scopolamine-induced apoptosis represented by the levels of Bax, Bcl-2, and activated caspase-3 in: A frontal cortex, B hippocampus, and C temporal cortex. Each data bar (n=3) is expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs control group; #p < 0.05, ###p < 0.001 vs scopolamine-treated group.

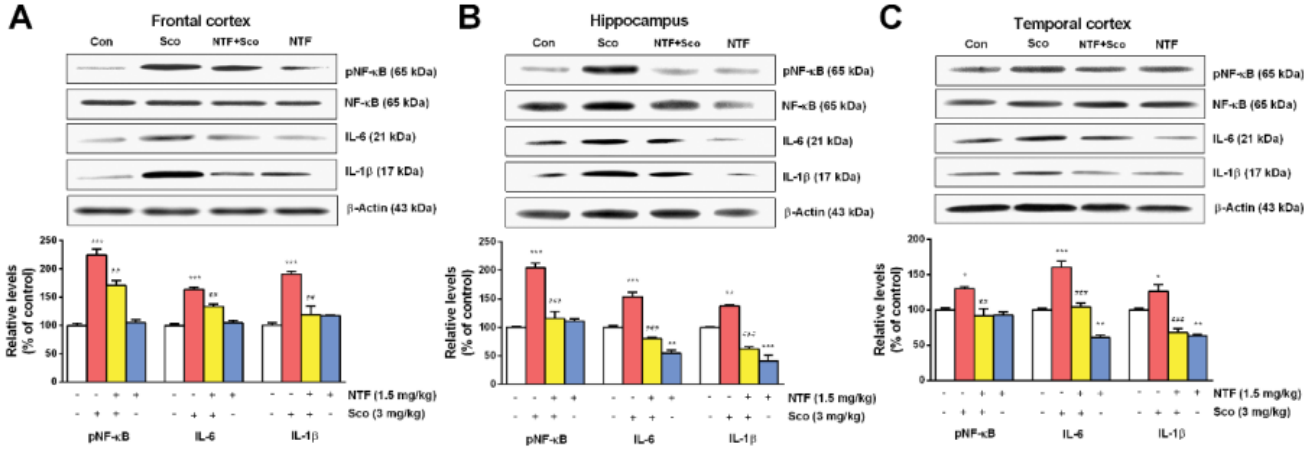

Fig 5. Effects of NTF on scopolamine-induced inflammatory responses indicated by the levels of p-NF-κB, IL-6, and IL-1β in: A frontal cortex, B hippocampus, and C temporal cortex. Each data bar (n=3) is expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs control group; #p < 0.05, ##p < 0.01, ###p < 0.001 vs scopolamine-treated group.

DISCUSSION

Scopolamine has been demonstrated to increase oxidative stress including ROS production in the mouse cortex and hippocampus, which could be reversed by pretreatment with sodium tanshinone IIA sulfonate, a radical-scavenging antioxidant.[26] The current study shows that administration of NTF was able to prevent scopolamine-augmented ROS production in the frontal and temporal cortices and hippocampus.

Oxidative stress contributes to the mechanisms of multiple neurodegenerative disorders including AD.[27] Both oxidative damage and cholinergic dysfunction have been implicated in cognitive impairment in rat dementia models. Treatment with antioxidant *Terminalia chebula* extract attenuated scopolamine-induced ROS generation and reduction in ChAT and ACh levels in mouse hippocampal tissue.[5] BACE1 is an enzyme necessary for the production of Aβ42 and Aβ40 peptides in AD.[28] Consistent with our finding, an antioxidant ipriflavone decreased BACE1 expression in the hippocampus of a scopolamine-treated rat.[29] Furthermore, NTF treatment alone significantly decreased apoptosis through downregulation of activated caspase-3 and Bax and upregulation of Bcl-2 in the hippocampus. Similarly, treatment with an antioxidant sodium tanshinone IIA sulfonate in a scopolamine-injected mouse significantly decreased Bax:Bcl-2 expression ratio and downregulated expression of activated caspase-3 in the hippocampus and cortex.[26]

Scopolamine also induces cytokines and NF-κB phosphorylation;[30] all of these changes were restored to normal levels in the rat cortex and hippocampus by NTF as described in the current study. Pretreatment

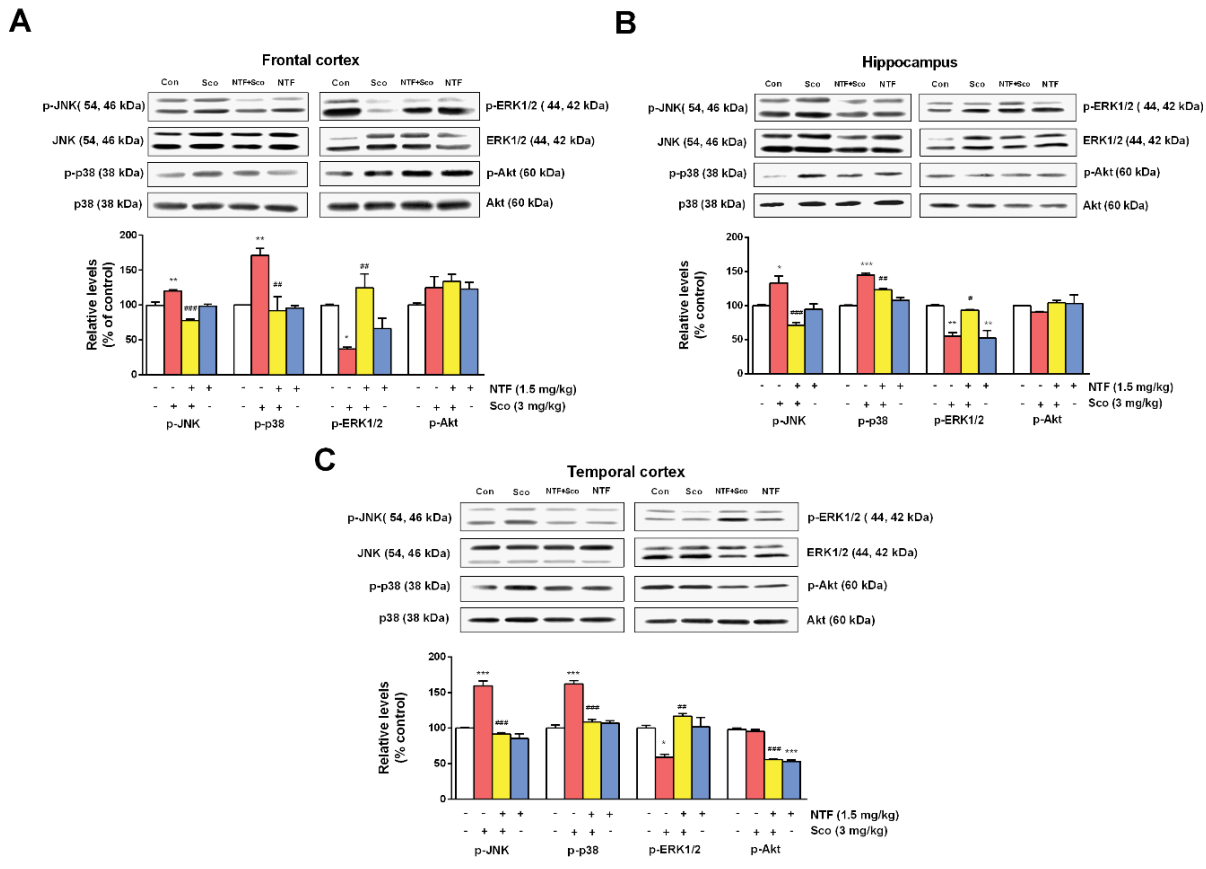

Fig 6. Effects of NTF on scopolamine-induced dysfunction in signaling pathways indicated by the levels of p-JNK, p-p38, p-ERK1/2 and p-Akt in: A frontal cortex, B hippocampus, and C temporal cortex. Each data bar (n=3) is expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs control group; ##p < 0.01, ###p < 0.001 vs scopolamine-treated group.

with a water-soluble ginseng oligosaccharide extract decreased scopolamine-induced expression of IL-1β and IL-6 mRNAs in the murine hippocampus and significantly prevented scopolamine from inducing spatial memory impairment during the water maze test.[31] In addition, antioxidant isoflavones from soybean-fermented food tempeh restored ACh level and suppressed scopolamine-induced IL-1β expression in the rat brains.[32]

The effects of scopolamine treatment on phosphorylation of the four signaling proteins (JNK, p38, ERK1/2, and Akt) were consistent across the three brain regions. Whether NTF could restore these changes could not be predicted based on the effects of NTF treatment alone in each brain tissue type. However, where scopolamine or NTF alone resulted in similar effects to a cell signaling protein, e.g. p-Akt (stimulated in the frontal cortex but unaffected in the temporal cortex and hippocampus), the results of their combination were as expected. In scopolamine-treated rats, NTF inhibited phosphorylation of JNK and p38 in these three brain regions. Previous studies have reported that JNK inhibitor SP600125 increased the number of surviving cells after ischemia in the rat hippocampus[33] and that SB202190 p38 inhibitor provided neuroprotection against cell apoptosis in mice.[34]

Scopolamine-decreased p-ERK1/2 levels in the three brain regions were accompanied by elevated BACE1 levels. Congruently, the expression and activity of BACE1 are negatively regulated by the ERK pathway.[35] Similar to our study, treatment of an antioxidant ipriflavone restored ERK1/2 phosphorylation and decreased BACE-1 expression and subsequent Aβ pathologies in a scopolamine-administered rat.[15] ERK1/2 inhibitor U0126 also blocked alleviating effects of amyrin, a triterpene with antioxidant activity, on scopolamine-induced memory deficits.[36]

CONCLUSION

This preliminary result demonstrates that NTF was able to attenuate scopolamine-induced impairment of the spatial cognitive performance, oxidative stress, inflammation, and apoptosis in the brain cortex and hippocampus, but its effects on cell signaling pathways in these brain tissues remained ambiguous. The utility of NTF as a potential preventive and therapeutic candidate for AD requires further experimentation. In a future study,

TABLE 1. Effects of NTF treatment on western blot protein expression in the frontal, hippocampus and temporal cortex in scopolamine-treated rats. Expression of target proteins is represented as the percentage of control. Results are expressed as mean ± SEM of three independent experiments.

	Frontal cortex			Hippocampus			Temporal cortex		
NTF (1.5 mg/kg)	-	+	+	-	+	+	-	+	+
Sco (3 mg/kg)	+	+	-	+	+	-	+	+	-
ChAT	76.9 ± 0.90*	125.9 ± 4.70###	101.1 ± 4.51	57.7 ± 1.41**	137.4 ± 0.73###	141.6 ± 12.38**	52.9 ± 3.78**	119.4 ± 10.98###	104.1 ± 4.11
BACE1	147.8 ± 4.45**	99.3 ± 2.43##	114.3 ± 11.36	145.0 ± 1.23**	68.6 ± 9.74###	80.7 ± 0.60	137.0 ± 6.40**	95.1 ± 8.43##	81.8 ± 2.13
Bax	137.7 ± 0.67***	112.33 ± 5.82##	81.1 ± 2.90***	188.5 ± 15.4***	105.6 ± 7.41###	42.3 ± 0.50**	143.0 ± 6.87***	104.2 ± 0.19###	76.2 ± 0.61*
Bcl-2	68.4 ± 4.11**	109.8 ± 6.23##	98.9 ± 5.14	58.2 ± 4.01***	189.7 ± 3.88###	143.5 ± 5.11***	54.0 ± 1.22***	96.6 ± 1.94###	119.8 ± 0.86***
Act. casp 3	211.2 ± 17.09***	163.5 ± 1.85#	97.2 ± 3.42	186.8 ± 2.32***	112.2 ± 0.92###	42.7 ± 0.83***	148.1 ± 8.3**	83.4 ± 5.29###	69.6 ± 5.92*
p-NFkB	224.8 ± 10.48***	170.2 ± 9.14##	105.3 ± 4.77	204.2 ± 7.93***	115.1 ± 12.8###	110.9 ± 3.90	130.1 ± 2.59*	91.1 ± 10.58##	92.2 ± 5.04
IL-6	163.5 ± 4.31***	133.7 ± 3.71##	104.8 ± 4.28	153.6 ± 7.63***	80.1 ± 2.50###	53.9 ± 6.29**	160.2 ± 8.74***	103.7 ± 6.28###	60.6 ± 4.00**
IL-1β	190.5 ± 5.52***	118.7 ± 15.4##	117.2 ± 0.79	137.5 ± 2.36**	62.2 ± 3.73###	40.7 ± 10.02***	126.2 ± 9.26*	67.6 ± 5.94###	63.4 ± 1.57**
p-JNK	120.2 ± 3.86**	77.8 ± 3.84###	98.5 ± 3.10	133.6 ± 9.39*	70.8 ± 4.67###	94.8 ± 7.94	159.7 ± 6.84***	91.3 ± 2.56###	85.6 ± 6.68
p38	171.2 ± 9.98**	91.9 ± 20.2##	96.1 ± 3.33	145.2 ± 2.44***	123.2 ± 2.26##	107.9 ± 4.02	162.0 ± 4.81***	109.0 ± 3.58###	107.1 ± 3.53
p-ERK	36.9 ± 2.68*	124.7 ± 20.27##	66.8 ± 14.29	55.1 ± 5.05**	93.4 ± 0.88#	52.3 ± 11.29**	58.8 ± 4.43*	116.9 ± 3.28##	101.7 ± 13.36
p-Akt	125.1 ± 16.05	134.0 ± 9.97	123.2 ± 9.56	90.3 ± 1.05	104.3 ± 3.20	103.2 ± 12.61	92.9 ± 4.52	55.5 ± 0.75###	54.4 ± 2.02***

*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ compared to control; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.01$ compared to scopolamine-treated group.

the effect of NTF on the altered activities of different cell signaling pathways and their mechanisms in rat brain tissues (frontal cortex, hippocampus, and temporal cortex) are needed to be investigated and the sample size should be expanded in order to acquire more conclusive results.

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