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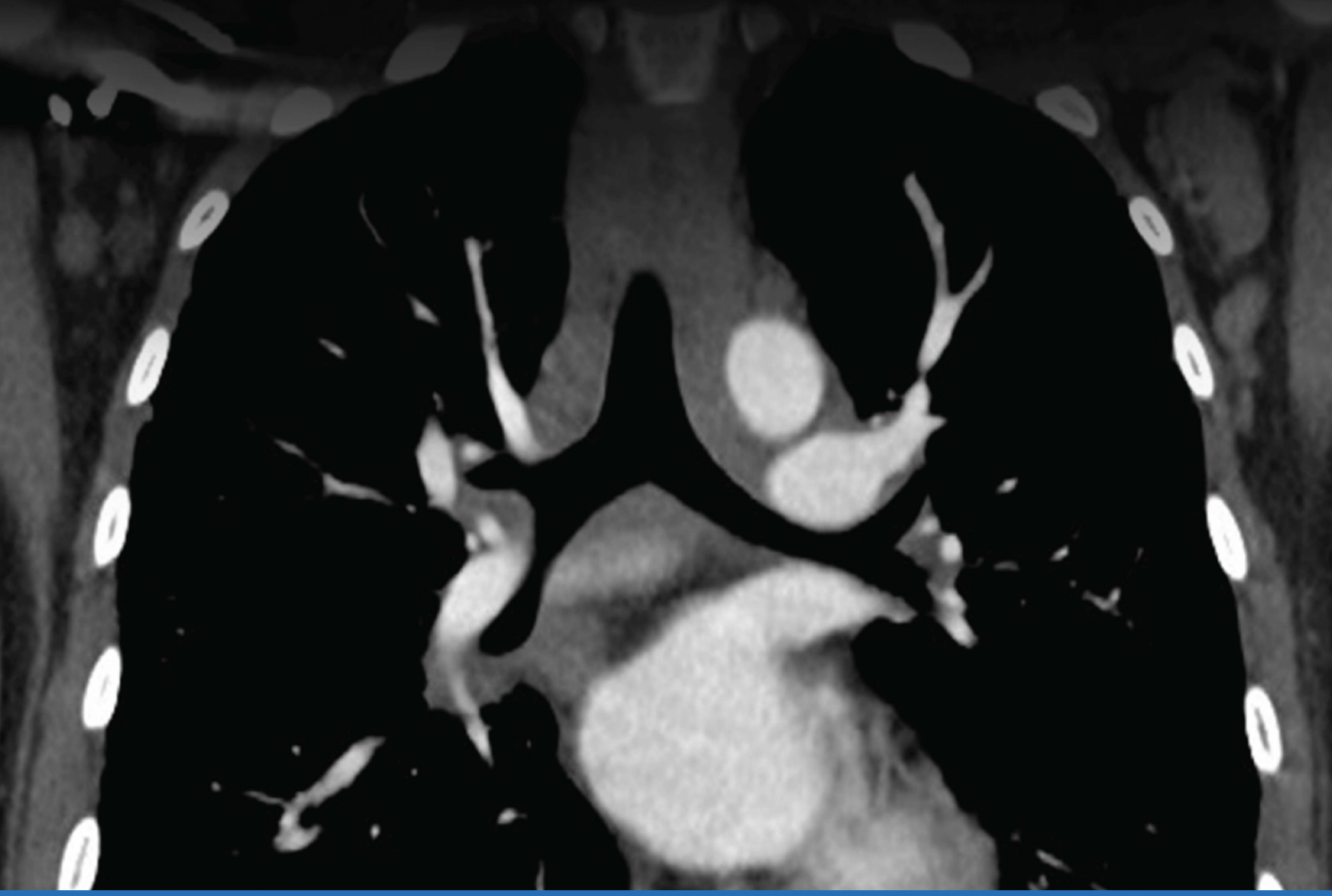

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The Research Medical Journal (RMJ) (formerly Ramathibodi Medical Journal) has been in continuous operation since 1978. This peer-reviewed journal aims to disseminate research findings, academic progress, and innovations in medicine, biomedical science, public health, and medical education to medical personnel and researchers domestically and abroad.

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Original Article

Factors Influencing the Decision on the Selection of Allied Health Careers Among Singapore Residents

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Abstract

Background: Singapore's ageing population has created significant healthcare workforce shortages, especially in allied health. This called for the urgent need to increase the local manpower to mitigate this issue.

Objective: To understand the factors influencing career choices in allied health among Singaporean youths.

Methods: This study utilized an online questionnaire to collect data. The questionnaire was distributed via social media, targeting Singaporean residents between the ages of 16 and 30 years, excluding existing allied health students/professionals. A subgroup analysis was also done by splitting respondents into 3 age groups (16-18, 19-24, and 25-30 years).

Results: Among the 305 responses collected, the most preferred allied health professions were psychologist, dietitian or nutritionist, and counsellor. Across all age groups, the top deterring factors were long hours, exhaustion, or working overtime, and low paying profession. For ages 16-18 and 19-24 years, increased salary and flexible work schedule were the top enticing factors. For respondents aged 25-30 years, flexible work schedule was the top factor instead, followed by increased salary.

Conclusions: Generally, the top enticing factors included increased salary, flexible work schedule and positive work environment, while the top deterring factors were long hours, exhaustion, working overtime, low paying profession, and financial issues incurred from allied health education. The subgroup analysis revealed some deviations of priorities between the different age groups which could be suggestive of the difference in perceptions between age groups. Larger, comparative studies are required to understand the needs and perceptions of young Singaporean residents more comprehensively.

Keywords: Allied health, Career choices, Singapore, Subgroup analysis, Influences

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Introduction

In Singapore, the proportion of citizens aged 65 and above has increased to 18.4% in 2022 from 11.1% in 2012.[1] This indicates a rapidly ageing population. Consequently, there has been a shortage of human resources in various sectors, particularly in healthcare. This is worsened by the rapidly increasing demand for healthcare and the rising prevalence of chronic illnesses within Singapore's ageing population. According to the Ministry of Health in Singapore, there currently are 58 000 healthcare workers, but 82 000 will be required by 2030.[2] In response to Singapore's healthcare manpower shortage,

attempts to retain and employ more local and foreign healthcare workers have been made. Despite the government's efforts, staffing shortages still persist as a major issue in the healthcare sector.[3] This study aimed to identify the factors that influence Singapore residents when choosing allied health as a career.

Methods

Most of the respondents were within the surveyors' personal contacts. This study's inclusion criteria included Singaporean residents (inclusive of permanent residents) who were between the ages of 16 and 30 years. This study's exclusion criteria included respondents who were existing allied health professionals or students enrolled in allied health courses.

In this study's surveys, it was emphasized that participation was voluntary and that by choosing to participate, respondents consented to the use of their data for research purposes. Participants were also assured that all collected data would be kept strictly confidential. For biographic data, this study only gathered respondents' age, gender, and occupation. This helped to reduce their identifiability and ensured anonymity.

This study employed a quantitative online questionnaire to gather data. The questionnaire was designed to assess respondents' preferences and deterrents regarding careers in allied health professions. The online questionnaire was distributed through various social media platforms such as Instagram. Data were collected from 23 November 2022 to 29 December 2022.

The survey had a total of 2 sections. The first section of the survey gathers the respondents' biographic data, including their ages, gender, and occupations. The second section of the survey collected information regarding the respondents' preference in various allied health occupations. Additionally, it aimed to identify the top factors that entice and deter them to work in these professions.

Respondents were initially presented with several individual allied health professions and groups of allied health professions. Descriptions of the main job scope for each individual allied health profession and each group of allied health professions were given to ensure a more comprehensive understanding of the roles and responsibilities of the allied health professions (Supplementary S1). For example, for the counsellor group, which included genetic counsellors, mental health counsellors, and family therapist counsellors, the main job scope was to provide professional advice to individuals, families, and healthcare providers. Furthermore, they were asked to pick up to 3 professions that they would prefer the most and up to 3 that they would prefer the least. Subsequently, they were asked to identify up to 3 factors that would most deter them and up to 3 that would most entice them to pursue a career in allied health.

Results

Sociodemographic

Out of 305 respondents, 150 were aged 16-18 years, 90 were aged 19-24 years, and 65 were aged 25-30 years. In terms of occupation, 201 of the respondents were students, 10 were serving National Service in Singapore (NSF), 19 were unemployed, 2 were self-employed, 1 was part-time employed, and 72 were full-time employed.

Allied Healthcare

When it came to which allied health professions respondents would prefer the most, psychologist was the top choice, with 148 out of 305 respondents selecting it. This was followed by dietitian, or nutritionist (n = 91) and counsellor (n = 83). Respondents aged 16-18 years most frequently chose psychologists (n = 76), counsellor (n = 42), and child life therapist (n = 41). In the 19-24 age group, the most popular options were psychologist (n = 48), dietitian or nutritionist (n = 29), and child life therapist (n = 20). Most chosen options from respondents aged 25-30 years were psychologist (n = 24), dietitian or nutritionist (n = 23), and counsellor (n = 22) (Table 1).

When it came to allied health professions that respondents would prefer the least, emergency medical personnel was the top choice, with 92 out of 305 respondents selecting it. This was followed by dental personnel (n = 79) and child life therapist (n =76). Respondents aged 16-18 years most frequently chose dental personnel (n = 42), emergency medical personnel (n = 39), and child life therapist (n = 38). In the 19-24 age group, the most popular options were child life therapist (n = 27), expressive art therapist (n = 27), dental personnel (n = 25), and emergency medical personnel (n = 25). Most chosen options from respondents aged 25-30 years were emergency medical personnel (n = 28), dental personnel (n = 12), child life therapist (n = 11), and counsellor (n = 11) (Table 2).

Table 1. Top 5 Preferred Allied Health Professions Among Respondents

Profession*	No. of Respondents			Total
	Age, y			
	16-18	19-24	25-30	
Psychologist	76	48	24	148
Dietitian or nutritionist	39	29	23	91
Counsellor	42	19	22	83
Child life therapist	41	20	20	81
Eye care specialist	28	14	15	57

*Each respondent could select up to 3 professions.

Table 2. Top 5 Least Preferred Allied Health Professions Among Respondents

Profession*	No. of Respondents			Total
	Age, y			
	16-18	19-24	25-30	
Emergency medical personnel	39	25	28	92
Dental personnel	42	25	12	79
Child life therapist	38	27	11	76
Expressive art therapist	36	27	8	71
Counsellor	32	22	11	65

*Each respondent could select up to 3 professions.

When it came to factors that entice respondents to become allied health professionals, increased salary emerged as the leading choice, with 189 out of 305 respondents identifying it as the most compelling reason to pursue a career in allied health. This was followed by flexible work schedule (n = 162) and positive work environment (n = 129). Respondents aged 16-18 years most frequently chose increased salary (n = 100), flexible work schedule (n = 86), and positive work environment (n = 71). In the 19-24 age group, the most popular options were increased salary (n = 60), flexible work schedule (n = 42), and more leaves (paid or unpaid) (n = 32). Most chosen options from respondents aged 25-30 years were flexible work schedule (n = 34), positive work environment (n = 29), and increased salary (n = 29) (Table 3).

When it came to factors that deter respondents from becoming allied health professionals, long hours, exhaustion, or working overtime emerged as the leading choice, deterring 201 out of 305 respondents. This was followed by low paying profession (n = 104) and financial issues (eg, debt from school) (n = 86). Respondents aged 16-18 years most frequently chose long hours, exhaustion, or working overtime (n = 105), financial issues (n = 55), and low paying profession (n = 51). In the 19-24 age group, the most popular options were long hours, exhaustion, or working overtime (n = 55), low paying profession (n = 37), and inadequate career progression (n = 30). Most chosen options from respondents aged 25-30 years were long hours, exhaustion, or working overtime (n = 41), health risks (n = 21), and inadequate career progression (n = 17) (Table 4).

Table 3. Top 5 Factors That Entice Respondents for Allied Health Profession

Factor*	No. of Respondents			Total
	Age, y			
	16-18	19-24	25-30	
Increased salary	100	60	29	189
Flexible work schedule	86	42	34	162
Positive work environment	71	29	29	129
More leaves	50	32	15	97
More emphasis on personal well-being	38	27	14	79

*Each respondent could select up to 3 professions.

Table 4. Top 5 Factors That Deter Respondents for Allied Health Profession

Factor*	No. of Respondents			Total
	Age, y			
	16-18	19-24	25-30	
Long hours, exhaustion, or working overtime	105	55	41	201
Low paying profession	51	37	16	104
Financial issues	55	21	10	86
Inadequate career progression	25	30	17	72
Health risks	29	14	21	64

*Each respondent could select up to 3 professions.

Discussion

Sociodemographic

Generally, it is expected that the younger the respondents, the less experienced and knowledgeable they are in the workforce, and the more their views are affected by their family members, friends, teachers or other social views as they have little experience in the workforce.[4-5] Given that 240 out of 305 (78.7%) of this study's respondents were under the age of 25 years, this survey predominantly captured the perspectives of younger individuals, which could also reflect broader societal perspectives. Given that 201 respondents were students and only 72 of the respondents were full-time employed, this further emphasized the youth-centric nature of this investigation, targeting young individuals who may not have entered the labour force. The low number of respondents in categories like self-employed, part-time, and NSF indicated that these groups were not the primary focus and had limited representation in our data.

Allied Healthcare

A significantly higher proportion of respondents aged 16-18 years (50.7%) and 19-24 years (53.3%) picked psychology to be one of the top 3 allied health professions. However, a smaller proportion (36.9%) picked psychology to be one of the top 3 allied health professions for those aged 25-30 years. Possibly, this trend could be attributed to the increasing emphasis on mental health in recent years, which might have raised awareness of this issue and consequently elevated the profile of psychologists among the younger generation.[6]

Interestingly, dental personnel were voted amongst the top 2 least attractive allied health professions in Singapore, despite dental hygienists being one of the highest paying allied health professionals.[7] Similarly, occupational therapists, radiation therapists, genetic counsellors, and audiologists have some of the higher salaries, but were not one of the top 5 most attractive allied health professions. This may be due to respondents' lack of awareness about salaries, as such information was not provided; thus, choices were likely based on job descriptions alone. Due to a lack of literature investigating the barriers to entering allied health professions among young Singapore residents, comparison with literature targeting respondents from different demographics was conducted. A literature review conducted by Wallis et al[8] agreed that financial costs of training pose a potential barrier, while a lack of awareness and misconceptions about allied professions remain the key obstacles.

Across all 3 age groups, the factor that deterred most respondents from becoming allied healthcare professionals was long hours, exhaustion, or working overtime with 201 selections, while low paying profession was second at 104 selections. The substantial difference between long hours, exhaustion, or working overtime and low paying profession could suggest that certain respondents might prefer a job that pays lower but is less demanding in terms of workload. Moreover, this could also suggest that respondents perceived the salaries in allied healthcare professions as inadequate given the high amounts of workload in allied health professions. Notably, this perception of allied healthcare as extremely demanding is supported by factual evidence. A 2019 study conducted in a Singapore tertiary hospital found that the self-reported burnout levels among allied health professionals were relatively high, with 67.4% of participants experiencing emotional exhaustion and/or depersonalization.[9] Similarly, high burnout levels were also observed among physicians and nurses.[10-11] These findings generally align with this study, which identified long hours, exhaustion, or working overtime was

the strongest deterring factor for young Singaporeans considering a career in healthcare. The widespread awareness of burnout among healthcare professionals, as documented in existing literature, may have contributed to shaping these perceptions. This suggests that the high prevalence of burnout not only contributes to existing challenges within the profession, but also serves as a significant deterrent for prospective entrants into the field.

Among respondents aged 16-18 years and those in the 19-24 age group, increased salary emerged as the primary factor motivating them (100 and 60, respectively), while the second most enticing factor was flexible work schedule (86 and 42, respectively). Contrastingly, the top factor chosen by respondents aged 25-30 years was flexible work schedule, followed by increased salary. This might have possibly indicated a shift in priorities and career motivations as individuals progress into their late twenties as although financial considerations, represented by an increase in salary, remained significant, the emphasis on work flexibility became the dominant factor for respondents in this age bracket. This may be because individuals in their late twenties often take on greater responsibilities, such as starting a family. A flexible work schedule would therefore become increasingly important for achieving a better work-life balance, allowing one to meet both professional obligations and personal commitments.

Limitations

Firstly, an overwhelming proportion of respondents were students aged 19-24 years. As a result, the data disproportionately reflects the perspectives of students aged 19-24 years compared to other groups. Therefore, the results obtained might not reflect the perspectives of other groups accurately. However, this imbalance could be viewed as a strength, given that adolescents in this age range are often grappling with crucial career decisions.[12] Recognizing their concerns and preferences holds paramount importance for governmental bodies and educational institutions striving to allure and retain skilled individuals in allied healthcare fields.

Secondly, information on respondents' education levels were not collected. This omission is notable as educational attainment can greatly influence one's perspectives. The absence of this data might have resulted in overlooking a pivotal factor that could have contributed to our data. Consequently, extrapolating the study findings to encompass all students aged 19-24 years in Singapore might pose challenges. Further research is necessary to investigate the correlation between education levels and their priorities when it comes to choosing a career in allied health.

Thirdly, allied healthcare professions that were found to share significant similarities in their job scopes were grouped together as one option. As such, investigating the difference between the job scopes of allied health professions in the same group was not possible

Lastly, many responses to the open-ended questions were vague — for example, describing occupations as simply interesting. However, more detailed responses were used for our analysis

Future Research

Further research in this area could extend to investigating the correlation between education levels and their priorities when it comes to choosing a career in allied health. This could help governmental bodies and educational institutions to better cater to adolescents based on their education levels and increase the likelihood of them choosing allied health as their career. Additionally, examining the struggles faced by specific

vulnerable population groups, such as migrant allied health professionals, can yield valuable insights. These insights not only can be utilized to increase the allied health workforce, but also to enhance retention rates among existing allied health professionals.

Conclusions

The primary factors deterring Singaporean residents from pursuing careers in allied health professions were identified as long hours, exhaustion, or working overtime, low paying profession, and financial issue, while the factors that would entice them the most to pursue a career in allied health were increased salary, flexible work schedule, and positive work environment. Further research is strongly encouraged to explore these issues more deeply and support a better understanding among young Singaporeans. Such endeavours hold the potential to provide invaluable insights that can inform future initiatives aimed at addressing the challenges within allied health professions.

Additional Information

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Ethics Approval: Ethical approval is not required as the data collected from our surveys were anonymous, nonsensitive, nonidentifiable, and the nature of the research posed no health risk to participants.

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Formal Analysis: Donovan Wong, Stephen Chang
Methodology: All authors
Writing – Original Draft: Donovan Wong, Stephen Chang
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Supplementary Material: Download Supplementary S1 from the following link: https://he02.tci-thaijo.org/index.php/ramajournal/article/view/271376/186670

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RMJ

Original Article

Tuberculin Skin Test as a Diagnostic Tool for Sarcoid Uveitis: A Retrospective Analysis at Ramathibodi Hospital, Thailand

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Abstract

Background: Sarcoid uveitis is rarely diagnosed in tuberculosis-endemic countries like Thailand, despite similar clinical presentations to tuberculous uveitis. Differentiating between the 2 conditions is challenging.

Objectives: To identify the diagnostic value of the tuberculin skin test (TST) for diagnosing sarcoid uveitis and use high-resolution computed tomography (HRCT) chest as a gold standard.

Methods: A retrospective review of medical records was conducted for patients who visited the uveitis clinic between January 2006 and December 2021. The inclusion criteria is the patients who underwent both TST and HRCT as part of the diagnostic workup. TST result < 10 mm, which excluded tuberculous uveitis, was strongly considered for diagnosing sarcoid uveitis, with HRCT finding serving as the gold standard due to the limitation of performing tissue biopsy.

Results: The study included 48 uveitis patients, with 19 (39.6%) showing sarcoidosis on HRCT. Among these, 18 (94.7%) had TST < 10 mm, while 1 (5.3%) had TST ≥ 10 mm. In contrast, of those in the non-sarcoidosis group, 13 (44.8%) had TST < 10 mm and 16 (55.2%) had TST ≥ 10 mm. Statistical analysis revealed a significant association between TST and HRCT ($P < .001$). The TST's sensitivity, specificity, positive predictive value, and negative predictive value for sarcoid uveitis was 94.7%, 55.2%, 58.1%, and 94.1%, respectively.

Conclusions: This study suggested that TST is a potential noninvasive adjunctive diagnostic tool for sarcoid uveitis. Using TST as a screening tool, due to its high sensitivity, could improve early detection and appropriate management of patients, leading to better clinical outcomes.

Keywords: Sarcoid uveitis, Noninvasive tool, Tuberculin skin test, High-resolution computed tomography chest, Tuberculous uveitis

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Introduction

Sarcoidosis is a multisystem inflammatory disease that can affect various organs in the body with unknown etiology characterized by the presence of noncaseating granulomas in the affected organs. Sarcoidosis affects people worldwide. The incidence of sarcoidosis can vary across the world. Sarcoidosis is generally more common in the western countries rather than the eastern countries. For example, the annual incidence of sarcoidosis in African-Americans is as high as 30-70 per 100 000 population, followed by the Europeans is 10-20 per 100 000 population, but as low as 1-3 per 100 000 population

among Asians and Hispanics.[1-11] However, in the recent years, there has been an increase in incidences among Asian countries like India,[2] Japan,[3] and Korea.[4] It is important to note that accurate data on the global incidence for sarcoidosis are limited due to variations in diagnostic practices, underreporting, and regional differences in disease awareness.

The most affected sites are the lungs and intrathoracic lymph nodes (more than 90% of patients.)[5-12] Including the eyes, sarcoidosis uveitis, also known as ocular sarcoidosis, is a specific manifestation of sarcoidosis that primarily affects the intraocular tissue. A recent retrospective series reported that uveitis was the first presentation of sarcoidosis in 80% of presumed or biopsy-proven cases.[13] Patients with sarcoidosis uveitis can present with blurred vision, redness, floater, or discomfort. Clinical manifestations are acute or chronic granulomatous uveitis, which can be any type of uveitis (anterior, intermediate, posterior, or panuveitis). Typical biomicroscopic findings include mutton-fat keratic precipitates, Koeppe and Busacca iris nodules at the pupil margin and in the iris stroma, respectively, and white clumps of cells (snowball) in the anterior vitreous. Moreover, typical radiological findings are bilateral hilar lymphadenopathy, or parenchymal lung changes consistent with sarcoidosis. Definite diagnosis requires the presence of noncaseating granuloma or only minimal amount of focal necrosis from biopsy tissue. A combination of clinical, radiological, and histopathological criteria is used to diagnose sarcoidosis. However, biopsy procedures such as diagnostic pars plana vitrectomy (PPV), transbronchial lymph node and lung biopsies are invasive procedures.[14, 15]

Clinical, radiological, and histopathological similarities with tuberculosis (TB) make the differential diagnosis of 2 conditions difficult especially in countries with endemic area of TB including Thailand.

In Thailand, sarcoid uveitis has rarely been diagnosed. It is not known whether the rarity of sarcoid uveitis in Thailand is genuine or whether sarcoid uveitis in this area remains underdiagnosed. However, a recent retrospective cohort study from Siriraj Hospital[16] showed an increase in diagnosis of sarcoidosis in Thailand. The study found that from 2005 to 2018, 89 confirmed cases of sarcoidosis were identified. Most patients had intrathoracic disease (81 cases [91%]). Sarcoid uveitis was the most common extrathoracic disease (35 cases [39.3%]), which is far higher than previous reports from Europe and North America.[6, 7, 17, 18] It may be the clue that the actual number of sarcoid uveitis is higher than we expected. So, we need to know what the noninvasive diagnostic tools to differentiate sarcoid uveitis from tuberculous uveitis.

Tuberculin skin test (TST), also known as the Mantoux test, is primarily used to diagnose TB infection. This test is cost-effective and feasible in almost every hospital. It involves injecting a small amount of purified protein derivative (PPD) 0.1 mL, a substance derived from *Mycobacterium tuberculosis* (MTB), into the skin and then measures the reaction at the injection site after 48-72 hours. The test measures the immune response to the PPD antigen, which can indicate previous exposure to TB. So, we can use TST as a tool to exclude TB infection.

The aim of this study was to evaluate sensitivity, specificity, positive predictive value, and negative predictive value of TST as a diagnostic tool to diagnose sarcoid uveitis. We used high-resolution computed tomography (HRCT) of the chest as a gold standard. Indicative findings of sarcoid uveitis from chest HRCT such as bilateral hilar lymphadenopathy, mediastinal lymphadenopathy, lung parenchymal involvement of micronodules with a perilymphatic distribution.[2, 19]

Methods

Data Source

All data were kept confidential in our database. We retrospectively reviewed the electronic medical records of 1628 subjects who were diagnosed with uveitis at Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand between January 2006 and December 2021.

Subject Selection

In this study, we aimed to assess the diagnostic utility of TST compared to HRCT of the chest as a noninvasive tool for identifying sarcoid uveitis. Patients may or may not have undergone a biopsy.

Besides the performed TST and HRCT, inclusion criteria consisted of clinical signs corresponding with sarcoidosis uveitis following the International Workshop on Ocular Sarcoidosis (IWOS)[15] by 2 of the following: mutton-fat keratic precipitates (large and small) and/or iris nodules at pupillary margin (Koeppe) or in stroma (Busacca), trabecular meshwork nodules and/or tent-shaped peripheral anterior synechia, snowballs/string of pearls vitreous opacities, multiple chorioretinal peripheral lesions (active and atrophic), nodular and/or segmental periphlebitis (candle wax drippings) and/or macroaneurysm in an inflamed eye, optic disc nodule(s)/granuloma(s) and/or solitary choroidal nodule, or bilaterality (assessed by ophthalmological examination including ocular imaging showing subclinical inflammation). Exclusion criteria consisted of HIV infected patients, immunocompromised patients or on immunosuppressive drugs, and concurrent active pulmonary or other TB disease.

Interpretation

TST results were considered positive for the diagnosis of sarcoid uveitis if the induration diameter measured less than 10 mm (negative results for tuberculosis infection). Cut point of 10 mm in the endemic area where TB is common, including Thailand according to the Centers for Disease Control and Prevention guideline. In this study, interpretation was performed in an ophthalmology outpatient department at 48-72 hours after the PPD injection. Chest HRCT findings indicative of sarcoidosis[2, 19] included bilateral hilar lymphadenopathy, mediastinal lymphadenopathy, lung parenchymal involvement of micronodules with a perilymphatic distribution were used as a gold standard.

A standardized case record form was used to record demographics and disease characteristics. Analyses were performed using Stata software, version 17 (StataCorp. Version 17. College Station, TX: StataCorp LLC; 2021). The mean (SD), median, and range were used for descriptive statistics. The chi-squared test was used to analyze the statistics. *P* values < .001 were considered statistically significant. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated by using Stata software.

Results

Demographic Data and Clinical Characteristics

From 2006 to 2021, a total of 48 patients with uveitis met the inclusion criteria. There were 23 (48%) men and 25 (52%) women. The mean (SD) age at presentation was 48.2 (16.0) years (range 10-77 years). Most cases (43.75%) were in the middle-age range of 40-59 years. In terms of laterality, bilateral involvement (29 patients [60.4%]) was more

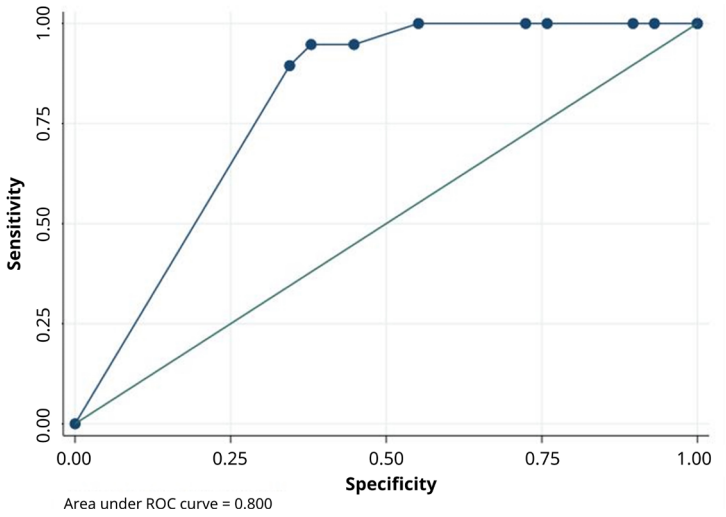
common than unilateral involvement (19 patients [39.6%]). Anatomically, panuveitis was the most common type of uveitis, seen in 34 cases (70.8%), followed by posterior uveitis 7 cases (14.6%), anterior uveitis 6 cases (12.5%), and intermediate uveitis 1 cases (2.1%).

The study included 48 uveitis patients, with 19 (39.6%) showing findings consistent with sarcoidosis on HRCT chest. Among patients diagnosed with sarcoid uveitis based on chest HRCT, 18 (94.7%) exhibited TST < 10 mm, while 1 (5.3%) showed TST ≥ 10 mm. In contrast, of those in the non-sarcoid uveitis on chest HRCT, 13 (44.8%) displayed TST < 10 mm, while 16 (55.2%) had TST ≥ 10 mm (Table 1). Statistical analysis demonstrated a significant association between TST results and chest HRCT findings ($P < .001$). The sensitivity, specificity, positive predictive value, and negative predictive value of TST for sarcoid uveitis, using chest HRCT as the gold standard, were 94.7%, 55.2%, 58.1%, and 94.1%, respectively, and the areas under the receiver operating characteristic curve (AUC) was 0.800 (95% CI 0.698-0.903) (Figure 1).

Table 1. The Results of Tuberculin Skin Test as a Diagnostic Tool for Sarcoid Uveitis

Value	No. of Patients		
	Sarcoid Uveitis	Non-Sarcoid Uveitis	Total
< 10 mm (positive diagnostic test for sarcoid uveitis)	18	13	31
≥ 10 mm (negative diagnostic test for sarcoid uveitis)	1	16	17
Total	19	29	48

Figure 1. Areas Under the Receiver Operating Characteristic Curve Plotted to Determine the Diagnostic Values of Tuberculin Skin Test



A total of 48 patients with uveitis met the inclusion criteria. The number of sarcoid uveitis was 19 cases (definite 5 case, presumed 14 cases). The most common cause of non-sarcoid uveitis was tuberculous uveitis 18 cases, followed by 5 cases of Vogt-Koyanagi-Harada disease, 1 case of systemic lupus erythematosus, 1 case of Behcet's disease, 1 case of granulomatosis with polyangiitis, 1 case of Sjögren's syndrome, and 2 cases of idiopathic uveitis (Table 2).

Definite Sarcoid Uveitis

A 28-year-old male with bilateral granulomatous anterior uveitis, TST 0 mm, chest HRCT showed bilateral hilar lymphadenopathy, peribronchovascular nodularity (Figure 2A). Transbronchial needle aspiration (TBNA) detected nonnecrotizing granulomatous inflammation with Schaumann bodies.

A 31-year-old female with bilateral panuveitis, TST 0 mm, chest HRCT showed extensive small pulmonary nodules in both lungs with multifocal patchy ground-glass opacities in both upper lobes with extensive lymphadenopathy (Figure 2B). Cervical lymph node biopsy detected nonnecrotizing granulomatous lymphadenitis.

A 61-year-old female with bilateral panuveitis, TST 0 mm, chest HRCT showed bilateral hilar lymphadenopathy, multiple nodular and patchy ground-glass opacities at right upper lobe and right lower lobe (Figure 2C). TBNA detected nonnecrotizing granulomatous inflammation.

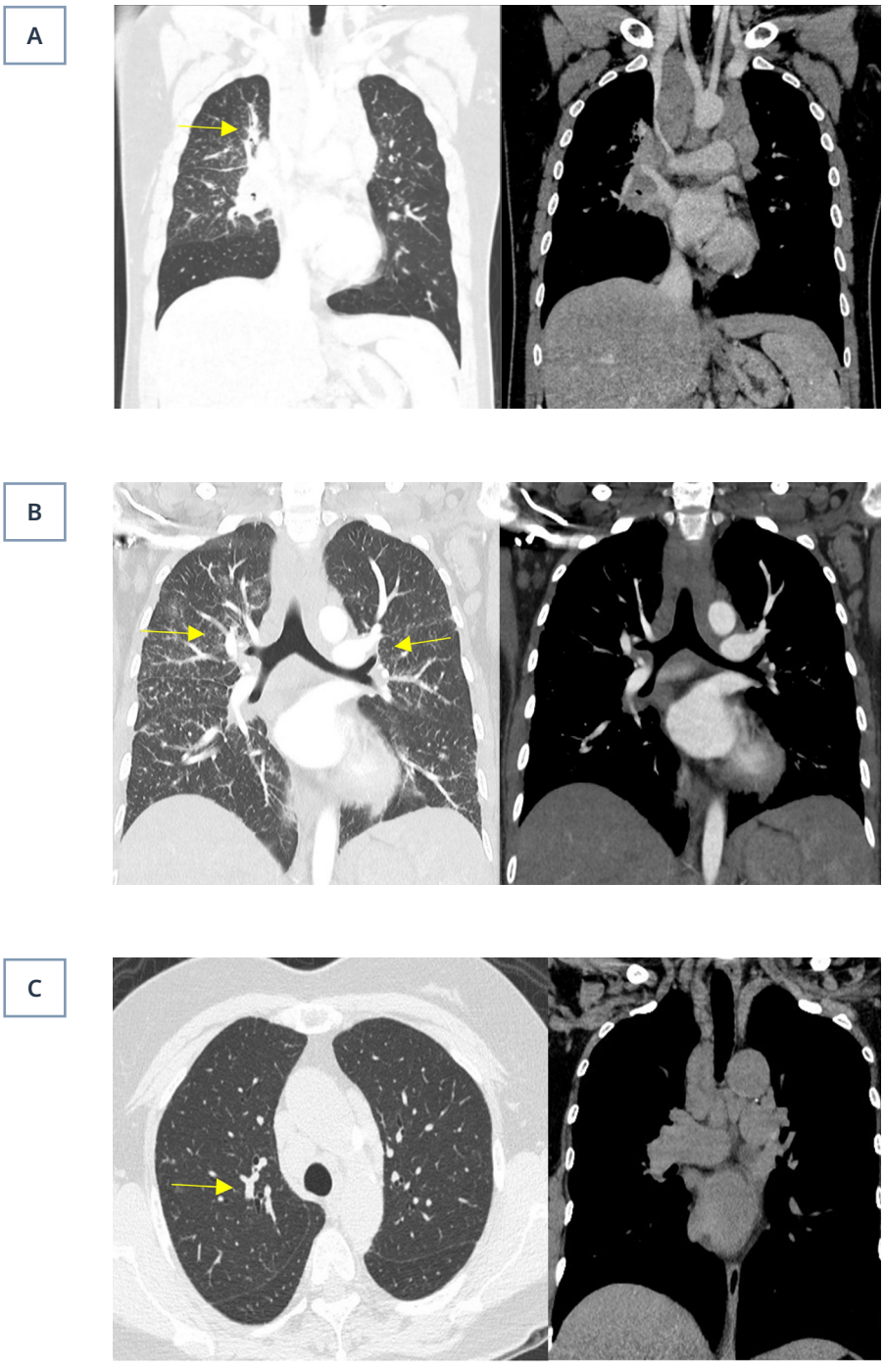
Presumed Sarcoid Uveitis

A 54-year-old male with right multifocal choroiditis with snowball and snowbank (Figure 3A), TST 5 mm, chest HRCT showed bilateral calcified hilar lymphadenopathy with '1-2-3 sign', peribronchovascular patchy ground-glass opacities both lungs (Figure 3B). TBNA detected no evidence of granuloma or carcinoma was seen.

Table 2. Causes of Uveitis

Cause	No. (%)
Sarcoid uveitis	19 (39.6)
Non-sarcoid uveitis	
Tuberculous uveitis	18 (37.5)
Vogt-Koyanagi-Harada disease	5 (10.4)
Systemic lupus erythematosus	1 (2.1)
Behcet's disease	1 (2.1)
Granulomatosis with polyangiitis	1 (2.1)
Sjögren's syndrome	1 (2.1)
Idiopathic	2 (4.1)

Figure 2. High-Resolution Computed Tomography of the Chest in Definite Sarcoid Uveitis

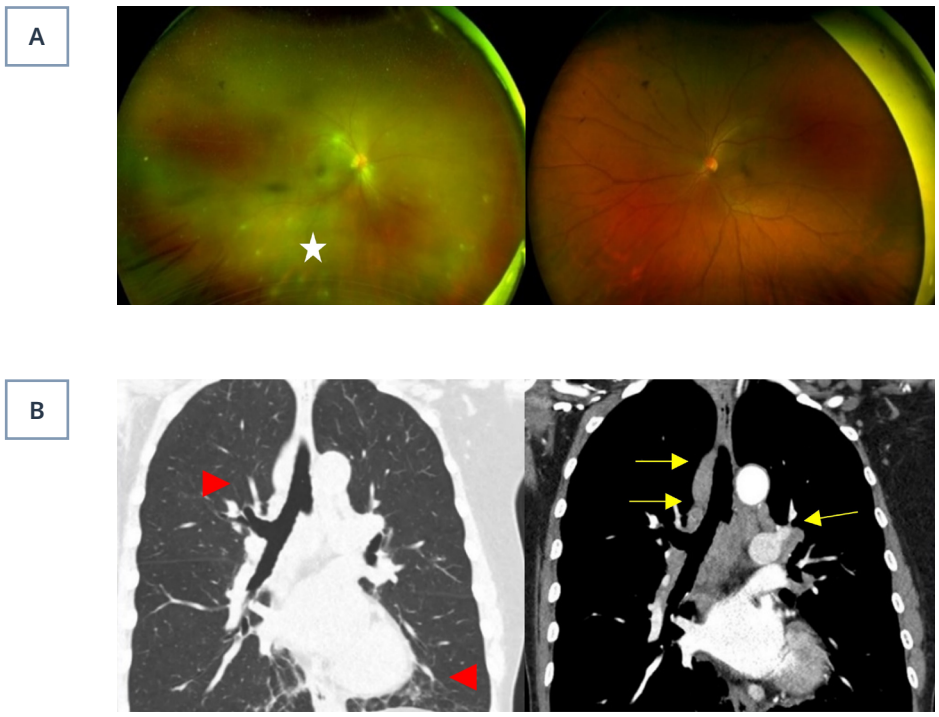


A, Bilateral hilar lymphadenopathy with peribronchovascular nodularity (arrow).

B, Extensive small pulmonary nodules with multifocal patchy ground-glass opacities in both upper lobes with extensive lymphadenopathy (arrow).

C, Bilateral hilar lymphadenopathy with ground-glass opacities at right upper lobe and right lower lobe (arrow).

Figure 3. Ultra-Widefield Fundus Camera of Right Eye and High-Resolution Computed Tomography of the Chest in Presumed Sarcoid Uveitis



A, Ultra-widefield fundus camera of right eye showed multifocal choroiditis with snowball and snowbank (star).

B, Chest HRCT showed bilateral calcified hilar lymphadenopathy with '1-2-3 sign' (arrow), peribronchovascular patchy ground-glass opacities both Lungs (arrow head).

Discussion

This study showed that TST had high sensitivity and negative predictive value which can be used as a screening tool to rule out sarcoid uveitis. Contrastingly TST had low specificity and positive predictive value which are not specific to sarcoid uveitis.

Sarcoidosis is a granulomatous disease that can sometimes resemble TB clinically, radiologically, and histologically. Therefore, it is important to rule out active TB infection, as it can present with similar symptoms and findings. The TST can help determine if a person has been exposed to TB in the past by measuring the immune response to TB antigens. This study chose TST as a study test due to feasibility, cost-effectiveness, and safety.

HRCT of the chest in which thin-slice chest images are obtained and post-processed in a high-spatial-frequency reconstruction algorithm. This technique is performed to visualize small structures of the lung and detect enlarged lymph nodes, which can be suggestive of various conditions, including sarcoidosis. That otherwise may be difficult to assess on a conventional chest imaging.[20]

To the diagnosis of sarcoid uveitis, both the TST and chest HRCT can be helpful. The diagnosis of sarcoid uveitis is typically based on a combination of clinical evaluation, ocular examination, imaging studies, laboratory tests (eg, serum ACE), and sometimes

a biopsy of affected tissues. The histopathological finding of sarcoidosis is the presence of noncaseating granulomas. These granulomas tend to be most prevalent around the bronchovascular area and the fibrous septate containing pulmonary veins.[21] While caseating granulomas are usually found in ocular tuberculosis, in conjunction with positive culture for MTB such as polymerase chain reaction (PCR) for MTB.[22]

In our study, there was a limitation in tissue biopsy for some patients. Tissue biopsy was attempted to confirm diagnosis as definite sarcoidosis uveitis in only 5 patients. The other 14 patients were diagnosed as presumed sarcoidosis uveitis. According to the revised the IWOS criteria[15] in 2017, ocular sarcoidosis was determined as definite, presumed, and probable.

Due to the retrospective nature of the study, it was not feasible to conduct TST or chest HRCT in all patients exhibiting clinical features consistent with sarcoid uveitis. Consequently, the number of patients included in this study is likely underestimated. Nonetheless, further prospective studies with larger sample sizes and extended follow-up periods are warranted to validate the utility of TST and HRCT of the chest. Additionally, it's important to acknowledge limitations associated with TST, such as the potential for false-positive results due to previous Bacillus Calmette-Guérin (BCG) vaccination or nontuberculous mycobacterial infections. Therefore, if feasible, the use of interferon-gamma release assay might be considered as an alternative to TST. For cases of highly suspected sarcoidosis uveitis with a positive TST, we recommend obtaining a tissue biopsy that presents pathological evidence of noncaseating granulomas to prevent missing a diagnosis of sarcoid uveitis.

However, the prevalence of the disease in the tested population has a significant impact on the positive and negative predictive values. This study conducted in Thailand, where sarcoidosis has a low prevalence. As the prevalence of the disease decreases, the positive predictive value experiences a decline, indicating a lower probability of correctly identifying true positive cases. Conversely, the negative predictive value shows an upward trend, suggesting a higher likelihood of correctly identifying true negative cases. These findings underscore the critical role that disease prevalence plays in determining the accuracy and reliability of predictive values in diagnostic testing.

The results of this present study correlate with a prior study[23] which suggested that TST may be a useful tool for diagnosing sarcoid uveitis. However, the diagnosis and management of sarcoid uveitis would typically involve a multidisciplinary approach, including ophthalmologists, pulmonologists, and other relevant specialists. The diagnosis may be based on a combination of clinical evaluation, medical history, imaging tests (such as chest x-ray or CT scan), laboratory investigations (such as TST, QuantiFERON TB, serum lysozyme or serum ACE), and sometimes a biopsy of affected tissues.[24-27]

Conclusions

This study suggested that the TST may be a potential noninvasive adjunctive tool for sarcoid uveitis. TST < 10 mm showed a significant association with the presence of sarcoidosis on chest HRCT. Using TST as a screening tool, due to its high sensitivity, could improve early detection and appropriate management of patients, leading to better clinical outcomes. Specifically, highly suspected sarcoidosis uveitis with common ocular signs includes mutton-fat keratic precipitates, iris nodules and snowballs/string of pearls vitreous opacities.

Additional Information

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Ethics Approval: This retrospective cross-sectional study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (MURA2022/290 on 9 May 2022), which waived the need for written informed consent from the subjects.

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RMJ

Original Article

The Association Between Oral Health Literacy and Oral Health Behaviors Among Thai Older Adults in Health Region 6, Thailand

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Abstract

Background: Older individuals are susceptible to oral diseases, which are among the most prevalent diseases worldwide. Proper oral health behaviors (OHB) can prevent or manage most oral diseases.

Objective: To investigate the associations between oral health literacy (OHL) and OHB among older adults in Eastern (Health Region 6) Thailand.

Methods: A cross-sectional study was conducted to collect data from 385 older adults. A multistage sampling technique was used for sample selection. The questionnaires comprised 3 sections, including demographic characteristics, an OHL questionnaire, and an OHB questionnaire. Data was obtained through face-to-face interviews with participants. Multiple logistic regression was used to analyze variables that were associated with OHB.

Results: A total of 385 older adults, with a mean (SD) age of 64.78 (3.03) years, participated in the study. Only 32.2% demonstrated good OHB. In the adjusted models, good OHB scores significantly correlated with secondary school education level (OR [95% CI], 3.20 [1.62-6.45]), bachelor's degree and higher education level (OR [95% CI], 1.73 [1.01-2.98]), ever receiving oral health information (OR [95%CI], 2.53 [1.13-6.19]), alcohol drinking (OR [95% CI], 0.24 [0.07-0.70]) and an adequate level of OHL within 2 of 6 domains: media literacy (OR [95% CI], 1.73 [1.11-2.70]) and self-management skill (OR [95% CI], 2.45 [1.53-3.96]).

Conclusions: This study revealed that older adults in Health Region 6 have a low OHB level. Therefore, it is essential to increase the appropriate promotion of OHB, with behavioral modification programs for improving OHL, particularly in the areas of media literacy and self-management skills.

Keywords: Oral health behaviors, Oral health literacy, Older adults, Thailand

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Introduction

One of the most prevalent diseases and one of the main health concerns worldwide is poor oral health, particularly among older people.[1] Oral diseases are an important factor affecting their overall health and quality of life.[2, 3] Common oral health problems in older adults are periodontal disease, dental caries, tooth loss, xerostomia, and oral precancerous and cancerous conditions.[4] Poor oral health among older people can negatively affect daily activities, leading to impaired chewing, nutritional deficiencies, social relations, and loneliness.[1, 5] According to the World Health Organization (WHO), oral diseases are among the most common noncommunicable diseases worldwide, and they are related to a range of chronic diseases, such as diabetes mellitus, cardiovascular disease, certain cancers, and pneumonia.[6]

Oral health behaviors (OHB) clearly influence oral diseases, and an increase in good oral health behaviors leads to a decrease in their prevalence rate.[7, 8] The Encyclopedia of Public Health describes oral health behavior as a complex interplay of oral hygiene habits, nutritional preferences, and the pattern of a person's utilization of dental services, all of which affect individual oral health.[9] Ou et al[10] investigated older adult Chinese oral health behaviors and found that individuals who had never used dental floss and had irregular dental check-ups have a lower number of remaining teeth than those who floss at least once a day and have frequent dental check-ups. Likewise, Pan et al[11] investigated adult Taiwanese oral health behaviors and found that individuals who had never used dental floss have a lower number of remaining teeth than those who floss at least once a day.

Maintaining functional teeth is critical for supporting healthy aging. Successful oral aging is associated with adequate function and comfort. Older adults retaining more than or equal to 20 teeth are less likely to experience poorer health.[12] According to a 2023 report from the Thailand National Oral Health Survey (TNOHS), a nationally representative oral health survey, 48.6% of Thais aged 60-74 years in Eastern (Health Region 6) Thailand had at least 20 active permanent teeth, compared to the country's average of 60.9%. The report also found that older adults were experiencing oral health problems, due mainly to improper oral health behaviors, such as not brushing teeth before bed without eating any food, not using brushing accessories, and not getting dental services at least once a year. It is the leading cause of tooth loss in older adults. The older adults have improper OHB, limited access to oral health services, and communication problems with dentists.[13]

Oral health literacy (OHL) is correlated with poor OHB, as suggested by Alzeer et al.[14] They found that adolescents in the Kingdom of Saudi Arabia with low OHL often have inappropriate OHB. Further, OHL has been suggested to be associated with oral health status such as those proposed by Adil et al.[15] They found that parents' OHL scores were significantly associated with dental caries in Malaysian children. Low OHL has been identified as a major barrier to dental care, such as dental service utilization, daily brushing frequency, and urgent care utilization for a dental problem.[16, 17]

Oral health literacy is the degree to which individuals have the capacity to obtain, process, and understand basic oral health information and services needed to make appropriate health decisions.[18] According to this definition, OHL for older adults is very crucial because it may influence their oral health. Inadequate oral health literacy leads to reduced utilization of services, poor outcomes, improper behaviors, and increased risk of oral disease.[19, 20] Improving OHL is essential for enhancing oral health outcomes and reducing disparities.[21] For that reason, investigating the associations between OHL and OHB in older adults is essential.

Some factors, such as socioeconomic conditions, that influence individuals' OHB for the prevention and control of oral disease are unmodifiable.17 On the other hand, the 6 competencies and skills of individuals for promoting oral health literacy — access skills toward oral health information and services, cognitive skills, communication skills, self-management skills, media literacy skills, and decision-making skills — are all modifiable factors that can be improved to serve as a strategy for the prevention of oral disease.[22, 23] Accordingly, measuring and identifying the association between these modifiable variables might be of significant help in improving oral health. Older adults had a high prevalence of limited oral health literacy[24] although research into and promotion of the dimensions of

oral health literacy in older adults is still scarce, and the association between OHB and associated factors remains inadequately investigated.

According to the TNOHS 2023 report, there was a high prevalence of oral disease among older adults, as well as a lack of information on the efficacy of oral health programs. Therefore, this study aimed to investigate the associations between OHL and OHB among older adults in Eastern (Health Region 6) Thailand. This study has provided essential data for organizing an improved OHL program to improve individuals' adoption of proper OHB.

Methods

Participants and Setting

This research was a cross-sectional study that was conducted in the Eastern (Health Region 6) Thailand. The sample size was calculated using a finite population for testing proportions with a 95% confidence level.[25] The sample size was 369, and after adjusting the sample size for a dropout rate of ten percent,[26] the final sample size was 406. A multistage sampling technique was used for selecting the sample units. Based on a Gross Provincial Product (GPP) level that represents economic and social indicators at the provincial level, the 8 provinces in the Eastern (Health Region 6) Thailand were divided into 2 groups: high and low.[27] One province was randomly selected from each GPP province group. In the second step, 2 distinct individuals were randomly selected to represent the province. In the third step, the research was performed at a health-promoting hospital run by the Ministry of Public Health, where oral hygienists could provide services. Participants were selected through consecutive sampling. After the data had been organized and verified, participants were then selected from 4 districts across high GPP, which was Chon Buri, and low GPP, which was Chanthaburi province.

Instruments

An interview questionnaire was used to collect research information. In the process of data collection, the trained interviewers collected data on demographic characteristics, OHB, and OHL variables through face-to-face interviews while participants were waiting to receive typical health services at a health-promoting hospital, and the duration of each interview was approximately 20 minutes. The questionnaire consisted of 3 sections. The first section collected demographic characteristics about the participants, including gender, age, marital status, education level, occupation, financial status, chronic health conditions, smoking, alcohol consumption, betel nut use, receiving information about dental health, and experience with the replacement of natural teeth.

The second section of the questionnaire was an OHL questionnaire, adapted from the previous study by Jittimanee et al.[22] The OHL questionnaire was a comprehensive assessment tool for identifying self-perceived oral health care competencies. This study developed 6 domains of OHL for the older Thai adult population: 1) cognitive skills; 2) accessing skills; 3) communication skills; 4) self-management skills; 5) media literacy skills; and 6) decision-making skills regarding oral health. The scale comprised 35 items with a rating scale. Oral health literacy scores were interpreted and classified in terms of percentile 75, with inadequate OHL (< percentile 75) and adequate OHL (≥ percentile 75).[28, 29]

The third section of the questionnaire was the oral health behaviors questionnaire, which was modified from the 8th Thailand Oral Health Survey 2017 to be suitable for the context of older adult people living in Thailand.[30] The questionnaire focused on oral

hygiene habits, nutritional preferences, and utilization of dental services.[7,9] The questionnaire comprised 13 items, and older adult participants could choose only one answer for activities they did during the previous week. The questionnaire was characterized by the scale levels: always practiced (5-7 times/week), sometimes practiced (2-4 times/week), and never practiced. The following classification was applied to the scoring criteria: The scores of 32-39 indicated good oral health behaviors, while the scores of 13-31 indicated low oral health behaviors that required improvement.

In a pilot study, the overall questionnaires were tested on 30 older adult people from the Eastern (Health Region 6) Thailand who had similar characteristics. This study conducted a reliability test on sections 2-3 of the questionnaires, achieving Cronbach's α coefficients of 0.83 and 0.86, respectively. Three experts in oral health behavioral research qualified the questionnaire to confirm its validity and comprehensiveness, with the item-objective congruence index (IOC) showing content validity above 0.5.

Statistical Analysis

Statistical analysis was performed using the Jamovi version 2.3.28 program software package. This study used percentage and mean (SD) in descriptive statistics, and analyzed with multiple logistic regression using the enter method to identify the independent variables associated with oral health behaviors. The initial univariate analyses led this study to include variables with a *P* value of less than .25 in the multivariate analysis. As a result, the final model was considered significant with a *P* value less than .05.

Results

Demographic Data and Clinical Characteristics

A total of 385 older adults participated, the mean (SD) age of participants was 64.78 (3.03) years, and over half of them were female (72.7%). Most participants (71.4%) were uneducated or obtained only primary school education, and 65.7% were married. Just over a third (46.0%) of participants had self-reported sufficient income. For health behaviors, 14.3% smoked cigarettes, 8.8% used betel nuts, and approximately one fifth of participants drank alcohol. The majority (91.7%) had received oral health information (Table1).

Table 1. General Characteristics of Participants

Characteristic	No. (%)
Gender	
Female	280 (72.7)
Male	105 (27.3)
Age, y	
60-64	169 (43.9)
65-69	216 (56.1)
Marital status	
Single	50 (13.0)
Married	253 (65.7)
Separated/divorced/windowed	82 (21.3)

Table 1. General Characteristics of Participants (Continued)	
Characteristic	**No. (%)**
Education level	
Uneducated and primary school	275 (71.4)
Secondary school	65 (16.9)
Bachelor's degree and higher	45 (11.7)
Occupation	
Agriculturists	97 (25.2)
Personal business	163 (42.3)
Unemployed/retired	125 (32.5)
Financial status	
Insufficient	103 (26.7)
Sufficient, but no savings	177 (46.0)
Sufficient and savings	105 (27.3)
History of chronic disease	
No	142 (36.9)
Yes	243 (63.1)
Smoking	
No	330 (85.7)
Yes	55 (14.3)
Alcohol drinking	
No	312 (81.0)
Yes	73 (19.0)
Betel nut use	
No	351 (91.2)
Yes	34 (8.8)
Experience for the replacement of natural teeth	
Never	196 (50.9)
Ever	189 (49.1)
Receiving information about dental health	
No	32 (8.3)
Yes*	353 (91.7)
By village health volunteers	100 (28.3)
By family member/relatives	56 (15.9)
By friends	26 (7.4)
By health personnel	239 (67.7)
By online media	92 (26.1)

* Each participant could select more than 1 option.

The majority of participants (75.1%) had inadequate OHL and the mean (SD) score of OHL was 100.44 (19.04). In terms of OHB, the majority of older adults (67.8%) demonstrated OHB at low level and the mean (SD) score of OHB was 29.23 (4.49) (Table 2).

In multivariate analysis, the results from the final model showed that the significant variables associated with good oral health behaviors were education level, alcohol consumption, receiving oral health information, and 2 domains of oral health literacy (self-management skills and media literacy skills). Older people with a secondary school education level were 3.20 times more likely to have good oral health behaviors than those with uneducated and primary levels (OR [95% CI], 3.20 [1.62-6.45]; *P* = .001). The results also showed that older people who were alcohol drinkers were less likely to have good oral health behaviors than those in the reference group (OR [95% CI], 0.24 [0.07-0.70]; *P* = .013). Older people who had ever received oral health information were 2.53 times more likely to have good oral health behaviors than those who had never received oral health information before (OR [95% CI], 2.53 [1.13-6.19]; *P* = .030). In addition, older people with adequate self-management skills and media literacy skills were 2.45 (95% CI, 1.53-3.96; *P* < .001) and 1.73 (95% CI, 1.11-2.70; *P* = .015) times more likely to have good oral health behaviors than those in the reference group, respectively (Table3).

Table 2. Oral Health Literacy and Oral Health Behaviors of Older Adults

Variable	No. (%)
Oral health literacy	
Inadequate (score ≤ 112)	289 (75.1)
Adequate (score > 112)	96 (24.9)
Mean (SD) [range], score	100.11 (19.04) [33-135]
Cognitive skills	
Inadequate (score ≤ 8)	232 (60.3)
Adequate (score > 8)	153 (39.7)
Mean (SD) [range], score	7.98 (1.59) [1-10]
Accessing skills	
Inadequate (score ≤ 20)	256 (66.5)
Adequate (score > 20)	129 (33.5)
Mean (SD) [range], score	18.56 (4.38) [5-25]
Communication skills	
Inadequate (score ≤ 16)	288 (74.8)
Adequate (score > 16)	97 (25.2)
Mean (SD) [range], score	14.55 (3.75) [4-20]
Self-management skills	
Inadequate (score ≤ 25)	280 (72.7)
Adequate (score > 25)	105 (27.3)
Mean (SD) [range], score	22.94 (4.58) [6-30]

Table 2. Oral Health Literacy and Oral Health Behaviors of Older Adults (Continued)	
Variable	**No. (%)**
Media literacy skills	
Inadequate (score ≤ 23)	221 (57.4)
Adequate (score > 23)	164 (42.6)
Mean (SD) [range], score	20.85 (6.33) [6-30]
Decision-making skills	
Inadequate (score ≤ 17)	282 (73.2)
Adequate (score > 17)	103 (26.8)
Mean (SD) [range], score	15.56 (3.34) [4-20]
Oral health behaviors	
Low (score ≤ 31)	261 (67.8)
Good (score > 31)	124 (32.2)
Mean (SD) [range], score	29.23 (4.49) [15-39]

Table 3. Univariate and Multivariate Logistic Regression Analyses of Oral Health Behaviors of Participants

Variable	Good OHB, No. (%)	Univariate Analysis		Multivariate Analysis	
		Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Education level					
Primary school	73 (58.9)	1 (Reference)	NA	1 (Reference)	NA
Secondary school	25 (20.1)	3.78 (1.97-7.24)	< .001	3.20 (1.62-6.45)	.001
Bachelor's degree or higher	26 (21.0)	2.18 (1.01-4.74)	.047	1.73 (1.01-2.98)	.045
Alcohol drinking					
No	108 (87.1)	1 (Reference)	NA	1 (Reference)	NA
Yes	16 (12.9)	0.53 (0.29-0.96)	.039	0.24 (0.07-0.70)	.013
Receiving oral information					
No	3 (2.4)	1 (Reference)	NA	1 (Reference)	NA
Yes	121 (97.6)	5.04 (1.50-16.88)	.009	2.53 (1.13-6.19)	.030
Oral health behaviors					
Self-management skill					
Inadequate	68 (54.8)	1 (Reference)	NA	1 (Reference)	NA
Adequate	56 (45.2)	3.56 (2.22-5.70)	< .001	2.45 (1.53-3.96)	< .001
Media-literacy skill					
Inadequate	50 (40.3)	1 (Reference)	NA	1 (Reference)	NA
Adequate	74 (59.7)	2.81 (1.81-4.36)	< .001	1.73 (1.11-2.70)	.015

Abbreviations: NA, not applicable; OHB, oral health behaviors.

Discussion

A majority of the participants (67.8%) demonstrated OHB at low level, which included not flossing daily, performing oral self-examination, limiting sugar intake, and not getting regular checkups. However, they had good OHB, such as brushing their teeth with fluoride toothpaste, and brushing their teeth twice daily. Additionally, this study revealed the variables significantly associated with good oral health behaviors were education level, alcohol consumption, receiving oral health information, and 2 domains of oral health literacy (self-management skills and media literacy skills).

This study's findings about the OHB of participants were in agreement with a study conducted among older adults in Nakhon Pathom by Subbowon,[31] where only 18.4% of the older adults underwent annual check-ups or oral examinations. This might have been due to increased medical expenses, long wait times, and fear of COVID-19 infection, which could hinder regular checkups.

This study found a substantially significant association between oral health behavior level, demographic characteristics, and some domains of oral health literacy among older adults in Thailand.

In terms of demographic characteristics, the study found that education level, receiving oral health information, and drinking alcohol had statistically significant associations with oral health behaviors. This was similar to reports by Piyakhunakorn et al[32] and Khamrin et al[24] who have reported that higher education levels may lead to good oral health behaviors, but education alone is not a reliable predictor of these behaviors. Therefore, to encourage older adults to manage their oral health care effectively, oral health care professions should provide simple, understandable, and context-appropriate information, ensuring they can make informed decisions about their oral health.

A study found a significant relationship between oral health information and older adults' oral health behaviors. This agreed with a study conducted in older adults by Kongsri[33] in Khon Kaen. They found that social support influenced the older adults' attitude towards oral healthcare and self-management skills. Limited access to dental health information led to inappropriate behaviors and increased oral health problems. The increasing trend of older adult people using the internet, particularly on personal smartphones, can increase the potential to actively collect oral health information.[13, 34] Therefore, incorporating oral health information into social media and enhancing information search skills can foster and sustain healthy oral health habits in older adults.

This study found a significant correlation between alcohol consumption and oral health behaviors, leading to increased oral health issues.[35] Alcohol drinkers may experience psychological effects and personality changes, affecting the patient-dentist relationship, leading to reduced interest in dental care and noncompliance. Additionally, alcohol dependents may experience dry mouth at night and neglect both personal and professional oral health care, increasing their risk of caries.[31, 35] Therefore, obtaining an alcohol history is crucial for effective preventative measures.

In terms of OHL, the majority of the participants (75.1%) demonstrated OHL at an inadequate level. This was is consistent with the study by Khamrin et al[24] that investigated oral health literacy, self-efficacy, social support, and factors associated with the oral health behaviors of older adults in the rural areas of northern Thailand. That research found that the participants (85%) had OHL at inadequate level. However, this study differed from the result of Piyakhunakorn et al[32] that studied the associations

between OHL and OHB among community-dwelling older adults in Thailand. That study found just over half of the participants (51.3%) had OHL at high level.

This study examined 6 competencies and skills for promoting oral health literacy: cognitive skills, accessing skills, communication skills, self-management skills, media literacy skills, and decision-making skills. This study aimed to measure and identify the association between these domains of oral health literacy and oral health behaviors to improve oral health behaviors among older adults in Eastern Thailand. This study's analysis of factors revealed that self-management skills, media literacy skills, and 2 domains of oral health literacy, significantly correlated with oral health behaviors.

This study's finding about OHL of participants were in agreement with a study by Li et al,[36] that investigated adolescents' Chinese oral health self-management ability. That study found that the poor oral health self-management ability of adolescents was due to lack of awareness, lack of knowledge, and inability to solve oral problems. Self-management skills are crucial for managing symptoms, treatments, and oral health behaviors, enhancing dental care, and preventing problems. Furthermore, it is considered that adolescents usually take responsibility for personal care such as oral hygiene care, making decisions about eating meals during school hours and reporting abnormality or a symptom related to their teeth. It is evident that OHL appears to impact effective self-care oral behaviors in adolescents and older populations. Inadequate self-management skills among older adults can lead to decreased decision-making and increased oral health problems. Further, this study found that media literacy had statistically significant associations with oral health behaviors. This was similar to reports by Geraee et al[37] who investigated an adolescent Iranian media literacy training program. That study found media literacy training programs were effective on the participants' knowledge and behavioral intention. Therefore, encouraging older adult individuals to recognize the importance of self-management and media literacy skills can help develop good OHB, enhancing their ability to independently manage care. It is essential to enhance the promotion of appropriate OHB. Behavioral modification programs should be implemented to improve OHL, particularly in the areas of self-management and media literacy skills.

To the best of this study team' knowledge, this was the first study performed in the Health Region 6 assessing OHL and its association with the OHB. This study has provided essential data for organizing an improved OHL program to improve individuals' adoption of proper OHB. Additionally, the OHL questionnaire used in this study is an instrument that measures all 6 competencies and skills of individuals for promoting OHL and is suitable for the context of older adult people living in Thailand. However, there were limitations. Initially, the variable outcomes reported in this study are specific to oral health behaviors; so future research could concentrate on other outcomes, such as oral health status. The second limitation is this study used self-reported questionnaires, which are prone to bias due to participants forgetting some information.

Conclusions

This study has revealed that the older adults in Thailand have low levels of OHB. A statistically significant association was found between oral health behavior and 2 domains of OHL. Therefore, it is essential to enhance the promotion of appropriate OHB through behavioral modification programs aimed at improving OHL, particularly in media literacy and self-management skills.

Additional Information

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Ethics Approval: This study was conducted under the highest ethical standard as outlined by the Burapha University-Institutional Review Board for Protection of Human Subject in Research (BUU-IRB), Thailand (IRB3-021/2565 on 21 March 2022). The participants' identifying information remained confidential throughout.

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RMJ

Original Article

Development of Enzyme-Linked Immunosorbent Assay and Lateral Flow Strip Assay for *Trimeresurus albolabris* Venom Detection

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Abstract

Background: Snakebite symptoms (eg, neurological signs, local swelling, nonclotting blood) can overlap among different snake types. Accurate venom identification is crucial for selecting the appropriate antivenom against hemotoxic, neurotoxic, or cytotoxic effects. In Thailand, the common snakes *Daboia siamensis, Calloselasma rhodostoma*, and *Trimeresurus albolabris* possess hemotoxic venoms, which can cause symptoms such as pain, swelling, bruising, and bleeding. Although enzyme-linked immunosorbent assay (ELISA) is widely employed for snake venom detection due to its high sensitivity, it is time-consuming. It requires a well-equipped laboratory and specialized skills, whereas the later flow strip assay (LFA) is easy to use and significantly reduces the time required; however, it is typically used for qualitative detection. However, both ELISA and LFA are valuable for snakebite diagnosis. Enhancing the sensitivity, accuracy, and reliability of these assays, particularly for low-abundance targets, remains a critical objective.

Objectives: To develop sandwich ELISA and LFA for detecting *T. albolabris* venom and to enhance the specificity of horse immunoglobulin G (HIgG) against *T. albolabris* venom for use in ELISA and LFA, thereby reducing the likelihood of cross-reactivity in detection.

Methods: Specific HIgG against *T. albolabris* venom was purified using an affinity column. The cross-reactivity of snake venoms was demonstrated through Western blotting. Snake venom detection was quantified by ELISA and visually assessed using LFA.

Results: The sandwich ELISA assay for *T. albolabris* venom detection yielded a coefficient of determination greater than 0.99, a limit of detection at 11.37 ng/mL, and a limit of quantification at 34.45 ng/mL, without any cross-reaction with the venom of *C. rhodostoma* and *D. siamensis*. The LFA can detect *T. albolabris* venom at 25 ng/mL, showing no cross-reaction and no positive test in the test line for either *C. rhodostoma* or *D. siamensis* venom.

Conclusions: The developed sandwich ELISA assay and the LFA could distinguish *T. albolabris* venom from *C. rhodostoma* and *D. siamensis* venom.

Keywords: *Trimeresurus albolabris*, Lateral flow strip assay, ELISA, Snake venoms

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Introduction

Each year, venomous snakebites result in the deaths of approximately 81 410 to 137 880 people worldwide.[1] Snakebites remain a significant public health concern in many tropical and subtropical countries. Southeast Asia is one of the regions most affected by

numerous venomous snakes.[2] In 2007, approximately 700-18 000 people died of venomous snakes in 8 countries of Southeast Asia, including Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Thailand, and Vietnam.[3] Thailand is located in the tropics, with lowlands, forests, and mountains, which makes it a place where snakes are abundant.

The effects of snake venoms are broadly classified into hemotoxic, neurotoxic, or myotoxic categories. Snake venoms can cause symptoms such as neurological signs, nonclotting blood/spontaneous systemic bleeding, local swelling, and tissue damage. Sometimes, it leads to permanent disability and limb amputation.[1] Identifying snake species is crucial for clinicians to select an appropriate antivenom and treatment.[5] A doctor will diagnose a patient bitten by an unknown venomous snake based on the expression of the patient's symptoms and laboratory tests.[4] *Daboia siamensis, Calloselasma rhodostoma, and Trimeresurus albolabris* are commonly found in Thailand, and their venoms affect the blood system, classified as hemotoxic venoms. Pain, swelling, blistering, bruising, nausea, vomiting, and bleeding are common symptoms following a bite from these snakes. Due to their overlapping symptoms, it may cause diagnostic confusion; however, a *D. siamensis* bite can be confirmed by testing Factor V and X levels. Besides, a bite by *T. albolabris*, known as the White-lipped Pit Viper, which is found throughout Thailand, might be mistakenly considered a cobra, *Naja kaouthia*, bite because of their similar swelling and inflammation around the wound,[6-8] in the case that neurological symptoms have not appeared. Therefore, it is beneficial to test for snake type confirmation.

Immunological tests for detecting snake venom have been identified as important clinical applications. Snake venom detection by enzyme-linked immunosorbent assay (ELISA) is currently in use worldwide. It provides specificity and sensitivity to detect and identify differentiated envenomation even with small venom quantities. Although the ELISA reagent is inexpensive and stable, it is time-consuming and requires a well-equipped laboratory and skills.[9] The same or similar proteins in snake venoms were found among the closely related snake species, referred to as cross-reactions, in the ELISA tests for snake venom.[10] The indirect ELISA test used to diagnose snakebites for *Bungarus multicinctus* and *Naja atra* (neurotoxic snake venoms) demonstrated strong cross-reactivity with the venoms of *Trimeresurus stejnegeri* and *Protobothrops mucrosquamatus*, which are hemotoxic snake venoms. Conversely, ELISA tests for *T. stejnegeri* and *P. mucrosquamatus* showed slight cross-reactivity with the venoms of *B. multicinctus* and *N. atra*.[11] Cross-reactivity tests with ingroup and outgroup samples are crucial for validating the specificity of ELISA. It is essential to ensure that antibodies target the correct antigen, thereby minimizing false positives and enhancing the test's reliability. In case of *T. albolabris* venom detection, *N. kaouthia*, a neurotoxic snake venom with distinct toxin profiles and mechanisms compared to hemotoxic snake venom, can serve as an outgroup sample, whereas *C. rhodostoma* and *D. siamensis* venoms are considered to be ingroup samples. These snake venoms can be utilized to evaluate whether the assay can detect *T. albolabris* venom without cross-reactivity, thereby enhancing the test's accuracy and reliability. To reduce or eliminate cross-reactivity and improve the discrimination capability of ELISA for the accurate identification of snake species, protein purification using immobilized venom proteins that cross-react with the relevant antibodies through affinity columns may be helpful.[12] Although the ELISA test has several advantages and is used for various applications, the later flow strip assay (LFA) is easier to use as a point-of-care test. Indeed, LFA requires minimal personal training to interpret results, with lower infrastructure needs in healthcare and the ability to provide quick outcomes for immediate decision-making, compared to ELISA.

Unfortunately, the complexities of snake venom have resulted in fewer efforts in Thailand to develop sensitive assays for its detection. Therefore, this study aimed to develop a sandwich ELISA and LFA for detecting *T. albolabris* venom. Moreover, affinity column chromatography was employed to enhance the specificity of HIgG against *T. albolabris* venom for use in ELISA and LFA, thereby reducing the likelihood of cross-reactivity in detection.

Methods

Snake Venoms, Hyperimmune Horse Plasma, and Normal Human Serum

The freeze-dried powder of *D. siamensis*, *C. rhodostoma*, *T. albolabris*, and *N. kaouthia* venom was obtained from the Queen Saovabha Memorial Institute (QSMI) Snake Farm, Thai Red Cross Society, Bangkok, Thailand (stored at 4 °C). Hyperimmune horse plasma against *T. albolabris* venom obtained from the horse farm, QSMI, Thai Red Cross Society, Prachuap Khiri Khan, Thailand. The plasma was stored at -20 °C before use. Pooled normal human serum was purchased from Sigma, USA (collected from healthy human donors) and stored at -20 °C before use.

Monovalent Antivenom for *Trimeresurus albolabris* Venom (Green Pit Viper Antivenom)

Monovalent antivenom against *T. albolabris* venom was obtained from QSMI. It was prepared from equine serum. Each 1 mL contains specific immunoglobulin that can neutralize 0.7 mg of Green Pit Viper venom.

Horse Immunoglobulin G (HIgG)

To obtain HIgG, 50 mL of hyperimmune horse plasma against *T. albolabris* venom was precipitated with 35% ammonium sulfate at 4 °C for 30 minutes. Then, it was centrifuged at 5000 rpm for 30 minutes and dialyzed overnight against 2 liters of binding buffer (10 mM Tris-HCl, pH 7.5) at 4 °C.

Preparation of an Affinity Column for Purifying HIgG Against *Trimeresurus albolabris* Venom

CNBr-activated Sepharose 4B was used to purify the specific antibody, as it enables the highly selective and efficient isolation of the target antibody from complex mixtures, such as serum or horse plasma, by covalently binding it to the Sepharose beads. As such, 1 g of CNBr-activated Sepharose 4B (per venom sample) (Cytiva, USA) medium was weighed and washed with 200 mL of 1.0 mM HCl (pH 3.0) using a sintered glass filter (porosity G3) and then washed with coupling buffer (0.1 M $NaHCO_3$, pH 8.3). Each of *D. siamensis*, *C. rhodostoma*, and *T. albolabris* venom (freeze-dried powder) was weighed at 10 mg separately and dissolved in 5 mL of coupling buffer. Each dissolved venom was added to swollen Sepharose 4B medium and incubated overnight at 4 °C. Then, each venom medium was washed several times with a coupling buffer on a sintered glass filter. Blocking any remaining active groups, each venom medium was transferred to 0.1 M Tris-HCl buffer, pH 8.0, and left to stand for 2 hours at room temperature. Washing the medium with at least 3 cycles of alternating pH by at least 5 medium volumes of each buffer on a sintered glass filter. Each cycle consisted of a wash with 20 mL of 0.1 M acetic acid/sodium acetate, pH 4.0, containing 0.5 M NaCl, followed by a wash with 20 mL of 0.1 M Tris-HCl, pH 8, containing 0.5 M NaCl. Then, each medium venom was packed into a column and equilibrated with a binding buffer (10 mM Tris-HCl, pH 7.5).

Purification of Specific HIgG Against *Trimeresurus albolabris* Venom

The 10-mg HIgG in 10 mM Tris-HCl at pH 7.5 was administered into the *C. rhodostoma* venom Sepharose 4B column, as the first column, that had been equilibrated with binding buffer and washed with binding buffer (2.5 column volumes [CV]). Then, the column was eluted with a stepwise gradient of 0.1 M glycine, pH 2.7, from 0% to 100% (4 CV). Eluted fractions were pooled and dialyzed overnight with 2 liters of binding buffer. HIgG obtained from the first column was concentrated and passed through the second column, the *D. siamensis* venom Sepharose 4B column, followed by the third, *T. albolabris* venom Sepharose 4B column. The purification method steps in the second and third columns were identical to those in the first. The specific HIgG for *T. albolabris* venom was dialyzed in phosphate-buffered saline (PBS), pH 7.3. Before use, the specific HIgG for *T. albolabris* venom was stored at -20 °C. The AKTA pure (GE, USA) system performed all purification steps at a flow rate of 0.5 mL/min. The purified specific HIgG to *T. albolabris* venom was displayed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Western Blot Analysis Between Antivenom (QSMI) and HIgG to *Trimeresurus albolabris* Venom

Accordingly, 20 µg of each snake venom (*D. siamensis*, *C. rhodostoma*, and *T. albolabris*) was processed using 12% SDS-PAGE. After electrophoresis, proteins were transferred from the gel onto polyvinylidene difluoride (PVDF) blotting membranes (GE, USA) using electroblotting. The membrane was blocked from nonspecific binding sites by incubating it with a blocking buffer (3% BSA) for 1 hour. Then, the monovalent antivenom (QSMI) or the specific HIgG at the final concentration of 0.03 mg/mL in 3% BSA buffer was added to the membrane and incubated overnight at 4 °C. The membrane was washed several times with Tris-buffered saline containing 0.1% Tween 20 (TBST) to remove unbound antibody. The anti-horse IgG conjugated to HRP (Sigma, USA) was diluted 1:1000 in 3% BSA buffer and added, followed by incubation for 1 hour at room temperature. The membrane was washed with TBST, and the substrate solution, consisting of 4-chloro-1-naphthol (Sigma, USA) and hydrogen peroxide (H_2O_2), was applied to the blotting membrane to develop the target bands.

Conjugation of HIgG Against *Trimeresurus albolabris* Venom With Horseradish Peroxidase (HRP)

The processing was followed by the HOOK™ HRP PLUS labeling kit (G-Biosciences, USA). Briefly, 1 mg/ml of HIgG against *T. albolabris* venom was coupled with HRP in HOOK™ HRP PLUS vial using the conjugation reagent given by the kit. Then, 5 M sodium cyanoborohydride was added to the vial and incubated at room temperature for 15 minutes. After that, the quenching buffer was added to the vial, and the mixture was incubated with shaking for 15 minutes. The HIgG solution from the previous step was entered into the SpinOUT™ GT-600 column to remove sodium cyanoborohydride. To collect the conjugate of HIgG against *T. albolabris* venom, the column was eluted with PBS, pH 7.3. The HRP-conjugated HIgG against *T. albolabris* venom was aliquoted and stored at -20 °C before use.

Sandwich ELISA Assays for *Trimeresurus albolabris* Venom Detection

The specific HIgG for *T. albolabris* venom detection (2 µg/mL, 50 µL) was diluted in coating buffer (0.1 M sodium carbonate, pH 9.5) and placed on 96-well polystyrene microplates (Costar™ 96-Well, USA). The plates were then incubated for 3 hours at 37 °C.

After that, the wells were washed 3 times with PBS, pH 7.3, containing 0.05% Tween 20 (PBST), using a microplate washer (Hydro Flex, Tecan, USA). The wells were then blocked with 1% BSA in PBS for 30 minutes at 37 °C. Then, the wells were rewashed. To establish a standard concentration curve, 2-fold serial dilutions of *T. albolabris* venom in human serum, ranging from 7.8 to 500 ng/mL, were prepared in 50 µL volumes and added to the wells. After incubation at room temperature for 30 minutes, the plate was washed and supplemented with 50 µL of HRP-HIgG to *T. albolabris* venom (1:5000 in PBS, pH 7.3), followed by incubation at 37 °C for 30 minutes. Next, the plate was washed, and the substrate of peroxidase, O-phenylenediamine (OPD), and 35% H_2O_2 (100 µL) were added. The mixture was incubated in the dark at room temperature for 30 minutes. Finally, 50 µL of 3 M H_2SO_4, serving as a stopping reagent, was added to the wells to terminate the OPD reaction. The specific bound complex was detected by optical density (OD) measurement at 492 nm, with a reference at 620 nm, using a microplate reader (Sunrise, Tecan, USA). The determination of the limit of detection (LOD) was calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels approximating the LOD, as follows: LOD = 3.3 × (Sy/S). The limit of quantification (LOQ) was calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula: LOQ = 10 × (Sy/S), where the standard deviation of the response was determined based on the standard deviation of the y-intercepts of regression lines. The spiking experiment assessed known concentrations of *T. albolabris* venom at 31.25, 62.5, 125, and 250 ng/mL to determine whether variations in the diluent of the standard curve influenced analyte detection. To investigate the cross-reaction of the specific HIgG against *T. albolabris* venom, not only *D. siamensis* and *C. rhodostoma*, which served as ingroup samples, but also *N. kaouthia*, which served as an outgroup sample, was utilized at high concentrations of 250, 500, and 1000 ng/mL.

Colloidal Gold-Labeled HIgG to *Trimeresurus albolabris* Venom Preparation

The 40 nm gold nanoparticle solution (OD = 1) from Serve Science Company, Bangkok, Thailand, was adjusted to a pH of 8.0 with 0.2 M sodium carbonate. The activated gold solution was mixed in a 1:1 (v/v) ratio with the HIgG to *T. albolabris* venom at a concentration of 2 mg/mL in PBS, pH 7.3. The reaction was incubated for 10 minutes at room temperature with gentle shaking or rotation to promote the binding of the antibody to the gold particles. After conjugation, 0.5% BSA was added to block any unreacted sites on the nanoparticle surface, and the mixture was incubated for an additional 15 minutes to prevent nonspecific binding. Subsequently, the gold solution was centrifuged at 10 000 rpm and 4 °C for 30 minutes, and the gold pellets were collected. The gold pellets were then suspended in PBST containing 1% BSA, and this step was repeated once. Finally, the gold-labeled HIgG pellets were suspended in 1 mL of storage buffer (20 mM sodium carbonate, pH 8.0, 2% sucrose, 5% trehalose, 0.1% sodium azide) and stored at 4 °C until use. The method was modified based on Kumar et al.[12]

Lateral Flow Strip for *Trimeresurus albolabris* Venom Detection

The strips consisted of nitrocellulose membranes (Unisart CN140), sample pads (Ahstrom 8964), conjugate pads (GF33 Glass Fiber), and absorbent pads (Ahlstrom 222). Before assembly, the conjugate pads were saturated with 10 OD of HIgG against *T. albolabris* venom-conjugated colloidal gold, sprayed (10 µL/cm) using a sprayer machine

(XYZ3060, BioDot, USA), and allowed them to dry for an hour at 37 °C. In the test line, 1 mg/mL of HIgG against *T. albolabris* venom in PBS, pH 7.3, was sprayed (1 µL/cm) on nitrocellulose membranes. For the control line, 1 mg/mL of an anti-horse IgG whole molecule (Sigma, USA) in PBS, pH 7.3, was also sprayed (1 µL/cm). The membrane was dried at 37 °C for 1 hour before assembly. The nitrocellulose membranes, conjugated pads, sample pads, and absorbent pads were pasted onto the cardboard, with each pad overlapping the adjacent ones. A cutter machine (CM5000, BioDot, USA) cut the assembled strips into pieces measuring 0.4 x 6.0 cm each. Sensitivity and specificity of the lateral flow strips were tested. The spiked *T. albolabris* venom in pooled human serum, at concentrations of 12.5, 25, 50, and 100 ng/mL, was applied to the sample pads (n = 5) in a volume of 60 µL. The results were demonstrated within 15 minutes at room temperature. The lateral flow strip for *T. albolabris* venom detection was also examined for reaction with the venom of *D. siamensis*, *C. rhodostoma*, as ingroup samples, and *N. kaouthia* venom as an outgroup sample (n = 5). The intensity of the test line and control line was measured using a RapidScan ST5 (USA) without a measurement unit.

Protein Concentration

In all experiments, the Qubit Protein kit (Thermo Fisher Scientific, USA) was used for measuring protein concentration.

Statistical Analysis

Data were presented as mean (SE) and analyzed using one-way analysis of variance, followed by Bonferroni's multiple comparisons tests with PRIMER of Biostatistics software, version 6.0. Differences were considered significant at $P < .05$.

Results

Western Blot Analysis Between Antivenom (QSMI) and HIgG to *Trimeresurus albolabris* Venom

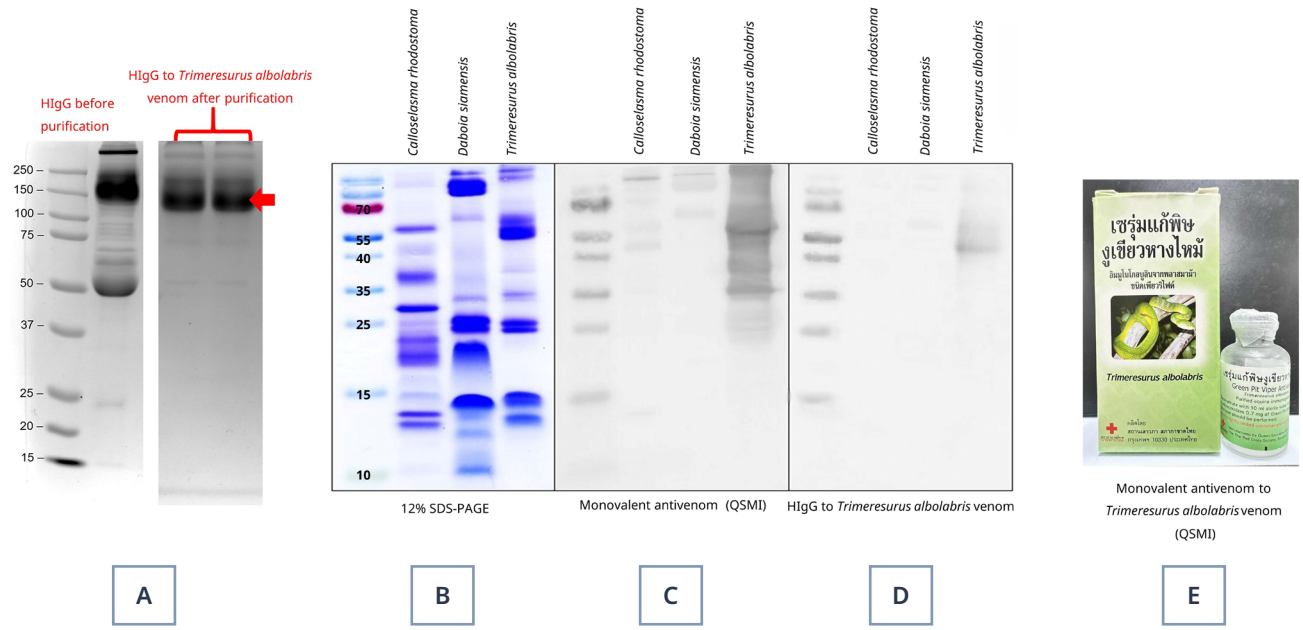
Specificity evaluation of the purified HIgG to *T. albolabris* venom was determined by Western blot analysis compared to monovalent antivenom, and the cross-reaction among hemotoxic snake venoms was considered, as well. As such, HIgG against *T. albolabris* venom was more specific to *T. albolabris* venom (Figure 1D) than monovalent antivenom (QSMI), which showed a high cross-reaction in both *C. rhodostoma* and *D. siamensis* venom (Figure 1C). However, the result of purified HIgG against *T. albolabris* venom showed slightly protein bands of *D. siamensis* venom at high molecular weights above 55 kDa. In contrast, the *C. rhodostoma* venom lane was nearly invisible (Figure 1D). The Western blot analysis revealed that the purified specific HIgG against *T. albolabris* venom exhibited less cross-reaction with *C. rhodostoma* and *D. siamensis* venom than the monovalent antivenom (QSMI).

Sandwich ELISA Assays for *Trimeresurus albolabris* Venom Detection

The conjugated HRP-HIgG against *T. albolabris* venom (a capture antibody) and OPD with 35% H_2O_2 (a detection antibody) were used to determine the sensitivity and specificity of the ELISA assay. Parallelly, pooled human serum was serially diluted to test for the presence of *T. albolabris* venom, cross-reactivity to *C. rhodostoma* and *D. siamensis* venom, and to generate a standard curve. For *T. albolabris* venom detection, LOD was 11.37 ng/mL, and LOQ was 34.45 ng/mL, with a coefficient (R^2) value greater than 0.99 (Figure 2A), with

the potential to identify and quantify *T. albolabris* venom in pooled human serum. Notably, the LOQ was passed for the standard criteria (identification, precision, and trueness), supporting the relative accuracy and precision of the assay.[13] The use of *T. albolabris* venoms (31.25, 62.5, 125, and 250 ng/mL) in a spike experiment to find the percentage recovery, recovery (%) = (observed concentration at dilution/predicted spike concentration after dilution) × 100, was performed. An acceptable recovery range of 80% to 120% signifies the absence of any matrix effect,[14] whereas a recovery percentage outside this range suggests potential interference from sample components. All concentrations of *T. albolabris* venoms were in the acceptable range of the % recovery (Figure 2B). Meanwhile, the HIgG against *T. albolabris* venom showed no cross-reactivity with the venoms of *C. rhodostoma* and *D. siamensis* at concentrations ranging from 0 to 1000 ng/mL. However, slight cross-reactivity was observed at higher concentrations of *N. kaouthia* venom at 500 and 1000 ng/mL, but not at the lower concentration of 250 ng/mL (Figure 2C).

Figure 1. Purification of HIgG Against *Trimeresurus albolabris* Involved Western Blot Analysis Using Hemotoxic Snake Venoms, Alongside Monovalent Antivenom (QSMI) and HIgG Specific to *Trimeresurus albolabris* Venom



Abbreviations: HIgG, horse Immunoglobulin G; QSMI, Queen Saovabha Memorial Institute; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

A, The crude HIgG (20 µg per well) was analyzed before and after the purification of HIgG against *T. albolabris* venom, with duplicate samples (10 µg per well each) run on a 10% SDS-PAGE under nonreducing conditions.

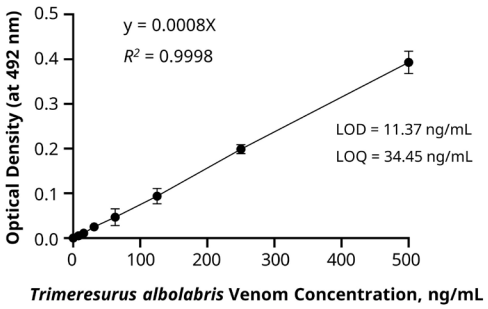
B, The Western blot analysis of crude snake venoms on 12% SDS-PAGE.

C and D, The Western blot analysis along with monovalent antivenom (QSMI) and HIgG against *T. albolabris* venom was performed using hemotoxic snake venoms (20 µg per well for each), *C. rhodostoma*, *D. siamensis*, and *T. albolabris* venoms.

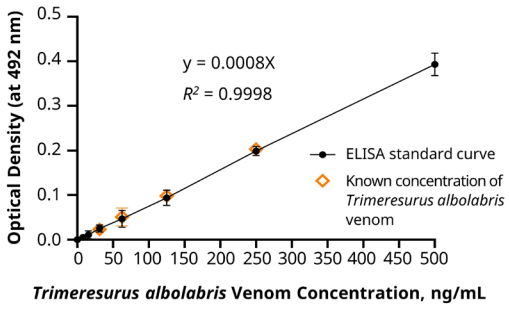
E, The commercially available green pit viper antivenom product from QSMI was displayed.

Figure 2. Development of Sandwich ELISA Assay to Detect *Trimeresurus albolabris* Venom, Including Spiking Tests With Pooled Human Serum and an Assessment of the Cross-Reactivity of HIgG With *Trimeresurus albolabris* Venom

A

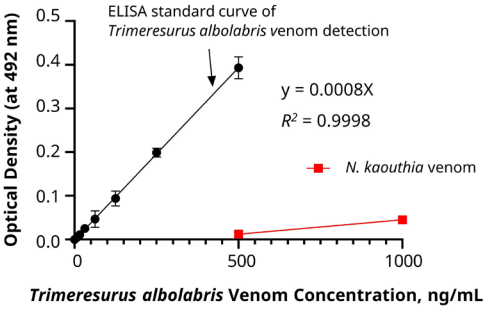


B



Concentration of *Trimeresurus albolabris* Venom on the Standard Curve, ng/mL	Spiking test of *Trimeresurus albolabris* Venom, Mean (SE), ng/mL (n = 3)	% Recovery
250	253.33 (0.011)	98.82
125	122.50 (0.012)	98.00
62.5	63.33 (0.020)	101.33
31.25	29.17 (0.005)	93.34

C



Concentration, ng/mL	Cross-Reactivity to HIgG *Trimeresurus albolabris* Antibody, Mean (SE), ng/mL (n = 3)		
	Calloselasma rhodostoma Venom	*Daboia siamensis* Venom	*Naja kaouthia* Venom
250	Under the cut-off value	Under the cut-off value	Under the cut-off value
500	Under the cut-off value	Under the cut-off value	15.00 (0.006)
1000	Under the cut-off value	Under the cut-off value	56.67 (0.010)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HIgG, horse Immunoglobulin G; LOD, limit of detection; LOQ, limit of quantification.

A, The standard curve of ELISA for *T. albolabris* venom.

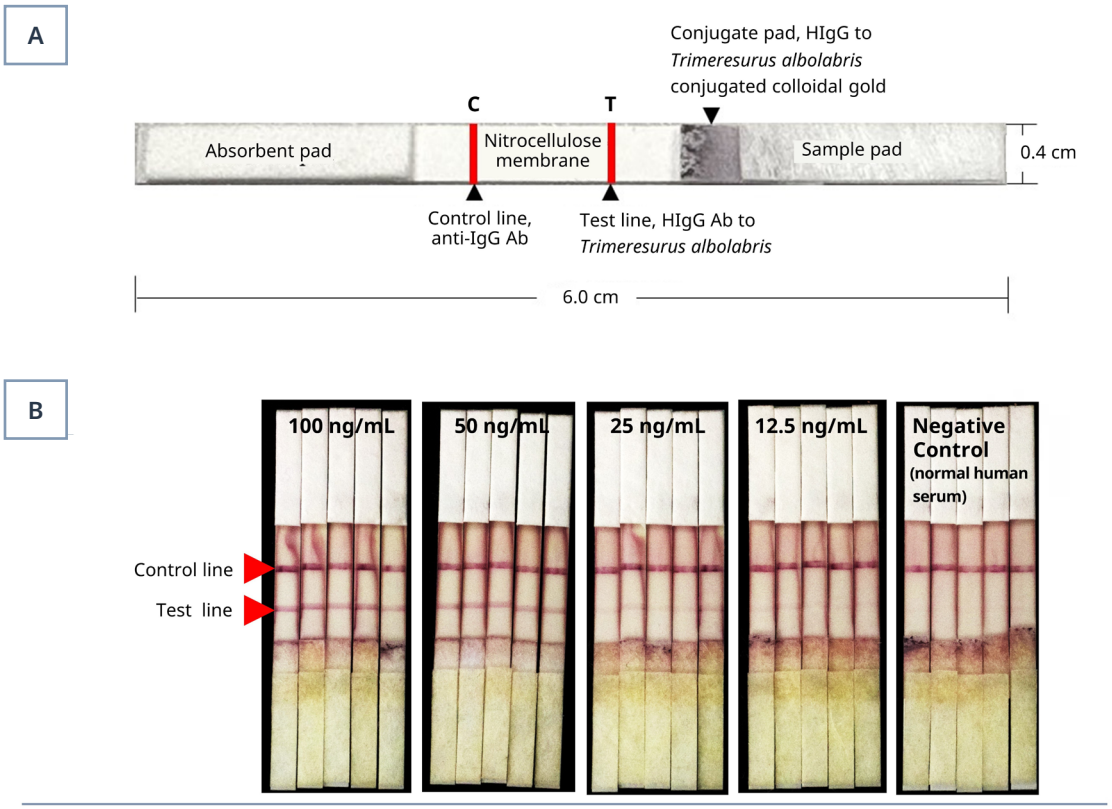
B, The *T. albolabris* venom spiking test using pooled human serum.

C, The cross-reactivity of the HIgG against *T. albolabris* venom with related snakes.

Lateral Flow Strip Test for *Trimeresurus albolabris* Venom Detection

A lateral flow strip for *T. albolabris* venom detection, which was composed of a sample pad, conjugated colloidal gold pad, absorbent pad, and nitrocellulose membrane with HIgG against *T. albolabris* venom (test line; T), and anti-IgG antibody (control line; C), was produced (Figure 3A). Sensitivity and specificity tests using various concentrations of *T. albolabris* venom in pooled human serum, along with a visual inspection of the positive test line, were clear at concentrations above 25 ng/mL but ambiguous at 12.5 ng/mL (Figure 3B). The pooled normal human serum (undiluted), serving as a negative control test, showed a low-intensity line (0-75) (Table in Figure 3B). The test line intensity for venom detection correlated well with the venom concentrations (Table in Figure 3B). To evaluate cross-reactivity, lateral flow strip tests were used to test the venom of *D. siamensis* and *C. rhodostoma*, which had hemotoxic venoms (similar to *T. albolabris*), and *N. kaouthia* (outgroup), a neurotoxic snake.

Figure 3. Development of Lateral Flow Strip Tests to Detect *Trimeresurus albolabris* Venom



Venom of *Trimeresurus albolabris*	100 ng/mL	50 ng/mL	25 ng/mL	12.5 ng/mL	Negative Control
Test line intensity, range	1544-2291	1375-1546	462-917	317-482	0-75

A, The design of a lateral flow strip for detecting *T. albolabris* venom.

B, The results of detecting *T. albolabris* venom on the strips using various venom concentrations.

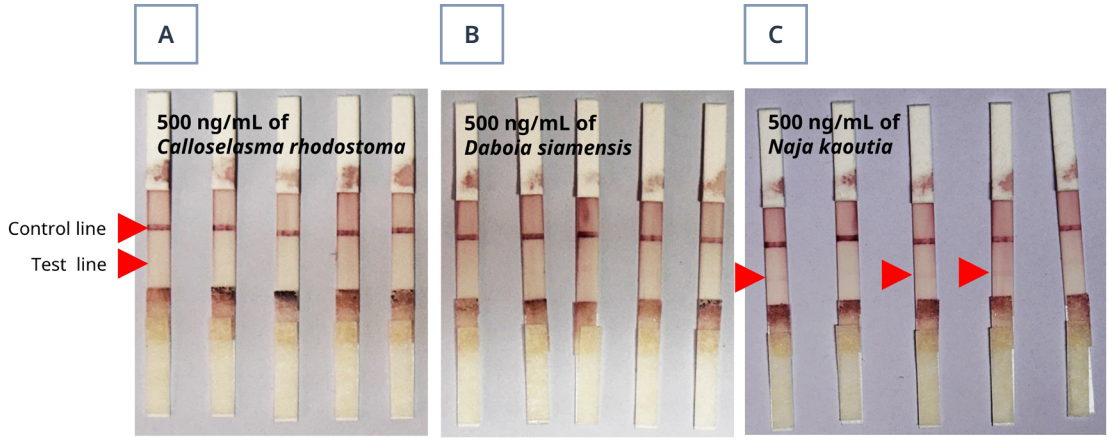
The lateral flow strip test on *C. rhodostoma* and *D. siamensis* venom yielded negative results in the test lines at concentrations of 500 ng/mL (Figure 4A and 4B), suggesting that the developed strip assay did not exhibit sufficient cross-reactivity to produce unclear results at high-dose concentrations. At a high concentration of 500 ng/mL, slight cross-reactivity was observed on the test line for *N. kaouthia* venom (Figure 4C).

When *C. rhodostoma* and *D. siamensis* venoms on the test line intensity were measured, it was found that *C. rhodostoma* venom gave intensity range values at 0-34 (Figure 4A), while *D. siamensis* venom scores were 0-87 (visually negative) (Figure 4B). At 500 ng/mL, we observed some cross-reactivity on the test line for *N. kaouthia* venom, resulting in an intensity range of 176-275 (indicated by the red arrow in Figure 4C). The visually detectable intensity surpassed 100.

Discussion

ELISA, a valuable tool for diagnosing snake envenomation by detecting the presence of specific snake venoms in blood, relies on antibodies that specifically react with venom proteins and hospital laboratory machines. Meanwhile, lateral flow strips make it easier to identify envenomation without requiring sophisticated equipment and less trained personnel for field use.

Figure 4. Evaluation of Cross-Reactivity Using Lateral Flow Strip Tests With Hemotoxic Snake Venoms



Venom (500 ng/mL)	*Calloselasma rhodostoma*	*Daboia siamensis*	*Naja kaoutia*
Test line intensity, range	0-34	0-87	176-275

A and B, The cross-reactivity detection on the lateral flow strip tests using hemotoxic snake venoms (*C. rhodostoma* and *D. siamensis*).

C, Neurotoxic snake venom (*N. kaouthia*) at 500 ng/mL.

T. albolabris bites cause local and systemic envenomation, producing painful and similar local symptoms to those of *C. rhodostoma*, *D. siamensis*, and *N. kaouthia*, including local swelling (the dominant symptom), hemorrhage formation, local damage and necrosis, which can be challenging to distinguish from those of these snake species. Although *C. rhodostoma* often causes multiple haemorrhage blebs, *D. siamensis* frequently forms blisters around the wound with necrosis, and *N. naja* is neurotoxic,[6-8] the more rapid snake identification provides a better treatment outcome. Indeed, common antigens in venom are structurally or functionally similar across multiple snake species, often leading to some degree of cross-reactivity between the venoms. Minimizing venom cross-reactivity in ELISA assays is challenging, especially for those within the same family.

To isolate the specific HIgG that recognized *T. alborabris* venom and reduced cross-reactivity, crude HIgG was purified using an affinity column bound separately with *C. rhodostoma* and *D. siamensis* venom. After purification, the specific HIgG for *T. alborabris* venom primarily comprised proteins with an approximate molecular weight of 150 kDa, with only a minimal presence of proteins of lower molecular weights (Figure 1A). Indeed, the cross-reactivity of a single-valent *T. albolabris* antivenom (QSMI) toward *C. rhodostoma* and *D. siamensis* venoms was demonstrated through Western blot analysis (Figure 1B), especially in high molecular weight fractions. With affinity purification using *C. rhodostoma* and *D. siamensis* medium columns, a better specificity of anti-*T. albolabris* HIgG was shown by Western blot (Figure 1C). Although the cross-reactivity limits the use of antibodies for snake identification, it was beneficial for neutralizing venoms from several types of snakes. For example, anti-VIPMYN (Fab2H) fraction from *Crotalus durissus* and *Bothrops asper* (North American snakes) was effective in neutralizing the hemorrhagic activity of 8 venoms (*Agkistrodon piscivorus piscivorus*, *B. asper*, *Crotalus adamanteus*, *C. durissus durissus*, *C. horridus atricaudatus*, *C. h. horridus*, *C. atrox*, and *C. molossus molossus*).[15] However, cross-reactivity may not cover all fractions of the venoms, such as anti-FabO from *C. atrox*, *C. adamanteus*, *C. scutulatus scutulatus*, and *A. p. piscivorus* neutralized only the gelatinase activity of *A. p. piscivorus*, *C. d. durissus*, and *C. m. molossus* venoms.[16]

With a more specific antibody against *T. albolabris* venom, the LOD from the ELISA standard curve met the criteria for identification, precision, and accuracy above the background noise.[13] The primary purpose of LOD is to confirm the presence of a presenting analyte without precisely measuring its concentration. The LOQ, at 34.45 ng/mL, indicated that the ELISA was assayed with relative accuracy and precision at 34.45 ng/mL (Figure 2A) and could be confidently reported as a reliable value. Knowing the quantifiable range from LOQ can help guide sample preparation decisions to fall within the assay's accurate range, improving result reliability. In ELISA, measuring the LOQ sets the lowest point of accurate quantification, which is essential in sensitive research and diagnostic settings. Due to HRP enzyme labelling in this study, which gave LOQ of 34.45 ng/mL, the biotin-streptavidin might enhance sensitivity (reduced LOQ value) as indicated in the ELISA against neurotoxic (*B. multicinctus* and *N. atra*) and hemotoxic venoms (*T. stejnegeri* and *P. mucrosquamatus*) at the LOQ of 0.39 and 0.78 ng/mL, respectively.[11] The higher sensitivity of the biotin-streptavidin system over HRP-based direct conjugates is partly due to the multivalent binding ability.[17, 18] Each biotinylated antibody can bind multiple streptavidin molecules, leading to multivalent signal amplification and enabling more enzyme molecules per target site. In contrast, HRP is usually directly conjugated to the primary or secondary antibody, meaning each antibody carries only one or a limited number of HRP molecules. This single-layered approach limits the total signal that can be produced. However,

HRP is known for its simplicity, cost-effectiveness, and effectiveness in detection. It is also widely used for standard assays with acceptable sensitivity for many substrates. Therefore, the sensitivity of the developed ELISA may be enhanced by biotin-streptavidin conjugation.

Additionally, the recovery percentage (% recovery) is used to evaluate the accuracy of ELISA by relying on the known concentration of the analyte that has been spiked into the sample. Here, the recovery percentage ranged from 93.4% to 101.33% (Figure 2B), indicating that the detection was acceptable. Notably, a good recovery percentage indicates the validity and reliability of ELISA. A recovery percentage within an acceptable range (typically 80%-120%) suggests minimal interference.[14] Here, our ELISA distinguished *T. albolabris* from *C. rhodostoma* and *D. siamensis* venoms, even at the high concentration of 1000 ng/mL, without the cross-reactivity to the *T. albolabris* HIgG. The OD at 429 nm from *C. rhodostoma* and *D. siamensis* venoms was lower than the background cut-off from pooled human sera (negative result). However, there was a slight cross-reactivity with *N. kaouthia* venom (a neurotoxic snake venom) at a concentration of 500 ng/mL or higher (Figure 2C). To reduce cross-reactivity with *N. kaouthia* venom, further purification using *N. kaouthia*-based affinity columns will enhance the specificity of HIgG against *T. albolabris* venom.

Despite the high specificity and sensitivity of our sandwich ELISA assay, the waiting time may be too long for practical use. Therefore, we created a lateral flow strip (a sandwich-based immune strip) using 40 nm gold nanoparticles (the larger gold nanoparticles exhibit more potent plasmonic properties), which could be easily detected by a color change due to enhanced optical properties. Besides, gold nanoparticles are more stable in solution than the smaller particles and are easily aggregable.[19] The specificity and sensitivity of our lateral flow strip, using the diluted *T. albolabris* venoms at 12.5, 25, 50, and 100 ng/mL in pooled human serum, were tested to see if the high viscosity of the serum could cause nonspecific binding or background noise on the test strip. Although there was low noise in our lateral flow test (no visible band by serum alone), the interference was easily reduced by diluting the serum in real clinical use with several strips in the same tests.[11] However, this strip test was possibly more suitable for the undiluted serum sample because the sample pad (Ahlstrom 8964) allows liquid samples to move across the membrane with a high flow rate (80-135 seconds per 4 cm).

A report investigating the presence of green pit viper venom in the bloodstream of snakebite victims found that the average half-life of the venom was approximately 27.5 hours during the first 3 days and extended to over 50 hours between days 5 and 7 post-bite. Notably, in about 14.8% of patients, venom remained present in the bloodstream up to day 14, and this persistent antigenaemia was associated with prolonged thrombocytopenia and coagulopathy.[20] The study on Russell's viper (*D. russelii*) venom levels in the serum of snakebite victims in Burma assessed venom concentrations using an ELISA. Serum venom levels ranged from less than 10 ng/mL to 290 ng/mL before antivenom treatment. Following antivenom administration, venom levels significantly decreased; however, residual venom was still detected in some cases. For example, one patient exhibited 11.5 ng/mL of venom 66 hours after receiving antivenom. Fatal cases had serum venom levels of 95 ng/mL and 185 ng/mL.[21]

In the LFA against *B. atrox* and *L. muta* venoms, spiked plasma and urine levels were detected at 10-50 ng/mL.[22] Additionally, the LFA identified hemotoxic snake venoms (*T. stejnegeri* and *P. mucrosquamatus*) in human serum at concentrations below 50 ng/mL, within 15 minutes.[11] The developed LFA demonstrated that the lowest concentration of

T. albolabris venom that could be visually detected was 25 ng/mL (Figure 3B), showing a slight cross-reaction with a high level of *N. kaouthia* venom (Figure 4C), but no cross-reaction with *C. rhodostoma* or *D. siamensis* venoms (Figure 4A and 4B). The cross-reactivity observed in ELISA and LFA between *T. albolabris* venom and *N. kaouthia* venom may be attributed to similarities in their protein components. Both venoms contain proteins that could share homologous epitopes, regions recognized by antibodies. These shared epitopes might cause antibodies developed for one venom to bind to components of the other, resulting in false positives or reduced assay specificity.

Conclusions

The sandwich ELISA assay demonstrated its ability to detect *T. albolabris* venom, with a LOD of 11.37 ng/mL and LOQ of 34.45 ng/mL. Additionally, the 3-column purification could enhance the specificity of HIgG against *T. albolabris* venom, which exhibits no cross-reactivity with the venoms of *C. rhodostoma* or *D. siamensis* venom, both of which belong to the same group of hemotoxic snake venoms. However, a slight cross-reaction with a high concentration of *N. kaouthia* venom occurred at levels of 500 ng/mL or higher, which was not present at 250 ng/mL.

The extended processing time of ELISA presents challenges for practical applications. To address this limitation, LFA was developed for rapid venom detection, utilizing enhanced optical properties and stability. The LFA could detect *T. albolabris* venom at 25 ng/mL, distinguishing it from the venoms of *C. rhodostoma* and *D. siamensis*, without producing false-positive test lines. However, a slight cross-reaction was observed at the test line when the sample was tested with 500 ng/mL of *N. kaouthia* venom.

Further optimization of the sandwich ELISA and LFA is necessary, particularly through validation with clinical samples from patients who have received snakebites. Despite this, the study highlights the feasibility of developing ELISA and LFA for venom detection in Thailand, marking an important step toward improving diagnostic tools for snakebite management.

Additional Information

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Ethics Approval: To clarify the ethical considerations of this study, we confirm that the research was approved by the Institutional Review Board of the Queen Saovabha Memorial Institute (QSMI-ACUC-01-2024 on 31 January 2024). This approval pertains explicitly to the current research involving the use of snake venom and hyperimmune horse plasma. This study did not involve testing in animal models or human subjects. Pooled normal human serum used in this study was obtained commercially from Sigma-Aldrich (USA).

Clinical Trial Consideration: This study does not report on a clinical trial.

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RMJ

Original Article

Evaluating the Validity and Reliability of the Thai Translated Psychological Vulnerability Scale for Graduate Students

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Abstract

Background: Psychological vulnerability plays a pivotal role in mental health issues, marked by distorted thinking patterns leading to maladaptive coping behaviors.

Objective: To adapt the concise and user-friendly Psychological Vulnerability Scale (PVS) into Thai while assessing its psychometric properties.

Methods: This study employed purposive sampling, selecting 384 Thai graduate students from various academic disciplines, ensuring the inclusion of specific subgroups relevant to the study's focus on mental health and psychological vulnerability. Data were collected using an online survey that included the Thai version of the PVS, the Thai Mental Health Questionnaire (TMHQ), and demographic information. A pilot test was conducted with 30 students prior to the main survey to assess the clarity and reliability of the instruments.

Results: These findings indicated the translated Thai PVS's robust content validity and revealed 2 factors explaining 56.7% variance through exploratory factor analysis. Pearson correlation coefficients showed a significant moderate relationship between Thai PVS, TMHQ, and vulnerability perception. Furthermore, the Thai PVS showed a strong correlation with the original version and had acceptable internal consistency reliability, with Cronbach α at 0.668 and McDonald ω at 0.672.

Conclusions: These results affirm the Thai PVS's robust psychometric properties, making it a valuable tool for screening mental health issues among graduate students, addressing a critical need in this population.

Keywords: Psychological vulnerability, Psychometric property, Graduate students

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Introduction

Understanding the role of psychological vulnerability in mental health disorders is pivotal. The vulnerability-stress model, a fundamental concept in psychology, suggests that vulnerability lowers the threshold for mental disorder onset.[1] Some individuals exhibit heightened susceptibility to negative outcomes under stress, while others display resilience. Sinclair et al[2] describe psychological vulnerability as cognitive tendencies involving false beliefs leading to maladaptive coping. Vulnerability is essential in understanding mental disorders[3] and is central to comprehending conditions like depression.[4] Despite extensive research, diverse interpretations and definitions persist.

Psychological vulnerability is a significant area of research in psychology. Psychological Vulnerability Scale (PVS) provides a well-constructed instrument for assessing this aspect.[2] The PVS focuses on specific cognitive paradigms related to self-concept, including dependency perception, perfectionism, negative attributions, and the need for external validation.

Distortions in these constructs are linked to unfavorable mental health outcomes. However, a universally agreed-upon definition remains elusive.

The PVS, initially developed for individuals with rheumatoid arthritis,[2] has been adapted for general population, such as adolescents and university students. Studies involving 1875 secondary school students and 267 Portuguese higher education students demonstrated the scale's one-factor structure, good internal consistency, test-retest reliability, and convergent validity with mental health measures.[5-6] These findings confirm that the PVS is a reliable and valid tool for assessing psychological vulnerability in nonclinical populations. In Thailand, there has been limited research on psychological vulnerability and its impact on student populations, particularly graduate students. Globally, graduate students face higher levels of depression and anxiety, largely due to the stress and changing responsibilities they encounter.[7] They are 6 times more likely to experience these issues than the general population.[8] Graduate students also face a higher risk of suicidal thoughts and actions, often accompanied by a sense of loss of control, eating disorders, hopelessness, desperation, and clinical depression.[9] However, there is a lack of instruments specifically adapted to assess psychological vulnerability within this population in Thailand.

This study aims to address this gap by adapting the PVS into Thai version, assessing its psychometric properties, and introducing it to Thai graduate students. By doing so, the study seeks to enhance mental health awareness and support preventive interventions for this critical population. A comprehensive understanding of psychological vulnerability will contribute to more targeted mental health initiatives, improving support systems for graduate students in Thailand.

Methods

Study Design

A cross-sectional validation study, it encompassed 2 primary phases: translating and adapting the PVS into Thai and assessing its psychometric attributes. Authorization for this translational work and subsequent assessment came from Vaughn Sinclair, a renowned expert from Vanderbilt University's psychiatric nursing department. The translation process adhered to the World Health Organization's (WHO) guidelines, following their established protocol for instrument translation, including forward and back translations to ensure methodological rigor and linguistic fidelity.[10] The study then scrutinized the psychometric properties of the Thai PVS, focusing on Thai graduate students.

Participants

The study included 414 participants, recruited through purposive sampling with specific inclusion criteria, and divided into 2 groups. The first group consisted of 30 graduate students from Mahidol University who met the following criteria: 1) enrollment in a graduate program, 2) proficiency in both Thai and English languages, with passing scores on the English proficiency exams required for graduate programs at Mahidol University, and 3) willingness to participate. This group was used for the pretest during the adaptation of the Thai version of the PVS, in accordance with Perneger's recommendations.[11] The second group included 384 graduate students who were actively enrolled in graduate programs at various universities in Thailand. The inclusion criteria for this group were: 1) enrollment in a graduate program in Thailand, 2) being a native Thai speaker with

the ability to read and understand Thai language, and 3) willingness to participate. This larger sample size was determined using the Cochran formula, with a 95% confidence interval and a 5% margin of error, to conduct a comprehensive examination of the Thai PVS's psychometric properties. Data from the first group were analyzed to initially evaluate parallel form reliability and semantic equivalence between the Thai and original versions of the PVS after establishing content validity. The second group participated by completing an online questionnaire with no missing data. Participants provided consent by action, which was recorded when they clicked the link to complete the questionnaire. Participants who did not provide consent or were not graduate students were automatically excluded. All questionnaires were distributed, and data were collected from August 2020 to February 2021.

Instrument

The PVS consists of 6 questions, each rated on a 5-point scale from 1 (does not describe me at all) to 5 (describes me very well). The original version of the PVS underwent internal consistency testing, yielding Cronbach α coefficients ranging from 0.71 to 0.87 across different populations. Additionally, test-retest reliability was evaluated twice, resulting in correlations of $r = 0.83$ at a 6-week interval and $r = 0.81$ at 3 months.[2] The PVS is a self-report instrument with clear instructions: 'Consider how well the following statements describe your behavior and actions on a scale from 1 to 5. Mark an X on the number that best reflects your behavior'. The scale is designed to assess psychological vulnerability by asking participants to rate how much each statement reflects their behavior and feelings. Example questions include: 'I am frequently aware of feeling inferior to other people' and 'I need approval from others to feel good about myself'. Scoring for the PVS involves summing the responses to all 6 questions, where a higher total score indicates greater psychological vulnerability in the individuals. Following the back-translation process, the Thai version of the PVS was subjected to content validity assessment. The revised scales were then administered to a group of 30 graduate students for the examination of the psychometric properties of the translated scale.

The Thai Mental Health Questionnaire (TMHQ) serves as a practical tool designed for screening mental health symptoms within the Thai general population. Its construct validity has been established through factor analysis, and it exhibits strong reliability, with a Cronbach α coefficient of 0.89. Comprising a total of 70 questions, TMHQ is an ordinal rating scale and a self-administered instrument that individuals can complete independently. The instructions for TMHQ, located at the top of the scale, are designed to be comprehensible to readers with a basic level of literacy. An example question from the scale is, 'In the past month, have you experienced any of these symptoms?'. Responses are rated on a scale of 0 = not at all, 1 = a little, 2 = somewhat, 3 = usually, and 4 = frequently. The sum of scores reflects 5 domains of mental health: somatization, anxiety, depression, psychotic, and social function. Scoring and interpreting TMHQ results require an answer sheet with specific instructions for calculating the raw score.[12] These 5 domains of TMHQ are relevant to mental health symptoms that may positively correlate with psychological vulnerability, especially within the depression domain. Given its robust psychometric properties and applicability to the Thai general population, TMHQ is an appropriate tool for assessing the criterion validity of the PVS.

The perception of vulnerability survey question is a single-item survey designed to explore the relationship between participants' self-perceived psychological vulnerability

and their scores on the PVS. Participants are asked to rate their perception of psychological vulnerability on a scale ranging from 1 (not at all vulnerable) to 5 (extremely vulnerable). A higher score indicates a stronger perception of psychological vulnerability.

The demographic survey collects personal information from participants, encompassing details such as age, gender, marital status, year of study, field of study, university type, and university location.

Statistical Analysis

All statistical analyses for this study were performed using SPSS version 29.0 (IBM SPSS Statistics for Windows, Version 29.0. Armonk, NY: IBM Corp; 2022) and Jamovi version 2.4 program software package. The initial stage of the research focused on adapting the PVS from its original language to Thai through forward and backward translation processes. This adaptation phase also involved assessing content validity based on the index of items objective congruence (IOC) and ensuring parallel reliability between the 2 different language versions by examining Pearson correlation coefficients. In the second phase of the study, construct validity was evaluated using exploratory factor analysis (EFA), where the number of factors was determined by examining the scree plot and eigenvalues greater than 1. Principal component analysis (PCA) was used as the extraction method, and an oblimin rotation with Kaiser normalization was applied. Criterion-related validity was assessed through the Pearson correlation coefficient to examine the relationships between the Thai PVS, perception of vulnerability, and the TMHQ. Additionally, internal consistency reliability was determined using Cronbach α and McDonald ω. *P* values less than .05 were considered statistically significant.

Results

Descriptive Analysis of Psychological Vulnerability Scale Scores and Demographic Variations

The adaptation of the PVS among 30 Thai graduate students (73.3% female) resulted in a mean (SD) Thai PVS score of 16.90 (5.55), with 66.7% passing the English proficiency exams (MUGrad test). Psychological vulnerability among 384 Thai graduate students with a mean (SD) age of 27.54 (4.79) years was assessed through a descriptive analysis of their scores on the Thai PVS (mean [SD], 18.32 [4.4]; median [range], 19 [7-30]). The range of scores on the Thai PVS spanned 23 points, and the interquartile range (IQR) was 6, suggesting a normal distribution of the data. In terms of interpretation, a mean (SD) PVS score of 18.32 (4.44) indicated a moderate level of psychological vulnerability, in accordance with the guideline that higher scores reflect greater vulnerability.[2] Furthermore, it's worth noting that female students reported slightly higher mean PVS scores compared to male students (mean [SD], 18.36 [4.31] vs 17.96 [5.52]), while students who chose not to identify their gender reported the highest mean PVS scores (mean [SD], 20.06 [5.52]). However, there were no significant differences in PVS mean scores based on gender, marital status, university location, or university type. Significant variations in PVS scores were observed among age groups, different fields of study and years of study, as determined by post hoc pairwise comparisons. The sample aged between 41-50 years reported the lowest PVS score (mean [SD], 14.85 [3.53]) and showed a significant difference compared to those aged 21-30 years and over 50 years. Business administration and law students tended to report the lowest PVS scores (mean [SD], 16.48 [4.68]), and this score was significantly

different from the group of students studying art and humanities (mean [SD], 19.21 [4.17]). Additionally, students in their fourth year of graduate studies reported the highest PVS scores (mean [SD], 20.28 [4.68]), which significantly differed from students in the first year of study (mean [SD], 17.51 [4.58]) (Table 1).

Table 1. Participants' Demographic Information and Psychological Vulnerability Scale Score

Characteristic	No. (%)	PVS Score, Mean (SD)	Statistics	Post Hoc Test
The adaptation of the PVS sample group	30 (7.2)	16.90 (5.55)	NA	NA
Gender				
Male	6 (20)	NA	NA	NA
Female	22 (73.3)	NA	NA	NA
Chose not to identify	2 (6.7)	NA	NA	NA
English proficiency test				
MUGrad (≥ 60)	20 (66.7)	NA	NA	NA
IELTS (≥ 5)	6 (20)	NA	NA	NA
TOEFL iBT (≥ 54)	3 (10)	NA	NA	NA
TOEFL ITP (≥ 480)	1 (3.3)	NA	NA	NA
The psychometric properties sample group	384 (92.8)	18.32 (4.44)	NA	NA
Gender				
Male	97 (25.3)	17.96 (4.58)	*F* (2, 381) = 1.475, *P* = .230	NA
Female	272 (70.8)	18.36 (4.31)		
Chose not to identify	15 (3.9)	20.06 (5.52)		
Age, y				
21-30	320 (83.3)	18.51 (4.26)	*F* (3, 380) = 2.769, *P* = .042	(21-30 vs 41-50) (41-50 vs >50)
31-40	54 (14.1)	17.48 (5.35)		
41-50	7 (1.8)	14.85 (3.53)		
> 50	3 (0.8)	21.33 (2.08)		
Marital status				
Single	359 (93.5)	18.33 (4.39)	*F* (2, 381) = 0.175, *P* = .839	NA
Married	23 (6.5)	18.43 (5.30)		
Divorced	2 (0.5)	16.50 (4.94)		
Fields of study				
Education	19 (4.9)	19.78 (4.44)	*F* (7, 376) = 2.764, *P* = .008	(Arts and humanities vs Business and law)
Arts and humanities	56 (14.6)	19.21 (4.17)		
Social sciences	94 (24.5)	17.90 (4.31)		
Business and law	58 (15.1)	16.48 (4.68)		
Natural sciences	38 (9.9)	19.21 (4.39)		
Information technologies	14 (3.6)	19.64 (3.10)		
Engineering and architecture	21 (5.5)	19.23 (3.61)		
Health science and welfare	84 (21.9)	18.30 (4.65)		

Table 1. Participants' Demographic Information and Psychological Vulnerability Scale Score (Continued)

Characteristic	No. (%)	PVS Score, Mean (SD)	Statistics	Post Hoc Test
Years of study				
1	131 (34.1)	17.51 (4.58)	*F* (4, 379) = 2.876, *P* = .023	(1 vs 4)
2	142 (37.0)	18.75 (4.09)		
3	61 (15.9)	18.31 (4.36)		
4	28 (7.3)	20.28 (4.68)		
≥ 5	22 (5.7)	18.00 (4.91)		
Location of university				
Bangkok and metropolitan areas	366 (95.3)	18.32 (4.47)	*t* (382) = -0.114, *P* = .910	NA
Other provinces	18 (4.7)	18.44 (3.83)		
Types of university				
Public university	338 (88.0)	18.30 (4.54)	*F* (3, 380) = 0.178, *P* = .911	NA
Autonomous university	5 (1.3)	19.00 (3.08)		
Rajabhat university	4 (1.0)	19.75 (5.31)		
Private university	37 (9.6)	18.27 (3.61)		

Abbreviations: NA, not applicable; PVS, Psychological Vulnerability Scale.

Validity

Content Validity: In assessing content validity, a series of steps were followed. First, a backward translation was conducted, and necessary adjustments were made. Subsequently, IOC analysis was performed as the final evaluation. This analysis involved input from 3 language and psychology experts who assessed the Thai translation of the PVS after the completion of the backward translation process and item revisions. The outcome of this content validity assessment revealed that all items in the scale received IOC scores ranging from 0.67 to 1.00, which fell within the acceptable range (IOC > 0.5) as outlined by Turner et al.[13] This indicated that the translation and adaptation of the PVS into Thai maintained a high level of content validity, reflecting the alignment between the original and translated versions of the items.

Construct Validity: To assess construct validity for the Thai version of the PVS, 2 main methods were employed: EFA and a known-groups comparison. The data were found to be suitable for EFA, meeting the necessary criteria with a significant Bartlett's test of sphericity and a Kaiser-Meyer-Olkin (KMO) result of 0.75, which exceeded the commonly recommended value of 0.6 (KMO = 0.705, Bartlett's $\chi^2 = 325.361$, $df = 15$, $P < .01$). EFA revealed 2 components (Table 2 and Figure 1). Component 1 consisted of 3 items from a 6-item scale, explaining 37.9% of the variance with factor loadings ranging from 0.603 to 0.718. Component 2 also comprised 3 items from the same scale, explaining 18.7% of the variance with factor loadings ranging from 0.565 to 0.811. Together, these 2 components accounted for a cumulative variance of 56.68%. The individual item variance (h^2) ranged between 0.438 and 0.692, all of which met the acceptable threshold of 0.4 suggested by Costello et al[14], indicating suitability for retaining these variables. However, item 2 had a lower corrected item-total correlation value (below 0.3), suggesting that it might not be strongly correlated with the overall scale, as per Cristobal et al.[15]

Lastly, the known-group method was employed to confirm construct validity by comparing PVS scores between 2 groups: students reporting no mental health symptoms (T-score < 65) and students at risk for mental health issues (T-score ≥ 65). The independent sample t test results demonstrated that the mean PVS score for students reporting at risk for depressive symptoms (mean [SD], 21.13 [3.74]) was significantly higher than that of students without depressive symptoms (mean [SD], 17.00 [4.12]; $t(261.11) = 9.764$; $P < .01$). This significant difference also applied to all domains of the TMHQ, except for the social function domain, which did not show a significant difference in mean PVS scores between the 2 groups (Table 3).

Criterion-Related Validity: Criterion-related validity was assessed through data analysis, which involved calculating bivariate Pearson correlation coefficients between the translated Thai-PVS and the TMHQ. Positive and moderate correlations between PVS scores and the scores across all domains of the TMHQ were found. Notably, the strongest correlation was observed between PVS scores and depression ($r = 0.537$, $P < .01$). Furthermore, significant, positive, and moderate relationships were found between PVS scores and other TMHQ domains, with correlations ranging from $r = 0.481$ (anxiety) to $r = 0.349$ (psychotic). These results indicated the ability of the PVS to measure psychological vulnerability effectively in relation to various mental health domains. Additionally, a positive and moderate correlation was identified between PVS scores and the perception of vulnerability ($r = 0.504$, $P < .01$) (Table 4).

Reliability

Reliability analysis was performed, and results are presented with correlation coefficient and mean (SD), for both PVS and its Thai translation with 30 initial sample groups. Notably, the correlation coefficient between the Thai and the original English version was robust at 0.835. This strong correlation was further supported by individual item correlations between the two versions, which ranged from 0.685 to 0.916, all of which were statistically significant ($P < .01$) (Table 5). Importantly, there were no significant differences in the means for each paired item across the scale, confirming the excellent parallel reliability of the Thai PVS. Furthermore, internal consistency reliability was assessed with a dataset of 384 participants. The Cronbach α and McDonald ω coefficients for the total score of the Thai translated version of the PVS were 0.668 and 0.672 respectively, indicating an acceptable level of internal consistency reliability. These coefficients reflected the degree to which the items within the scale measure the same underlying construct of psychological vulnerability consistently.

Table 2. Descriptive Data and Results of the Analysis of Psychological Vulnerability Scale Items

PVS Items	Mean (SD)	Corrected Item-Total Correlation	If Item Deleted		h^2	Factor Loading	
			α	ω		Component 1	Component 2
1) I am frequently aware of feeling inferior to other people.	2.74 (1.21)	0.403	0.642	0.631	0.438	NA	0.565
2) I tend to set my goals too high and become frustrated trying to reach them.	3.34 (1.23)	0.292	0.663	0.666	0.607	NA	0.811
3) I feel entitled to get better treatment from others than I generally receive.	2.40 (1.13)	0.362	0.638	0.645	0.509	0.718	NA
4) If I don't achieve my goals, I feel like a failure as a person.	3.25 (1.27)	0.463	0.602	0.617	0.622	NA	0.759
5) I often feel resentful when others take advantage of me or ignore my feelings.	3.55 (1.22)	0.383	0.632	0.635	0.692	0.854	NA
6) I need approval from others to feel good about myself.	3.04 (1.18)	0.487	0.595	0.601	0.533	0.603	NA
PVS total score	18.3 (4.44)	NA	NA	NA	NA	NA	NA
The variance explained, %	NA	NA	NA	NA	NA	37.9	18.7

Abbreviations: NA, not applicable; PVS, Psychological Vulnerability Scale.

Figure 1. The Scree Plot Showing Components and Eigenvalue of the Factor Analysis of Psychological Vulnerability Scale Items

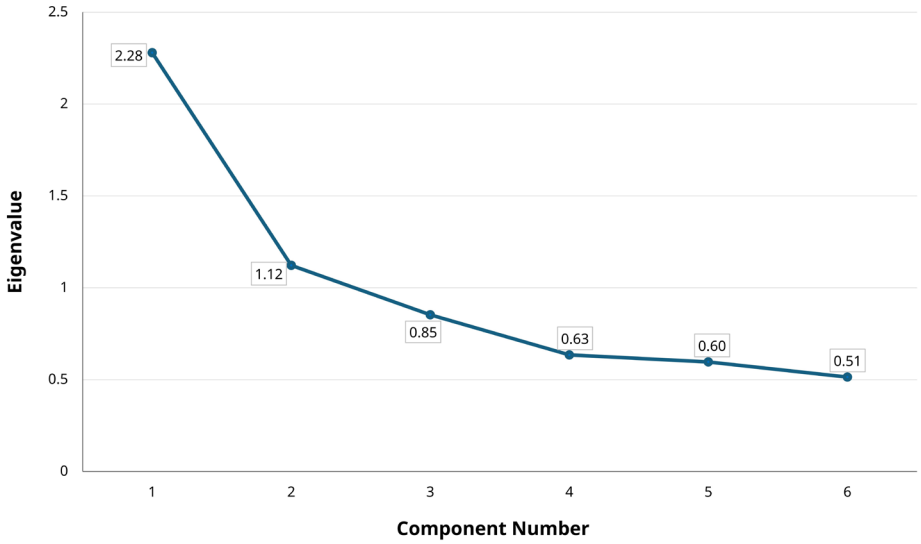

Table 3. Comparison of Psychological Vulnerability Scale Scores Between Samples Without Mental Health Problems and Those at Risk for Mental Health Problems

Mental Health Problem	No. (Mean [SD]) PVS Scores		Statistics	Cohen *d* Effect size
	No Mental Health Problems	At Risk for Mental Health Problems		
Somatization	237 (17.10 [4.20])	147 (20.30 [4.10])	t (382) = 7.327, P < .01	0.77
Depression	261 (17.00 [4.12])	123 (21.13 [3.74])	t (261.11) = 9.764, P < .01	1.05
Anxiety	246 (17.04 [4.34])	138 (20.62 [3.63])	t (327.26) = 8.633, P < .01	0.89
Psychotic	376 (18.25 [4.41])	8 (21.75 [4.52])	t (382) = 2.212, P = .028	0.78
Social function	378 (18.29 [4.42])	6 (20.33 [5.42])	t (382) = 1.114, P = .266	0.41

Abbreviations: PVS, Psychological Vulnerability Scale.

Table 4. Criterion Validity of the Thai Version of the Psychological Vulnerability Scale

Scale	TMHQ					POV
	Somatization	Depression	Anxiety	Psychotic	Social Function	
PVS	0.471	0.537	0.481	0.349	0.444	0.504

Abbreviations: POV, Perception of psychological vulnerability score; PVS, Psychological Vulnerability Scale; TMHQ, Thai Mental Health Questionnaire.

Table 5. Parallel Form Reliability of the Psychological Vulnerability Scale Items With 30 Graduate Students From the Translation and Adaptation Phase

Item of the Thai PVS	Correlation	Mean (SD)	
		English	Thai
1	0.732	2.77 (1.00)	2.60 (1.13)
2	0.897	2.90 (1.24)	3.03 (1.27)
3	0.685	2.53 (1.10)	2.37 (1.09)
4	0.902	2.87 (1.30)	2.90 (1.42)
5	0.880	3.40 (1.32)	3.30 (1.36)
6	0.916	2.80 (1.34)	2.70 (1.31)
PVS total score	0.835	17.27 (4.75)	16.90 (5.55)

Abbreviations: NA, not applicable; PVS, Psychological Vulnerability Scale.

Discussion

Significance of PVS Scores

The mean score of the PVS in this study was 18.32, which aligned with previous research on psychological vulnerability within populations with higher education, suggesting a moderate level of psychological vulnerability.[6, 16-19] Notably, no established norms exist for differentiating score levels among the Thai university population, making this study the first of its kind to investigate PVS scores among Thai graduate students. Additionally, it is important to highlight that >30% of the participants reported high levels of mental health symptoms, including somatization, anxiety, and depression, suggesting a need for professional assistance to address their mental health concerns.

Evidence of Validity

Content validity was assessed using the IOC, with values ranging from 0.67 to 1.00, exceeding the 0.5 threshold and indicating language equivalence.[13] Differences in translation preferences led to a score of 0.67 for items 2 and 3, particularly concerning the translation of frustrated and generally. All experts were psychology professionals fluent in both Thai and English languages. After careful evaluation of translation options, the final selections were made to ensure accuracy.

To assess the construct validity of the Thai PVS and determine if it measures its intended theoretical concept, EFA was employed. To ensure the suitability of the data for this analysis, Bartlett's test of sphericity and the KMO test were employed, confirming that the data met the requirements for factor analysis. The aim of the factor analysis was to either validate the PVS as a unidimensional scale or identify potential components if it deviated from this structure. Surprisingly, the factor analysis revealed the presence of 2 components, despite previous literature supporting the unidimensional structure of the PVS.[2, 20-23] These 2 components had eigenvalues of 2.28 and 1.12, surpassing the threshold of 1, indicating their significance.[24] Component 1 included items 3, 5, and 6, explaining 37.9% of the variance, while Component 2 comprised items 1, 2, and 4 from the same scale, accounting for 18.7% of the variance. Together, these 2 components explained a total variance of 56.68%, slightly below the 60% threshold. However, it's worth noting that Peterson[25] suggested in the meta-analysis that variance analysis in EFA that the average percentage of variance accounted for was 56.6%.

To further examine the 2 identified factors, the PVS item keywords were instrumental in identifying variables that align with both components: 1) the need for approval from others, and 2) self-criticism. The need for approval from others was evident in items 3, 5, and 6, all of which highlighted a reliance on others for one's self-esteem ('I feel entitled to better treatment from others than I generally receive', 'I often feel resentful when others take advantage of me or ignore my feelings', and 'I need approval from others to feel good about myself'). On the other hand, items 1, 2, and 4 reflected self-criticism, involving thoughts that place blame on oneself ('I am frequently aware of feeling inferior to other people', 'I tend to set my goals too high and become frustrated trying to reach them', 'If I don't achieve my goals, I feel like a failure as a person'). It is noteworthy that both factors are related to low self-worth and a negative attribution style, as initially described by Sinclair et al,[2] and they align with the work of Nogueira et al,[6] which found that the PVS consists of 2 key components reflecting self-criticism and social approval. While both components signify unhealthy cognitive patterns or psychological vulnerability, they appear to convey somewhat distinct meanings in the context of this study. The 2-factor structure of the Thai PVS can be particularly useful in identifying specific areas where individuals may need psychological support. For example, individuals with high scores in the need for approval from others may benefit from interventions focused on improving self-esteem and fostering greater independence from external validation. In contrast, those with higher self-criticism may require strategies that promote self-compassion, realistic goal setting, and reducing the tendency to excessively blame themselves. These insights can be applied in both clinical settings and preventive programs to target and address specific cognitive vulnerabilities, ultimately helping to improve overall mental health outcomes.

Furthermore, the communality value, which predicts the cumulative total variance of the scale ranging from 44% to 69%, signifies the percentage of a variable's variance explained by all other factors. However, the presence of 2 factors instead of one factor requires a more profound exploration within the cultural context. For instance, the inclination toward seeking approval and depending on others is not uncommon among Thai students. It's conceivable that in the Thai educational system, students lean more toward collectivism than individualism. This assumption is evident in various educational settings, from schools to universities, where group interests often take precedence over individual ones.[26] Furthermore, in pursuit of testing the validity of the Thai version of the PVS, an additional step was taken by employing the known group method. This method serves the purpose of confirming that the Thai PVS scale effectively distinguishes between 2 well-defined groups. In this study, these groups encompassed students who reported high levels of mental health issues in domains such as depression, anxiety, somatization, and psychosis, as well as those who did not report such issues. Significantly divergent PVS scores were observed across all domains of the TMHQ, except for the social function domain. This exception could be attributed to the fact that the capacity and inclination for effective social interaction may not align with the concept of psychological vulnerability as defined in this context.

To assess the suitability of the Thai version of the PVS as a predictive tool for mental health issues within the Thai population, criterion validity testing was conducted, examining the correlations between the PVS and 2 measurements: the TMHQ and participants' self-reported perception of psychological vulnerability. The results revealed significant correlations between PVS scores and all domains of the TMHQ ($P < .01$), with correlations of $r = 0.537$ for depression, $r = 0.481$ for anxiety, $r = 0.471$ for somatization,

r = 0.444 for social function, and r = 0.349 for psychosis. This suggests that higher PVS scores are associated with higher scores in each TMHQ domain and vice versa. Additionally, the PVS exhibited a positive and moderate relationship with participants' self-reported perception of psychological vulnerability (r = 0.504, P < .01). These findings align with a study by Nogueira et al[6] among higher education students, where the PVS demonstrated weak to moderate correlations with various subscales, including psychoticism (r = 0.61, P < .001), depression (r = 0.60, P < .001), somatization (r = 0.28, P < .05), and perception of vulnerability (r = 0.51, P < .0001). This convergence of results supports the conclusion that the PVS is a suitable tool for measuring psychological vulnerability, which is intricately linked to mental health problems.

In summary, the Thai version of the PVS demonstrated its appropriateness and credibility in terms of content, criterion, and construct validity. These findings affirm that the tool effectively measures its intended target, which is psychological vulnerability.

Evidence of Reliability

The reliability testing was conducted initially among a group of 30 graduate students proficient in English to assess the parallel reliability of the scale. The results confirmed a high correlation coefficient of 0.835 between the Thai translation of the PVS and the English version, signifying the equivalence of both versions. Furthermore, the correlations between the 6 items on the 2 questionnaires were notably strong, with values of 0.732, 0.897, 0.685, 0.902, 0.880, and 0.916. These findings suggest a high level of consistency and agreement between the original and Thai versions, as demonstrated by the absence of significant differences in mean scores between the 2 versions for the same group of graduate students (17.27 vs 16.90). This indicates that individuals who score higher in the Thai version also tend to score higher in the original version, affirming that the Thai-translated version effectively conveys the same concepts, even across 2 distinct languages. However, it's worth noting that the correlation for item number 3 was 0.685, which was slightly lower than the correlations observed for other pairs, suggesting slightly less confidence in the choice of vocabulary for that specific item. Nonetheless, overall, the translated version maintained consistent meaning with the English version following the backward translation process.

Cronbach α and McDonald ω were used to evaluate the internal consistency of the Thai-translated PVS. The resulting alpha coefficients of 0.668 and 0.672 are lower than the original PVS, which had α values ranging from 0.71 to 0.86 across various sample groups[2], and the Portuguese version, which reported a Cronbach α coefficient of 0.73 among higher education students.[6] Although a lower α suggests reduced reliability[27], several factors may explain this outcome. First, Taber[28] suggests that an α value of ≥ 0.6 is often considered acceptable in research, particularly when evaluating internal consistency. Additionally, while the PVS was originally designed as a unidimensional scale, our factor analysis revealed 2 components, suggesting that the translated scale may measure multiple constructs related to psychological vulnerability, even if they fall under the same broader concept. This aligns with Sinclair and Wallston's assertion[2] that PVS items could represent distinct psychological variables under the general umbrella of vulnerability. Furthermore, the Thai graduate student population in this study represents a new sample group that has not been examined with the PVS. Recent research has questioned whether Cronbach α is the best measure of reliability, as it primarily assesses homogeneity, which is only one aspect of internal consistency.[29] Dunn et al[30] also highlighted the limitations of

Cronbach α and suggested that McDonald ω is a more robust and comprehensive measure of internal consistency, particularly in multidimensional scales. This supports the rationale for presenting McDonald ω alongside Cronbach α and McDonald ω is considered a more reliable measure, especially in cases where the scale may measure multiple constructs or show multidimensionality. Omega is less sensitive to the assumptions of unidimensionality that Cronbach α assumes, making it particularly useful in scales with complex structures, such as the one found in this study. Several additional factors may explain the lower α coefficient observed in this study. Cultural differences between the Thai sample and the original population could lead to differences in how participants interpret and respond to the items. The adaptation of the PVS to the Thai context may have introduced variations in the understanding of some items, therefore impacting the responses. Additionally, the small sample size could have contributed to higher variability in the data, which might have affected the reliability coefficient. The translation process, although rigorous, may have also led to subtle discrepancies in meaning, which could further influence how participants responded to the items. For Item 2, the corrected item-total correlation was below 0.3. However, our analysis indicated that deleting this item did not increase Cronbach α and McDonald ω, which remained nearly unchanged. The α if item deleted value was 0.663 (0.666 for ω if item deleted), showing that the removal of Item 2 did not enhance the scale's internal consistency. Furthermore, the individual item variance values for all items ranged from 0.438 to 0.692, all exceeding the acceptable threshold of 0.4 suggested by Costello et al.[14] This suggests that each item contributes significantly to the overall scale, justifying the retention of all items in the final version of the Thai PVS. Finally, the method of data collection, including the use of an online questionnaire, may have influenced participants' willingness to provide honest and accurate responses, potentially leading to a lower internal consistency. Although the method was convenient and allowed for broader participation, it may have also introduced certain biases or factors that influenced the quality of the responses.

In summary, while the Thai version of the PVS does not exhibit excellent reliability, the scale's internal consistency remains acceptable according to some standards. Taber[28] suggests that an α value above 0.6 is acceptable, but a value below 0.7 may be considered poor or unreasonable. George et al[31] indicate that while a Cronbach α below 0.7 may be questioned, it can still be acceptable in certain contexts, such as when measuring complex or multidimensional constructs. Ultimately, the reliability of the translated PVS remains a subject of varying opinions in the literature, and further research is needed to better understand its consistency in different populations. Factors such as cultural differences, sample size, translation processes, and data collection methods should be considered in future adaptations of the scale to enhance its reliability and validity.

Several limitations should be considered in this study. Firstly, the participant sample, although intended to represent Thai graduate students from across the country, was predominantly located in Bangkok and metropolitan areas. This geographical concentration raises concerns about the generalizability of the findings to the entire population. Secondly, most participants were enrolled in public universities, which may have led to an uneven distribution of questionnaire responses. Additionally, since this study relied on an online self-report survey, it is susceptible to biases inherent in such data collection methods. For future research, it is advisable to reexamine the scale's psychometric properties to confirm its construct validity and internal consistency. Expanding the study to different populations that could benefit from the scale's use would also be valuable. Moreover,

further investigations into measuring psychological vulnerabilities can enhance our understanding of their impact on mental health. This could involve adapting the scale for specific populations, exploring additional aspects of psychological vulnerability, or even developing new scales to broaden the scope of this research area.

Conclusions

The results indicate that the Thai version of the PVS is a valid screening tool for identifying cognitive vulnerabilities related to mental health issues, especially depression, among graduate students. This study underscores the importance of using the PVS for early detection and intervention, tailoring psychotherapy to target cognitive vulnerabilities, and fostering mental health awareness among Thai graduate students to promote a healthier self-perception of vulnerability in the university setting.

Additional Information

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Ethics Approval: All procedures performed in this study involving human subjects were in accordance with the ethical standards of the institutional research committees at the Institutional Review Board of the Faculty of Medicine Siriraj Hospital at Mahidol University, before being conducted (Si 179/2020 on 4 March 2020) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Review Article

MicroRNA-Mediated Regulation of Granulosa Cell Apoptosis: A Review

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Abstract

MicroRNAs (miRNAs) play a critical role in regulating granulosa cell apoptosis, a fundamental process in ovarian follicular development and atresia. This review synthesizes current evidence indicating that miRNAs coordinate granulosa cell fate through 3 primary mechanistic pathways. First, the mitochondrial pathway, where miRNAs such as miR-484, miR-15a-5p, and miR-26b modulate BCL2 family proteins and cytochrome C release. Second, cell signaling cascades, particularly through the transforming growth factor-β (TGF-β) pathway, where miR-33b, miR-142, miR-423, miR-383, and miR-320 regulate various signaling components including transforming growth factor beta receptor 1 (TGFBR1) and protecting mothers against decapentaplegic homolog (SMAD) proteins. Third, metabolic regulation, where miR-34a-5p, miR-19a-3p, and miR-19b-3p influence cellular metabolism and survival through pathways such as phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) and glycolysis. Dysregulation of these miRNA-mediated processes has significant implications for reproductive disorders, particularly polycystic ovary syndrome (PCOS) and premature ovarian failure. Awareness about these complex regulatory networks not only advances the knowledge of follicular development but also indicates potential therapeutic targets for treating ovarian disorders characterized by abnormal granulosa cell apoptosis.

Keywords: MicroRNA, Granulosa cell apoptosis, Ovarian follicular development, Reproductive disorders

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Introduction

Accumulated evidence has shown the microRNAs (miRNAs) to contribute effectively in regulating granulosa cell apoptosis, a critical process in ovarian follicular development and atresia.[1] These small, noncoding RNA molecules, typically 18-24 nucleotides in length, function as post-transcriptional regulators by binding to specific sequences in the 3′-untranslated region (UTR) of target messenger RNAs, leading to either mRNA degradation or translational repression.[2] In the context of granulosa cells, which are essential for nourishing oocytes and regulating follicular development, miRNAs orchestrate complex molecular pathways that determine cell fate.[3] The process is particularly significant given that in mammals, more than 99% of ovarian follicles undergo atresia, with only a small fraction reaching ovulation.[4] Multiple miRNAs have been identified as key regulators in this process, notably including the let-7 family, miR-23-27-24 cluster, miR-183-96-182 cluster, and miR-17-92 cluster.[5-8] These miRNAs primarily function through targeting crucial genes involved in cell survival and death pathways, such as protecting

mothers against decapentaplegic homolog 4 (SMAD4), SMAD5, SMAD7, mitogen-activated protein kinase kinase kinase 1 (MAP3K1), and various components of the transforming growth factor-β (TGF-β) signaling pathway.[6, 9-11] The regulatory network is more complicated by the fact that one miRNA can target hundreds of different mRNAs, while a single mRNA can be targeted by multiple miRNAs, creating an intricate web of molecular interactions that precisely control granulosa cell fate.[12, 13]

The dysregulation of miRNA-mediated control of granulosa cell apoptosis has significant pathological implications, particularly in conditions like polycystic ovary syndrome (PCOS) and premature ovarian failure (POF).[14-17] Research has showed that in PCOS patients, several key miRNAs show altered expression patterns; for instance, miR-99a is significantly downregulated while insulin-like growth factor-1 receptor (IGF-1R) is upregulated, leading to abnormal follicular development.[18] The pathological process typically manifests through 2 distinct mechanisms: first, through direct regulation of apoptotic pathways, where miRNAs like miR-23a and miR-27a promote granulosa cell apoptosis by targeting SMAD5 through the Fas ligand-Fas (FasL-Fas)-mediated pathway; and second, through modulation of steroidogenesis, where miRNAs such as miR-378 and miR-320 affect estradiol synthesis and granulosa cell proliferation by targeting key enzymes like aromatase (CYP19A1).[6, 19-21] This complex relationship becomes particularly obvious in early folliculogenesis, where the relatively lower apoptosis rate and higher proliferation rate than normal in PCOS account for the simultaneous development of multiple follicles.[22] However, as development progresses beyond the mid-antral stage, the pathological process shifts, leading to increased granulosa cell apoptosis, progressive accumulation of follicular fluid, and eventual formation of thin-walled cysts characteristic of polycystic ovaries.[22]

The clinical manifestations of disrupted miRNA-mediated granulosa cell apoptosis regulation present significant implications for female reproductive health and fertility treatment options.[23] In clinical settings, these disruptions commonly manifest as irregular menstrual cycles, infertility, and hormone imbalances, particularly evident in conditions like PCOS where patients exhibit elevated luteinizing hormone (LH) levels (approximately 11.52 IU/L compared to 6.71 IU/L in healthy individuals) and significantly higher anti-Müllerian hormone (AMH) levels (12.22 ng/mL versus 4.57 ng/mL in controls).[18] Research has revealed that specific miRNA expression profiles could serve as probable diagnostic biomarkers; for instance, differential expression of miR-146a, miR-182-5p, miR-509-3p, and miR-149-5p between mural granulosa cells and cumulus cells has diagnostic value.[24] These findings have opened new therapeutic possibilities, particularly in the context of assisted reproductive technologies.[25]

miRNAs in Granulosa Cell Apoptosis

The miRNAs, as extensively elucidated in the literature, contribute to the granulosa cell apoptosis via various mechanisms (Table 1). The pathways and mechanisms could be summarized as follows:

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis

miRNA	Study Type	Method	Result	Conclusion	Reference
Mitochondrial pathway					
miR-484	Clinical and experimental	• Analysis of granulosa cells from follicular fluid (n = 114 women) • Transfection experiments • Luciferase assays • Western blot • RT-PCR • Flow cytometry	• miR-484 was highly expressed in GCs from DOR patients • miR-484 repressed GC proliferation and induced apoptosis • miR-484 directly targeted YAP1 • miR-484 induced mitochondrial dysfunction	miR-484 contributes to DOR by regulating granulosa cell function via YAP1-mediated mitochondrial function and apoptosis	Li et,[26] 2022
miR-15a-5p	Clinical and experimental	• Analysis of follicular fluid from POR and non-POR patients (n = 45) • miRNA sequencing • RT-PCR • Western blot • Cell culture experiments	• miR-15a-5p significantly elevated in young POR group • Repressed granulosa cell proliferation through PI3K-AKT-mTOR pathway • Promoted apoptosis via BCL2 and BAD	miR-15a-5p levels correlate with poor ovarian response and may serve as a potential biomarker	Zhang et al,[27] 2017
miR-26b	Experimental	• miRNA profiling • Cell culture • Flow cytometry • DNA break analysis	• miR-26b was upregulated during atresia • miR-26b increased DNA breaks by targeting ATM	miR-26b promotes granulosa cell apoptosis through ATM targeting during follicular atresia	Lin et al,[31] 2012
miR-26b	Experimental	• Cell culture • Luciferase assays • Western blot • Flow cytometry	miR-26b targeted SMAD4 and promoted granulosa cell apoptosis	miR-26b functions as a proapoptotic factor by targeting SMAD4 in porcine follicular granulosa cells	Liu et al,[10] 2014
Cell signaling					
miR-33b	Clinical and experimental	• Granulosa cell collection from PCOS patients • RT-PCR • Luciferase reporter assays • Western blot • Cell proliferation assays	• miR-33b significantly upregulated in PCOS patients • Directly targeted TGFBR1 • Suppressed TGF-β signaling pathway • Promoted cell proliferation and reduces apoptosis	miR-33b contributes to PCOS pathogenesis through altered TGF-β signaling in granulosa cells	Li et al,[15] 2015

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-142	Clinical and experimental	• Granulosa cell collection from PCOS patients • RT-PCR • Luciferase reporter assays • Western blot • Cell cycle analysis	• miR-142 significantly upregulated in PCOS patients • Directly targeted TGFBR1 • Inhibited TGF-β signaling via reduced SMAD2/3 phosphorylation • Altered cell cycle regulation	miR-142 dysregulation contributes to PCOS through disrupted TGF-β signaling and cell cycle control	Li et al,[15] 2015
miR-383	Experimental	• Granulosa cell culture • Transfection experiments • Target validation • Hormone measurements	• miR-320 regulated by miR-383 • Affected granulosa cell function • Targeted E2F1 and SF-1	miR-320 regulates granulosa cell functions through targeting specific transcription factors	Yin et al,[19] 2014
miR-383	Experimental	• Granulosa cell culture • Transfection experiments • Luciferase assays • Western blot • RT-PCR • ChIP assays	• miR-383 promoted estradiol release • Directly targeted RBMS1 • Transcriptionally regulated by SF-1 • Did not affect cell proliferation or apoptosis	miR-383 regulates steroidogenesis by targeting RBMS1, mediated by SF-1 transcriptional activation	Yin et al,[28] 2012
miR-383	Experimental	MicroRNA array assay of healthy, early atretic and progressively atretic follicles in porcine ovary	miR-383 could not be accurately measured due to low abundance in most follicle samples analyzed	No definitive conclusions could be drawn about miR-383's role due to its low expression levels	Schauer et al,[29] 2013
miR-423	Experimental	• RNA sequencing • Cell culture • Flow cytometry • Luciferase assays	• miR-423 was downregulated during atresia • it suppressed early apoptosis by targeting SMAD7	miR-423 inhibits follicular atresia initiation and early granulosa cell apoptosis	Li et al,[32] 2023

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-423	Clinical and experimental (in vitro)	• Follicular fluid collection from PCOS patients and controls • Real-time PCR for miRNA quantification • Dual luciferase assay • Western blot	• miR-423 expression was downregulated in PCOS patients • miR-423 directly repressed SMAD7 expression • Affected granulosa cell apoptosis pathway	miR-423 regulates granulosa cell function through targeting SMAD7 and may be involved in PCOS pathogenesis	Li et al,[15] 2019
miR-144	Experimental (in vitro and in vivo)	• Mouse granulosa cell culture • Luciferase reporter assays • Western blot • qRT-PCR • Flow cytometry	• miR-144 targeted E2F1 and SF-1 • Regulated granulosa cell proliferation and steroidogenesis • Affected PGE2 production	miR-144 regulates granulosa cell functions by targeting E2F1/SF-1 and modulating the PGE2 pathway	Zhou et al,[33] 2017
miR-144	Clinical and experimental	• RNA sequencing • Cell proliferation assays • Luciferase reporter assays • Western blot	miR-144 regulated granulosa cell function through multiple pathways (cell proliferation and steroidogenesis pathways)	miR-144 plays a role in granulosa cell regulation	Chen et al,[34] 2023
miR-320	Clinical and experimental (in vitro)	• Collection of human follicular fluid • Real-time PCR • Embryo quality assessment • Cell culture experiments	• miR-320 levels correlated with embryo quality • Affected granulosa cell function • Regulated steroidogenesis	miR-320 could serve as a biomarker for embryo quality and impacts follicular development	Yin et al,[19] 2014
miR-320	Clinical and experimental (in vitro)	• Follicular fluid analysis • miRNA expression profiling • Cell proliferation assays • Hormone analysis	• miR-320 expression affected by melatonin levels • Impacted embryo development • Regulated cell proliferation	miR-320 participates in melatonin-mediated regulation of embryo development	Khan et al,[35] 2021

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-873	Experimental	• Cell culture experiments • RT-PCR • Western blot • Luciferase assays	miR-873 regulated granulosa cell function and survival through specific signaling pathways	miR-873 serves as a regulatory factor in granulosa cell function and follicular development	Sontakke et al,[36] 2014
Metabolic regulation					
miR-34a-5p	Clinical and experimental	• Human KGN cells analysis • Clinical sample analysis • Metabolic pathway studies • Western blot • RT-PCR	• Directly targeted LDHA, impaired glycolysis through upregulation of proapoptotic factors • Reduced energy availability in granulosa cells through upregulation of proapoptotic factors • Promoted apoptosis through upregulation of proapoptotic factors	miR-34a-5p orchestrates a complex regulatory network linking metabolic dysfunction to cell death in granulosa cells	Cui et al,[37] 2024
miR-19a-3p	Clinical and experimental	• Follicular fluid analysis • miRNA profiling • Cell culture experiments • RT-PCR • Pathway analysis	• miR-19a-3p differentially expressed in PCOS patients • Downregulated upon copper exposure in granulosa cells • Targeted genes in PI3K-Akt and FOXO pathways • Altered expression precedes apoptosis	miR-19a-3p works as a molecular mediator in stress response pathways affecting granulosa cell survival	Chen et al,[34] 2023; Cui et al,[54] 2021
miR-19b-3p	Clinical and experimental	• Exosomal miRNA profiling • KEGG pathway analysis • RT-PCR • Functional assays	• Significantly upregulated in PCOS patients • Associated with metabolic pathway regulation • Participated in PI3K-Akt and MAPK signaling • Influenced granulosa cell apoptosis	miR-19b-3p functions as a regulatory molecule in metabolic pathways affecting granulosa cell fate	Xie et al,[55] 2016; Ye et al,[56] 2021

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-99a	Clinical and experimental	• Analysis of granulosa cells from PCOS patients • Cell culture experiments • Target validation studies	• miR-99a was significantly downregulated in PCOS patients • IGF-1R was upregulated • Abnormal follicular development	miR-99a dysregulation contributes to PCOS pathogenesis through altered IGF-1R signaling	Geng et al,[18] 2019
miR-27a-3p	Experimental	• MicroRNA profiling • Cell culture studies • Luciferase assays	• miR-27a-3p was upregulated during follicular atresia • It promoted granulosa cell apoptosis by targeting ATM gene	miR-27a-3p functions as a proapoptotic factor in porcine granulosa cells through ATM regulation	Tao et al,[30] 2023

Abbreviations: ATM, ataxia telangiectasia mutated; BAD, BCL2-associated agonist of cell death; ChIP, chromatin immunoprecipitation; DOR, diminished ovarian reserve; E2F1, E2F transcription factor 1; GCs, granulosa cells; IGF-1R, insulin-like growth factor-1 receptor; LDHA, lactate dehydrogenase A; PGE2, prostaglandin E2; PCOS, polycystic ovary syndrome; PI3K-Akt, phosphoinositide 3-kinase-protein kinase B, POR, poor ovarian response; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RBMS1, RNA binding motif single stranded interacting protein 1; RT-PCR, reverse transcription-polymerase chain reaction; SF-1, steroidogenic factor 1; SMAD, mothers against decapentaplegic homolog; TGF-β, transforming growth factor-β; TGFBR1, transforming growth factor beta receptor 1; YAP1, Yes-associated protein 1.

1) Mitochondrial Pathways

miR-484

Two significant studies have particularly emphasized the importance of miR-484 in this process through distinct but related mechanisms. Wang et al[39] demonstrated that miR-484 mediates oxidative stress-induced ovarian dysfunction by targeting SESN2, leading to increased mitochondrial reactive oxygen species (ROS) production, compromised mitochondrial membrane potential, and reduced ATP levels in granulosa cells. The study showed that miR-484 overexpression disrupted mitochondrial dynamics and activated the mitochondrial apoptotic pathway, evidenced by increased cytochrome C release and elevated expression of proapoptotic proteins. In addition, Li et al[26] revealed another mechanism through which miR-484 impacts mitochondrial function by targeting Yes-associated protein 1 (*YAP1*), showing that increased miR-484 expression leads to mitochondrial dysfunction characterized by fragmented mitochondrial networks and depolarized mitochondrial membrane potential. Both studies demonstrated that rescuing the respective targets (SESN2 or YAP1) could restore mitochondrial function and prevent apoptosis, suggesting that miR-484 serves as a central regulator of mitochondrial-mediated granulosa cell (GC) apoptosis through multiple pathways. These findings have recognized miR-484 as a key mediator of mitochondrial-dependent granulosa cell death and suggest that targeting this miRNA or its downstream effectors could provide therapeutic opportunities for treating ovarian disorders characterized by excessive GC apoptosis.

miR-15a-5p

Another regulator is miR-15a-5p, which has been reported in several studies. Of particular significance, Zhang et al[27] demonstrated through miRNA sequencing and functional studies that elevated miR-15a-5p levels in poor ovarian response patients significantly impact the intrinsic apoptotic pathway. They showed that high levels of miR-15a-5p repressed granulosa cell proliferation and promoted apoptosis through direct regulation of BCL2 and BCL2-associated agonist of cell death (BAD), which are key regulators of mitochondrial-mediated apoptosis. Using KGN cell line experiments, they demonstrated that miR-15a-5p mimic transfection significantly decreased BCL2 expression while increasing proapoptotic markers. This finding was further supported by Wang et al[39] who, although studying preeclampsia, revealed that miR-15a-5p regulates the expression of apoptosis-related proteins including cleaved-caspase-3, BCL2-associated X protein (BAX), and BCL2, suggesting a conserved role in mitochondrial apoptotic signaling across different reproductive pathologies. Especially, both studies identified the PI3K/AKT pathway as a fundamental mediator of these effects, with Zhang et al,[27] showing that miR-15a-5p inhibits this survival pathway in granulosa cells, leading to increased mitochondrial-dependent apoptosis.[27] This mechanism was further elucidated by Naji et al[40] in their PCOS study, where they observed differential expression of miR-15a-5p between granulosa cells and follicular fluid, suggesting a complex regulatory network in the control of cell survival and death pathways. Collectively, these findings establish miR-15a-5p as a critical regulator of the mitochondrial apoptotic pathway in granulosa cells, primarily through its modulation of BCL2 family proteins and the PI3K/AKT signaling axis, contributing to our understanding of how microRNA dysregulation may lead to reproductive pathologies through altered granulosa cell survival.

miR-26

Numerous studies examining the molecular mechanisms of granulosa cell apoptosis have identified miR-26b as a significant regulator of cell death pathways. Liu et al[41] presented compelling evidence that miR-26b functions as a proapoptotic factor in porcine granulosa cells through mechanisms involving the mitochondrial death pathway. Using flow cytometry and molecular analyses, they demonstrated that miR-26b overexpression significantly altered the balance of key mitochondrial pathway proteins, decreasing antiapoptotic Bcl-2 levels while promoting expression of the proapoptotic protein BAX. Through detailed mechanistic studies, they revealed a novel HAS2-HA-CD44-Caspase-3 pathway through which miR-26b mediates its apoptotic effects, ultimately leading to activation of the intrinsic mitochondrial apoptotic cascade. Importantly, inhibition of miR-26b protected against granulosa cell apoptosis by preserving the expression of antiapoptotic factors, with a demonstrated increase in BCL2 and decrease in caspase-3 activation. Other studies have shown that miR-26 family members can promote granulosa cell apoptosis through different mechanisms; such as targeting *DHCR24* or *Ezh2*.[41, 42] These findings have shown miR-26b as a regulator of the intrinsic apoptotic pathway in granulosa cells, although further research is still needed to fully elucidate its interactions with other components of the mitochondrial death machinery.

Studies have demonstrated that miR-26b plays a significant role in promoting granulosa cell apoptosis through mitochondrial-dependent pathways. Liu et al[41] offered compelling evidence that miR-26b functions as a proapoptotic factor by directly targeting the *HAS2* gene, leading to downstream activation of the mitochondrial apoptotic pathway.

Through flow cytometry and molecular analyses, they demonstrated that miR-26b overexpression significantly decreased antiapoptotic BCL2 levels while promoting expression of the proapoptotic protein BAX. The study revealed a novel HAS2-HA-CD44-Caspase-3 pathway through which miR-26b mediates its apoptotic effects, ultimately leading to mitochondrial membrane permeabilization and cytochrome C release. This mechanism was further supported by Zhang et al[43] who showed that miR-26b's proapoptotic effects involve targeting *DHCR24*, which result in similar alterations in the BCL2/BAX ratio and subsequent activation of the intrinsic mitochondrial apoptotic pathway. Obviously, both studies demonstrated that inhibition of miR-26b protected against granulosa cell apoptosis by preserving mitochondrial integrity through maintenance of antiapoptotic protein expression. The importance of miR-26b in mitochondrial-mediated apoptosis was additionally corroborated by Huo et al[42] who showed that miR-26 family members (particularly miR-26a) regulate the expression of key apoptotic mediators involved in the mitochondrial death pathway. Together, these findings have shown miR-26b as a central regulator of the intrinsic apoptotic pathway in granulosa cells, suggesting its potential as a therapeutic target for disorders involving abnormal follicular atresia or granulosa cell death.

2) Cell Signaling

miR-33b

Research has revealed significant contributions of miR-33b to granulosa cell signaling pathways and survival mechanisms, particularly in the context of PCOS. In 2019, Li et al[15] reported that miR-33b is significantly upregulated (P = .032) in granulosa cells of PCOS patients and directly targets the transforming growth factor beta receptor 1 *(TGFBR1)* through specific binding to its 3′ UTR region. This targeting leads to suppression of the TGFB signaling pathway, which normally regulates cell proliferation and apoptosis. When combined with other miRNAs dysregulated, elevated miR-33b levels promote granulosa cell survival by increasing cell proliferation (P = .0098) and significantly reducing apoptotic rates (P = .027). This antiapoptotic effect is accompanied by cell cycle modifications, specifically an increase in S phase cell numbers (P = .0036), indicating enhanced cell division. Previous research has also linked miR-33b to metabolic regulation in PCOS, with Yang et al[44] showing its involvement in glucose transport through glucose transporter type 4 (GLUT4) regulation, suggesting a multifaceted role in PCOS pathogenesis. The dysregulation of miR-33b thus appears to contribute to the abnormal follicular development characteristic of PCOS through both direct effects on granulosa cell survival and broader metabolic impacts.

miR-142

The miR-142 is also reported in modulating granulosa cell apoptosis through complex interactions with the TGF-β signaling pathway. In a study by Li et al[15] they found that miR-142 is significantly upregulated in granulosa cells of PCOS patients (P = .021) and directly targets *TGFBR1* through binding sites in its 3′ UTR, as confirmed through dual luciferase reporter assays. This targeting relationship was shown to have functional consequences, as elevated miR-142 levels led to decreased TGFBR1 protein expression and subsequent inhibition of TGF-β signaling, evidenced by reduced phosphorylation of downstream effectors SMAD2 and SMAD3. The functional significance of this regulatory axis was demonstrated through cell-based assays, where miR-142 overexpression promoted granulosa cell proliferation and suppressed apoptosis, particularly by increasing

the proportion of cells in S-phase. These findings aligned with previous work showing miR-142's role as a TGF-β signaling repressor in other cell types,[45-47] but uniquely established its importance in ovarian function. Mechanistically, the disruption of TGF-β signaling by elevated miR-142 was found to alter the expression of key cell cycle regulators, with significant downregulation of *CDKN1A* and *CDKN2B* and upregulation of c-*MYC*, creating a molecular environment that favors cell survival over apoptosis. This pathway appears particularly relevant to PCOS pathogenesis, as patient samples consistently showed this pattern of elevated miR-142, reduced TGFBR1, and altered downstream signaling, suggesting that targeting this axis could have therapeutic potential in treating PCOS-related ovarian dysfunction.

miR-423

Several recent studies have supported evidence for miR-423's critical role in regulating granulosa cell function and apoptosis through complex signaling pathways. One study showed that miR-423 acts as a key inhibitor of granulosa cell apoptosis, particularly in the early stages, by directly targeting and repressing SMAD7 expression through interaction with its 3'UTR region.[32] This was further supported by Xu et al[48] who showed that miR-423 expression was significantly downregulated in granulosa cells from PCOS patients, leading to dysregulated cell proliferation and survival. The mechanistic roles were expanded by Xie et al[49] who revealed that miR-423 modulates granulosa cell function through the CSF1 pathway, affecting both cell survival and estradiol synthesis. Collectively, these studies have established a regulatory network where miR-423 functions as a central mediator in the TGF-β signaling pathway by targeting multiple components; most notably SMAD7, which has been consistently identified across studies as a direct target. When miR-423 levels are reduced, as observed in pathological conditions like PCOS, the resulting increase in SMAD7 expression leads to enhanced granulosa cell apoptosis and disrupted follicular development. This has been conclusively demonstrated through various experimental approaches, including luciferase reporter assays, flow cytometry analyses, and functional studies using miRNA mimics and inhibitors, which showed that restoring miR-423 levels could effectively suppress granulosa cell apoptosis and normalize TGF-β signaling. These findings not only illuminate the molecular mechanisms underlying granulosa cell regulation, but also suggest potential therapeutic strategies targeting the miR-423/SMAD7/TGF-β axis for treating ovarian disorders characterized by abnormal granulosa cell apoptosis.

miR-383

Several studies have provided compelling evidence for the critical role of miR-383 in regulating granulosa cell apoptosis through complex signaling pathways, particularly in the context of polycystic ovary syndrome (PCOS). Remarkably, Yin et al[19] demonstrated that miR-383 expression was significantly downregulated in TGF-β1-treated mouse granulosa cells, where it targeted E2F transcription factor 1 (E2F1) and steroidogenic factor 1 (SF-1) to regulate cell proliferation and steroidogenesis. Building on this foundation, Li et al[50] revealed a novel regulatory axis involving miR-383-5p and cold-inducible RNA binding protein (CIRP) in human granulosa cells. Their research showed that miR-383-5p expression was markedly decreased in PCOS patients' granulosa cells, exhibiting negative correlations with clinical parameters including body mass index (BMI), luteinizing hormone (LH) levels, and testosterone concentrations. Through sophisticated molecular analyses including

luciferase reporter assays and Western blotting, they established that miR-383-5p directly targets CIRP, which subsequently modulates the PI3K/AKT signaling pathway. The researchers demonstrated that overexpression of miR-383-5p enhanced granulosa cell apoptosis by suppressing CIRP expression, leading to decreased phosphorylation of PI3K and AKT, and increased expression of proapoptotic proteins including BAX and cleaved caspase-3. This mechanistic understanding was further validated through rescue experiments showing that CIRP overexpression could partially reverse the proapoptotic effects of miR-383-5p.[50] Collectively, these studies establish miR-383 as a master regulator of granulosa cell fate through its intricate modulation of multiple signaling pathways, including both the classical PI3K/AKT pathway and steroidogenic regulatory networks, suggesting its potential as both a diagnostic marker and therapeutic target in PCOS treatment.

miR-320

Based on the comprehensive analysis of recent studies, miR-320 has emerged as a critical regulator of granulosa cell function and survival through multiple signaling pathways. A study showed that miR-320 directly targets the runt-related transcription factor 2 (RUNX2) in cumulus granulosa cells, thereby modulating the expression of steroidogenic enzymes CYP11A1 and CYP19A1.[21] This regulation was shown to be IGF-1-dependent, as their in vitro studies revealed that IGF-1 stimulation significantly upregulated miR-320 expression in normal granulosa cells after 24 hours of treatment. Furthermore, Yin et al[19] established through luciferase reporter assays that miR-320 also targets E2F1 and SF-1, key transcription factors involved in cell cycle regulation and steroidogenesis. Their functional studies have demonstrated that miR-320 overexpression significantly increased granulosa cell apoptosis, as evidenced by enhanced cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP) proteins. More recent clinical research by Liu et al[51] corroborated these findings, and showed that altered miR-320 levels in granulosa cells correlate with cellular function and survival outcomes. Through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of 195 patient samples, they found that higher miR-320 expression levels were associated with increased apoptotic markers and reduced cell viability. To conclude, these studies have established a complex regulatory network where miR-320 influences granulosa cell fate through multiple mechanisms: direct regulation of steroidogenic pathways via RUNX2/CYP11A1/CYP19A1 signaling, modulation of cell cycle progression through E2F1 targeting, and regulation of apoptotic pathways through caspase-dependent mechanisms. This multilayered control emphasizes miR-320's crucial role in maintaining granulosa cell homeostasis and suggests its potential as a therapeutic target for reproductive disorders characterized by aberrant granulosa cell function.

miR-144

Research on miR-144's role in granulosa cell function also showed significant impact on cell survival and steroidogenesis through complex regulatory mechanisms. Zhou et al[33] found that miR-144 directly targets transcription factors E2F1 and SF-1, which are key regulators of granulosa cell proliferation and steroidogenic activity. Their research showed that miR-144 expression is regulated by the transcription factor CP2 and influences prostaglandin E2 (PGE2) production, which is important for ovulation and luteinization. The study utilized both in vitro and in vivo approaches, including luciferase reporter assays and flow cytometry, to establish that miR-144 modulates granulosa

cell functions through multiple pathways. This finding was corroborated by additional research[34] that confirmed miR-144's regulatory effects on granulosa cell proliferation and steroidogenesis. The multi-targeted nature of miR-144 suggests its possible role as a master regulator in ovarian function, coordinating both cell cycle progression and hormone production.

3) Metabolic Regulation

miR-34a-5p

Recent studies have provided compelling evidence for miR-34a-5p's central role in metabolic regulation and granulosa cell apoptosis, particularly in the context of ovarian dysfunction. Han et al[52] demonstrated through in vitro studies using chicken follicular granulosa cells that miR-34a-5p promotes both autophagy and apoptosis by targeting lymphoid enhancer-binding factor 1 (LEF1) and modulating the Hippo-Yes-associated protein (YAP) signaling pathway. This finding was corroborated by Fabová et al[53] who utilized porcine ovarian granulosa cells to show that miR-34a-5p inhibits cell proliferation while promoting apoptosis through the regulation of key proteins including BAX and caspase-3. Most recently, Cui et al[37] provided crucial mechanistic insights through their work with human KGN cells and clinical samples, demonstrating that miR-34a-5p directly targets lactate dehydrogenase A (LDHA), leading to impaired glycolysis and reduced energy availability in granulosa cells. Their research revealed a clear metabolic pathway where elevated miR-34a-5p levels, particularly evident in PCOS patients, suppress glycolytic enzyme activity (including HK2 and PKM2) and reduce pyruvate-to-lactate conversion, ultimately promoting granulosa cell apoptosis through the upregulation of proapoptotic factors and downregulation of antiapoptotic BCL2. Together, these studies have established a comprehensive understanding of how miR-34a-5p orchestrates a complex regulatory network that links metabolic dysfunction to cell death in granulosa cells, suggesting its potential as both a diagnostic marker and therapeutic target in ovarian disorders characterized by aberrant follicular development and increased granulosa cell apoptosis.

miR-19a-3p

Some other studies have investigated miR-19a-3p and confirmed it as significant regulator in granulosa cell apoptosis and metabolic pathways, particularly under copper-induced stress conditions. One of these is the 2021 Cui et al's[54] study which proved through microRNA profiling of follicular fluid that miR-19a-3p was differentially expressed between women with and without PCOS, suggesting its potential role in ovarian dysfunction. This finding was further substantiated by Chen et al[34] who observed that copper exposure significantly downregulated miR-19a-3p expression in human luteinized granulosa cells across multiple treatment concentrations (0.5-10.0 μg/mL). Through bioinformatic analyses and pathway mapping, they revealed that miR-19a-3p targets genes involved in critical signaling cascades, including the phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) and FOXO signaling pathways, which are fundamental to cell survival and apoptotic regulation. The researchers validated these findings using RT-qPCR, demonstrating that the downregulation of miR-19a-3p coincided with increased expression of apoptotic markers and activation of the caspase-dependent pathway. Notably, the altered expression of miR-19a-3p was observed to precede visible signs of cellular apoptosis, suggesting its potential role as an early molecular mediator in the stress response pathway.

This regulatory relationship indicates that miR-19a-3p may serve as a critical molecular switch in determining granulosa cell fate, potentially through its modulation of metabolic and survival pathways. Understanding this mechanism could provide valuable insights into both the pathogenesis of ovarian disorders and potential therapeutic approaches for maintaining granulosa cell health.

miR-19b-3p

Several studies have explained the probable role of miR-19b-3p in metabolic regulation pathways leading to granulosa cell apoptosis. It was hypothesized, through RNA sequencing and RT-qPCR validation, that copper exposure significantly altered miR-19b expression patterns in ovarian granulosa cells, with the miRNA being involved in the activation of the caspase-dependent apoptotic signaling pathway.[34] This finding was corroborated by Xie et al[55] who identified miR-19b-3p as one of the differentially expressed miRNAs in granulosa cells of ovarian hyperresponders, with their pathway analysis revealing its involvement in metabolic processes and cell proliferation regulation. Particularly, Ye et al[56] provided further evidence through exosomal miRNA profiling of follicular fluid, showing that miR-19b-3p was significantly upregulated (log_{FC}: 1.724336734, P = .041056) in PCOS patients and was associated with metabolic pathway regulation through KEGG pathway analysis. The study identified that miR-19b-3p participated in crucial metabolic signaling networks, including the PI3K-Akt and MAPK pathways, which are known regulators of granulosa cell survival and apoptosis. Interestingly, Nagata et al[57] found that miR-19b was highly abundant in the follicular fluid of young cows and demonstrated its importance in oocyte development, suggesting an age-dependent regulatory role. Collectively, these studies have established miR-19b-3p as a key regulatory molecule in metabolic pathways that influence granulosa cell fate, particularly through its involvement in apoptotic signaling cascades, making it a potential therapeutic target for ovarian disorders characterized by aberrant granulosa cell apoptosis.

miR-99

Reports on miR-99a have supported its vital role in regulating metabolic pathways and granulosa cell survival in the context of PCOS. Geng et al[18] conducted a comprehensive study demonstrating that miR-99a is significantly downregulated in granulosa cells of PCOS patients compared to controls. Through rigorous molecular analyses, they established that miR-99a directly targets IGF-1R, a key component of metabolic signaling pathways in ovarian cells. The researchers observed that the diminished expression of miR-99a corresponded with increased IGF-1R levels, leading to dysregulated cell proliferation and abnormal follicular development characteristic of PCOS. Functionally, their in vitro experiments showed that restoring miR-99a levels effectively reduced IGF-1R expression and normalized granulosa cell function, including proliferation rates and apoptotic patterns. This regulatory relationship was further validated through target validation studies, confirming that miR-99a's effects were specifically mediated through IGF-1R targeting. The study's findings have established miR-99a as a possible biomarker and therapeutic target for PCOS.

miR-27a-3p

Research on miR-27a-3p has revealed it plays a significant role in granulosa cell apoptosis through multiple pathways. According to Nie et al,[6] miR-27a promotes human

granulosa cell apoptosis by directly targeting SMAD5, activating the FasL-Fas signaling pathway that increases levels of Fas, FasL, cleaved caspase-8, and cleaved caspase-3. This finding has been complemented by Tao et al,[30] who demonstrated that miR-27a-3p inhibits mouse granulosa cell proliferation by targeting Vangl1 and Vangl2, which are key components in the Wnt signaling pathway. Their research showed that overexpression of miR-27a-3p significantly suppressed granulosa cell proliferation, while silencing it had the opposite effect. The myogenic differentiation (MyoD) transcription factor was identified as a regulator that binds to and activates the miR-27a-3p promoter. Together, these studies establish miR-27a-3p as an important regulator in ovarian follicle development, functioning primarily as a proapoptotic factor in granulosa cells across multiple species by targeting different genes that ultimately affect cell survival pathways.

Discussion

The involved regulation of granulosa cell apoptosis by microRNAs is a complex molecular network, that coordinates follicular development and atresia through multiple distinct but interconnected pathways. Current evidence demonstrates that these regulatory molecules primarily operate through 3 major mechanistic axes: the mitochondrial pathway, cell signaling cascades, and metabolic regulation. In the mitochondrial pathway, key players such as miR-484, miR-15a-5p, and miR-26b have been shown to modulate the expression of critical apoptotic proteins, particularly members of the BCL2 family, ultimately controlling cytochrome C release and downstream caspase activation. The significance of this regulation is particularly evident in pathological conditions, where dysregulation of these miRNAs leads to abnormal apoptotic patterns and subsequent follicular development abnormalities. For instance, studies have demonstrated that miR-484 simultaneously targets multiple proteins including SESN2 and YAP1, creating a sophisticated regulatory network that fine-tunes mitochondrial function and cellular survival.[26, 38] Also, it regulates activities of miR-15a-5p through BCL2 and BAD, and miR-26b via the HAS2-HA-CD44-Caspase-3 pathway.[27, 41] These findings together support the remarkable complexity and precision of miRNA-mediated regulation in granulosa cell apoptosis (Figure 1).

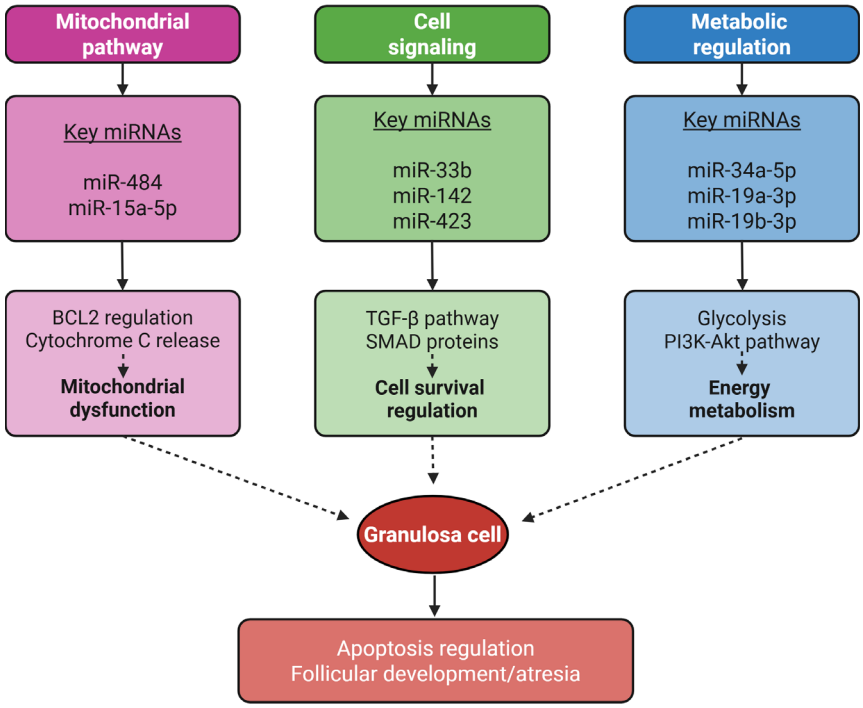
The diagram illustrates the mitochondrial pathway (involving miR-484 and miR-15a-5p, regulating BCL2 and cytochrome C), cell signaling pathway (including miR-33b, miR-142, and miR-423, modulating TGF-β and SMAD proteins), and metabolic regulation pathway (featuring miR-34a-5p, miR-19a-3p, and miR-19b-3p, affecting glycolysis and PI3K-Akt signaling). These pathways converge to control granulosa cell fate through apoptosis regulation and follicular development/atresia.

The cell signaling dimension of miRNA-mediated granulosa cell apoptosis regulation has revealed an equally sophisticated network, predominantly centered around the TGF-β signaling pathway and its associated molecules. Several key miRNAs, including miR-33b, miR-142, miR-423, miR-383, and miR-320, have been identified as crucial regulators of these signaling cascades. Of particular significance is the coordinated regulation of the TGF-β pathway through multiple miRNAs targeting different components of the signaling mechanism. For instance, both miR-33b and miR-142 directly target TGFBR1, leading to suppressed TGF-β signaling and altered cell survival outcomes in PCOS patients.[15] This regulatory complexity is further exemplified by miR-423's role in modulating SMAD7 expression and subsequent TGF-β pathway activity.[32, 48] Furthermore, miR-383 has

demonstrated the interconnected nature of these pathways through its regulation of the PI3K/AKT signaling cascade via CIRP targeting, while miR-320's simultaneous control of steroidogenic enzymes and cell cycle regulators denotes the complicated nature of these regulatory networks.[50, 51]

The metabolic dimension of miRNA-mediated granulosa cell apoptosis adds another layer of complexity to this regulatory network, particularly through the actions of miR-34a-5p, miR-19a-3p, and miR-19b-3p. Recent evidence has revealed that these miRNAs serve as critical mediators between cellular metabolism and apoptotic pathways. Most notably, miR-34a-5p has been shown to directly influence glycolytic metabolism through LDHA targeting, demonstrating how metabolic disruption can trigger apoptotic cascades in granulosa cells.[37] The importance of these metabolic regulations is further emphasized by the differential expression patterns of miR-19a-3p and miR-19b-3p observed in various pathological conditions, particularly their involvement in PI3K-Akt and FOXO signaling pathways.[34, 56] These findings collectively support the remarkable complexity and precision of miRNA-mediated regulation in granulosa cell apoptosis.

Figure 1. Three Major Pathways in microRNA-Mediated Regulation of Granulosa Cell Apoptosis



Abbreviations: SMAD, mothers against decapentaplegic homolog; TGF-β, transforming growth factor-β.

Conclusions

This review focused on the complex nature of microRNA-mediated regulation in granulosa cell apoptosis as well as its fundamental importance in ovarian function and fertility. Through the detailed examination of 3 primary regulatory mechanisms: mitochondrial pathways, cell signaling cascades, and metabolic regulation; it can be concluded that miRNAs act as necessary molecular switches controlling granulosa cell fate. The complex interaction between these pathways, coordinated by specific miRNAs targeting multiple components within each mechanism, has revealed an advanced regulatory network that maintains proper follicular development and atresia. Of particular significance is the developing evidence that dysregulation of these miRNA-mediated processes contributes significantly to reproductive disorders, especially PCOS and premature ovarian failure. However, and due to the topic's importance, future research should focus on translating these molecular targets into practical clinical applications, particularly in developing targeted treatments that can modulate specific miRNA pathways to restore normal ovarian function in pathological conditions.

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Review Article

Conceptualizing Functional Ability in Heart Failure: A Concept Analysis and Implications for Nursing Practice

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Abstract

The review article discussed the concept of functional ability. It is a critical outcome in patients with heart failure that reflects the prognosis of the disease. Therefore, a deeper understanding of this term's meaning, components, and operational definition is required in nursing practice. This paper aims to clarify the concept of functional ability for people with heart failure. This term is used in many ways, but its meaning, components, and assessment may vary. Consequently, the concept analysis was used to develop an operational definition, critical attributes, antecedents, consequences, and empirical referents. Functional ability encompasses physical, social, and psychological components essential for independent living in patients with heart failure. Determining the defining attributes includes physical ability, social ability, and psychological ability. This paper also defines the antecedents of functional ability, consisting of musculoskeletal capacity and muscle strength, the ability to move, good communication skills, and good cognition. Consequences include quality of life and well-being, health status, vital lung capacity, disease prognosis, good relationship, depression, dependence, fall, and hospitalization. In addition, the article provides empirical references for the measurement of functional abilities, such as the assessment of physical activity in daily life, mobility and work, the assessment of behavior in social activities, interaction and communication with others, and the assessment of emotion and feelings. This review provides a clearer understanding of the concept of functional ability and a new emphasis on the importance of targeting interventions to promote functional ability for patients with heart failure.

Keywords: Functional ability, Heart failure, Concept analysis

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Introduction

Patients with heart failure exhibit reduced functional abilities, which have been linked to their prognosis, clinical outcomes, and consequent loss of quality of life.[1] Functional ability is significant for the cardiovascular, muscular, and physiological assessments in chronic disease, including heart failure. It has a crucial role in predicting disease prognosis.[2-4] Functional ability has several meanings and is often used in operational definition in the health care research.[5, 6] The literature lacks clarity regarding the meaning and components of functional ability. Most definitions rely on functional domains assessed using various tools, primarily focusing on physical function and the ability to perform activities of daily living (ADL).[7-9]

Based on the literature, functional ability has several components including physical activity,[5] physical ability,[10, 11] ADL,[5, 12] and combined abilities.[2, 13-15] Good functional ability is

associated with good clinical outcomes, prognosis, health status, and reduced disease severity.[14] Functional ability is often confused with functional status and capacity, though they differ.[16-18] Functional status refers to patient-centered health outcomes,[19] while functional capacity is defined by maximal oxygen uptake.[17, 20]

Although efforts have been made to clarify what functional ability is, there is still considerable confusion.[7] In both clinical practice and research studies, it is difficult to improve functional ability when there are still unknown definitions, meanings, components, and appropriate instruments. The literature review also revealed that operational definitions and instruments vary across studies.[13, 21, 22] In addition, no study has conducted a systematic review and meta-analysis (SRMA) of functional ability in heart failure. Existing evidence was only found in the SRMA on functional status [23] and functional capacity in patients with heart failure.[24] To achieve consistent scientific communication about functional ability, this paper aimed to generate an analysis of the concept of functional ability especially with the relation to heart failure. Clarifying a vague concept and providing an accurate meaning, operational definition, components, and measured instrument of functional ability would be useful to determine prognosis, readmission rate, hospitalization, and mortality in patients with heart failure.[25] Walker and Avant's[26] method of concept analysis was used to guide this study as it provides a systematic and structured approach to clarify and define concepts which is essential for understanding and advancing knowledge in nursing and other disciplines. In addition, this approach is easy to understand, so that it can be readily applied by the researchers.[27]

Concept Analysis of Functional Ability

The 8-step of concept analysis according to the approach of Walker et al[26] are as follows: 1) selection of a concept, 2) determining the aims or purposes of the analysis, 3) identification of all concept uses, 4) determining the defining attributes, 5) identifying a model case, 6) identifying borderline cases, related cases, and contrary cases, 7) identifying antecedents and consequences, and 8) defining empirical referents.

Selection of Concept

The concept of functional ability was chosen to conduct the concept analysis because it is widely used in nursing, and it has various definitions, components, and instruments to measure that has been used in nursing research and practice.[16, 28] Furthermore, this concept is not a primitive umbrella term, but it is important for functional assessment and reflection of the quality of care, quality of life, clinical outcomes, and prognosis of patients with heart failure.

Determining the Aims or Purposes of the Analysis

The methods could be used to clarify the meaning and examine components of functional ability, develop an operational definition and select the appropriate instrument for assessing functional ability that can be used in nursing research on patients with heart failure patients.[26]

Identification of All Concept Uses

The term 'functional ability' has several meanings. The Medical Dictionary defines it as the ability to perform activities of daily living, including bathing, dressing, and other

independent living skills, such as shopping and housework.[29] The Cambridge Dictionary splits this term into 2 words: functional and ability,[30] 'functional' refers to working in the expected or necessary way, while 'ability' is the physical or mental strength or skill needed to do something. The Encyclopedia describes functional ability as the ability to manage daily life, including basic functions that enable people to socialize, work, or engage in a variety of other productive and social activities, including self-care activities and ADL that reflect a person's ability to live independently in the community.[31] In addition, the World Health Organization (WHO) states that functional ability is a combination of the individual's intrinsic ability, relevant environmental characteristics, and interaction between them.[32]

Functional ability was identified as a keyword for searches in the Google Scholar, PubMed, CINAHL, ProQuest, ThaiJo, and Cochrane databases. The word 'functional ability' is included either in the title, content, or keyword and serves as a criterion for the selection of research articles. The literature was searched in English for the ones published up to 2024. The authors used meanings from various fields, including nursing, medicine, and others.[26] In many articles, the term was not clearly defined. Thus, the literature search revealed 38 articles that clearly defined functional ability (Table 1) and presented the number of articles appearing in each attribute (Table 2).

Definition of Functional Ability

Based on the literature review, several keywords for functional ability cover a wide variety of disciplines, including physiology, psychology, medicine, and nursing. It can be summarized that functional ability consists of 3 aspects: physical, social, and psychological abilities, and the definition of functional ability can be refers to the physical, social, and psychological ability to perform independent ADL.

Table 1. Definitions of Functional Ability

Author	Field	Definition
Avlund et al,[5] 1995	Nursing	Ability to perform ADL
Han et al,[8] 2022	Nursing	Ability to perform ADL
Mandelli et al,[9] 2020	Nursing	Ability to perform ADL
Elsawy et al,[12] 2011	Nursing	Ability to perform ADL
Lara-Ruiz,[21] 2019	Nursing	Ability to perform ADL
Sablonnière et al,[33] 2021	Nursing	Ability to perform ADL
Oliveira et al,[34] 2020	Nursing	Ability to perform ADL
Kalpana et al,[10] 2021	Nursing	Ability to perform ADL
Rodrigues et al,[35] 2008	Nursing	Ability to perform ADL
Yaqoob et al,[36] 2018	Nursing	Ability to perform ADL
Elżbieciak,[37] 2017	Nursing	Ability to perform ADL
Nielsen et al,[38] 2014	Nursing	Ability to perform ADL
Ohenewa et al,[39] 2021	Nursing	Ability to perform ADL
Advinha et al,[40] 2021	Nursing	Ability to perform ADL
Chinchai et al,[41] 2017	Nursing	Ability to perform ADL and communication

Table 1. Definitions of Functional Ability (Continued)

Author	Field	Definition
Gialanella et al,[42] 2022	Nursing	Ability to perform ADL and muscle strength of lower limbs and range of motion of lower limbs
Purdy,[43] 2002	Nursing	Ability to perform ADL, linguistic abilities, cognitive abilities, and communication
Finkel,[44] 2020	Nursing	Performance in 3 factors: balance, fine motor skills, and flexibility
Lopes et al,[45] 2020	Nursing	The intrinsic abilities of the individual (physical, mental, and psychosocial abilities), the environment in which they live, and the interaction between the individual and this environment
Lučkin et al,[16] 2021	Nursing	The ability of a person to perform all daily activities ensures an adequate quality of life, including biological, psychological, and social functions
Kaushik et al,[46] 2020	Nursing	Grip strength, flexion, and extension range of motion
Martins et al,[47] 2020	Nursing	The confidence in the ability to perform the exercise
Memel,[2] 2008	Nursing	Physical function (ability to work, perform ADL, mental health, and social functioning
Okiljević et al,[48] 2017	Physiology	Ability to engage in regular physical exercise
Stankoviç et al,[49] 2019	Physiology	Ability to physically exercise
Paulsamy et al,[50] 2021	Nursing	Isometric and isotonic quadriceps contraction exercise
Qureshi et al,[28] 2021	Nursing	Quadriceps strengthening exercise
Fallatah et al,[51] 2021	Nursing	Ability to perform active range of motion exercises, flexed and extended neck slowly without holding at the end ranges
Sporis et al,[52] 2011	Nursing	Ability to walk on a platform
García-Garro et al,[53] 2020	Nursing	Verbal fluency, executive function, functional flexibility, and lower-body strength
Su et al,[15] 2021	Nursing	Cognitive, emotional, interpersonal, and physical functional abilities
Kim et al,[13] 2019	Nursing	Physical. psychological function, communication, and cognition
Tippett et al,[54] 2013	Nursing	Task performance and cognitive-motor integration abilities
Moreno-Agostino et al,[3] 2021	Nursing	Activities and participation (mobility, hand and arm use, self-care, sensory functioning, interpersonal relationships, cognition)
Migaj et al,[1] 2022	Medicine	Ability to perform everyday life activities that require specific endurance and physical fitness
Gravenstein et al,[6] 2024	Medicine	The subject's ability to perform everyday executive function tasks for independent living, and the associated availability of resources to complete the tasks

Abbreviation: ADL, activities of daily living.

Table 2. The Number of Articles That Occur in Each Attribute of the 38 Articles

Attribute	No. of Articles
Physical ability	38
Social ability	11
Psychological ability	7

Determining the Defining Attributes

Attributes of a concept are the features of the concept that appear repeatedly in the evidence.[55] Walker et al[26] explained that the attributes of a concept are the heart of concept analysis because this step was written to explain the group of attributes that are most frequently associated with the concept and that provide the analyst with a broader insight into the concept (Table 3).

Identifying a Model Case

According to Walker et al[26] the fifth step of concept analysis is the identification of a model case. A model case is an example of the use of the concept that shows all the defining attributes of the concept. For example, the model case is specified as follows:

Miss A, 55 years, patient with heart failure, enrolled to be treated in the heart failure clinic for 6 months. She goes to see the doctor at every appointment, takes medicines regularly, exercises at least 3-5 days per week, limits the amount of salty diet, and measures the fluid intake and output every day, including weight measurement. She walks across the footbridge to go marketing every day (physical ability). She goes to make merits at the temple on Buddhist holy days and talks to others about the development of the village after the completion of religious ceremonies (social ability). She feels happy to join with other people, and she is not worried about heart disease (psychological ability).

This model case includes all 3 important attributes: physical, social, and psychological abilities.

Identifying Additional Cases

The sixth step of concept analysis is identifying additional cases consisting of a borderline case, a related case, a contrary case, an invented case, and an illegitimate case.[26] The additional cases are as follows:

Borderline Case: This case is an example that contains most of the defining attributes of the concept being examined, but not all. An example is shown as follows:

Table 3. Attributes of the Functional Ability Concept

Attribute	Explanation
Physical ability	• Ability to do independent physical activity in daily living • Mobility • Ability to work
Social ability	• Ability to maintain relationships and engage in social activities • Interaction and communication with others • Participation in community activities
Psychological ability	• Feeling good • Less worry, stress, and depression. • Not feeling down

Mr. B is receiving care from the heart failure clinic for 1 month. Today, he has a follow-up at the clinic. A nurse provides him with a 6-minute walk test, and he can walk 700 meters without dyspnea with an oxygen saturation of 96% (physical ability). He can tell the dietitian the menu that he has eaten in the past week. Moreover, a psychologist found that over the last 2 weeks, he has also interested or pleased in doing things in daily life, and he has not felt down, depressed, or hopeless (Patient Health Questionnaire-2: negative) (psychological ability). On the other hand, his daughter said that her father didn't hang out with friends as often as before because he was tired (incomplete social ability).

In this case, there are proper physical and psychological abilities that are the most crucial attributes of functional ability, but the social ability is not complete.

Related Case: This case is also an instance of a concept that is related to the concept being studied but does not contain all defining attributes. The related case is similar to the model case but is not them.

Mr. C, 60 years, has congestive heart failure. He was hospitalized with volume overload, orthopnea, crepitation in both lungs and ankle edema. Chest x-ray shows pulmonary congestion and cephalization in both lungs, and echocardiography shows left ventricular ejection fraction of 35%. Today he feels comfortable and happy, talks with other patients in a friendly manner. In addition, his son helps Mr. C with his daily activities such as feeding, taking a bath, dressing, walking, and practicing hygiene because Mr. C sometimes gets exhausted.

Mr. C has a good quality of life that is similar to the concept of functional ability but he cannot do all activities by his own and there is suboptimal physical ability in this case.

Contrary Case: This case is a clear example of the functional ability concept.

Miss D, 55 years, diagnosed with heart failure for 10 years, is unable to walk, eat, take a bath, or dress by herself. She doesn't do anything, she wants to be alone, and she sometimes cries. Thus, today her daughter takes her to see a doctor. While she is waiting to see the physician, she has dyspnea, an oxygen saturation of 80%, respiratory rate (RR) of 32 breaths/minute, pulse rate of 90 beats/minute, and blood pressure of 90/60 mmHg. Then, she is intubated with mechanical ventilator setting of a pressure control mode, RR 12, positive end-expiratory pressure (PEEP) 5, fraction of inspired oxygen (FiO_2) 0.4, on fentanyl (10:1) rate 5 mL/hour, and the Richmond Agitation-Sedation score (RASS) -3 under sedative drug.

There are no significant attributes of the functional ability concept in this case because the case study cannot function in terms of physical, psychological, and social ability. Therefore, this situation is the opposite condition of the concept of functional ability.

Identifying Antecedents and Consequences

Identifying antecedents and consequences is the seventh step in a concept analysis of functional ability. Antecedents are those events that must have taken place prior to the occurrence of the concept. Consequences are incidents that occur as a result of the occurrence of the concept or the outcome of the concept, and they contain positive and negative outcomes for patients (Figure 1).[26]

Antecedents: Literature revealed several antecedents related to the concept of functional ability. These include musculoskeletal capacity and muscle strength,[5, 42, 45, 46, 48, 51, 52, 54, 55, 56] ability to move,[5, 28, 43, 45, 47, 48, 51, 54] good communication skills,[15, 21, 42, 44, 57] and good cognitive function.[13, 15, 44, 55]

Consequences: Consequences consist of positive consequences and negative consequences. The positive consequences include quality of life or well-being,[15, 16, 22, 38] good health status,[2, 5, 9-10, 12, 16, 21, 22, 33-34, 36-38, 40, 42, 43, 44, 46, 51] normal vital lung capacity, good disease prognosis, and good relationships.[15, 46, 48] While, depression,[2, 15] dependence,[12, 39, 42] fall,[5, 42, 45, 46, 48, 51, 52, 54, 55, 56] and hospitalization[25] are the negative consequences.

Defining Empirical Referents

The last step in a concept analysis is empirical referents which are categories of actual phenomena that, by their presence, demonstrate the occurrence of the concept itself. If we are to measure the functional ability concept, we must assess 3 attributes, including physical ability, social ability, and psychological ability (Table 4).

Figure 1. Antecedents, Critical Attributes, and Consequences of Functional Ability

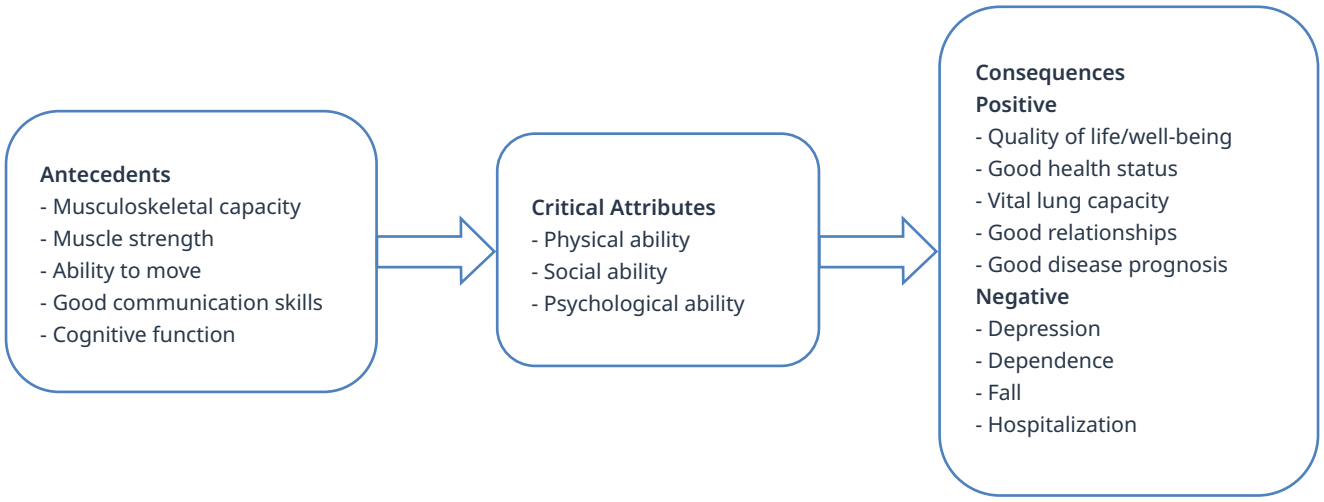

Table 4. The Empirical Referents of the Functional Ability Concept

Attribute	Empirical Referent
Physical ability	• Ask about physical activity in daily living, mobility, and work • Assess the patient's ability to perform daily activities, mobility, and work by using questionnaires • Assess patients' ability through methods created by the health care providers and observe the behaviors of the patients' instructions
Social ability	• Observe the behaviors of social activities, interaction, and communication with others • Assess the patient's ability to participate in social events by using questionnaires
Psychological ability	• Self-reported mood scales and clinical patient engagement and emotional expression observations • Mental health assessed by using standardized questionnaires

Instruments for Functional Ability Assessment

There are several instruments for the evaluation of functional ability. Most of the studies measure physical aspects of functional ability, consisting of the basic ADL,[9, 12, 22, 33, 35, 38, 43] instrumental ADL,[5, 6, 9, 12, 33, 38, 43, 54] Timed Up and Go Test,[11, 28, 47] Living Skills and Resources Revision 2,[6] Five Times Sit-to-Stand Test,[11] 6-minute walk test,[11] 10-meter walk test,[11] Functional Activities Questionnaire,[33] spontaneous behavior interview-basic activities of daily living (SBI-BADL),[9] Pfeffer Functional Activities Questionnaire,[34] Kujala score,[10] Michigan Hand Questionnaire,[46] Step Test,[47] Stage Balance Test Modified,[47] Lawton instrumental activities of daily living scale,[12, 37, 38] Care dependency scale,[37] Katz Index of Independence in ADL,[12, 37, 38, 42] and vital lung capacity.[48, 49] Physical ability and social abilities can be assessed by the Functional Independence Measures,[41] the Direct Assessment of Functional Status,[21] and the Health Assessment Questionnaire,[2] which assesses ADL. For social ability, the Activities and Participation Profile related to Mobility[47] and the Porch Index of Communicative Ability[43] can be used. In addition, the tools can be assessed for psychological ability are Mini-Mental State Examination for depression[37] and self-efficacy for exercise.[47]

In conclusion, the instruments can be used to measure all 3 critical attributes of concepts, including long-term care assessment evaluation, the 36-item Short Form Health Survey (SF-36) (use subscales),[15] and the WHO Quality of Life (use subscales).[36] Most studies measure functional ability only in the physical domain.[9, 10, 11, 12, 28, 33, 34, 35, 39, 42, 44, 46, 48, 49, 51, 53, 56] Furthermore, some literature found that the researchers used a combination of instruments to measure functional ability.[2, 15, 16, 21, 22, 37, 38, 41, 43, 45, 47, 54, 57]

Implications for Nursing Practice

Analyzing the concept of functional ability in older adults and patients with heart failure has excellent implications for the development of nursing knowledge and practice. Concept analysis of this concept provides a clear understanding of the meaning, attributes, antecedents, and consequences, including assessment of the outcomes of the concept. The findings are used to assess the physical and social abilities that indicate disease prognosis, quality of life, well-being, and health status. In addition, the clinical implications of this review can guide nurses in providing care interventions that improve the functional abilities of patients with heart failure.

Implications for Nursing Research

The implications of this report for research are that 1) this concept analysis can be used as a developmental instrument to directly measure functional ability for persons with heart failure, and 2) nursing interventions are needed to increase the functional ability of people with heart failure.

Conclusions

The concept of functional ability analysis produces the attributes of physical, social, and psychological abilities. These results provide a clearer understanding of the concept of functional ability in terms of meaning, antecedents, important attributes, consequences, and empirical referents that are significant in nursing. This concept analysis offers a valuable perspective on functional ability that guides the nursing profession and clinical research in providing better interventions to improve functional ability in

patients with heart failure. Moreover, the results of the analysis provide a definition and characteristics of functional ability that are useful for measuring nursing outcomes. In addition, it is also an advantage in the development of research tools.

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Case Report

Single-Incision Laparoscopic Common Bile Duct Exploration and Cholecystectomy: An Innovative Transcystic Technique

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Abstract

Background: Common bile duct stones may be managed via 2-stage endoscopic retrograde cholangiopancreatography followed by single-incision laparoscopic cholecystectomy, or 1-stage laparoscopic common bile duct exploration and cholecystectomy (LCBDE+LC). This study reports early experience and technique in 1-stage single-incision laparoscopic common bile duct exploration and cholecystectomy (SILCBDE+SILC).

Case Presentation: This study analyzed 10 consecutive cases of choledocholithiasis that underwent SILCBDE+SILC from April 2022 to December 2023. The surgical technique involved the innovative use of an Endoscopic Applicator to better stabilize the choledochoscope for cystic duct cannulation. The mean (SD) operative time was 99 (34) minutes. All patients had 100% stone clearance rate and cholecystectomies with no complications. No conversion to multiport or open surgery was noted. Mean postoperative hospital stay was 1.5 days. Postoperative morbidity and mortality were 0%.

Conclusions: 1-stage SILCBDE+SILC is safe. This study's technique involving the use of an Endoscopic Applicator addresses the difficulties of manipulating the choledochoscope for cystic duct cannulation, despite its flexible nature and the extra distance between its entry port-site and the cystic ductotomy.

Keywords: Stones, Choledocholithiasis, Bile duct, Single-incision, Transcystic

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Introduction

Common bile duct (CBD) stones are estimated to be present in about 1% to 15% of individuals with gallstones. They can result in symptoms and complications such as biliary colic, jaundice, cholangitis, or pancreatitis.[1]

In the past, the treatment of choledocholithiasis involved an open CBD exploration and cholecystectomy in the same setting. With the first laparoscopic cholecystectomy (LC) performed by Mühe in 1985,[2] together with the option of removing bile duct stone via endoscopic retrograde cholangiopancreatography (ERCP), the strategy of performing 2-stage preoperative ERCP followed by LC became appealing. Subsequently, with the advancement in the laparoscopic technique, 1-stage multiport laparoscopic common bile duct exploration and cholecystectomy (LCBDE+LC) became a viable option. In 2 separate randomized controlled trials by Rogers et al[3] and Cuschieri et al,[4] both approaches demonstrated comparable success and adverse events, with shorter hospital stay for the latter.

Conventional LC utilizes 4 transabdominal ports – the first port is inserted at the umbilical region for the laparoscope, and the remaining 3 ports allow for abdominal access of laparoscopic instruments to perform the surgery. In conventional LCBDE+LC, the choledochoscope can be introduced via one of the 3 ports; instruments through one of the remaining ports are used to manipulate it. No additional port is typically required.

In recent years, single-incision laparoscopic cholecystectomy (SILC) via the umbilical region has been developed to minimize the number of transabdominal ports in an attempt to further reduce the pain associated with abdominal access as well as to improve cosmetic satisfaction.[5, 6] Anecdotally, it has also been observed that some patients with persistent pain at the site of one or more of the 3 instrument ports in conventional LC, which may be related to an underlying subcostal nerve injury, experience persistent neuralgia. With fewer port-sites in SILC, such nerve injury may be avoided and there is also a theoretically reduced risk of surgical site infection.

The main technical difficulties for SILC stem from the increased distance between the port-site and the cystic duct, limited angulation, and an inability to provide retraction in the conventional way due to the procedure's distinctive single axis approach of all instruments. Techniques have been described to overcome some of these difficulties.[5]

In the case of CBD stones, there is often a dilemma of deciding between forgoing the benefits of single-incision surgery with conventional LCBDE+LC or resorting to a 2-stage procedure with ERCP followed by SILC. In 2014, Chuang et al[7] reported safe and successful 1-stage single-incision laparoscopic common bile duct exploration with cholecystectomy (SILCBDE+SILC) in a comparative study of 34 patients with conventional LCBDE+LC via both transductal and transcystic approaches. With the need to introduce and manipulate a choledochoscope into the bile duct, a good control of it in the abdomen is thus required.

This study series describes 10 patients who underwent 1-stage SILCBDE+SILC via the transcystic approach with successful outcomes, and demonstrates how good control of the choledochoscope was obtained.

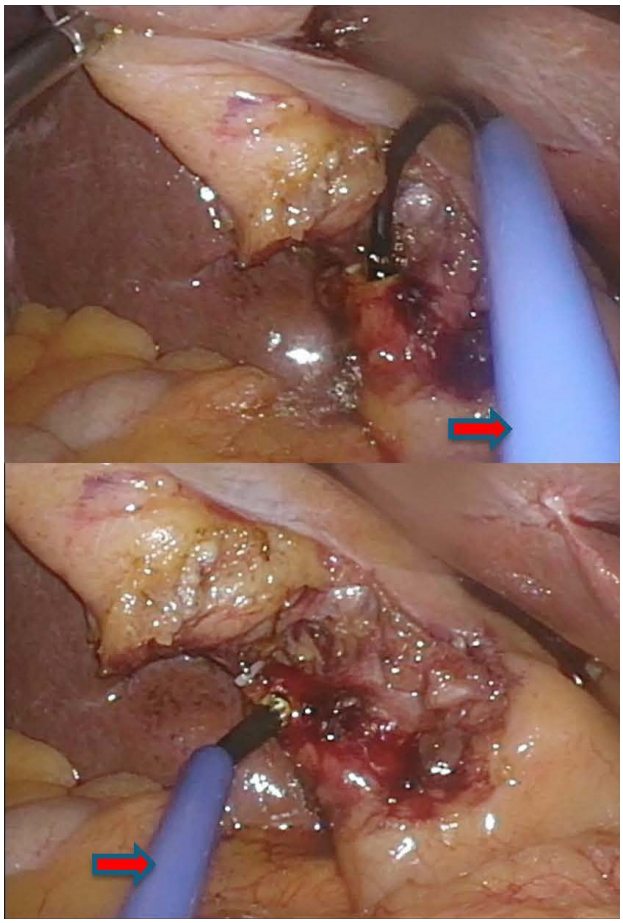
Case Presentation

Retrospective data analysis was conducted for 10 consecutive cases of choledocholithiasis that underwent 1-stage SILCBDE+SILC via the transcystic approach, performed by a surgeon (CSKY)-nurse team from April 2022 to December 2023. Patient demographics, clinical presentations, and operative results were recorded. Operative time was defined as the interval between initial skin incision and skin closure. Postoperative length of hospital stay (PLOS) was defined as the number of days between the day of surgery and the day of discharge.

A single 10-mm (8/10) or 15-mm transumbilical incision was made with open technique and an improvized multichannel glove port (9/10) or a TriPort+™ port (Olympus, Hachioji-shi, Tokyo, Japan) was inserted. Abdominal access and pneumoperitoneum were established. The gallbladder fundus and neck were each retracted with a laparoscopic grasper, Calot's triangle was dissected, and the cystic duct and cystic artery were identified (critical view of safety established). The distal cystic duct was secured with a hemolock clip and a cystic ductotomy was performed proximal to it. The cystic duct was then cannulated with a LithoVue™ Single-Use Digital Flexible Ureteroscope (Boston Scientific, Maple Grove, Minnesota, USA) through a Floseal™ Endoscopic Applicator (Baxter, Deerfield, Illinois, USA), which in turn was passed through one of the port channels. The Endoscopic Applicator

was employed in all 10 cases and served as a guide to direct the endoscope towards the cystic duct (Figure 1 and Supplementary S1). CBD stones were visualized with the endoscope and removed with a stone basket. Both the upper and lower tracts of the biliary duct were also explored to ensure complete stone clearance. Thereafter, the proximal cystic duct was clipped with 2 of 10-mm hemolocks and transected. The cystic artery was likewise clipped with hemolock and transected. Finally, the gallbladder was dissected off the cystic plate with care and placed in an endobag. With adequate haemostasis inspected, bile leak checked, and abdomen irrigated, the port was removed with the gallbladder specimen in the endobag. Local anaesthetic was infiltrated into the preperitoneal region of the single wound. The linea alba defect was closed with Polysorb™ 2/0 (Covidien, Mansfield, Massachusetts, USA) using 2 of figure-of-8 stitches and skin closure was performed with Biosyn™ 4/0 (Covidien, Mansfield, Massachusetts, USA).

Figure 1. Use of Endoscopic Applicator (Red Arrow) to Assist in Control and Manipulation of the Choledochoscope



Postoperatively, all patients were transferred to the general ward. Minimal analgesia was usually required. All patients were discharged with no complications and followed up at 3 days, 1 month, and 6 months later with biochemical tests (full blood count and liver function test) at the clinic where possible.

Most patients were female (6/10), of Chinese ethnicity (8/10), and had a mean (range) age of 47 (22-67) years. Two patients were referred to this study's surgeon after undergoing ERCP with stenting and had CBD stones in situ. None had prior gallbladder related procedures (Table 1). Eight patients had comorbidities and/or past surgical procedures, of which 5 had hepato-pancreato-biliary conditions or surgeries that involved the abdominal wall: the first had fibroid surgery in 2005, the second had ovarian adenocarcinoma surgery in 2019, the third had single-incision laparoscopic appendectomy 43 days before the SILCBDE+SILC procedure, the fourth had hepatitis B, had undergone total hysterectomy bilateral salpingo-oophorectomy in 2013, and had been on hormonal therapy after a breast-conserving surgery for breast cancer in 2016, and the fifth had lower segment caesarean section in 1982.

The majority of this study's patients presented with epigastric pain or discomfort (6/10), jaundice (6/10), and had raised liver function tests (8/10), marginally dilated CBD (range 7.0-12.8 mm) (8/10), and CBD stones less than 10-mm in diameter (9/10). Data for the remaining CBD diameters of 2 patients were unavailable, with 1 of the 2 patients recently passing a CBD stone prior to the procedure. As a result, the diameter of this patient's CBD stone could not be determined. One patient had acute cholangitis, and 1 patient had acute pancreatitis secondary to small CBD stones in the ampullary region. None presented with fever, although 1 patient reported experiencing chills. Clinical presentations, imaging results, and values of all biochemical tests were obtained prior to the procedure (Table 2). Two patients were asymptomatic with 1 having a normal biochemical test. Both were referred to this study's surgeon for incidental findings of gallstones and CBD stones from previous imaging for other purposes.

The mean (SD) operating time was 99 (34) minutes (range 60-159 minutes), with the majority (6/10) between 60 minutes and 90 minutes inclusive. Four patients had longer operative times ranging from 110 minutes to 159 minutes. All patients had a 100% stone clearance rate and uneventful cholecystectomies, with minimal blood losses and no complications. There was no conversion to multiport or open surgery.

Postoperatively, pain was well-controlled with intravenous and oral analgesics. Mean (SD) PLOS was 1.5 (0.92) days (range 1-4 days). Most patients had a PLOS of 1 day (7/10). Among the 3 patients who had a PLOS of more than 1 day, the first had a PLOS of 4 days due to a small fluid collection at the gallbladder bed and raised liver enzymes on a downward trend; the second had a PLOS of 2 days for nausea and vomiting; and the third had a PLOS of 2 days for pain management. All patients were well upon discharge, recovered without complications, and reported satisfaction with the procedure. Postoperative morbidity and mortality were 0.0%: no recurrences of biliary colic or complications of bile leak, wound infection, or hernia were noted.

Table 1. Patient Characteristics	
Characteristic	**No. of Patients (N = 10)**
Age, mean (range), y	47 (22-67)
Sex, female/male	6/4
Ethnicity	
Chinese	8
Others	2
Nationality	
Singaporean	8
Indonesian	2
BMI, mean (range), kg/m^2	23.6 (19.4-30.0)
Past medical history/past surgical history	
Heart, kidney, or liver issue	1
Previous cholecystectomy, CBDE, or ERCP	2
Other condition(s), operation(s), or treatment(s)	8

Abbreviations: BMI, body mass index; CBDE, common bile duct exploration; ERCP, endoscopic retrograde cholangiopancreatography.

Table 2. Biochemistry Results	
Liver and Biliary Profile – Selected*	**No. of Abnormal Results (N = 10)**
Total bilirubin	5
AST	7
ALT	7
GGT	8
ALP	6

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase.

* Normal reference intervals were total bilirubin < 26 μmol/L, AST male < 51 U/L, AST female < 36 U/L, ALT male < 51 U/L, ALT female < 36 U/L, GGT male < 60 U/L, GGT female <40 U/L, ALP 39-117 U/L.

Discussion

There has been much debate over the benefits that single-port surgeries offer in exchange for the added challenges in performing the procedures safely.[5] Many single-port studies on SILC are available, with limited data on SILCBDE+SILC.[7-17] With the sharing of best practices in performing surgeries via the single-port approach, a greater number of variations have been added to the current types of surgeries that can be performed.[6, 9]

This study has demonstrated that SILCBDE+SILC is safe and feasible. The mean (SD) operating time of 99 (34) minutes for SILCBDE+SILC is comparable with commonly reported timings for multiport LCBDE+LC.[7, 8, 14, 18] Operative time is dependent on the surgeon's experience level, surgical technique, and patient characteristics.[9]

Before moving to the 1-stage SILCBDE+SILC procedure, this study's surgeon had operated on more than 500 patients with the single-port approach for LC.

The range of operative times for 4 patients (110-159 minutes) was noted to be longer than the rest (60-90 minutes). Those values may reflect the management of the following: the first had dense adhesions of the liver to the anterior abdominal wall and underwent subsequent diagnostic laparoscopy; the second had multiple small black pigmented CBD stones and underwent subsequent colonoscopy; the third had adhesions of the proximal transverse colon to the right hypochondrial anterior abdominal wall, an irregular 10-mm CBD stone, and fragments of black CBD stones; and the fourth had an acutely inflamed gallbladder which increased the dissection duration slightly.

In transcystic SILCBDE, this study noted that the most challenging part of the procedure was the cannulation of the scope into the cystic duct. First, the choledochoscope (or a similar scope) was introduced through the abdomen via the umbilicus which is further from the cystic duct than the conventional epigastric port or subcostal port. In conventional LCBDE, the epigastric or subcostal port, through which the scope is introduced, can be used to manipulate the flexible scope through the cystic ductotomy. However, with transcystic SILCBDE, due to the increased distance of the port to the cystic duct, the steeper angle when approaching the latter, and the scope being flexible in nature, an adequate control of the scope was thus difficult to attain. As such, an additional firm 'equipment' to stabilize and direct the flexible scope towards the cystic duct was required. Appropriate control of the scope was achieved by adopting the use of a Floseal™ Endoscopic Applicator, which is stiffer and relatively long. The Endoscopic Applicator's internal diameter was sufficiently large to enclose the scope firmly without slipping, and this study's surgeon could adjust the scope further in or out by holding its end together with the Endoscopic Applicator. In this study authors' opinion, the control of the scope is crucial, particularly for the transcystic approach, and this study's case series demonstrates the use of the Endoscopic Applicator in achieving this.

To the study authors' knowledge, the SILCBDE procedure has been published in 8 studies[7, 8, 10-15] and 3 reviews.[9, 16, 17] All but 2 authors have reported using the transductal approach only.[8, 11-13] Between the 2 authors, Chuang et al[7, 14] predominantly employed the transductal approach, with occasional use of the transcystic approach, while Yeo et al[10] only operated transcystically with the use of a 5.5-Fr Nathanson basket kit (Cook Australia, Eight Mile Plains, Australia) under image intensification guidance where the use of a scope was not mentioned.

In the articles reported by Chuang et al[7, 14] an atraumatic grasper was highly recommended to manipulate the scope, with Steri-Strips™ (3M Corporation, St Paul, Minnesota, USA) wrapped around its distal end to protect the scope's coating. In this study authors' opinion, this method may still damage the scope and the transcystic approach seems to remain difficult. It is also unclear how the cystic duct was stabilized in the transcystic approach with the fine adjustments required for successful ductotomy and cannulation. In this study's series, as the Endoscopic Applicator was inserted via one of the working port channels, the same port channel was subsequently reused to introduce the scope into the abdomen. One remaining port channel was thus available for a grasper to retract the gallbladder neck laterally to stabilize the cystic duct during its cannulation. Finally, the longitudinal cystic ductotomy for the scope insertion was performed differently in Chuang's series and this study. Chuang et al[14] created a ductotomy up to the cystocholedochal junction and conducted subsequent repair with interrupted

figure-of-8 sutures, whereas this study's surgeon created a ductotomy just large enough for the scope cannulation, proximal to the distal hemolock clip. Once complete stone clearance in the biliary tree was obtained under direct visual guidance, one or more hemolock clips were secured to the proximal cystic duct and the cystic duct was then transected. Therefore, there was no need for repair of the cystic duct-bile duct junction.

The authors acknowledge that this study has a small sample size of 10 patients who underwent 1-stage SILCBDE+SILC, and more data is required to further validate the effectiveness of the procedure and this method.

Conclusions

1-stage SILCBDE+SILC is safe and produces similar clinical outcomes to conventional multiport surgeries. The use of an Endoscopic Applicator has helped this study to overcome the difficulties of managing a flexible scope when covering the extra distance between its port-site and the cystic duct with limited angulation, obtaining successful cystic duct cannulation, and performing the bile duct exploration itself.

Additional Information

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Informed Consent: This study was deemed not required for review by the Institutional Review Board in Singapore as it is a retrospective review of results of deidentified patients and verbal consent was obtained from each patient during consultation. Past medical and surgical history, relevant imaging, and biochemical test results were accessed. Personal information such as age, sex, ethnicity, nationality, and body mass index were collected and anonymized. Inclusion into the study was made known to the patient as entirely voluntary, and any question could be directed to the corresponding author by email.

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