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Original Article

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Principal and Secondary Diagnoses Related to Eye Injury: Characteristics from a tertiary center in Northern Thailand

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ABSTRACT

OBJECTIVE This study aimed to explore the epidemiological characteristics of primary and secondary diagnoses of eye injuries requiring hospitalization in a tertiary referral center.

METHODS The medical records of 726 patients with diagnoses of eye injury who were admitted between January 2016 and December 2019 were reviewed.

RESULTS Four hundred and thirty-six of the patients (60.1%) were hospitalized with a principal diagnosis related to eye injury, while 290 patients (39.9%) were admitted with secondary diagnoses. Over the four-year period of study, the proportion of admissions with a principal diagnosis stayed consistently in the range of 54.0% to 63.9%. Most of the patients in the principal diagnosis group were age between 41 and 60 years. Patients in the secondary diagnosis group, however, were mostly between the age of 21 and 40 years. The majority of patients in the principal diagnosis group (267 patients, 61.2%) had eye injuries which had occurred at work, whereas in the secondary diagnosis group most (230 patients, 79.3%) had eye injuries resulting from traffic accidents.

CONCLUSIONS Work-related accidents and traffic accidents were the two main types of at-risk behaviors in both the primary and secondary diagnosis categories. Initiatives for public health education related to eye injuries should pay more special attention to these two primary causes of severe eye injuries.

KEYWORDS principal diagnosis, secondary diagnosis, eye injury, hospitalization

INTRODUCTION

Although many regulations and/or programs have been established to reduce the incidence of eye injury in at-risk populations, eye injuries remain one of the leading causes of acquired visual impairment worldwide (1-8). Reviews of previous publications have shown that the study setting, time-trend period, and geographic location can significantly impact the incidence and characteristics of the eye injury. For acute eye injury patients with minor or insignificant ocular damage, treatment at outpatient clinics was mostly successful. On the contrary, individuals with significant eye injuries alone or in combination with other systemic

illnesses required hospital admission for emergency medical attention (9-13). In-hospital patients had a higher risk of visual loss and decreased quality of life. However, other clinical variables, apart from the severity of the eye injury, can also have an impact on the clinical profiles of patients, including the criteria for hospital admission which can potentially change with the development of new medical and surgical advancements. Thus, understanding of shifts in epidemiological patterns and characteristics of eye injuries necessitating hospitalization should be taken into consideration when evaluating the impact of an eye injury.

This study aimed to identify trends in patients who were admitted to hospital with either principal or secondary diagnoses of eye injury. This information could be useful in the prevention of impaired vision from eye injury in a tertiary care context and in improving understanding the epidemiology of severe eye injury.

METHODS

This retrospective observational study was approved by the Hospital Research Ethics Committee, Faculty of Medicine, Chiang Mai University Hospital (Study Code: OPT 2562 06958). As the review of medical records was conducted without having direct contact with the patients, informed consent was waived. All study processes were performed in accordance with the Declaration of Helsinki. The data was recorded in Microsoft Excel spread sheets and was then deidentified to maintain patient confidentiality.

The medical records of patients who had been admitted between January 2016 and December 2019 with principal or secondary diagnoses related to eye injury (under the standard International Classification of Diseases, Tenth Edition, Clinical Modification (ICD-10 -CM) codes of S001-2, S011, S021, S023, S041-4, S050-9, T150-1, T158-9, and T260) were identified. All patients with a principal diagnosis as well as those with any one of the first four secondary diagnoses of any eye injury-related codes were recruited. In the case of patients who had multiple admissions as a consequence of the same eye injury, only the initial admission was reviewed. In cases where individuals suffered repeated occurrences of an eye injury during the study period, either in the originally injured eye or in the other eye, only the first event was included in the analyses.

Statistical analysis

For descriptive analyses, continuous data is presented as means (standard deviation, SD) or medians (interquartile range, IQR), while frequencies are shown as percentages. The Wilcoxon rank sum test was used to compare continuous data, and the Chi-square test to compare categorical data. The data was analyzed by the STATA® software, version 17 (StataCorp, College Station, TX, USA). *P* values less than 0.05 were considered statistically significant.

RESULTS

In total, there were 726 patients, with 436 patients (60.1%) in the principal diagnosis group and 290 patients (39.9%) in the secondary diagnosis group. The mean (SD) age of patients in the principal diagnosis group was 42.9 (18.8) years, whereas in the secondary diagnosis group it was 32.7 (16.5) years ($p < 0.001$). Despite a downward trend over the four-year study period, there was no statistically significant difference in the proportion of patients in the principal vs the secondary diagnosis groups in any year: 63.3% (114/180) in 2016, 63.9% (110/172) in 2017, 59.8% (104/174) in 2018, and 54.0% (108/200) in 2019; $p = 0.165$. Patients in the principal diagnosis group were more likely to be older (between the ages of 41 and 60 years), whereas those in the secondary diagnosis group tended to be younger (between the ages of 21 and 40 years). Females had a greater diversity in age distribution in each study year and in diagnosis than males (Figures 1 and 2).

Among the 436 patients in the principal diagnosis group, the three most common conditions related to eye injuries were open wound of the eyeball (277 patients, 63.5%), contusion of the eye and adnexa (67 patients, 15.4%), and open wound of eyelid and periocular area (44 patients, 10.1%). Over the four study years, there was no difference in the proportion of injury conditions in the principal diagnosis group ($p = 0.752$). Among the 290 patients in the secondary diagnosis group, open wounds of the eyelid and periocular area (67 patients, 23.1%), fractured orbital walls (63 patients, 21.7%) and contusions of the eye and adnexa (54 patients, 18.6%) were the most common conditions. Among patients in the secondary diagnosis group, a trend of an increasing proportion of fractured orbital walls and a decreasing proportion of contusion of the eye and adnexa were observed, but

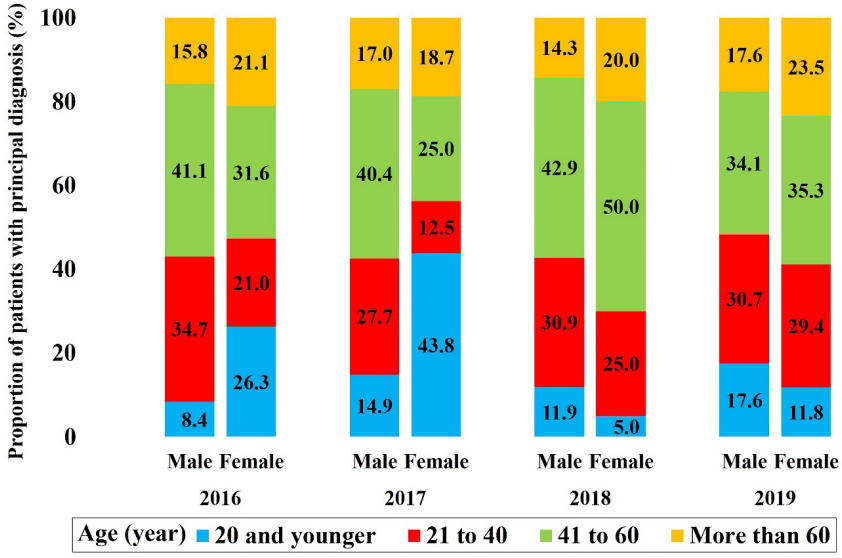

Figure 1. The distribution of age by gender and calendar year in patients with principal diagnoses related to eye injury subgroup

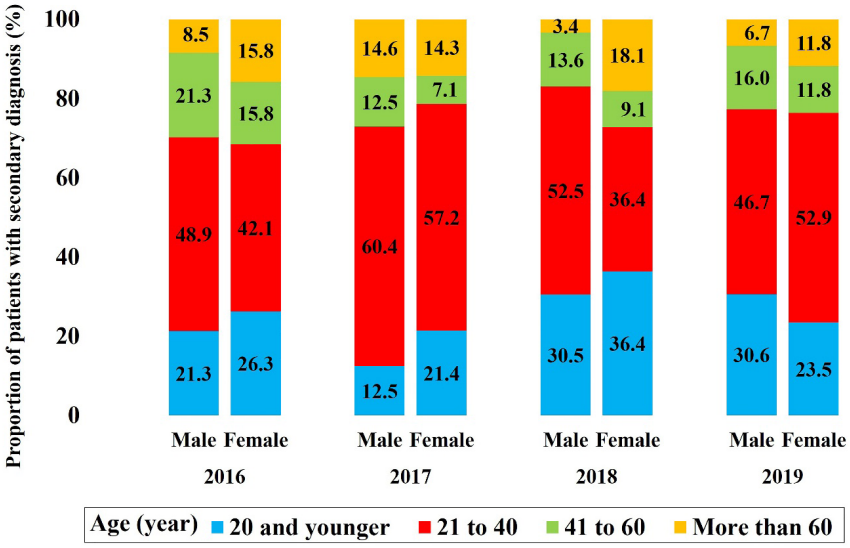

Figure 2. The distribution of age by gender and calendar year in patients with secondary diagnoses related to eye injury subgroup

the differences were not statistically significant ($p = 0.076$) (Figures 3 and 4).

Regarding the circumstances that led to the eye injury, the majority of the patients in the principal diagnosis group (267 patients, 61.2%) of the eye injuries occurred at work whereas in the secondary diagnosis group (230 patients, 79.3%) most of the eye injuries were a result of traffic accidents. Among the 285 patients in both groups who suffered injuries in traffic accidents, 242 (84.9%) of those accidents were related to motorcycles. The most common cause of injuries was blunt objects with 168 patients (38.5%) in the principal diagnosis group and 256 patients (88.3%) in the secondary diagnosis group. Of the bilateral eye injuries, 14 patients belonged to the primary diagnostic group, while 82 patients were in the secondary diagnosis group. The predominant causes of primary and secondary diagnoses of eye injuries were similar: traffic – 60 patients (73.2%) in the primary and 5 patients (35.7%) in the secondary diagnosis group; assault – 7 patients (8.5%) in the primary and 3 patients (21.4%) in the secondary diagnosis group, and work-related – 6 patients

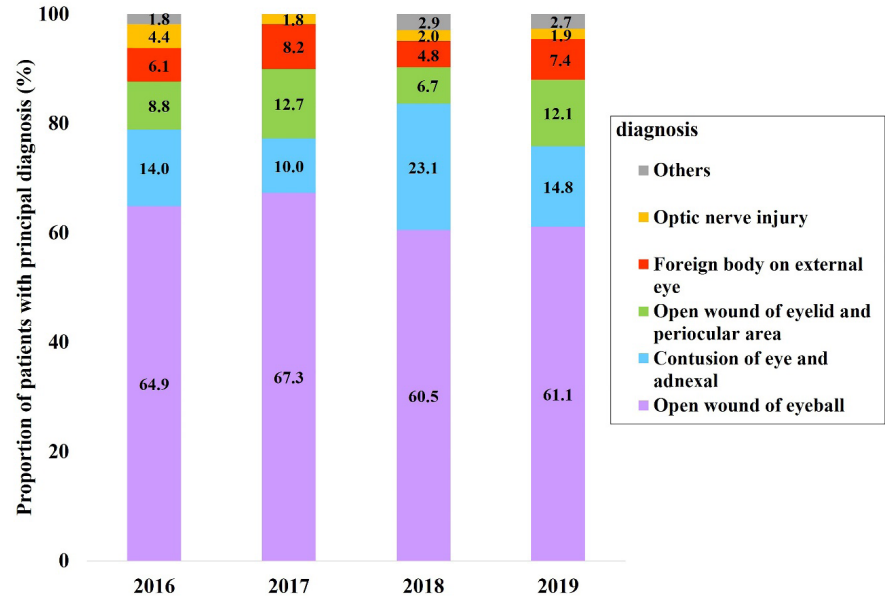

Figure 3. The distribution of eye injury diagnosis by calendar year in patients with principal diagnoses related to eye injury subgroup

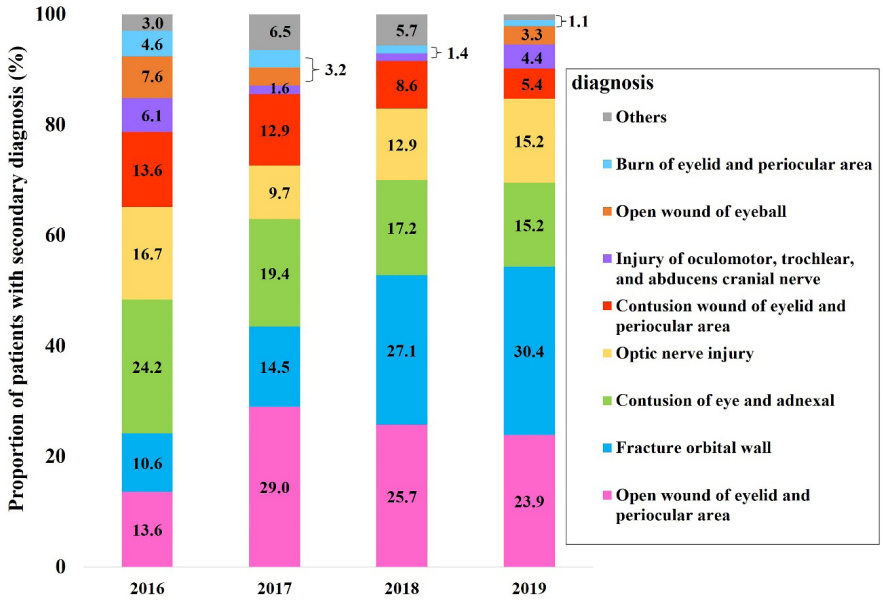

Figure 4. The distribution of eye injury diagnosis by calendar year in patients with secondary diagnoses related to eye injury subgroup

(7.3%) in the primary and 2 patients (14.3%) in the secondary diagnosis group. Table 1 summarizes patients' demographics, circumstances of and mechanisms/objects causing eye injury by eye injury-related diagnosis and calendar year.

Regarding ophthalmic interventions, 393 patients (90.1%) in the principal diagnosis group and 40 patients (13.8%) in the secondary diagnosis group required ophthalmic surgical treatment (Table 2). Additionally, 52 patients (11.9%) in the principal diagnosis group had other systemic involvements. Notably, 165 patients (56.9%) in the secondary diagnosis group received medical care from more than two subspecialties compared to only 8 patients (1.8%) in the primary diagnosis group.

A large proportion (174 patients, 60.0%) of patients in the secondary diagnosis group lost to follow-up. In the principal diagnosis group, 122 patients (36.5%) had final vision of worse than 20/200, and 39 patients (11.7%) were lost to follow-up. Table 2 summarizes ophthalmic management and final visual acuity by eye injury-related diagnosis.

Table 1. The distribution of demographics, circumstances and mechanisms/objects causing eye injury by eye injury-related diagnosis of in-patients and calendar year

Characteristics	Total, n (%)	Year, n (%)			
		2016	2017	2018	2019
Principal diagnosis	436	114	110	104	108
Demographics					
Male, n (%)	364 (83.5)	95 (83.3)	94 (85.4)	84 (80.8)	91 (84.3)
Age (year), n (%)					
20 and younger	63 (14.4)	13 (11.4)	21 (19.1)	11 (10.6)	18 (16.7)
21 to 40	129 (29.6)	37 (32.5)	28 (25.5)	31 (29.8)	33 (30.5)
41 to 60	170 (39.0)	45 (39.5)	42 (38.1)	46 (44.2)	37 (34.3)
More than 60	74 (17.0)	19 (16.6)	19 (17.3)	16 (15.4)	20 (18.5)
Unilateral eye injury	422 (96.8)	114 (100.0)	104 (94.6)	101 (97.1)	103 (84.5)
Circumstances related to injury					
Work-related activities	267 (61.2)	71 (62.3)	68 (61.8)	66 (63.5)	62 (57.4)
Traffic-related activities	55 (12.6)	14 (12.3)	15 (13.6)	10 (9.6)	16 (14.8)
Household-related activities	37 (8.5)	11 (9.7)	7 (6.4)	9 (8.7)	10 (9.3)
Assault	22 (5.1)	7 (6.2)	5 (4.6)	4 (3.8)	6 (5.5)
Others	55 (12.6)	11 (9.5)	15 (13.6)	15 (14.4)	14 (13.0)
Mechanisms/objects related to injury					
Hit by blunt objects	168 (38.5)	45 (39.5)	42 (38.2)	36 (34.6)	45 (41.7)
Hit by sharp objects	81 (18.6)	25 (21.9)	18 (16.4)	21 (20.2)	17 (15.7)
Hit by high velocity objects	145 (33.3)	34 (29.8)	41 (37.3)	38 (36.5)	32 (29.6)
Exposed to explosive, chemical, or thermal-related objects	29 (6.6)	6 (5.3)	5 (4.6)	8 (7.7)	10 (9.3)
Others	13 (3.0)	4 (3.5)	4 (3.5)	1 (1.0)	4 (3.7)
Secondary diagnosis	290	66	62	70	92
Demographics					
Male, n (%)	229 (79.0)	47 (71.2)	48 (77.4)	59 (84.3)	75 (81.5)
Age (year), n (%)					
20 and younger	73 (25.2)	15 (22.7)	9 (14.5)	22 (31.4)	27 (29.4)
21 to 40	147 (50.7)	31 (47.0)	37 (59.7)	35 (50.0)	44 (47.8)
41 to 60	43 (14.8)	13 (19.7)	7 (11.3)	9 (12.9)	14 (15.2)
More than 60	27 (9.3)	7 (10.6)	9 (14.5)	4 (5.7)	7 (7.6)
Unilateral eye injury	208 (71.7)	46 (69.7)	45 (72.6)	51 (72.9)	66 (71.7)
Circumstances related to injury					
Work-related activities	19 (6.6)	6 (9.1)	4 (6.5)	5 (7.1)	4 (4.4)
Traffic-related activities	230 (79.3)	52 (78.8)	51 (82.3)	54 (77.1)	73 (79.4)
Household-related activities	13 (4.5)	3 (4.6)	1 (1.6)	3 (4.3)	6 (6.5)
Sport-related activities	1 (0.3)	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)
Assault	16 (5.5)	4 (6.1)	1 (1.6)	6 (8.6)	5 (5.4)
Others	11 (3.8)	1 (1.5)	4 (6.5)	2 (2.9)	4 (4.4)
Mechanisms/objects related to injury					
Hit by blunt objects	256 (88.3)	60 (90.9)	50 (80.7)	60 (85.7)	86 (93.5)
Hit by sharp objects	9 (3.1)	1 (1.5)	3 (4.8)	3 (4.3)	2 (2.2)
Hit by high velocity objects	4 (1.4)	1 (1.5)	0 (0.0)	2 (2.9)	1 (1.1)
Exposed to explosive, chemical, or thermal eye injuries	12 (4.2)	3 (4.6)	2 (3.2)	5 (7.2)	2 (2.2)
Others	9 (3.0)	1 (1.5)	7 (11.3)	0 (0.0)	1 (1.1)

DISCUSSION

This study explored patterns of eye injury necessitating hospitalization in a university hospital in Northern Thailand based on principal and secondary diagnoses for eye injury at discharge. The results demonstrate that there were disparities between the two groups in terms of patient age, diagnostic conditions, and the injury-causing

Table 2. The distribution of in-patients by ophthalmic management and final visual acuity by eye injury-related diagnosis

	Total, n (%)	Eye Injury-related diagnosis, n (%)		*p*-value
		Principal diagnosis (n=436)	Secondary diagnosis (n=290)	
Ophthalmic management (n=726)				
Observation	56 (7.7)	2 (0.5)	54 (18.6)	< 0.001
Medical treatments	168 (23.1)	26 (6.0)	142 (49.0)	
Minor interventions	69 (9.5)	15 (3.4)	54 (18.6)	
Operational interventions	433 (59.6)	393 (90.1)	40 (13.8)	
Final snellen visual acuity (n=624)				
20/200 and better	259 (41.5)	173 (51.8)	86 (29.7)	< 0.001
Worse than 20/200	152 (24.4)	122 (36.5)	30 (10.3)	
Undetermined	213 (34.1)	39 (11.7)	174 (60.0)	

events. Young males who sustained orbital and adnexal injury in traffic accidents made up a significant number proportion of cases in the secondary diagnosis group, whereas middle-aged or adult men who had received open globe injuries at work made up the majority of cases in the principal diagnosis group. For that reason, in developing eye injury prevention strategies, it is imperative to pay close attention to the key risks in different populations.

Notably, a large proportion of eye injuries are treated at an outpatient clinic or an emergency setting, whereas eye injuries of greater severity which had a higher risk of visual loss are more likely to receive inpatient care (14-18). The epidemiology of ocular injuries requiring inpatient care has been thoroughly investigated and updated in numerous publications covering various study designs, clinical situations, and study sites (2, 10, 19).

A study in the US that examined the National Inpatient Sample database discovered that although the overall incidence of eye injuries requiring hospitalization increased from 18 per 100,000 population in 2001 to 22 per 100,000 population in 2014, the proportion in which eye injury was the principal diagnosis declined. That study proposed that the rise in secondary diagnosis of eye injuries was due mainly to falls among the elderly (12). In another population-based study that compared data from the years 2000, 2005, 2010, and 2015 in Taiwan, the rate of eye injuries requiring inpatient care decreased from 1.34% in 2000 to 0.63% in 2015 (13). That study also found no discernible change in the proportion of patients with a principal diagnosis compared to a secondary diagnosis throughout the four study years (a maximum value of 30.0% in 2010 and a minimum value of 25.5% in 2015). The authors postulated that enforcement to use eye protection may have contributed to the decline in the rate of eye injuries requiring hospitalization.

A number of covariables can also affect the likelihood of eye injuries requiring hospitalization. In this study, the ratio of hospitalized patients in the principal diagnosis group to the secondary diagnosis group remained steady over the study period. Severe injuries were more likely to be transferred to tertiary care institutions, which might have contributed to the high percentage of patients in the principal diagnosis group.

Regarding gender distribution, the presence of more males than females in this study is consistent with earlier studies of eye injuries (10, 11) in which disparities in the age-specific distribution by gender were observed (11, 13). For example, according to a study that examined data from the Taiwan National Health Insurance Research Database, males between the ages of 20 and 34 and females between the ages of 70 and 74 had the highest incidence rates of eye injuries (13). Additionally, a population-based study that analyzed data from 2005 to 2014 in Western Australia reported that males in of working age were most likely to appear for emergency eye care or be admitted after eye injuries, whereas females in the elderly age range were more likely to be admitted following eye injuries and children were most likely to present at the emergency room (20).

In this study, the age distribution of males remained relatively constant over the course of

the study period, peaking at medium to older adult age in the principal diagnosis group and at the adolescent to young adult age range for the secondary diagnosis group. In contrast, the age distribution of females fluctuated more widely across specific years, despite a trend towards an increased proportion of older patients in the principal diagnosis group over the study period. The small number of females in this study, however, may have had a confounding effect on the findings. Further studies with larger samples and more females could provide additional information on the distribution of eye injury by gender and age.

This study demonstrates that eye injury sustained at work continues to be a risk factor for patients in the principal diagnosis group. Several studies have underlined the importance of the use of eye protection to prevent workplace-related eye injury regardless of the type of occupation (21-24). As a majority of injured patients in this study worked independently in the agricultural sector where the importance of eye protection may have been largely overlooked. This is supported by the fact that many patients in the principal diagnosis group were struck by blunt or high-velocity objects, suffered open globe injuries, and were identified as having open wounds of the eyeball. On the other hand, the main causes of injury in the secondary diagnosis group were related to traffic accidents, particularly those involving motorbikes. These results are consistent with the finding that a significant proportion of patients in the secondary diagnosis group were hit by blunt objects, had orbital and adnexal injuries, were diagnosed as having open wounds of eyelid and periocular area, and experienced orbital wall fractures. Studies in Taiwan report similar results of traffic accidents in terms of hospitalization for eye injuries (13-25). Although wearing protective equipment when performing high-risk tasks or using a motor vehicle are encouraged in Thailand, ocular injuries still occur frequently. It is essential to emphasize personal safety awareness when engaging in both work- and vehicle-related activities to help reduce eye injuries.

This study provides an epidemiological review of significant eye injuries requiring hospitalization in a tertiary hospital over four years. However, detailed data addressing the circumstances surrounding ocular damage, the overall rate of eye injury, and the use of eye protection devices or other safety equipment could not be extracted due to the retrospective nature of the study. Additionally, final vision outcomes could not be evaluated for patients who did not return for follow-up care or who were transferred back to their local hospitals for ongoing care. Lastly, due to socioeconomic structural changes, changes in lifestyle, and changes in ophthalmology admission criteria, particularly during the COVID 19 outbreak, further longitudinal in-depth research is essential to determine the trend of severe eye injuries requiring inpatient care.

CONCLUSIONS

Males made up the majority of patients needing inpatient care due to eye injuries. Work-related eye injuries in the principal diagnosis group and traffic-related injuries in the secondary diagnosis group reflect the typical at-risk circumstances. This study provides a better understanding of serious eye injuries and also highlights associated risks for eye injuries which could aid in the establishment of public health education programs.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ADDITIONAL INFORMATION

Authors contribution

P.A.: conceptualization, methodology, implementation, data analysis, writing, review, and editing manuscript; J.C.: conceptualization, methodology, data analysis, review manuscript, and supervision; S.C.: conceptualization, acquisition of data, interpretation of data, and review manuscript; C.P.: conceptualization, acquisition of data, and review

manuscript. All authors have read and approved the content of the final manuscript.

Data availability statement

The data analyzed in this study are not publicly available due to participant privacy but are available from the corresponding author upon reasonable request.

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Original Article

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Environmental Risk Factors Associated with Behavioral Problems among Children with Attention Deficit Hyperactivity Disorder: Maternal Perceptions

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ABSTRACT

OBJECTIVE This study aims to identify environmental risk factors associated with behavioral problems in children and adolescents with attention deficit hyperactivity disorder (ADHD).

METHODS A case-control study was conducted in children aged 4-15 years comprising 92 children diagnosed with ADHD and 92 controls without ADHD. Stratified sampling was used to select 184 participants residing in Chiang Mai and Lamphun provinces, Thailand. Data were collected from the mothers of the participants using a self-report questionnaire between May and August 2022. The questionnaire consisted of three parts: child characteristics (gender, age, underlying diseases, past illness history), mother's characteristics (age, income, occupational status, history of cigarette smoking and alcohol drinking), and maternal perceptions about caring for the child and the child's behavioral problems (exposure to chemicals/toxicants, frequent punishment, excessive use of digital media and games). Data were analyzed using descriptive statistics, chi-squared test, odds ratio (OR) and logistic regression using SPSS version 21.

RESULTS Most of participants were males (86.96%) while 13.04% were females. The results showed that the ADHD cases were significantly more likely than the controls to have underlying diseases such as G6PD, allergy, and asthma (p = 0.006). There was also a statistically significant correlation with smoking history in the mothers of ADHD cases (OR = 2.64; 95% CI: 1.36-5.13 p = 0.004). In terms of maternal perceptions, the mothers of ADHD children were significantly more likely than the controls to have frequently punished the child (OR = 6.26; 95% CI: 3.24-12.07; p < 0.001), to have children who were exposed to chemicals (pesticides and fertilizer) or toxicants (passive smoking) (OR = 4.14; 95%CI: 1.76-9.73; p = 0.001), and to have allowed their child to use digital media and play video games excessively (OR = 2.82; 95% CI: 1.55-5.16; p = 0.001). After adjustment for other environmental risk factors, frequent child punishment (ORadj = 4.32; 95% CI: 2.13-8.78; p < 0.001) and exposure of the child to chemicals/toxicants (ORadj = 2.66; 95% CI: 1.00-7.02; p = 0.049) were found to be significantly related with ADHD.

CONCLUSIONS Frequent child punishment was the most significant environmental risk factor related to behavioral problems among children with ADHD. This indicates the importance of healthcare providers developing strategies and/or interventions to enhance knowledge and awareness in mothers of ADHD which can improve treatment outcomes.

KEYWORDS ADHD, risk factors, behavioral problems, maternal perceptions

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is the most common neuro-behavioral developmental disorder. ADHD onset typically occurs in childhood and continues into adulthood. It substantially affects developmental, social, academic and occupational functioning of persons with ADHD (1). The main symptoms of ADHD often emerge in the preschool period and include hyperarousal and hyperactivity, disinhibited behaviors, emotional dysregulation, inattention, and executive dysfunction (2). The prevalence of ADHD varies worldwide and is increasing over time according to the American Psychiatric Association revised diagnostic criteria (DSM-V) released in 2013 which also establishes standards of clinical practice that are applicable to and valuable for in the diagnosis and management of ADHD (3). The result of a meta-analysis of 179 studies showed an estimated ADHD prevalence of 7.2% (4). Similarly, prevalence of parent-reported ADHD among U.S. children aged 2-17 years as reported by the National Survey of Children's Health (NSCH) was 9.4% (5). In Thailand, the ADHD prevalence was 8.1% (6).

ADHD is a condition resulting from the collective influence of genetic and environmental risk factors. It is difficult to identify the true causality of environmental risk factors in ADHD, as it relates to several affecting variables. Environmental factors during the prenatal and perinatal periods such as smoking and alcohol drinking (7-9) as well as factors during toddlerhood such as exposure to chemicals/toxicants, negative parenting, maltreatment, and excessive use of digital media (1, 10–13) may contribute to the risk of later onset of ADHD. Previous studies in Thailand have found that risk factors relating to ADHD among school-age children included male gender, a history of being punished by teachers, living with relatives, divorce or separation of father and mother, income insufficiency and family debt, and children who watch television, use a smartphone, and play video games more than 1-2 hours a day (14–16). Parents who care for children with ADHD may experience increased stress due to their children's problematic behaviors. These behaviors include less compliance, increased distraction as well as requiring more attention and assistance. These behaviors can lead to negative consequences: poor monitoring of children's behavior and emotions, increased punishment, decreased supportive attitude, and increased conflict in the parent–child relationship. It has been suggested that healthcare providers should address parental perceptions of ADHD as part of the treatment (17), as well as efforts to increase parents' knowledge and ADHD symptoms awareness as a means of improving the children's quality of life (18).

There have been few studies about environmental risk factors in children with ADHD in Thailand. The present study aims to investigate the environmental risk factors associated with behavioral problems in children with ADHD through analysis of mothers' perceptions. The study results should be beneficial for aiding prevention of ADHD and for promotion of appropriate care for children with ADHD.

METHODS

A case-control study was conducted in children with and without ADHD aged 4-15 years. Stratified sampling was used to select 184 participants residing in Chiang Mai and Lamphun, Thailand. The study was approved by the Research Ethics Committee of the Department of Mental Health, Ministry of Public Health, Thailand (DMH. IRB.COA 010/2565).

The sample size was calculated using G*power version 3.1.9.4 (19) using completed parameters with large effect size (0.5) as recommended by Cohen (20) and Schlesselman (21), alpha = 0.05, power = 0.95, and Df = 5. The calculated sample size was 80 participants per group. An additional 10% was added to minimize potential data collection errors, meaning 88 participants were needed in each group. The ADHD cases had been previously diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria. The names of the ADHD cases were retrieved from the electronic medical records of Rajanagarindra Institute of Child Development, with ICD-10 (International Classification of Diseases-10) code F90.0-F90.9.

The demographic characteristics of the controls without ADHD, including gender, age, and living in Chiang Mai and Lamphun, were similar to those of the ADHD cases. Data were collected from the mothers of participants between May and August 2022 using a self-report questionnaire. The questionnaire consisted of three parts:

child characteristics (gender, age, underlying diseases, past illness history), mother's characteristics (age, income, occupational, history of cigarette smoking and alcohol drinking), and maternal perceptions about child care (exposure to chemicals/toxicants, frequent punishment, excessive use of digital media and games) and child behavioral problems.

Statistical analysis was performed using SPSS version 21 software (IBM corp.) For descriptive statistics, categorical data are presented as frequency and percentage, while numerical data are presented as mean ± SD or median (IQR) depending on the underlying distribution. Odds Ratios and Confidence Intervals (22) were analyzed and a $p < 0.05$ was considered statistically significant.

RESULTS

Demographic characteristics of the case and control participants

A total of 184 participants in this case-control study, including 92 cases with ADHD and 92 controls without ADHD. Most of participants were male (86.96%) while 13.04% were female (Table 1). The mean age of the case group was 8.95 with a standard deviation of 2.88 while the mean age of the control group was 8.52 with a standard deviation of 2.85. There was no significant difference in past illness history of head trauma and seizure between the groups, but the cases were significantly more likely than the controls to have underlying diseases such as G6PD, allergy, and asthma ($p = 0.006$).

Regarding comparison of mothers' demographic characteristics, there were significant differences in both age ($p = 0.035$) and history of cigarette smoking ($p = 0.004$). However, there was no significant difference in sufficient income ($p = 0.624$), history of alcohol drinking ($p = 0.833$), or being unemployed ($p = 0.647$) (Table 2).

Comparison of maternal perceptions showed that compared to the controls, the case mothers were significantly more likely to have used frequent child punishment because of behavioral problems ($p < 0.001$), let their child use digital media and play games excessively ($p = 0.001$), and allowed their child to be expose to chemicals (pesticides and fertilizers) or toxicants (passive smoking) ($p = 0.001$). However, there was no significant difference in regularly leaving the child alone ($p = 0.236$).

Binary logistic regression model

In the final binary logistic regression model (Table 3), all environmental risk factors, including history of cigarette smoking, allowing their child to be exposed to chemicals/toxicants, frequent child punishment, and excessive use of digital media and gaming were adjusted. The results showed that the cases were significantly more likely than the controls to have frequent child punishment (ORadj = 4.32; 95% CI: 2.13-8.78; $p < 0.001$), and to let their child be exposed to chemicals (pesticides and fertilizer) or toxicants (passive smoking) (ORadj = 2.66; 95% CI: 1.00-7.02; $p = 0.049$).

DISCUSSION

Parenting and family environment have significant impact on child development and may lead to a risk of developmental disorders including

Table 1. Comparison of demographic characteristics of children between ADHD group and Control group (n=184)

Personal factors	ADHD (n=92) n (%)	Control (n=92) n (%)	*p*-value
Age (years) (mean±SD)	8.95±2.88	8.52±2.85	0.304[a]
Sex			
Male	80 (86.96)	80 (86.96)	1.000[b]
Female	12 (13.04)	12 (13.04)	
Past history			
Head trauma	10 (12.20)	6 (6.52)	0.433[b]
Seizure	8 (8.70)	4 (4.35)	0.371[b]
Other underlying disease (G6PD, allergy, asthma)	31 (33.70)	14 (15.22)	0.006[b]

[a]Independent t-test; [b]Fisher's exact test

Table 2. Comparison of mothers' demographic characteristics and perceptions about caring and child behavioral problems between ADHD group and Control group (n=184).

Factors	ADHD (n=92) n (%)	Control (n=92) n (%)	*p*-value	OR	95% CI
Mother's age (years) (mean±SD)	38.98±6.32	36.96±6.56	0.035[a]	-	-
Sufficient income	64 (69.57)	68 (73.91)	0.624[b]	-	-
Being unemployed	36 (39.13)	32 (34.78)	0.647[b]	-	-
History of cigarette smoking	36 (39.13)	18 (19.57)	0.004[b]	2.64	1.36-5.13
History of alcohol drinking	14 (15.22)	12 (13.04)	0.833[b]	-	-
Allowing child to be expose to chemicals (pesticides and fertilizers) or toxicants (passive smoking)	26 (28.26)	8 (8.70)	0.001[b]	4.14	1.76-9.73
Leaving child alone regularly	13 (14.13)	7 (7.61)	0.236[b]	-	-
Frequent child punishment because of their behavioral problems	57 (61.96)	19 (20.65)	<0.001[b]	6.26	3.24-12.07
Excessive use of digital media and gaming	63 (68.48)	40 (43.48)	0.001[b]	2.82	1.55-5.16

[a]Independent t-test; [b]Fisher's exact test

Table 3. Binary logistic regression model[a] of environmental risk factors associated with behavioral problems in children with ADHD

Risk factors	Adjusted OR[b]	95% CI	*p*-value
History of cigarette smoking	1.16	0.52-2.58	0.719
Letting their child be exposed to chemicals (pesticides and fertilizers) or toxicants (passive smoking)	2.66	1.00-7.02	0.049
Frequent child punishment because of their behavioral problems	4.32	2.13-8.78	<0.001
Excessive use of digital media and gaming	1.81	0.88-3.66	0.097

[a]Hosmer and Lemeshow Test Chi-square = 5.597 and Significance = 0.347; [b]Model was adjusted for history of cigarette smoking, letting their child be exposed to chemicals/toxicants, frequent child punishment and excessive use of digital media and gaming

ADHD. The results of this case-control study showed that two environmental risk factors were significantly associated with behavioral problems in children with ADHD based on maternal perceptions. The strongest significant factor was frequent child punishment with 4.3 times the risk of behavioral problems in the case group compared to the controls. The cases mothers reported that the main causes of punishment were hyperactive, disruptive, and aggressive behaviors. The punishment forms that the parents used almost every week were scolding, blaming, and withdrawal of favorite activities. Hitting was also sometimes used as punishment.

Consistent with this, previous studies have reported that parents of children with ADHD used significantly more corporal punishment than the control group parents (23). In addition, 95% of parents of children with ADHD physically abused their children (24). This evidence strongly supports that children with ADHD are at significantly increased risk of parent abuse. Such risk might be a result of emotional dysregulation (25) and co-occurring disorders in children with ADHD such as oppositional defiant disorder, conduct disorder, anxiety, and depression (26).

ADHD results in parents having more difficulty looking after their children and consequently punishing them more frequently. This is congruent with several Thai studies demonstrating that a history of being punished was correlated with ADHD in elementary school children (14–16). Another factor associated with behavioral problems in children with ADHD is allowing the child to be exposed to chemicals and toxicants. The kinds of chemicals reported by the case mothers were pesticides and fertilizer, but specific chemical names, routes of exposure, and duration of exposure could not be precisely identified. Exposure to toxicants from tobacco smoke from passive smoking by family members living together, was associated with 2.7 times the risk of behavioral problems compared to the controls. These results are consistent with a population-based retrospec-

tive cohort study (2005-2012) conducted in Spain which showed an association between ADHD occurrence and exposure to pesticides (27). Similarly, another study reported that the children of smokers were approximately 1.5–3 times more likely to have ADHD or ADHD symptoms than children of non-smokers (13). Previous studies also found that ADHD was associated with prenatal maternal active or passive smoking and/or postnatal tobacco smoke exposure (7, 8).

However, In the present study after adjustment for all variables, the results showed no relationship between the incidence of behavioral problems and excessive use of digital media and gaming in children with ADHD. This is in contrast to the result of a Thai case-control study which reported that children who watched television more than one hour a day were 3.2 times more likely to have ADHD or ADHD symptoms than children who watched television less than one hour a day. Playing video games and using a smartphone were not risks factors (14).

In addition, this study showed an association between risk of behavioral problems and personal factors of children with ADHD related to having underlying diseases such as G6PD, allergy, and asthma. These results support a previous study that investigated the coexistence of other health-related conditions in individuals with ADHD which found that asthma was more common in ADHD children (OR = 2.19; 95% CI = [2.16, 2.22]) (28). The results of the present study have direct implications for patient care, including diagnosis and individual therapy. A strength of this case-control study is the sufficient sample size that provides more reliable and accurate findings. Another strength is that the case and the control groups had almost exactly the same characteristics, especially living in similar communities, which reduced the possibility of confounding bias.

There were, however, also several limitations. The first limitation is generalizability as the samples do not represent the general population in Thailand. Additionally, this study could not identify associations between other related factors and behavioral problems due to lack of data, e.g., types of punishment, types and duration of exposure to chemicals or toxicants, participants' age at initial use of digital media, duration of digital media and game usage. Further studies investigating these aspects are recommended.

CONCLUSIONS

Frequent child punishment is the most significant environmental risk factor related to behavioral problems among children with ADHD. Allowing children to be exposed to chemicals (pesticides and fertilizers) or toxicants (passive smoking) appears to be related to ADHD through alteration of neurological development in early childhood (29). It is important for healthcare providers to develop strategies and interventions to enhance the knowledge and awareness of mothers of children with ADHD and contribute to improvement of treatment outcomes.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ADDITIONAL INFORMATION

Data availabilty statement

The data used in this study are not publicly available due to Rajanagarindra Institute of Child Development's policy, but are available from the corresponding author upon reasonable request.

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Original Article

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The Association of Lifestyle Factors and Attention Problems in Adolescents

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ABSTRACT

OBJECTIVE This study aims to explore the associations between lifestyle factors such as physical activity, sleep patterns, media usage, and diet, and attention problems in adolescents.

METHODS This cross-sectional study involved 4,370 adolescents aged 13-18 years in Chiang Mai, Thailand. Lifestyle factors were assessed using the Youth Risk Behavior Surveillance System questionnaire, while attention problems were evaluated with the Youth Self-Report (YSR) Attention Problems subscale. Associations were analyzed using univariable and multivariable regression analysis.

RESULTS Media use exceeding two hours per day was significantly associated with clinical attention problems (aOR = 3.21, 95% CI: 1.81-5.71). Additionally, increased media use (b = 0.24, 95% CI: 0.19-0.28, $p < 0.01$), and high soft drinks consumption (b = 0.19, 95% CI: 0.12-0.26, $p < 0.01$) were positively associated with higher attention problem scores. Conversely, active physical activity (b = -0.09, 95% CI: -0.13 to -0.05, $p < 0.01$), adequate sleep (b = -0.26, 95% CI: -0.33 to -0.19, $p < 0.01$), regular breakfast intake (b = -0.09, 95% CI: -0.12 to -0.05, $p < 0.01$), vegetable consumption (b = -0.07, 95% CI: -0.13 to -0.02, $p = 0.01$), and milk consumption (b = -0.06, 95% CI: -0.11 to -0.01, $p = 0.03$) were negatively associated with attention problems scores.

CONCLUSIONS The study highlights the significant impact of lifestyle factors on attention problems among adolescents. These findings support the integration of lifestyle modifications into the assessment and management of attention issues in this age group, particularly emphasizing the need to reduce excessive media use.

KEYWORDS lifestyle factors, dietary patterns, attention problems, ADHD, adolescence

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder in children and adolescents (1-3). The clinical features of ADHD include a persistent pattern of inattention and/or hyperactivity/impulsivity interfering with development or functioning and are frequently comorbid with other psychiatric disorders (4). ADHD may persist into adolescence and adulthood (5), contributing significantly to the global disease burden (2).

Pharmacological treatment, such as methylphenidate, effectively decreases ADHD symptoms (6). However, concerns have been raised about medication safety, adverse effects, and adherence

problems during long-term use, leading to a non-pharmacological approach as part of the multimodal treatment, including lifestyle modifications (7, 8).

Growing evidence suggests that dietary interventions might reduce ADHD symptoms, potentially mediated by the gut-brain axis (9-14). Notably, a diet rich in fruits and vegetables has been linked to symptom alleviation (15). However, research has predominantly focused on younger children, leaving a knowledge gap regarding adolescents (11, 16-19). Sleep disturbances, including difficulties in sleep onset, short sleep duration (<8 hours), and night awakenings, are commonly seen in ADHD patients (20). These sleep disturbances also aggravate ADHD symptoms (21). Furthermore, physical activity exceeding 150 minutes per week has been linked to a decrease in ADHD symptoms among late adolescents (22).

Beyond diet, sleep, and exercise, media use has emerged as a critical factor influencing attention problems. A meta-analysis has highlighted significant associations between excessive screen time and ADHD-related behaviors in children and adolescents (23). These studies underscore the consistent observation of the relationship between screen media use and ADHD-related behaviors across four decades (23, 24), pointing to the growing prevalence of digital media use among adolescents as a potential contributor to attention problems.

This study aimed to explore the associations between lifestyle factors including physical activity, media use, sleep behavior, and dietary habits, and attention problems in adolescents. We hypothesize that a healthy lifestyle could serve as a protective factor against attention problem symptoms in this age group.

METHODS

A cross-sectional study was conducted in Chiang Mai, Thailand, using two-stage cluster sampling. Initially, large schools with over one thousand students were selected. This was followed by random sampling of six secondary and vocational schools. Participants and their parents provided consent before the students completed an anonymous, self-administered web-based questionnaire after their computer class. The study received approval from the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (PED-2558-03436).

Participant demographics, including age, gender, school type, academic achievement, body weight, and height, were recorded. The weight and height of the participants were self-reported. BMI percentile categories were classified according to the World Health Organization (WHO) cut-off points (underweight, normal weight, overweight, and obesity) based on their age and gender (25).

The Youth Self Report (YSR) questionnaire's Attention Problems (AP) subscale assessed attention problems through nine items scored on a three-point scale, measuring inattention, hyperactivity, and impulsivity, with a higher score indicating more significant attention problems. The YSR is known to have good psychometric properties, with a Cronbach's alpha of 0.83 and test-retest reliability of 0.79 in the original version (26-28). T-scores of 70 or higher, representing two standard deviations above the mean, are considered to be in the clinical range for the clinical scales (29). Dichotomizing the outcome variable facilitated the identification of factors associated with clinically relevant levels of attention difficulties. Lifestyle behaviors were assessed using the Youth Risk Behavior Surveillance System developed by the Centers for Disease Control and Prevention for adolescents aged 13-18 years (30).

Descriptive statistics were used to assess the quantitative data. Bivariate and multivariable logistic regression analyses were conducted to calculate the odds ratio (OR) and adjusted odds ratio (aOR). Additionally, univariable and multivariable linear regression analyses were performed to explore the associations between lifestyle factors and attention problems, adjusting for potential confounders. Collinearity and interaction among the factors were tested. Significant lifestyle factors associated with attention problems were further analyzed by the response score group for lifestyle factors, using analysis of variance (ANOVA). All analyses were performed using SPSS software, version 22.0 (IBM Corp, Armonk, NY, USA) for Windows.

RESULTS

Of the 5,639 students enrolled, 4,370 completed the questionnaires, resulting in a response rate of 77.5%. Males comprised 50.9% of the respondents. According to the YSR-AP scores, 2% of participants were identified with clinical attention problems,

while 4% were on the borderline. The gender distribution was even across all groups. A higher percentage of older adolescents (16-18 years) were observed in the clinical attention problems group. The participant characteristics, stratified by YSR-AP cut points, are detailed in Table 1.

Bivariate logistic regression analysis, as shown in Table 2, revealed that adolescents attending

Table 1. Characteristics of participants stratified by the Youth Self Report-Attention Problems (YSR-AP) cut point score and the associations with clinical attention problems (n=4,370).

Characteristics, n (%)	Overall cohort (n=4,370)	Clinical attention problems (n=87)	Normal to borderline (n=4,283)	OR	aOR
Gender					
Male	2,223 (50.9)	48 (55.2)	2,175 (54.3)	1.19	0.82
Female	2,147 (49.1)	39 (44.8)	2,108 (45.7)	Reference	Reference
Age					
13-15	2,389 (54.7)	39 (44.8)	2,350 (54.9)	0.67	0.66
16-18	1,981 (45.3)	48 (55.2)	1,933 (45.1)	Reference	Reference
School					
Secondary school	3,404 (77.9)	60 (69.0)	3,344 (78.1)	0.62*	0.83
Vocational school	966 (22.1)	27 (31.0)	939 (21.9)	Reference	Reference
BMI percentile (n=4,356)					
Underweight	902 (20.7)	46 (17.8)	856 (20.9)	0.86	1.23
Normal	2,701 (62.0)	157 (60.9)	2,544 (62.1)	Reference	Reference
Overweight	427 (9.8)	27 (10.5)	400 (9.8)	1.10	1.35
Obese	326 (7.5)	28 (10.9)	298 (7.3)	1.12	1.32
Academic achievement (n=4,064)					
Mostly A's or B's	2,809 (69.1)	39 (53.4)	2,770 (69.4)	0.51**	0.59*
Grade C to D	1,255 (30.9)	34 (46.6)	1,221 (30.6)	Reference	Reference
Physical activity ≥60 min per day					
Not every day	3,791 (86.8)	73 (83.9)	3,718 (86.8)	0.79	0.79
Every day	579 (13.2)	14 (16.1)	565 (13.2)	Reference	Reference
Sleep time					
Inadequate sleep	3,210 (73.5)	69 (79.3)	3,141 (73.4)	1.39	1.15
Adequate sleep	1,159 (26.5)	18 (20.7)	1,141 (26.6)	Reference	Reference
Media use					
≥2 hours per day	2,416 (55.3)	72 (82.8)	2,344 (54.7)	3.97**	3.21**
<2 hours per day	1,954 (44.7)	15 (17.2)	1,939 (45.3)	Reference	Reference
Breakfast					
Skipped breakfast	1,749 (40.0)	49 (56.3)	1,700 (39.7)	1.96**	1.46
Breakfast everyday	2,621 (60.0)	38 (43.7)	2,583 (60.3)	Reference	Reference
Consume fruit					
Not everyday	3,141 (71.9)	64 (73.6)	3,077 (71.8)	1.09	1.34
≥1 time per day	1,229 (28.1)	23 (26.4)	1,206 (28.2)	Reference	Reference
Consume vegetable (N=4369)					
Not everyday	2,792 (63.9)	57 (65.5)	2,735 (63.9)	1.08	1.15
≥1 time per day	1,577 (36.1)	30 (34.5)	1,547 (36.1)	Reference	Reference
Consume juice					
Not everyday	3,618 (82.8)	73 (83.9)	3,545 (82.8)	1.09	0.78
≥1 time per day	752 (17.2)	14 (16.1)	738 (17.2)	Reference	Reference
Consume milk					
Not everyday	2,256 (51.6)	54 (62.1)	2,202 (48.6)	1.55	1.27
≥1 time per day	2,114 (48.4)	33 (37.9)	2,081 (51.4)	Reference	Reference
Consume soft drink					
≥1 time per day	3,188 (73.0)	22 (25.3)	1,159 (27.1)	0.91	0.92
Not everyday	1,181 (27.0)	65 (74.7)	3,123 (72.9)	Reference	Reference

OR, odds ratio; aOR, adjusted odds ratio

Model adjusted for gender, age, school, BMI percentile, academic achievement, physical activity, sleep duration, media use, breakfast, fruit, vegetable, juice, milk and soft drink consumption.

*$p < 0.05$; **$p < 0.01$

Table 2. Multiple linear regression analyses predicting attention problems in adolescents 13-18 years old (n=4,370).

Variable	Univariate analysis *p*-value	Multiple linear regression analysis					
		b	SE	β	t	*p*-value	95% CI
Gender	0.090	0.18	0.10	0.03	1.83	0.07	-0.01 - 0.37
Age	<0.001	0.12	0.03	0.07	3.70	<0.01	0.05 - 0.18
School	<0.001	0.10	0.13	0.01	0.74	0.46	-0.15 - 0.34
BMI percentile	<0.001	0.06	0.05	0.02	1.28	0.20	-0.03 - 0.15
Physical activity	<0.001	-0.09	0.02	-0.07	-4.26	<0.01	-0.13 - -0.05
Sleep time	<0.001	-0.26	0.04	-0.11	-7.12	<0.01	-0.33 - -0.19
Media use	<0.001	0.24	0.02	0.16	10.82	<0.01	0.19 - 0.28
Breakfast	<0.001	-0.09	0.02	-0.06	-4.30	<0.01	-0.12 - -0.05
Consume fruit	<0.001	-0.04	0.03	-0.02	-1.05	0.29	-0.10 - 0.03
Consume vegetable	<0.001	-0.07	0.03	-0.04	-2.54	0.01	-0.13 - -0.02
Consume juice	<0.001	-0.04	0.04	-0.02	-1.03	0.30	-0.11 - 0.03
Consume milk	<0.001	-0.06	0.03	-0.03	-2.13	0.03	-0.11 - -0.01
Consume soft drink	<0.001	0.19	0.03	0.08	5.54	<0.01	0.12 - 0.26

b, Unstandardized coefficients; SE, standard error; β, standardized coefficients; CI, confidence interval

vocational schools had a significantly higher likelihood of having clinical attention problems compared to those in secondary schools (OR = 0.62, 95% CI = 0.39-0.99). Lower academic achievement was also associated with a higher risk of clinical attention problems (OR = 0.51, 95% CI = 0.32-0.81). Adolescents who reported media use of 2 hours or more per day were nearly four times more likely to have clinical attention problems (OR = 3.97, 95% CI = 2.27-6.95). Additionally, skipping breakfast was significantly associated with clinical attention problems (OR = 1.96, 95% CI = 1.28-3.01).

In the multivariable logistic regression analysis, after adjusting for potential confounders, media use of 2 hours or more per day remained a significant predictor of clinical attention problems (aOR = 3.21, 95% CI = 1.81-5.71). Lower academic achievement also remained significantly associated with clinical attention problems after adjustment (aOR = 0.59, 95% CI = 0.36-0.96). However, the associations between school type and breakfast habits with clinical attention problems were attenuated and no longer statistically significant after adjustment.

Multivariable linear regression analyses revealed that older age, increased media use, and soft drinks consumption were positively associated with higher attention problem scores. Conversely, physical activity, adequate sleep, regular breakfast intake, and consumption of vegetables and milk showed significant negative associations with attention problems scores.

The analysis of lifestyle factors and their association with attention problem scores revealed several significant findings, as illustrated in Figure 1. Adolescents who engaged in daily physical activity had significantly lower mean YSR-AP scores compared to those physically active less than 3 days per week (4.27 vs 4.89, $p < 0.001$). Regarding sleep duration, those who slept 8-10 hours per night had significantly lower scores than those who slept 6-7 hours or less than 6 hours (4.03, 4.74, and 5.78 respectively, $p < 0.001$). Adolescents who reported media use of 5 hours or more per day had significantly higher scores compared to those who used media for 3-4 hours and less than 2 hours per day (5.83, 4.70, and 4.11 respectively, $p < 0.001$).

Analysis of dietary habits and their association with attention problem scores, as presented in Figure 2, found that regular breakfast consumption was associated with significantly lower mean attention problem scores compared to those who ate breakfast less than 3 days per week (4.37 vs 5.30, $p < 0.001$). Adolescents who consumed vegetables 1-3 times per day had significantly lower scores compared to those who did not consume vegetables daily (4.43 vs 4.91, $p < 0.001$). High soft drink consumption (more than 4 times per day) correlated with significantly higher attention problem scores compared to consuming soft drinks 1-2 times per day (6.08 vs 4.86, $p < 0.001$). Milk consumption 1-3 times per day was linked to lower mean scores compared to no daily milk consumption (4.49 vs 5.00, $p < 0.001$).

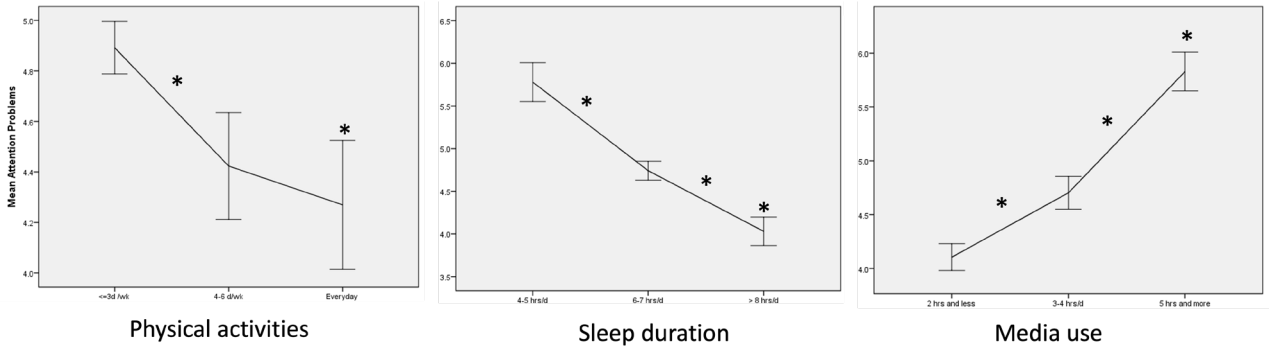

Figure 1. Attention problem scores according to the response scores for the lifestyle factors.
*Statistically significant difference of Youth Self Report-Attention Problems (YSR-AP) scores between 2 groups at $p < 0.05$

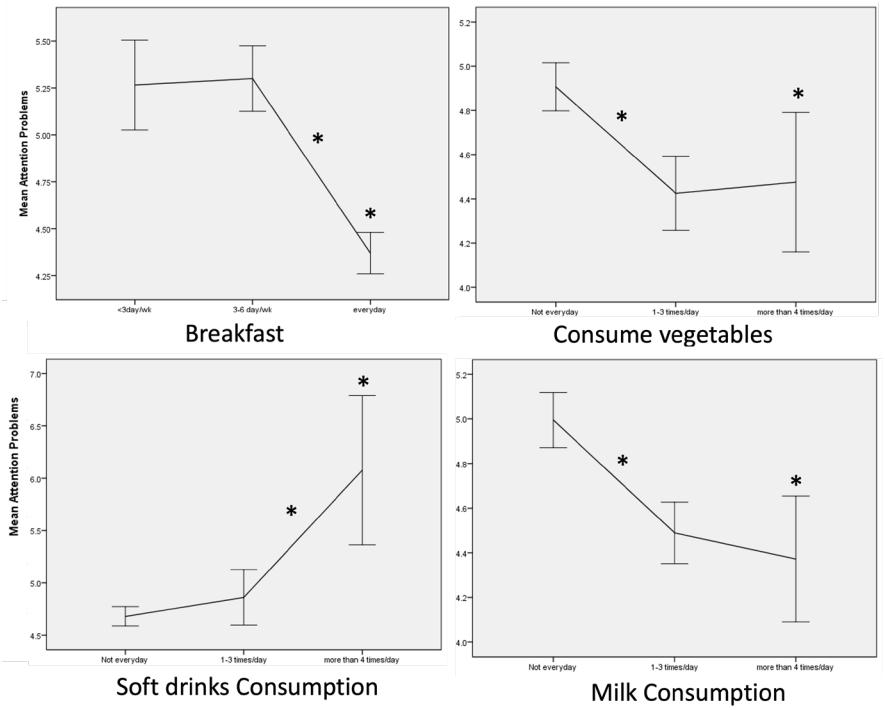

Figure 2. Attention problem scores according to the response scores for the dietary habits.
*Statistically significant difference of Youth Self Report-Attention Problems (YSR-AP) scores between 2 groups at $p < 0.05$

DISCUSSION

Our study reveals a concerning trend of unhealthy lifestyles among adolescents in Chiang Mai, characterized by limited physical activity, inadequate sleep, excessive media use, and poor dietary habits. Notably, we found that media use of 2 hours or more per day was significantly associated with clinical attention problems, even after controlling for potential confounders such as age, gender, BMI percentile, and other lifestyle factors.

This aligns with previous research (23, 24), which identified significant associations between screen media use and ADHD-related behaviors. Extending these findings, our study demonstrates how excessive media use correlates with clinically significant attention problems among Thai adolescents. Thorell et al. (31) suggested reciprocal associations between digital media use and ADHD symptoms, indicating that children with ADHD might be predisposed to developing problematic digital media habits, potentially exacerbating their symptoms and complicating their daily life. Although our cross-sectional design does not allow us to determine causality or the direction of these relationships, it underscores the need to consider the complex, bidirectional interactions between digital media use and ADHD symptoms.

Several mechanisms could potentially explain the link between excessive media use and attention problems. For instance, digital media may displace essential activities such as sleep, physical activity, and face-to-face social interactions, which are

vital for cognitive and behavioral development (31). Moreover, the fast-paced and stimulating nature of digital content could contribute to attentional difficulties and impulsivity in adolescents (32).

Our findings also corroborate previous research indicating significant relationships between various lifestyle factors such as skipping breakfast (33, 34), excessive media use (23, 24), insufficient sleep duration (21), and low physical activity levels (22) and increased attention problems. Notably, adolescents who engaged in daily physical activity and achieved adequate sleep (8-10 hours per night) had significantly lower mean YSR-AP scores than their less active or sleep-deprived counterparts, supporting the notion that insufficient sleep and inadequate physical activity are associated with heightened attention problems (35, 36).

We also discovered protective effects of regular breakfast consumption and the intake of vegetables and milk against attention problems. Adolescents consuming these items 1-3 times per day exhibited significantly lower attention problem scores than those who did not, echoing previous findings that associate high vegetable intake and regular breakfast consumption with reduced ADHD symptoms (18).

Furthermore, our analysis supports the link between high soft drink consumption (more than 4 times per day) and increased attention problems (37-39). Potential mechanisms for these associations might involve sugar intake dynamics, such as sugar tolerance, reactive hypoglycemia post-ingestion, and diminished consumption of essential micronutrients (40). The lack of awareness about healthy dietary habits among adolescents with attention problems, coupled with their propensity for risk-taking behaviors, might explain these patterns (41). Nevertheless, more longitudinal research is needed to verify the effectiveness of dietary interventions for ADHD in this demographic.

It is important to note that while our multivariate linear regression analysis identified associations between lifestyle factors and attention problem scores, these scores do not confirm that participants meet the criteria for clinical attention problems. Although lifestyle modifications should not replace medication as primary treatment, they could serve as a valuable adjunct in managing attention problems in adolescents. Our results have significant clinical implications for assessing and managing attention problems, suggesting that clinicians and parents should consider the potential adverse impacts of excessive media use and other unhealthy lifestyle factors. Implementing strategies to monitor and limit screen time, promote regular physical activity, ensure sufficient sleep, and encourage a balanced diet could form a comprehensive approach to managing attention difficulties.

The strengths of this study include its large, representative sample and the comprehensive assessment of various lifestyle factors associated with attention problems in adolescents. However, some limitations should be noted. Firstly, the cross-sectional design precludes causal inferences. Longitudinal studies are needed to establish the directionality of the observed associations and to evaluate the effectiveness of lifestyle interventions for managing attention problems. Secondly, the reliance on self-reported data may introduce recall bias and other response biases, although the anonymous web-based questionnaire might have mitigated this issue. Thirdly, the YSR-AP scale, while a reliable and valid tool, does not provide a clinical diagnosis of ADHD. Future studies should consider using more comprehensive diagnostic assessments. Lastly, the lack of data on participants' underlying medical conditions, such as pre-existing ADHD and their mood status, which may influence attention problems, should be acknowledged.

CONCLUSIONS

Our study highlights the critical role of lifestyle factors in the management of attention problems among adolescents. We strongly advocate for healthcare professionals to routinely screen for unhealthy lifestyle habits and provide comprehensive guidance on adopting healthier behaviors. This approach should be integral to the management strategies for attention difficulties in this demographic.

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CONFLICTS OF INTERESTS

The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this study.

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Factors Related to a Good Quality of Work Life for Employees in the Poultry Processing Industry in Northeastern Thailand

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ABSTRACT

OBJECTIVE This study aims to analyze factors related to a good quality of work life (QoWL) for employees in the poultry processing industry in northeastern Thailand.

METHODS This study was a cross-sectional analytical study. The sample size was 229 poultry processing workers with good and 458 with not-good QoWL. The Work-related Quality of Life Scale-2 Online Website (THQWL) questionnaire was used. A test of the reliability of the questionnaire found a Cronbach's alpha of 0.899. The principal analyses used the Chi-squared test, the Fisher exact test, and binary logistic regression. Confidence intervals were set at 95% (95% CI), and a p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS Factors significantly related to employees with a good QoWL were working in departments other than slaughtering departments (AOR = 15.58, 95% CI: 7.62, 31.83, $p < 0.001$), having less than a bachelor's degree education level (AOR = 6.65, 95% CI: 3.05, 14.49, $p < 0.001$), being a unit leader or in an upper level position (AOR = 7.26, 95% CI: 3.30, 15.98, $p < 0.001$) and working 40 or more hours per week (AOR= 1.92, 95% CI: 1.17, 3.16, $p = 0.010$).

CONCLUSIONS Not working in the slaughtering department showed the strongest association with good QoWL. Other factors significantly linked to good QoWL included an education level lower than a bachelor's degree, holding positions as unit leaders or in upper management, and working 40 hours or more per week. The study's findings can be applied to enhance and promote the QoWL of workers in the poultry processing and related industries as well as to help improve and reinforce the knowledge, skills, and other attributes important to the efficient performance of employees in the poultry processing industry.

KEYWORDS quality of work life, poultry processing industry, work-related quality of life scale, slaughtering

INTRODUCTION

Thailand aims to become a key player in the global food industry, particularly in poultry, currently ranking 12th in global food exports (1). The poultry sector is expected to grow by 2.5-3.5% annually from 2023 to 2025, driven by the full reopening of the country after COVID-19 boosting tourism and supporting economic recovery. Increased demand for chicken products in restaurants and food processing industries is also anticipated (2).

In the industrial field globally, more than 300 million individuals face mental health problems, substance abuse issues and other disorders affecting their work capacity (3). Work-related stress is associated with various health problems, including depression, heart disease and musculoskeletal disorders (4). The poultry processing industry encompasses specialized facilities designed for poultry slaughtering and dressing, including butchering and processing meat and other poultry products for human consumption. Workers in this industry face a variety of occupational health hazards, including physical risks such as noise and exposure to cold and heat. They also encounter ergonomic challenges including repetitive tasks, forceful exertion and awkward work postures. They are also exposed to chemical hazards from dust, disinfectants, and cleaning agents used in processing, as well as biological threats such as bacteria, viruses, and fungi. Additionally, they face psychosocial hazards, e.g., stress, long work hours, and shift work (5). According to international organizations including the United Nations International Labor Organization (ILO) and Human Rights Watch, poultry processing employees have one of the most dangerous jobs, facing risks greater than those in many other manufacturing processes and sectors (6). Globally, food production industries are adopting standards like ISO 22000 for food safety and ISO 45001 which include recommendations related to mental health (7). Beyond directly impacting worker health, exposure to working conditions in these industries may also affect the quality of employees' working life, absenteeism, work productivity and product quality (8, 9).

Quality of Working Life (QoWL) is a vital aspect which can help ensure employees have the capacity and willingness to succeed in their roles. This contrasts with management practices that often rely on monetary compensation, leading to the potential reduction of organizational management power, particularly in a knowledge-based economy (10). High-quality work life is crucial in the workplace and forms an essential part of an overall strategic plan for achieving long-term, highly productive and reliable company objectives. Organizations prioritizing the maintenance of a high quality work environment are likely to outperform their competitors, resulting in superior organizational performance and lower turnover (11). Previous research on QoWL has shown the significant impact it has on workers and their families (12). Inappropriate work environments can negatively impact motivation, morale, production and performance, and can also increase attrition and burnout (13, 14). There is a substantial correlation between QoWL factors and workforce benefits, including enhanced morale, reduced attrition, increased employee performance and productivity as well as reduced incidents of sickness, absenteeism, stress and burnout. Many organizations are implementing programs such as organizational structure and physical environment improvements, providing group support, supervision and job redesign to reduce job stress and improve QoWL. While previous studies have focused on physical and ergonomic hazards (5, 15), there is also a wealth of research on the association between QoWL and work-related issues (16-18).

This study's purpose was to analyze factors related to the quality of work life in the poultry processing industry in northeastern Thailand. The study assessed employees' views on organizational policies and management, identified areas for improvement, and developed recommendations to enhance the industry's working conditions.

METHODS

Study design, population and sample

This cross-sectional analytical study included 6,077 participants who were employees in the poultry processing industry in northeastern Thailand. The inclusion criteria were Thai nationality, age over 18 years, and a minimum work tenure of 3 months. The exclusion criteria were individuals who had participated in the preliminary item tryout or who had psychiatric diseases.

The sample size calculation used IBM Statistical Package for Social Sciences (SPSS) software, version 28 for Windows (IBM Corp., Armonk, NY; KKU network license), comparing the proportions of two independent groups with a significance level of 0.05 and a power of 80%. A sample ratio of 1:2 was used. Proportions from a study by Soonthornvinit et al. (19) were referenced for sample size calculation. The calculated required sample size was 229 with good and 458 with not-good QoWL. To account for a potential 10% dropout rate, 756 questionnaires were prepared

for distribution in December 2022. The initial step involved contacting human resources and safety officers to extend invitations to workers to participate in the study. Following this, the officers were provided a link to facilitate the distribution of questionnaires to the employees. The researcher used a stratified random sampling method by departments to select the samples.

Research tools

The questionnaire consisted of two parts: 1) the work-related quality of life scale-2 online website (THQWL) and 2) personal and occupational information, a total of 15 items. The self-administered Work-related Quality of Life Scale-2 Online Website (THQWL) questionnaire was used in this study. The researcher had received permission to use the online survey software on the website. Among multiple questionnaires related to QoWL, researchers chose THQWL due to its comprehensive coverage of 7 dimensions, along with its good validity and reliability. The content validity index for THQWL was determined to be 0.97 (20). The researcher tested the questionnaires with 30 samples (Try out) to determine the questionnaires' reliability by calculating the Cronbach's alpha coefficient. Testing the reliability revealed a Cronbach's alpha of 0.899. THQWL consisted of seven subscales, namely employee engagement (EET), control at work (CAW), home-work interface (HWI), general well-being (GWB), job and career satisfaction (JCS), working conditions (WCS) and stress at work (SAW), a total of 32 items. Each question was answered using a five-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree), including 5 negatively worded questions (reverse scored). The QoWL total scores were categorized into three levels: low (31 to 71 points), moderate (72 to 113 points) and high (114 to 155 points) (19). A higher total score indicated a good QoWL, while moderate and low levels indicated not good QoWL.

Statistical analysis

The principal analyses used descriptive statistics, presented as frequencies and percentages. Inferential statistics were then applied to analyze the factors influencing good QoWL using binary logistic regression. The Pearson Chi-squared test was used to compare characteristics between the two groups, with statistical significance set at a 95% CI ($p < 0.05$). The Fisher exact test was used when a cell had an expected count less than 5 and exceeded 20% of the total cell count. This analysis was comprised of two steps. First, univariate analysis examined the relationship of each variable, yielding crude odds ratios. In the second step, multivariate analysis was conducted to control for confounding factors, resulting in adjusted odds ratios. This study included all relevant independent variables, as there may be clinically important variables identified from the literature review. Variables with $p < 0.25$ were selected following the criteria established by Hosmer and Lemeshow (21). Multicollinearity was assessed using the variance inflation factor (VIF), with values greater than 10 and tolerance less than 0.1 indicating multicollinearity. All analyses were performed using the IBM Statistical Package for Social Sciences (SPSS) software, version 28 for Windows (IBM Corp., Armonk, NY; KKU network license).

Ethics

The Khon Kaen University Ethics Committee approved this study for Human Research based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (HE 651388). Participants who participated in the study and their personal identification information remained undisclosed. The data provided did not influence the participants' professional obligations.

RESULTS

Characteristics of the participants

The final sample comprised 687 employees with either good or not-good QoWL. The demographic characteristics that were similar between the groups included gender, monthly income, finances, extra income, and health hazards. The majority of the 229 employees with good QoWL were age ≥ 40 years (42.5%), female (34.5%), single or separated (30.5%), were from Nakhon Ratchasima (hometown) (36.5%), had less than a bachelor's degree (36.5%), were in unit leader or upper-level positions (44.9%), worked in a department other than the slaughtering department (44.8%), had worked 5 or more years in this industry (38.8%), and worked 40 or more hours per week (37.0%) (Table 1).

Table 1. Demographic characteristics of participants between the good and not-good QoWL groups (n=687)

Factors	Good (n=229) n (%)	Not good (n=458) n (%)	*p*-value
Age			< 0.001
≥ 40 yrs	91 (42.5)	123 (57.5)	
< 40 yrs	138 (29.2)	335 (70.8)	
Gender			0.306
female	171 (34.5)	325 (65.5)	
Male	58 (30.4)	133 (69.6)	
Marital status			0.032
Married	93 (38.6)	148 (61.4)	
Single/separated	136 (30.5)	310 (69.5)	
Hometown			0.005
Nakhon Ratchasima	180 (36.5)	313 (63.5)	
Other	49 (25.3)	145 (74.7)	
Education level			< 0.001
Lower than bachelor's degree	209 (36.5)	363 (63.5)	
Bachelor's degree or higher	20 (17.4)	95 (82.6)	
Monthly income (Baht)			0.356
≥ 20,000	23 (28.7)	57 (71.3)	
Up to 20,000	206 (33.9)	401 (66.1)	
Finances			0.231
No savings	207 (32.7)	426 (67.3)	
Savings	22 (40.7)	32 (59.3)	
Extra income			0.367
Yes	26 (38.2)	42 (61.8)	
No	203 (32.8)	416 (67.2)	
Position			0.023
Unit leader and upper-level	35 (44.9)	43 (55.1)	
Operations staff	194 (31.9)	415 (68.1)	
Department			< 0.001
Not slaughtering	219 (44.8)	270 (55.2)	
Slaughtering	10 (5.1)	188 (94.9)	
Years of work			0.017
≥ 5 yrs	101 (38.8)	159 (61.2)	
Up to 5 yrs	128 (30.0)	299 (70.0)	
Working hours			< 0.001
≥ 40 hrs	200 (37.0)	341 (63.0)	
< 40 hrs	29 (19.9)	117 (80.1)	
Health hazard			0.604
No	2 (50.0)	2 (50.0)	
Yes	227 (33.2)	456 (66.8)	

Binary logistic regression analyses of personal and work factors, and QoWL

The results of the univariate analyses revealed that the factors significantly associated with good QoWL were: age > 40 years (crude odds ratios (COR) 1.79, 95% CI: 1.28, 2.51, p = < 0.001); being married (COR 1.43, 95% CI: 1.03, 1.98, p = 0.032); Nakhon Ratchasima hometown (COR 1.70, 95% CI: 1.17, 2.46, p = 0.005); less than a bachelor's degree education level (COR 2.73, 95% CI: 1.64, 4.56, p < 0.001); being a unit or upper-level leader (COR 1.74, 95% CI: 1.08, 2.81, p = 0.023); not working in a slaughtering department (COR 15.24, 95% CI: 7.87, 29.52, p < 0.001); at least 5 years working in this industry (COR 1.48, 95% CI: 1.07, 2.05, p = 0.017); and working ≥ 40 hours per week (COR 2.36, 95% CI: 1.52, 3.68, p < 0.001).

The multivariable analysis revealed that not working in the slaughtering department (adjusted odds ratios (AOR) = 15.58, 95% CI: 7.62, 31.83, p < 0.001) had the strongest association with good QoWL. Other factors significantly related to good

QoWL included less than a bachelor's degree education level (AOR = 6.65, 95% CI: 3.05, 14.49, $p < 0.001$), being a unit leader or in an upper-level position (AOR = 7.26, 95% CI: 3.30, 15.98, $p < 0.001$), and working ≥ 40 hours per week (AOR= 1.92, 95% CI: 1.17, 3.16, $p = 0.010$) (Table 2).

Comparison of the level of WRQLS-2 (QoWL) in each of seven dimensions and overall (OVL) (n = 687)

Most employees in both groups had good scores in job and career satisfaction (good QoWL: 87.8%, not good QoWL: 32.8%) and good employee engagement (good QoWL: 89.1%, not good QoWL: 24.7%). The lowest scores were for stress at work (good QoWL: 26.6%, not good QoWL: 8.3%) and control at work (good QoWL: 56.8%, not good QoWL: 17.2%). (Figure 1)

DISCUSSION

This analytical study focused on identifying factors associated with a good QoWL for employees in the poultry processing industry in Northeastern Thailand. Determination of the sample size used

Table 2. Binary logistic regression analyses of personal and work factors & QoWL (n=687)

Factor	Good QoWL n (%)	Crude OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Age					
≥ 40 yrs	91 (42.5)	1.79 (1.28, 2.51)	< 0.001	1.40 (0.91, 2.15)	0.123
< 40 yrs	138 (29.2)	1		1	
Gender					
female	171 (34.5)	1.21 (0.84, 1.72)	0.306		
Male	58 (30.4)	1			
Marital status					
Married	93 (38.6)	1.43 (1.03, 1.98)	0.032	1.12 (0.75, 1.66)	0.582
Single/separated	136 (30.5)	1		1	
Hometown					
Nakhon Ratchasima	180 (36.5)	1.70 (1.17, 2.46)	0.005	1.28 (0.83, 1.97)	0.261
Other	49 (25.3)	1		1	
Education level					
Lower than bachelor's degree	209 (36.5)	2.73 (1.64, 4.56)	< 0.001	6.65 (3.05, 14.49)	<0.001
Bachelor's degree or higher	20 (17.4)	1		1	
Monthly income (Baht)					
≥ 20,000	23 (28.7)	0.79 (0.47, 1.31)	0.356		
Up to 20,000	206 (33.9)	1			
Finances					
No savings	207 (32.7)	0.71 (0.40, 1.25)	0.231		
Savings	22 (40.7)	1			
Extra income					
Yes	26 (38.2)	1.27 (0.76, 2.13)	0.367		
No	203 (32.8)	1			
Position					
Unit leader and upper-level	35 (44.9)	1.74 (1.08, 2.81)	0.023	7.26 (3.30, 15.98)	<0.001
Operations staff	194 (31.9)	1		1	
Department					
Not slaughtering	219 (44.8)	15.24 (7.87, 29.52)	< 0.001	15.58 (7.62, 31.83)	<0.001
Slaughtering	10 (5.1)	1		1	
Years of work					
≥ 5 yrs	101 (38.8)	1.48 (1.07, 2.05)	0.017	1.11 (0.74, 1.68)	0.608
Up to 5 yrs	128 (30.0)	1		1	
Working hours					
≥ 40 hrs	200 (37.0)	2.36 (1.52, 3.68)	< 0.001	1.92 (1.17, 3.16)	0.010
< 40 hrs	29 (19.0)	1		1	
Health hazard					
No	2 (50.0)	0.50 (0.07, 3.56)	0.604		
Yes	227 (33.2)	1			

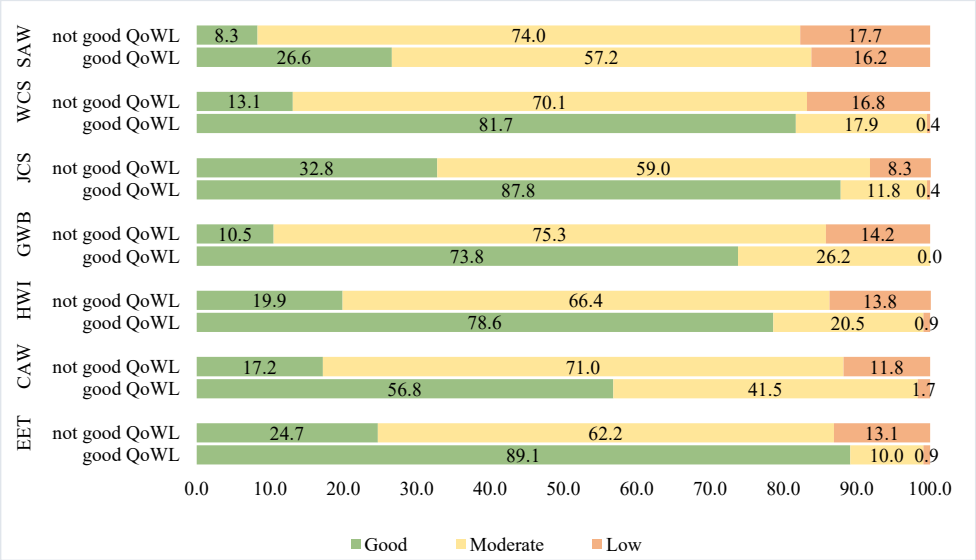

Figure 1. Comparison of the level of QoWL separated by seven dimensions (n=687)
EET, employee engagement; CAW, control at work; HWI, home-work interface; GWB, general well-being; JCS, job and career satisfaction; WCS, working conditions; SAW, stress at work

a formula for comparing two independent proportions and employed a probability sampling method. The sample was considered representative and contributed to the identification of factors related to QoWL. Potentially confounding factors such as gender, monthly income, financial status, and additional income were identified and addressed using multiple logistic regression. The THQWL questionnaire chosen for data collection consisted of sections on personal and occupational information along with the THQWL scale itself. Its reliability and validity were found to be satisfactory.

To ascertain the relative importance of these factors, we used multiple logistic regression. AOR reveal that not working in a slaughtering department exhibited the strongest association with good QoWL. Employees not in a slaughtering department reported higher QoWL ratings compared to those in a slaughtering department. This difference may stem from workers involved in slaughtering employing various coping strategies, both adaptive and maladaptive, to help mitigate negative aspects of the workplace environment and associated stressors. A study by Slade et al. sheds light on the role of slaughterhouse workers, highlighting the stress of authorized killing of living beings and its poorly understood impact on workers' well-being. That research reported a higher prevalence of mental health issues, particularly depression and anxiety, among slaughterhouse workers, along with supportive attitudes towards violence (22). In response to these findings, slaughterhouse management should adopt a holistic approach to meeting the needs of employees throughout all stages of their work, including extending well-being interventions to the broader communities associated with the slaughterhouse (23).

Employees working ≥ 40 hours per week had higher QoWL ratings compared to those working fewer than 40 hours per week. Despite the global manufacturing sector's typical weekly working hours ranging between 35 and 45, certain developing countries, including Thailand, exceed this norm, averaging over 59 hours per week (24). Even though labor laws in Thailand stipulate that workers must not exceed 48 hours for general work and no more than 42 hours for jobs posing physical health risks or welfare concerns (25). In spite of the legal limitations, Thai employees often work beyond these thresholds. In line with previous studies, the findings of the present study indicate that increased working hours and overtime do not necessarily lead to decreased satisfaction. On the contrary, longer working hours and overtime have positive effects on both life and job satisfaction, while a desire to reduce working hours tends to have a negative impact on satisfaction. Employees' lack of desire to reduce their working hours is primarily influenced by overtime pay. The rate of compensating for overtime significantly affects job satisfaction. Satisfaction

increases and hour mismatches decrease when employees are compensated for overtime. However, overtime compensation and expected overtime hours differ across various jobs (26).

Working ≥ 40 hours per week or being employed in non-slaughtering departments were identified as contributors to a positive QoWL, specifically due to the working conditions (WCS). The assessment focused on the extent to which employees were satisfied with the fundamental resources, working conditions, and security necessary for effective job performance. It is evident that physical working conditions, which influence employees' perceptions of health and safety, play a significant role in employee QoWL.

The correlation between individual components and QoWL among employees indicated that unit leaders and upper-level positions had higher QoWL ratings compared to operative staff positions. Operative staff workers tended to place a higher value on extrinsic motivators such as rewards, while leader workers were more focused on intrinsic motivators such as job content for achieving satisfaction (12). The results of the present study are consistent with previous research. For example, Kuster et al. highlighted that operative staff workers may face hazardous conditions and psychosocial pressures in the workplace, with over half reporting symptoms of high anxiety. This underscores the necessity to enhance attention on improving and promoting self-management (14). Anbari et al. found a significant relationship between job satisfaction and the quality of the staffs' working life. Challenges inherent in tasks, lack of opportunities to showcase talents, and limited participation in organizational decision-making were identified as factors negatively impacting job satisfaction. Previous studies have suggested that if the quality of working life is improved, staff can experience a sense of justice, security and success (27). In contrast, it has been observed that leader workers tend to focus more on enterprise-related aspects, whereas operative staff workers prioritize benefits. Attitudes towards what they consider decent work vary between these groups, with emerging evidence suggesting the increased importance of soft factors across both operative staff worker and leader levels (13). Tangchuang et al. reported that working life in an American-owned electronic factory exhibited distinctions between operators and supervisors, engineers and middle management. Some personnel working in potentially harmful conditions received additional compensation, emphasizing the importance of safety and good working conditions. The study also emphasized that qualified supervisors should possess qualities such as loyalty, hard work and efficient problem-solving (28). With the specific details depending on the context of the factory and the job descriptions.

In this study, individuals with less than a bachelor's degree exhibited higher QoWL ratings compared to those with a bachelor's degree or higher. Educational level is part of a complex set of factors, including attained education, social class, income and occupational position, the separate effects of which are not easily distinguishable. In general, professionals and managers tend to exhibit greater character strengths compared to other types of positions, particularly in terms of cognitive abilities. This is to be expected, given the higher cognitive demands and educational requirements associated with these roles. Therefore, the finding that cognitive strengths are more pronounced in managers and professionals is unsurprising and aligns with expectations (29). Previous studies strongly support the idea that meeting workers' expectations correlates positively with future work performance. For instance, having unmet expectations leads to decreased organizational identification and job involvement, higher turnover rates, reduced job satisfaction, increased distress, diminished commitment and weakened interpersonal trust. In terms of workplace integration, unmet expectations are more predictive of poor work adjustment than personal traits like self-efficacy and negative affectivity. While there may be some debate, overall, research indicates that unmet expectations forecast significantly lower work outcomes (30). On the other hand, highly educated individuals generally report better subjective health, greater work and functional abilities, and have a longer life expectancy. Among these factors, education level holds primary importance, remaining relatively constant throughout adult life, being easily measurable and strongly linked to health. Education is particularly significant as it imparts knowledge and skills while enhancing cognitive abilities (31). Additionally, a noteworthy correlation has been observed

between educational qualifications, the growth and development of employees, and self-motivation related to working conditions (32). Conversely, Krommuang and Lertthaitrakul's study of office employees in Bangkok revealed a different assessment of quality of work life based on educational levels. That research indicated that individuals with a master's degree experienced a higher QoWL compared to those with a bachelor's degree. This difference was attributed to higher education providing opportunities for advanced positions and higher returns, with private organizations prioritizing work performance and abilities over educational qualifications in promotions and rewards (33).

Unit leaders and individuals in upper-level positions with less than bachelor's degree were found to have a better QoWL due to their greater level of control at work (CAW). CAW, as a dimension of QoWL, involves several factors: the ability to voice opinions and influence changes in the workplace, involvement in decisions that affect individuals in their specific area of work, participation in decisions that directly impact members of the public, and having sufficient opportunities to question leaders regarding changes at work (34).

In the present study, both groups of employees exhibited high scores in job and career satisfaction (JCS) and employee engagement (EET), as depicted in Figure 1. In a previous study by Abadi et al., the researchers found that QoWL significantly influenced job satisfaction and employee performance, that employee engagement had a significant positive effect on job satisfaction and employee performance, and that job satisfaction significantly influenced employee performance. Moreover, QoWL and employee engagement were identified as having a significant effect on employee performance through job satisfaction (35). Industrial psychologists have noted that the degree of latitude employees have at work, particularly their control over job-related decisions, has a profound impact on their health, morale and ability to handle the workload. The lowest levels of job satisfaction were observed under conditions of high stress at work (SAW) and low control at work (CAW). Robert Karasek's research indicates that employees with high job demands but low control reported more post-work exhaustion, difficulty waking up in the morning, depression, nervousness, anxiety and disturbed sleep compared to their counterparts with more control. Increasing employee control or decision-making latitude has been suggested as a way to reduce job strain without necessarily reducing the workload (36). Hofstede previously suggested that the factors influencing QoWL may vary among employees at different organizational levels within a culture. For example, focusing on job content might be more relevant for professionals and managers, while emphasizing the social context could be more important for clerks and technicians (37).

Additionally, across different slaughterhouses, policies, procedures, and roles pertaining to QoWL should include conducting weekly safety training sessions or meetings to help empower workers in actively monitoring and implementing processes, thus fostering an employee-driven safety culture, ensuring employees take proper breaks throughout their shifts to avoid certain injuries resulting from fatigue, mandating the use of personal protective equipment (PPE) as needed, implementing workstation rotation to reduce the wear and tear on bodies caused by repetitive tasks, and promptly seeking medical attention in the event of cuts, scrapes, or bleeding.

A major strength of this study is that it was the first analytical study to shed light on factors related to QoWL in the poultry processing industry. Notwithstanding, limitations of this research include the possibility of imperfect recall due to the data collection method, as individuals may not accurately remember some details after two weeks. Additionally, the study's cross-sectional nature precludes the determination of direct causality. Nevertheless, initial associations can be clarified to generate hypotheses for analytical studies aimed at further investigating causes and outcomes. Additionally, it is crucial to assess the validity and reliability of tools before their utilization, given that factors influencing QoWL may vary among employees at different organizational levels within a culture.

CONCLUSIONS

The study examined factors influencing the quality of work life (QoWL) among poultry processing industry employees in Northeastern Thailand. Employees not working in the slaughtering department showed the strongest associa-

tion with good QoWL. Other factors significantly linked to good QoWL included having an education level lower than a bachelor's degree, holding a position as unit leader or in upper management, and working 40 hours or more per week. Improving QoWL requires slaughterhouse management to adopt a comprehensive approach to addressing employee needs across all work stages. In addition, implementing policies, procedures, and roles related to QoWL should be expanded to other slaughterhouses.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Original Article

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Treatment Patterns and Surgical Outcomes of Stage IA1 Cervical Carcinoma

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ABSTRACT

OBJECTIVE The primary objective was to determine the 5-year overall survival rate and 5-year disease-free survival rate of patients with CACx 1A1 treated at Chiang Mai University Hospital and the secondary objective was to describe treatment modalities as well as their associated complications.

METHODS Patients with stage IA1 cervical cancer diagnosed from January 2001 to June 2018 at Chiang Mai University Hospital were retrospectively reviewed. Inclusion criteria included previously untreated patients diagnosed with stage IA1 cervical cancer (2018 FIGO staging system). Exclusion criteria were patients with recurrent cervical cancer, other gynecologic malignancies, and those in a pregnant state. The analysis included treatment patterns, surgical types, and clinicopathologic variables, i.e., nodal metastasis, parametrial involvement, positive surgical margins, deep stromal invasion (DSI), lymph-vascular space invasion (LVSI), adjuvant treatment, 5-year disease-free survival, and 5-year overall survival. All pathologic slides were reviewed by gynecologic pathologists. The Kruskal-Wallis test, Fisher's exact test, the Kaplan-Meier method and log-rank test were used for statistical analysis.

RESULTS Of the 184 patients included in this study, simple hysterectomy was the most common treatment (57.3%), followed by modified radical hysterectomy (MRH) and radical hysterectomy (RH) (27.6% and 9.7% respectively). Conization and radiation were chosen in a few cases. At the median follow up time of 40.8 months, the 5-year disease free survival rate was 99.1% and the 5-year overall survival rate was 95.0%. Pelvic lymph node dissection was done in 62 cases (33.7%), but only one case (0.54%) had pelvic lymph node metastasis.

CONCLUSIONS The surgical and survival outcomes for women with stage IA1 cervical cancer are excellent. They can be effectively treated with less radical interventions such as simple hysterectomy and conization. Lymph node metastasis is rare at this stage (0.54%); therefore, lymphadenectomy may possibly be omitted.

KEYWORDS cervical cancer, IA1, treatment outcome

INTRODUCTION

Cervical cancer ranks as the fourth most frequently detected cancer and the fourth primary contributor to cancer-related fatalities among females globally (1). The majority of new cases and deaths stem from low and middle-income

countries, posing economic and social challenges for the Thai health system.

In recent years, there has been a notable increase in the adoption of cervical cancer screening programs incorporating PAP smear and/or high-risk human papillomavirus (HPV) testing. This trend has led to the earlier identification of preinvasive lesions, microinvasive, and early-stage cervical cancer. The implementation of this strategic approach has resulted in a significant reduction in the morbidity and mortality associated with cervical cancer.

In 2018, the updated staging criteria for carcinoma of the cervix uteri by the International Federation of Gynecology and Obstetrics classified stage IA1 cervical cancer as a microscopic carcinoma confined to the cervix uteri with stromal invasion of less than 3 mm in depth (2). Diagnosis necessitates microscopic examination of a conization encompassing the entire lesion. The treatment modalities for stage IA1 cervical cancer vary from conservative approaches (conization/trachelectomy), to hysterectomy (type 1–type 3), and radiation. The outcomes are excellent and comparable among the various modalities, with a five-year survival rate of 97.5% (3). The recurrence rate and death rate from the cancer are 0.8% and 0.2% respectively.

The choice of treatment is predominantly influenced by factors like fertility aspiration and patient compliance. Moreover, prognostic considerations, including lymph-vascular space invasion (LVSI), cell type, internal margin status, and pathology findings from post-conization endocervical curettage, significantly impact the treatment decision-making process (4).

The foremost determinant of survival is lymph node metastasis and is contingent on the depth of stromal invasion and LVSI. A study conducted by Benedet et al. (5) indicated that the rate of lymph node metastasis is 2.6% for LVSI-positive cases, decreasing to 0.3% for LVSI-negative cases.

As per recent guidelines regarding the management of microinvasive cervical cancer (6, 7), in cases where the tumor lacks LVSI, conization with negative margins or extrafascial hysterectomy is advisable for women who have completed childbearing. When LVSI is present, consideration should be given to pelvic lymphadenectomy, coupled with modified radical hysterectomy. For patients expressing a desire for fertility preservation, cervical conization with close monitoring is deemed reasonable. These recommendations reflect variations in the aggressiveness of treatment, potentially impacting patient morbidity. Notably, some recent studies indicate a very low risk of parametrial invasion at this stage, suggesting that a less radical surgery may be a viable option. Lee et al. similarly proposed conization alone with vigilant follow-up, irrespective of margin status or LVSI (8).

Though several studies on this issue have been published, more studies are still needed to improve future meta-analysis and to further study various aspects, especially rare cell type, rare modalities of treatment, and prognostic factors. Moreover, each center should develop its own data for evaluation of the treatment outcomes. Accordingly, we conducted this study with the primary objectives of determining the 5-year overall survival rate and the 5-year disease-free survival rate of patients with stage 1A1 cervical cancer treated at Chiang Mai University Hospital and secondarily to describe treatment modalities as well as their associated complications.

METHODS

A retrospective analysis was conducted on patients diagnosed with stage IA1 cervical cancer and treated at Chiang Mai University Hospital in Chiang Mai, Thailand, from January 2001 to June 2018. All cases were sourced from the clinical database of the Gynecologic Oncology Division, including comprehensive reviews of the clinical charts, operative reports, and pathology reports of each patient. Additionally, all histopathological reports underwent review by a specialist in gynecologic pathology.

Eligibility criteria included previously untreated patients diagnosed with stage IA1 cervical cancer, classified according to the 2018 FIGO staging system. For this study, stage IA1 cervical cancer, or microinvasive cancer, was defined as a microscopic tumor with stromal invasion not exceeding 3 mm in depth. The diagnosis should be established through examination of conization, hysterectomy, or trachelectomy specimens. Exclusion criteria encompassed patients with recurrent cervical cancer, other gynecologic malignancies, and those in a pregnant state.

Treatment strategies were categorized into three options: (1) conservative treatment by conization, (2) hysterectomy, and (3) radiation (vaginal brachytherapy). Within the hysterectomy strategy, irrespective of the surgical approach, groups were distinguished as follows: (a) simple hysterectomy group (extrafascial hysterectomy), (b) modified radical hysterectomy (MRH) group (class 2 Piver-Rutledge-Smith classification or type B Querleu-Morrow classification), and (c) radical hysterectomy (RH) group (class 3 Piver-Rutledge-Smith classification or type C Querleu-Morrow classification). The decision regarding pelvic lymph node dissection or sampling was based on the surgeon's discretion.

The procedure of MRH with pelvic node dissection (PND) was performed on patients exhibiting LVSI. RH and PND were conducted on patients with either the presence of LVSI or the identification of cancer cells in endocervical curettage specimens during the loop electrosurgical excision procedure (LEEP) or cold knife conization (CKC). Systematic pelvic lymphadenectomy involved the removal of all fatty tissue along both sides of the common iliac, external iliac, internal iliac vessels, and the lymphatic tissue in the obturator fossa. Para-aortic lymphadenectomy was only undertaken when gross metastasis to the common iliac nodes or para- aortic nodes was suspected.

Pathological variables of the cervical tumor were considered as follows: (i) histological cell type, (ii) depth of stromal invasion, (iii) LVSI was considered as positive if tumor cells were found in the lymph-vascular spaces, (iv) parametrial involvement, (v) lymph node status, (vi) residual tumor, defined as remaining tumor cells detected on hysterectomy specimens following cervical conization or biopsy, and (vii) vaginal margin status.

Following the primary surgical intervention, all patients underwent regular follow-up assessments at our hospital. The frequency of these assessments was established as every three months for the first year, every four months in the second year, every six months up to the fifth year, followed by yearly check-ups. The follow-up protocol, at the minimum, comprised cervical cytology and colposcopy, with directed biopsy performed when indicated. The time from the treatment date to either the recurrence diagnosis or to the last follow-up date was defined as the disease-free survival period. Overall survival was characterized as the time from the treatment date to the date of death. This study received approval from the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University.

All analyses were performed using Stata version 15 (StataCorp LLC, College Station, TX, USA). Continuous variables are expressed as mean ± SD or median (interquartile range) as appropriate and categorical variables are expressed as frequency (%). Comparisons among various groups were performed using the Kruskal-Wallis test and Fisher's exact test. The Kaplan–Meyer method was used in the analysis of survival rate, and differences were tested for statistical significance using the log-rank test. A p-value of less than 0.05 was considered to be significant.

RESULTS

Of the initial 193 patients with stage IA1 cervical cancer, eight were excluded due to missing data in the medical records and operative notes. Only one patient opted for conization due to fertility desires, leaving 184 patients eligible for this study. The study's flow chart is depicted in Figure 1, and Table 1 and 2 present treatment patterns and clinicopathological variables. The mean age of the study group was 48.7 years (SD: 8.8).

Among the 184 patients, 158 (85.9%) were diagnosed with stage IA1 cervical cancer through LEEP, 8 (4.3%) through CKC, and 18 (9.8%) through other methods (post-hysterectomy specimen, cervical biopsy, endo-cervical curettage). Surgical interventions included simple hysterectomy for 106 (57.6%) patients, MRH for 51 (27.7%), RH for 18 (9.8%), and radiation for nine (4.9%) patients. Twelve (6.5%) patients were HIV-infected, and only 1.4% had negative results from prior cervical cytology screening by PAP smear. The most common histology result was squamous cell carcinoma (85.9%), followed by adenocarcinoma (12.0%).

Lymph-vascular space invasion was present in 12 (7.2%) cases. Pelvic lymph node dissection was performed on 62 (33.7%) patients, with the mean number of nodes removed in MRH being 20 (range 10-27) and the mean RH being 24 (range 15-26). Only one case (0.6%) showed pelvic lymph node metastasis, and no parametrium involvement was found. Only one case had lymph node invasion at the obturator region, which was in

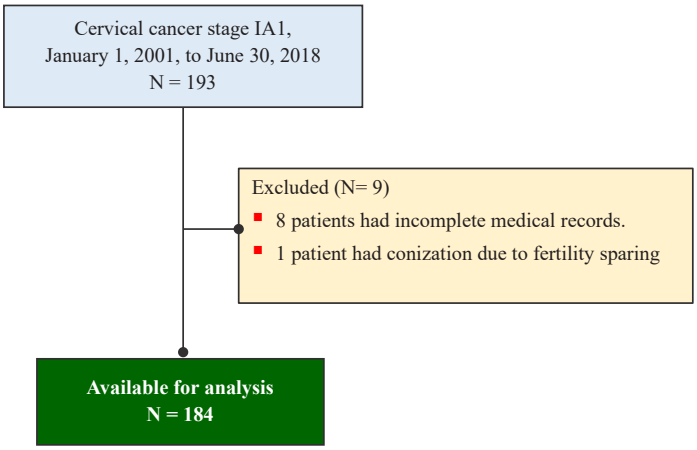

Figure 1. Flow chart of the study

Table 1. Baseline characteristics and outcomes of the patients in this study (n=184)

Characteristics	Treatment groups				Overall	*p*-value
	SH n=106 (57.6%)	MRH n=51 (27.7%)	RH n=18 (9.8%)	RT n=9 (4.9%)		
Age, mean ± SD (years)	47.7±8.1	50.4±8.7	47.6±7.6	54.7±15.4	48.7±8.8	0.140[a]
Histology						<0.001[b]
SCCA	102 (96.2)	38 (74.5)	10 (55.6)	158 (85.9)		
Adenocarcinoma	3 (2.8)	12 (23.5)	7 (38.9)	22 (12)		
Adenosquamous	1 (0.9)	1 (2)	1 (5.6)	3 (1.6)		
Mixed type	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)		
LVSI						<0.001[b]
Negative	105 (99.1)	44 (86.2)	14 (77.7)	172 (93.4)		
Positive	1 (0.9)	7 (13.8)	4 (22.3)	12 (6.6)		
Lymph node dissection						<0.001[b]
No	102 (96.2)	11 (21.6)	0 (0.0)	122 (66.3)		
Yes	4 (3.8)	40 (78.4)	18 (100)	62 (33.7)		
5-year disease-free survival rate (%)	98.6 (90.3-99.8)	100.0	100.0	99.1 (94.0-99.9)		0.88[c]
5-year overall survival rate (%)	94.2 (83.1-98.1)	97.1 (81.4-99.6)	100.0	95.0 (88.2-98.0)		0.26[c]
Follow-up time median (inter-quartile range), months	42.2 (20.2-77.2)	41.8 (15.4-81.4)	37.5 (23.6-61)	40.8 (20-74.8)		0.50[d]

[a], ANOVA test; [b], Fisher's exact test; [c], Log rank test; [d], Kruskal-Wallis test
MRH, modified radical hysterectomy; RH, radical hysterectomy; RT, radiation therapy; SH, simple hysterectomy

the MRH group. This patient received an adjuvant treatment of concurrent chemoradiation after surgery. Also, only one patient had a vaginal margin involvement of cancer, which was in the simple hysterectomy group, and she received laser vaporization after treatment. The number of residual tumors found during surgery after cervical conization or biopsy was not significantly different between the groups. There was one patient who requested fertility preservation management; she was a 30-year-old woman with an abnormal cytologic screening result. The histologic result from LEEP indicated squamous cell carcinoma of the cervix stage IA1 with positive margin for pre-invasive lesion. She refused hysterectomy, and re-LEEP was done as an alternative procedure with satisfactory results. She is currently doing well and has one child. The median follow-up time for all patients was 40.8 months (20.0-74.8).

Complications of surgical treatment, detailed in Table 3, included 4 (2.4%) cases with massive intra-operative blood loss (≥ 1,000 mL), 2 (1.2%)

Table 2. Clinico-pathological variables of surgical treatment patients (n=175)

Characteristics	Treatment groups				*p*-value
	SH	MRH	RH	Overall	
Number of lymph nodes removed	0 (0.0-0.0)	20 (10.0-27.0)	24 (15.0-26.0)	0 (0.0–18.0)	<0.001[a]
Positive lymph nodes*					0.39[b]
No	106 (100.0)	50 (98.0)	18 (100.0)	174 (99.4)	
Yes	0 (0.0)	1 (2.0)	0 (0.0)	1 (0.6)	
Vaginal margin*					1.00[b]
Not involved	101 (95.3)	49 (96.1)	18 (100.0)	168 (96)	
Vg HSIL	4 (3.8)	2 (3.9)	0 (0.0)	6 (3.4)	
Vg SCCA	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.6)	
Residual tumor*					0.430[b]
No	86 (81.1)	37 (72.5)	15 (83.3)	138 (78.9)	
Yes	20 (18.9)	14 (27.5)	3 (16.7)	37 (21.1)	

*Data are expressed as number (%); [a],Kruskal Wallis test; [b], Fisher's exact test
MRH, modified radical hysterectomy; RH, radical hysterectomy; SH, simple hysterectomy

Table 3. Complication of surgical treatment patients (n=175)

Variables	Treatment groups				*p*-value
	SH	MRH	RH	Overall	
Intra-operative blood loss > 1,000 mL*					0.140
No	100 (99.0)	48 (94.1)	18 (100.0)	166 (97.6)	
Yes	1 (1.0)	3 (5.9)	0 (0.0)	4 (2.4)	
Acute febrile*					0.040
No	96 (95.0)	43 (84.3)	18 (100.0)	157 (92.4)	
Yes	5 (5.0)	8 (15.7)	0 (0.0)	13 (7.6)	
Urinary tract injury*					-
No	101 (100.0)	51 (100)	18 (100.0)	170.0 (100)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Bowel injury*					0.160
No	101 (100.0)	49 (96.1)	18 (100.0)	168 (98.8)	
Yes	0 (0.0)	2 (3.9)	0 (0.0)	2 (1.2)	
Lymphedema*					0.040
No	100 (99.0)	48 (96.0)	16 (88.9)	164 (97.0)	
Yes	1 (1.0)	2 (4.0)	2 (11.1)	5 (3.0)	
Bladder dysfunction*					0.050
No	100 (99.0)	46 (92.0)	17 (94.4)	163 (96.4)	
Yes	1 (1.0)	4 (8.0)	1 (5.6)	6 (3.6)	

*Data are expressed as number (%). The *p*-values are based on Fisher's exact test.
MRH, modified radical hysterectomy; RH, radical hysterectomy; SH, simple hysterectomy

with bowel injury, and 13 (7.6%) with febrile morbidity. No urinary tract injuries were reported.

Late complications included significant lymphedema in 5 (3.0%) patients and bladder dysfunction in 6 (3.6%) patients, with recovery duration ranging from 6 weeks to 6 months.

Figures 2 and 3 present Kaplan-Meier survival probability plots for disease-free survival and overall survival. The 5-year disease-free survival rate was 99.1%, with no statistically significant difference between the groups (p = 0.88). There was only one case of recurrent cervical cancer in this series, occurring at the vaginal stump 9 months after simple hysterectomy. The patient was then given vaginal brachytherapy and is currently doing well. The 5-year overall survival rate was 95.0%, also without significant differences between the groups (p = 0.26).

DISCUSSION

Insights gained from this study include the following: (1) treatment outcomes were excellent in our series, resulting in a 5-year disease-free survival rate of 99% and 5-year overall survival

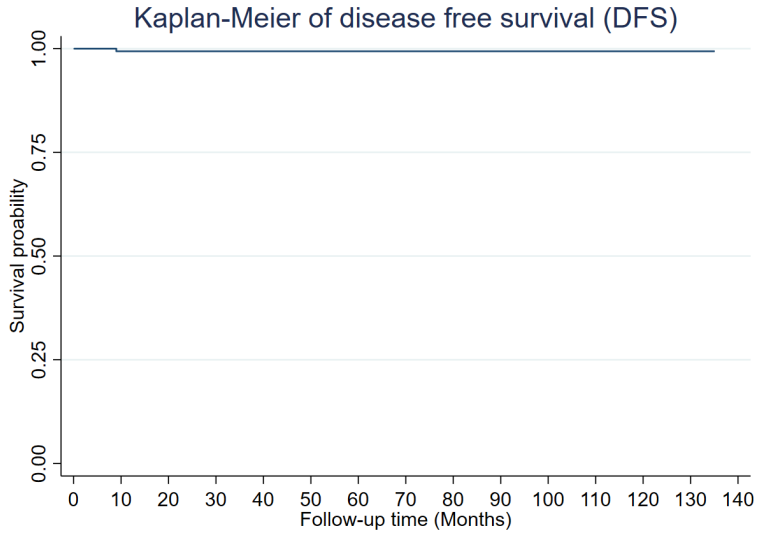

Figure 2. Kaplan-Meier disease-free survival (DFS)

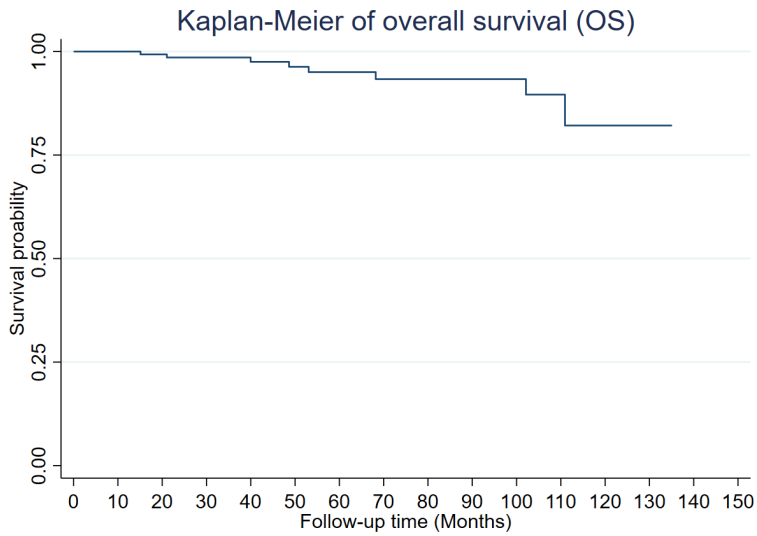

Figure 3. Kaplan-Meier overall survival (OS)

rate of 95%, regardless of treatment modalities. However, the more extensive surgery was associated with a higher rate of minor complications; (2) simple hysterectomy is the most common modality for stage 1A1, accounting for 57.6% of cases, followed by modified radical hysterectomy (27.7%), (3) the histological cell type adenocarcinoma tended to have a higher incidence of more extensive surgery (MRH, RH), though the choice of treatment was mainly based on surgeons' preference. 4) Lymph node metastasis was identified in only 1 out of 184 (0.54%). Because of the low prevalence of non-squamous cell types in this series, the favorable outcomes of this study are mainly reflective of stage 1A1 of squamous cell type. Note that the rates of lymph node metastasis and LVSI were very low and were equally distributed among the treatment subgroups. The low prevalence or small sample size might explain the lack of significant associations between the presence of lymph node metastasis, LVSI and survival outcomes. Note that the rates of modified radical and radical hysterectomy were relatively high in this study. This is attributed to the high rate of positive margins for preinvasive lesions identified in the histologic results of conization specimens.

The outcomes for stage IA1 cervical cancer patients in this study are highly favorable, with a 5-year disease-free survival rate of 99.1% and an overall survival rate of 95.0%. A study conducted by Wright et al. examined 1,409 stage IA1 cervical

cancer patients using SEER data in the USA (9). In their study, 40% of the patients underwent conization, while the remaining 60% underwent hysterectomy. The 5-year overall survival rates did not differ significantly between the two groups (98% and 99%, respectively). Their findings suggest the efficacy of conization, particularly for young women. However, it is important to note that the mean age of patients in our study was higher than that of the aforementioned research. Notably, only one patient in our study opted for conization for fertility preservation, and she has experienced no recurrence of the disease and continues to fare well.

Squamous cell carcinoma is the most common type of cervical cancer, and historically it has been perceived to have a more favorable prognosis than adenocarcinoma or adenosquamous cell carcinoma. Consequently, physicians tended to administer more aggressive treatments for microinvasive cervical cancer with adenocarcinoma cell types. As demonstrated in this study, patients in these groups often opted for more radical surgeries, such as MRH and RH. However, the treatment outcomes did not exhibit significant differences between these different cell types of cancer. Spoozak et al. reported that the adenocarcinoma cell type constituted 24.8% of microinvasive cervical cancer cases and was more prevalent in white and younger patients. Notably, conization was found to be a safe and effective treatment for this specific cell type (10). Furthermore, according to a study by Bean et al., the metastasis rate of the adenocarcinoma cell type to lymph nodes was 0.97%, compared to 0.72% observed in the squamous carcinoma cell type for stage IA cervical cancer (11). The authors suggested conservative treatments for patients with adenocarcinoma cell types in this stage.

Pelvic lymphadenectomy can lead to persistent morbidity for patients, including complications like lymphocyst, lymphedema, pelvic nerve injury, and potential increases in operation time and blood loss. Current cervical cancer treatment guidelines emphasize the assessment of LVSI as a crucial component of treatment planning (6, 7). If LVSI is identified, pelvic lymphadenectomy is recommended in conjunction with modified radical hysterectomy. A study by Benedet indicated that the rate of lymph node metastasis was 2.6% for LVSI-positive cases and 0.3% for LVSI-negative cases (5). However, studies by Spoozak, Haller, and Yahata et al. found no lymph node metastasis in stage IA1 cervical cancer, leading them to suggest the omission of lymphadenectomy in this cancer stage, regardless of LVSI status (10, 12, 13). Our study aligns with these findings. LVSI was detected in only 12 cases, and pelvic lymphadenectomy was performed in 62 cases (33.7%). Notably, only one case exhibited obturator lymph node metastasis, with no discernible association with LVSI status, and the patient remains in remission.

Both radical hysterectomy (RH) and modified radical hysterectomy (MRH) are considered as aggressive treatments for stage IA1 cervical cancer. However, our study revealed no statistically significant differences in overall survival rate and disease-free survival rate between radical surgery (RH and MRH) and simple hysterectomy. Given the very early stage of the disease, only one case exhibited tumor invasion into the vagina, and there were no cases of parametrial invasion. The residual tumors after conization were similar across all groups (70%-80%) and were not identified as a prognostic factor.

Baalbergen et al. and Yahata et al. conducted extensive studies on adenocarcinoma of the cervix stage IA over extended periods (80 months and 70.5 months, respectively) (13, 14). Baalbergen's study reported recurrence rates of 1.7% with conservative surgery and 2% with radical hysterectomy plus pelvic lymphadenectomy, which aligns with the findings from Yahata's study. Both studies concluded that conservative surgery, encompassing simple hysterectomy and conization, proved to be effective for stage IA1 cervical cancer.

Limited research exists on the efficacy of radiation therapy in microinvasive cervical cancer. In our study, utilization of that method was used primarily in cases with multiple comorbidities, accounting for 88.9% of such cases (8 of 9 cases). The radiation technique employed varied based on the preference of the oncologic radiologist and involved either pelvic external beam radiation or intracavitary radiation. Encouragingly, the outcomes in this patient subgroup were satisfactory, with no reported case of recurrence.

The strength of the present study is the inclusion of a large number of patients who were treated at a single institution, ensuring minimal variability in

operative techniques. Additionally, the thorough examination of all pathological specimens by expert gynecologic pathologists enhances the reliability of the findings. The weaknesses of this study are as follows: (1) the retrospective nature, leading to less reliability in some data, (2) heterogeneous modalities of treatment without specific criteria or indications for each modality but with decision making on treatment options based on surgeons' preference, (3) too small a sample size for subgroup analysis, for examples, the small number of cases with adenocarcinoma cell types, lymph node involvement, LVSI. Therefore, prognostic factors for survival rate could not be evaluated with high reliability.

CONCLUSIONS

In conclusion, our results support that stage 1A1 cervical cancer has favorable survival outcomes and that simple hysterectomy is the treatment of choice. More extensive surgery does not seem to increase 5-year overall and 5-year disease-free survival rates but may be associated with a higher rate of complications. Nevertheless, the results should be interpreted with caution since the sample size of the patients receiving treatments other than simple hysterectomy was relatively low, possibly causing the result of non-significance of added value of more aggressive treatment. Radiation and conization may also be considered in some selected cases. Cell type and LVSI status did not affect the outcomes.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ADDITIONAL INFORMATION

Author contributions

U.A.: conceptualization, proposal development, study design, data collection, manuscript writing; S.S.: conceptualization, study design, data collection/validation, manuscript editing; C.T.: conceptualization, study design, supervision, data collection/analysis, manuscript editing; K.T.: conceptualization, data collection, manuscript editing; P.S.: conceptualization, data collection, manuscript editing; J.S.: conceptualization, data collection, manuscript editing.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Research ID: OBG-2561-05847)

Informed consent statement

Not applicable

Data availability statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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Original Article

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Predictive Factors for Recurrence of Fistula-in-Ano After Surgery: A 10-year Experience in a Single Center Study

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ABSTRACT

OBJECTIVE This study aimed to evaluate the recurrence rate of fistula-in-ano treatment and factors associated with recurrence.

METHODS A retrospective cohort study was performed of cryptoglandular-cause fistula-in-ano patients who underwent surgery between January 2010 and June 2020. Cox's regression analysis was used to identify predictive factors for recurrence FIA.

RESULTS The study included 282 patients of whom 233 (82.6%) were male, 76 (27.0%) had previous anal fistula surgery, 77 (27.3%) had a complex type of fistula, 72 (25.5%) were high transphincteric, 3 (1.0%) were suprasphincteric, and 2 (0.7%) were extrasphincteric. Five types of operations were performed: 106 (37.6%) fistulotomies, 43 (15.2%) fistulectomies, five (1.8%) setons, six (2.1%) endorectal advancement flaps (ERAF), and 122 (43.3%) ligations of the intersphincteric fistula tract (LIFT). The recurrence rate was 20.1% (57 patients) among whom 54 (94.0%) were detected at follow-up within 12 months, 2 patients were detected in the second year, and 1 patient was detected in the third year. Four independent factors associated with the recurrence of FIA after surgery were identified: female gender (HR 2.67; 95% CI 1.34-5.34), BMI >25 kg/m^2 (HR 2.47; 95% CI 1.38-4.44), complex type of fistula (HR 2.02; 95% CI 1.02-3.97), and anterior opening (HR 2.14; 95% CI 1.12-4.10). Compared to the LIFT procedure, fistulotomy was the protective factor (HR 0.12; 95% CI 0.03-0.46) while ERAF had a higher rate of recurrence (HR 6.12; 95% CI 1.87-20.03).

CONCLUSIONS Patients with high BMI and female patients should be advised of the higher chance of recurrence after anal fistula surgery. More complex fistula-in-ano and sphincter-preserving surgery was also associated with a higher recurrence rate. The complete healing of the surgery should be monitored for two years after surgery.

KEYWORDS predictive factors, recurrence, fistula-in-ano, anal fistula

INTRODUCTION

Fistula-in-ano is one of the complex diseases for general and colorectal surgery. It is defined as the epithelialization of the tract that connects the perineal skin to the anal canal (1). There are varieties of fistula types involving different levels of anatomical complexity. The condition can result from various etiologies, e.g., cryptoglandular

infections, Crohn's disease, and iatrogenic causes, making its management complex and multifaceted. In Thailand, most cases are caused by cryptoglandular infection which is the focus of this study. While surgical intervention has been the cornerstone of treatment for fistula-in-ano, recurrence remains a significant concern, affecting the quality of life and well-being of afflicted individuals. Moreover, the treatment must be concerned about anorectal function and that the anal sphincter complex should be preserved. Numerous treatments for FIA have been proposed, but none has become the standard treatment. A number of novel surgical techniques and innovations for cryptoglandular anal fistula have been presented, but recurrence has remained around 2-28% (2-5). The recurrence of fistula-in-ano is a significant problem not only clinically but also in terms of the economic and psychological burden both on patients and healthcare systems. Patients with a recurrence of fistula-in-ano frequently suffer from recurrent pain, discharge, abscess formation requiring multiple surgeries and prolonged healing periods. Many studies have been conducted to address predictive factors for recurrence which can be identified before the surgery. Factors affecting recurrence can be divided into three groups: patient factors, e.g., age, gender, BMI, and illness, fistula-related factors, e.g., type of fistula, number and location of tracts, and treatment-related factors, e.g., operative techniques and use of antibiotics (6, 7). This study aimed to evaluate the recurrence rate of FIA after surgical treatment and to identify modifiable factors that can potentially affect the outcome of surgery.

METHODS

Patients

This retrospective cohort study was conducted at Chiang Mai University Hospital where data was collected on patients with cryptoglandular anal fistula who underwent curative surgery from January 2010 to June 2020. This study was approved by the Institutional Ethics Committee of the Faculty of Medicine Chiang Mai University (Study code: SUR-2566-0135, Ethical approval number 184/2566) with a waiver for informed consent. Patients with tuberculosis-related, Crohn's disease-related, or malignancy-related causes of FIA were excluded.

Fistula classification

As mentioned above, the complexity of FIA is determined by the pathway connecting the internal and external opening of the fistula tract to the anal sphincter. There are numerous classifications of FIA. This study focused on cryptoglandular etiology which divides FIA into a simple type (consisting of intersphincteric and low trans-sphincteric fistulas) and a complex type (determined by number of the tract, external sphincter involvement >30%, suprasphincteric type or extrasphincteric type) as well as patients' history of previous surgery and the location of the tract and the position of the opening using a clockface description). This classification system helps surgeons with operation planning and advising the patient.

Fistula surgery and treatment

In this study, the treatment approach for fistula management was determined through a combination of physical examination and intraoperative evaluation. If the physical examination indicated a complex type of FIA, additional imaging modalities such as endoanal ultrasonography or MRI were employed to assess the tract and assist in operative planning. In cases where the physical examination revealed the presence of an active abscess or an incompletely formed tract, the primary treatment methods involved drainage procedures or antibiotic therapy. Definitive surgery was deferred until the tract had become well-organized and more amenable to surgical intervention. The choice of definitive surgery was made based on the complexity of the case and the patient's preferences after receiving guidance and recommendations from the surgeon. On the operative day, patients were typically placed in a lithotomy or prone jackknife position. A proctoscopy examination was performed, and the internal opening (IO) was identified prior to the definitive surgery. For simple fistula types, fistulotomy (with or without marsupialization) and fistulectomy were the usual surgical procedures. In the case of complex FIA, the main curative treatments included LIFT (ligation of the intersphincteric fistula tract), anorectal advancement flap, and the use of a cutting seton.

Recurrence of fistula-in-ano

The complete or successful healing of a fistula is defined as the complete epithelization of the

external opening without any discharge from the previous external opening (EO) or surgical wound. The evaluation of successful healing was conducted three months after the definitive surgery. Failure of fistula surgery comprises two different definitions: persistence and recurrence (2, 6, 8). Persistence of an anal fistula is defined as failure of complete healing of the anal fistula more than six months after surgery. Recurrence is defined as the clinical reappearance of the fistula after complete healing of the surgical wound. Persistence and recurrence are the primary treatment outcomes of this study.

Data collection and follow-up protocol

The data were collected retrospectively from medical records in the Chiang Mai University Hospital Database. Patients who had never attended a follow-up clinic and those who could not be contacted by any means were excluded from the study.

Statistical analysis

Stata version 16 (Stata Corp., College Station, TX, USA) was used for statistical analysis. Continuous data are reported as mean ± standard deviation or median (interquartile range, IQR). Categorical data are given as numbers (percentages). Recurrence analysis used time-to-event analysis. Cox's regression (univariable and multivariable analysis) was used to investigate potential prognostic factors' effect on recurrence. Factors potentially associated with recurrent fistula based on a literature review and those with $p < 0.20$ from the univariable analysis were included in the multivariable analysis. Kaplan-Meier's Curve was used to describe recurrence for each potential prognostic factor including statistical significance. Statistical significance was set at $p < 0.05$.

RESULTS

Two hundred ninety-seven patients met the inclusion criteria of whom fifteen patients were excluded: 5 patients due to tuberculosis-related fistula, 4 patients with malignancy-related fistula, and 6 patients lost follow-up. Finally, 282 patients were analyzed in this study. Most of the patients were male (n = 233, 82.6%) with a mean age of 43 years. The average BMI was approximately 25.

Characteristics of the fistula

The most common type of fistula in this study was low transphincteric fistula (n = 126, 45%) followed by intersphincteric fistula (n = 79, 3%). Of the fistulas, 73% were categorized as a simple fistula. One-fourth of the patients had complex FIA, as shown in Figure 1.

In more than half the patients (n = 149, 52.8%) had the external opening was located posteriorly (3-9 o'clock), while 17 (37.1%) had only an anterior opening (10-2 o'clock) and 29 patients (10.3%) had multiple openings located both anteriorly and posteriorly. A total of 225 patients (80.0%) had only a single external opening.

Operations and treatments

The majority of the operations in this study were carried out under spinal anesthesia, with occasional general anesthesia. In our hospital, five common surgical procedures were performed to treat FIA. Among these procedures, the most frequently performed was the Ligation of the Intersphincteric Fistula Tract (LIFT), with 122 cases (67.0%) followed by fistulotomy with 106 cases (37.6%), and fistulectomy with 43 cases (15.2%). A smaller proportion of patients, six individuals (2.1%), underwent the Endorectal Advancement Flap procedure. The five remaining cases (1.7%) were treated with a Seton technique, as shown in Table 1.

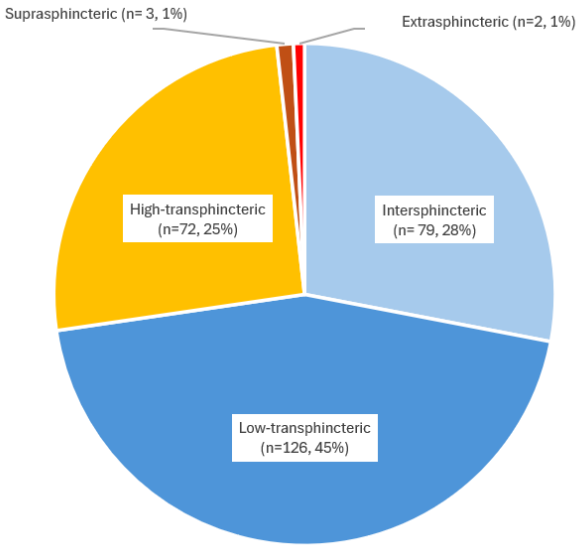

Figure 1. Types of anal fistula categorized by Park's Classification. The light blue and dark blue color represent the "Simple" fistula type. Yellow, orange, and red represent the "Complex" type of fistula

Table 1. Intra-operative factors

Parameters	Recurrence (n=57)	No recurrence (n=225)	*p*-value
Operation, n (%)			<0.001
Fistulotomy	3 (5.3)	103 (45.8)	
Fistulectomy	12 (21.1)	31 (13.8)	
ERAF	5 (8.8)	1 (0.4)	
Seton	0 (0.0)	5 (2.2)	
Lift	37 (64.9)	85 (37.8)	

ERAF, endorectal advancement flap

After surgery, 258 patients (91.5%) were prescribed oral antibiotics. The majority of these prescriptions (73.1%) consisted of a combination of third-generation oral cephalosporin or fluoroquinolone (ciprofloxacin) along with anaerobic antibiotics such as metronidazole or clindamycin. Another antibiotic used was oral amoxicillin and clavulanate, which accounted for 18.4% of the prescriptions and was favored by patients indicated by their better compliance.

Primary outcome

The primary outcome was the recurrence of the fistula which was defined above. The median follow-up time was 13 months (interquartile range, 3-34 months). Fifty-seven patients (20.2%) had a recurrent fistula in the same position or at a surgical wound. Fistulas occurring at a new location were excluded from this study. Notably, 54 patients (94.7%) were diagnosed with recurrence in the first 12 months following surgery, two were diagnosed in the second year and one in the third year after surgery (Figure 2).

The demographic data of patients in both the recurrence and non-recurrence groups are presented in Table 2. Individuals with a BMI exceeding 25 kg/m² experienced a significantly higher recurrence rate (54.4% compared to 45.6%). However, the presence of underlying illnesses (e.g., diabetes, HIV infection) or steroids usage and alcohol consumption did not reveal any significant differences between the two groups.

Furthermore, there was a statistically significant difference in the type of fistula between the two groups. The recurrence group had a higher percentage of complex fistulas (50.9% vs 21.3%), as well as a higher percentage of cases with multiple tracts (29.8% vs 17.3%). Additionally, anterior openings and combined openings were significantly higher in the recurrence group.

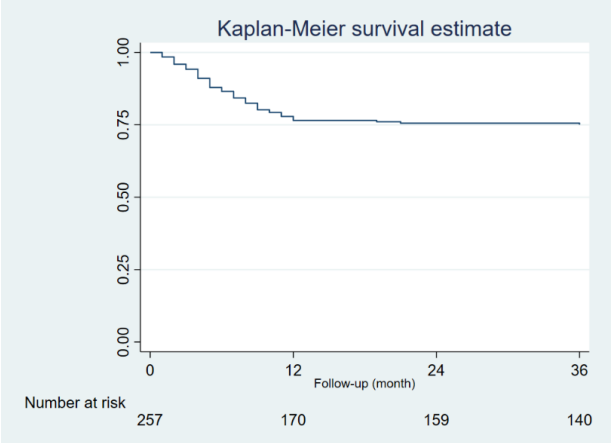

Figure 2. Kaplan-Maier survival curve of patients with fistula in Ano surgery after 36-month follow-up

Interestingly, there was no statistically significant difference observed between patients who did not use antibiotics and those who were prescribed Amoxicillin-Clavulanate or a combination of antibiotics.

A history of previous fistula surgery from another hospital occurred at a significantly higher rate in the recurrence group (42.1% vs 23.1%). Additionally, the choice of operative technique was found to have a significant impact on the treatment outcome. However, as can be seen in Table 3, the type of fistula itself played a pivotal role in determining the choice of operation.

For more complex types of fistulas, such as high transphincteric and suprasphincteric fistulas, the choice of operation did not significantly affect the treatment outcome. In these cases, the complexity of the fistula type often dictated the surgical approach, resulting in a reduced reliance on fistulotomy and fistulectomy. This underscores the importance of tailoring the surgical strategy to the specific characteristics of the fistula, especially when dealing with complex types, where the choice of operation may have less influence on treatment outcomes.

Table 2. Demographic data between recurrence and non-recurrence group of fistula after surgery

Parameters	Recurrence (n=57)	No recurrence (n=225)	*p*-value
Gender, n (%)			0.119
Male	43 (75.4)	190 (84.4)	
Female	14 (24.6)	35 (15.6)	
Age (years), mean (SD)	43.13 (13.6)	42.88 (15.2)	0.913
BMI (kg/m²), mean (SD)	25.61 (5.7)	24.52 (4.3)	0.108
BMI (kg/m²), n (%)			0.015
≤25	26 (45.6)	144 (64.0)	
>25	31 (54.4)	81 (36.0)	
Diabetes, n (%)	4 (7.0)	13 (5.8)	0.756
Smoking, n (%)	9 (15.8)	27 (12.0)	0.505
Alcohol, n (%)	24 (42.1)	82 (36.4)	0.447
Steroid use, n (%)	1 (1.8)	6 (2.7)	1.000
HIV infection, n (%)	0 (0.0)	4 (1.8)	0.586
Long sitting activities, n (%)	26 (65.00)	109 (70.8)	0.563
Types of fistula, n (%)			<0.001
Intersphincteric	5 (8.8)	74 (32.9)	
Low-transphincteric	23 (40.4)	103 (45.8)	
High-transphincteric	25 (43.9)	47 (20.9)	
Suprasphincteric	2 (3.5)	1 (0.4)	
Extrasphincteric	2 (3.5)	0 (0.0)	
External opening position, n (%)			0.001
Anterior tract	32 (56.1)	72 (32.0)	
Posterior tract	18 (31.6)	131 (58.2)	
Combined tract	7 (12.3)	22 (9.8)	
Number tract, n (%)			0.041
Single tract	40 (70.2)	186 (82.7)	
Multiple tracts	17 (29.8)	39 (17.3)	
Direction, n (%)			0.292
Straight tract	46 (80.7)	195 (86.7)	
Curved tract	11 (19.3)	30 (13.3)	
Pus or tract culture, n (%)			0.586
No	57 (100.0)	221 (98.2)	
Yes	0 (0.0)	4 (1.8)	
Pathological request, n (%)			0.011
No	16 (28.1)	107 (47.6)	
Yes	41 (71.9)	118 (52.4)	
Antibiotics use, n (%)			1.000
No	5 (8.8)	19 (8.4)	
Augmentin	10 (17.5)	42 (18.7)	
ATB	42 (73.7)	164 (72.9)	
History previous fistula, n (%)			0.007
No	33 (57.9)	173 (76.9)	
Yes	24 (42.1)	52 (23.1)	

BMI, body mass index; ERAF, endorectal advancement flap; LIFT, ligation of intersphincteric fistula tract

Factors affecting fistula recurrence

Factors reported in the literature and demographic data demonstrated to be statistically significant in the in this study were used in univariable and multivariable models as shown in Table 4. The results show that female gender, BMI > 25 kg/m², complex fistula group, higher number of tracts, anterior tract, the specific operative technique, and a history of previous fistula surgery are associated with a higher rate of fistula recurrence.

In multivariable analysis, the independent factors statistically significantly associated with fistula recurrence were female gender (HR 2.67; 95%CI,

Table 3. Demonstrate operation and recurrence rate in each type of fistula

Parameters	Recurrence (n=57)	No recurrence (n=225)	*p*-value
Intersphincteric, n (%)			0.041
Fistulotomy	1 (20.0)	52 (70.3)	
Fistulectomy	4 (80.0)	20 (27.0)	
Seton	0 (0.0)	2 (2.7)	
Low-transphincteric, n (%)			<0.001*
Fistulotomy	2 (8.7)	50 (48.5)	
Fistulectomy	6 (26.1)	10 (9.7)	
ERAF	2 (8.7)	1 (1.0)	
Seton	0 (0.0)	2 (2.0)	
LIFT	13 (56.5)	40 (38.8)	
High-transphincteric, n (%)			0.316
Fistulotomy	0 (0.0)	1 (2.1)	
Fistulectomy	1 (4.0)	1 (2.1)	
ERAF	2 (8.0)	0 (0.0)	
Seton	0 (0.0)	1 (2.1)	
LIFT	2 (88.0)	44 (93.7)	
Suprasphincteric, n (%)			1.000
Fistulectomy	1 (50.0)	0 (0.0)	
ERAF	1 (50.0)	0 (0.0)	
LIFT	0 (0.0)	1 (100.0)	

ERAF, endorectal advancement flap; LIFT, ligation of intersphincteric fistula tract

Table 4. Factors affecting recurrence of fistula in ano after surgery

Parameters	Univariable analysis		Multivariable analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Gender				
Male	1.00	[Ref]	1.00	[Ref]
female	1.77 (0.96-3.24)	0.065	2.67 (1.34-5.34)	0.005
Age (Year)	1.00 (0.98-1.01)	0.621	0.99 (0.97-1.01)	0.305
BMI (Kg/m²)				
≤25	1.00	[Ref]	1.00	[Ref]
>25	1.80 (1.06-3.04)	0.029	2.47 (1.38-4.44)	0.002
Steroid use	0.64 (0.09-4.64)	0.660	3.29 (0.41-26.58)	0.263
Alcohol	1.13 (0.67-1.92)	0.644	1.33 (0.74-2.37)	0.343
Diabetes	1.08 (0.39-3.00)	0.876	0.77 (0.25-2.36)	0.653
History previous fistula	2.12 (1.25-3.60)	0.006	0.84 (0.45-1.57)	0.595
Complex type				
Simple type	1.00	[Ref]	1.00	[Ref]
Complex type	3.31 (1.96-5.60)	<0.001	2.02 (1.02-3.97)	0.042
Direction				
Straight tract	1.00	[Ref]	1.00	[Ref]
Curved tract	1.48 (0.77-2.86)	0.244	0.65 (0.31-1.36)	0.251
External opening position				
Anterior opening	2.58 (1.43-4.66)	0.002	2.14 (1.12-4.10)	0.022
Posterior opening	1.00	[Ref]	1.00	[Ref]
Combined opening	2.16 (0.90-5.22)	0.086	1.60 (0.56-4.52)	0.379
Number tract				
Single tract	1.00	[Ref]	1.00	[Ref]
Multiple tracts	1.97 (1.11-3.48)	0.020	1.67 (0.81-3.45)	0.167
Operation				
Fistulotomy	0.07 (0.02-0.24)	<0.001	0.12 (0.03-0.46)	0.002
Fistulectomy	0.87 (0.45-1.67)	0.676	1.57 (0.70-3.56)	0.276
ERAF	3.17 (1.24-8.11)	0.016	6.12 (1.87-20.03)	0.003
LIFT	1.00	[Ref]	1.00	[Ref]

ERHR, hazard ratio; CI, confidence interval; BMI, body mass index; ERAF, endorectal advancement flap; LIFT, ligation of intersphincteric fistula tract

1.34-5.34), BMI > 25kg/m² (HR 2.47; 95% CI, 1.38-4.44), complex type of fistula (HR 2.02; 95% CI, 1.02-3.97), and anterior opening (HR 2.14; 95% CI, 1.12-4.10). When compared to the LIFT procedure, the operative technique with fistulotomy (HR 0.12; 95% CI, 0.03-0.46) had a lower rate of recurrence while ERAF (HR 6.12; 95% CI, 1.87-20.03) increased the chance of recurrence. The survival curve for gender, BMI, and complex fistula is shown in Figure 3.

DISCUSSION

This study included 282 patients with cryptoglandular fistula-in-ano. The recurrence rate was approximately 20 percent, the same as a report from Siriraj Hospital and Khon Kaen Hospital (2). Recurrence was defined to include a persistent fistula and the recurrence of the fistula in the same external opening and/or surgical wound which could have occurred in the LIFT procedure.

Most of the patients in this study were male which in concordance with several studies. This could be due to the fact that the lifestyles and occupations of male males generally include less hygiene care than those of females. However, females showed a higher recurrence rate, which may be explained by the fact that sphincter-saving procedures were often chosen for females to reduce the risk of incontinence (4, 9-12).

The classification defined by Park (3) can also predict the recurrence of FIA. High-transphincteric, suprasphincteric, and extrasphincteric fistulas are categorized as more the complex group (13). This complexity arises from the difficulty in eliminating the fistula tract and draining all infected tissue in these types of fistulas. Additionally, the formation of a horseshoe fistula presents a particular challenge for surgical intervention (14). Surgical techniques such as laying open or excising the entire tract may be limited due to the potential

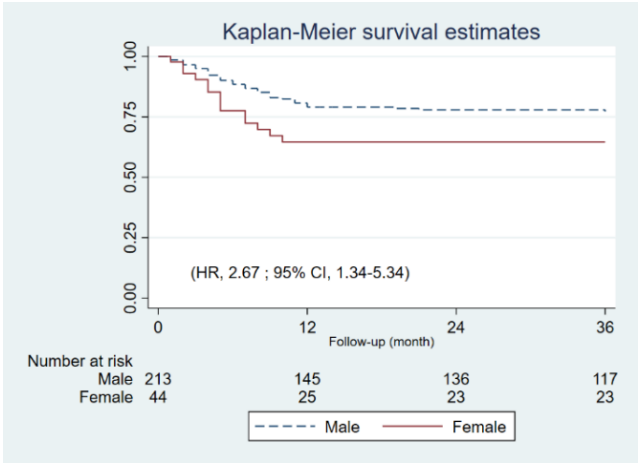

Figure 3.1

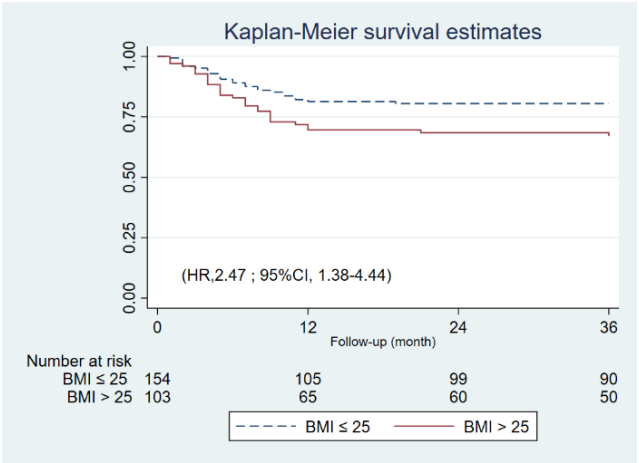

Figure 3.2

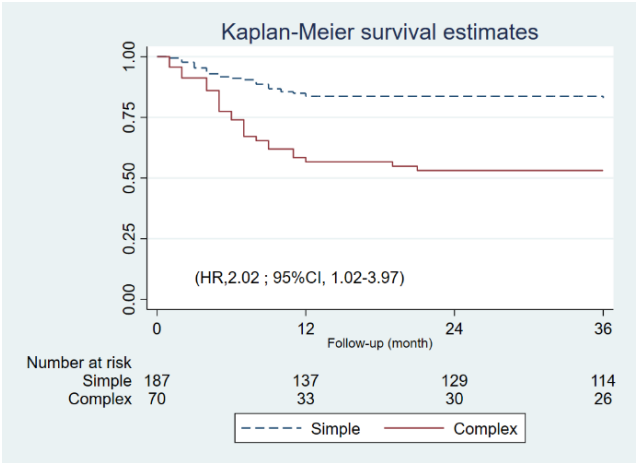

Figure 3.3

Figure 3. Kaplan-Meier survival curve shows survival without recurrence of anal fistula after surgery of gender (Figure 3.1), BMI (Figure 3.2), and complex fistula type (Figure 3.3)
HR, hazard ratio; CI, confidence interval; BMI, body mass index

risk of postoperative incontinence. As a result, sphincter-preserving surgical procedures are often preferred when dealing with this kind of complex fistula as was found to be the case in this study. Some studies have stated that the correlation between the complex type with a higher recurrence rate is due to unidentified or misidentified internal openings and tracts during the surgery. Preoperative high-quality imaging, such as 3-tesla MRI or 3D transrectal ultrasound, might help confirm the tract and improve the success of the operation (1).

In this study, co-morbidity did not show a significant association with fistula recurrence. However, existing evidence suggests that certain co-morbidities can impact the healing process and increase the risk of recurrence in FIA. For instance, diabetes mellitus has been reported to affect the healing process and increase the risk of recurrence (15). This is attributed to factors such as delayed tissue formation and the impairment of the host's immune response against infection in diabetic individuals. Similarly, the use of steroids can also suppress the host's immunity and has been linked to a higher recurrence rate in patients with FIA. Steroids can weaken the body's ability to combat infection and inflammation, potentially leading to treatment challenges and increased recurrence risk.

BMI was significantly associated with the recurrence of FIA. Lu et al. demonstrated a strong relationship between BMI and fistula recurrence (16). Additionally, Schwandner et al. identified obesity (BMI > 30 kg/m^2) as an independent factor for recurrence, with a hazard ratio of 3.35 (p < 0.02) (17). In this study, a similar relationship was observed, though with a slightly lower BMI threshold of > 25 kg/m^2 contributing to being associated with a higher recurrence rate. These results underscore the importance of considering BMI as a relevant factor when assessing the risk of FIA recurrence, planning appropriate treatment and advising patients.

In this study, it was observed that multiple openings appeared to have a potential relationship with recurrence. This finding is consistent with the categorization of multiple tracts as one of the criteria for a complex fistula type in the literature (1, 3, 18). However, Chadbunchachai et al. reported that having multiple tracts was not a risk factor for recurrence in their study (2). This highlights the complexity of assessing the impact of multiple openings on fistula recurrence and suggests that further research may be necessary to clarify its role as a predictive factor. There has been an ongoing debate regarding whether multiple simple fistulas should be categorized as part of the simple group. It is possible that excluding this subgroup might help increase understanding of factors associated with recurrence.

The operative technique employed in this study was found to have a significant impact on the treatment outcome. However, it is important to note that the choice of operative technique is often influenced by factors such as the complexity of the fistula and the surgeon's expertise. In cases of more complex fistulas, the preferred operative procedures may include Ligation of the Intersphincteric Fistula Tract (LIFT), Endorectal Advancement Flap (ERAF), and staged Seton placement. However, experienced coloproctologists may combine multiple procedures within a single operation while striving to preserve the anal sphincter function. This approach aims to achieve both effective treatment of the fistula and better functional outcomes for the patient. Ultimately, the choice of operative technique should be tailored to the specific characteristics of the fistula, patient factors, and the surgeon's expertise to optimize treatment outcomes while preserving anal function (19, 20). Further studies should focus on one technique in each fistula type so the outcomes could be compared to studies reporting results from the LIFT procedure (21, 22).

One year after surgery was the most beneficial time to evaluate the recurrence of fistula which accounted for 94% of cases in this study. However, another study reported recurrence in the second year (2) which suggests that ongoing follow-up beyond the first year after surgery is also essential to detect potential recurrences. Patients should be informed about the possibility of recurrence and the clinical symptoms associated with it. Surgeons should encourage patients to get timely medical attention as it can lead to more effective treatment outcomes.

Limitations

This study has several limitations. It was a retrospective investigation that relied on data collected since 2011 and thus may have introduced biases and limitations inherent in retrospective study designs. Furthermore, the study was conducted in a single institution, potentially limiting the generalizability of the findings to the general population with fistula-in-ano. The patient population and treatment approaches in this institution may not fully represent the normal incidence of fistula types and recurrence rates, as it primarily serves as a referral institution for more complex cases. The study did not include a detailed analysis of the factors and limitations specific to each type of FIA operation; future research could focus on individual fistula types and surgical techniques to provide a more nuanced understanding. Lastly, the study did not include an assessment of functional outcomes following FIA treatment, such as anal sphincter function and quality of life, which are important aspects of treatment evaluation.

CONCLUSIONS

Patients with high BMI and female patients should be advised of the higher risk of recurrence after anal fistula surgery. More complex Fistula-in-ano and sphincter-preserving surgery are associated with a higher recurrence rate. The completeness of healing after surgery should be monitored for two years.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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Original Article

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Stage-Affected Quality of Life in Endometrial Cancer Patients

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ABSTRACT

OBJECTIVE This study aims to compare the aspects of quality of life between early and advanced-stage endometrial cancer patients.

METHODS From January to February 2021, women diagnosed with endometrial cancer and treated at Maharaj Nakorn Chiang Mai Hospital were invited to participate. Two qualities of life questionnaires, the European Organization for Research and Treatment of Cancer Quality of Life QLQ-C30 questionnaire (EORTC QLQ-C30) and the CMU Endometrial Cancer Quality of Life (gastrointestinal, urinary, lymphatic, sexual, and hormonal domains) were used. The chi-squared test, the Mann-Whitney U test, and multivariable linear regression were used for statistical analysis.

RESULTS A total of 103 participants were divided into two groups: 65 individuals with early-stage disease and 38 with advanced-stage disease. All participants completed the quality-of-life questionnaires. The two groups were comparable in terms of global health status/QoL for EORTC QLQ-C30. However, the early-stage group had significantly higher functional scales and quality-of-life summary scores than the advanced-stage group. As for the CMU endometrial cancer QoL, the advanced-stage group had more negative issues related to the gynecologic and hormonal system compared to the early-stage group.

CONCLUSIONS Women with endometrial cancer generally have a good quality of life. Survivors of advanced stages may have a quality of life similar to those in the early stage but with a higher incidence of gastrointestinal, sexual, and hormonal complications.

KEYWORDS chemotherapy, endometrial cancer, oncology, quality of life

INTRODUCTION

The American Cancer Society in 2020 estimated that of the 893,660 women who would be diagnosed with cancer that year 7 percent would be diagnosed with endometrial cancer, of whom 4 percent would die of the disease. The report also stated that endometrial cancer has the highest incidence of gynecologic cancer in developed countries because of their aging population and changing lifestyles (1). The World Health Organization (WHO) reported that of 170,495 women newly diagnosed with cancer in Thailand, 2,671 (1.56%) had endometrial cancer, the third most common gynecologic malignancy following cervical and ovarian cancer. Additionally, the Age-Standardized Incidence Rate (ASR) of endometrial cancer was 16.4 (2). Due to advancements in medical technology, the 5-year survival rate for all stages globally is around 80 percent, but 65 percent have recurrence at the vaginal area and

18 percent have distance metastases (3).

Endometrial carcinoma is common in menopausal women age 45-65 years old, and is commonly diagnosed at the early stage (4). Approximately 95 percent of uterine cancers can be classified by histopathology into two groups: type 1 (grades 1 and 2 endometrioid adenocarcinomas) and type 2 (grade 3 endometrioid tumors and tumors of non-endometrioid histology).

The standard treatments of endometrial cancer are surgery, radiation, and chemotherapy. Surgery is the main treatment because it can define the stage of the disease and thus aid in the selection of postoperative treatments such as radiation and chemotherapy. Adjuvant therapy is considered for high-risk patients, e.g., patients age > 60 years, and those with deep myometrial invasion, endometrioid cell histology grade 3, serous or clear cell histology, or LVSI (5). These factors are prognostic of survival rate as well. However, patients who receive only surgical treatment have a better quality of life in terms of physical function, e.g., less constipation and less loss of appetite, than the patients receiving postoperative treatment, as well as in terms of social function (6). Moreover, menopausal patients who receive a total hysterectomy with bilateral salpingo-oophorectomy have lower libido and orgasm scores than the patients who receive only a total hysterectomy due to pain during intercourse (7). Obesity is another factor that determines quality of life. Studies have found that patients with BMI > 40 kg/m^2 have worse sleep quality than the patients who have BMI 25-29.99 kg/m^2 due to fatigue during daytime, increased stress and a lower quality of life. Patients who have BMI > 50 kg/m^2 in particular have greater problems with stress, physical and mental health (8). Fear of recurrence is also associated with a lower quality of life. Factors that can affect fear of recurrence include aging, physical illness, stress, and low quality of life (9).

The above information suggests that important factors for cancer patients include not only the treatment of the cancer but also the side effects after treatment, including being worried about recurrence. Researchers have noticed the importance of studying the quality of life of endometrial cancer patients treated at Maharaj Nakorn Chiang Mai Hospital and of efficiently using that quality-of-life information to benefit patients. The first objective of this research is to analyze factors affecting the quality of life of endometrial cancer patients treated at Maharaj Nakorn Chiang Mai Hospital using two quality of life questionnaires, the European Organization for Research and Treatment of Cancer Quality of Life QLQ-C30 questionnaire (EORTC QLQ-C30) and the CMU Endometrial Cancer Quality of Life questionnaire. The second objective is to compare the quality of life in early-stage (FIGO Stage I-II) and advanced stage (FIGO Stage III-IV) endometrial cancer patients.

METHODS

Study population

The study population was patients diagnosed with endometrial cancer who were treated by surgery, chemotherapy, or radiation and followed up at Maharaj Nakorn Chiang Mai Hospital during January and February 2021. All participants were given information about the research including the quality-of-life questionnaires which they would complete themselves. All participants provided written informed consent. For participants who were unable to complete the questionnaires, researchers conducted oral interviews and some patients were interviewed via telephone. Demographic data was obtained from hospital records or from interviews with the patient. Surgical and histopathological data was retrieved from medical records. The participants were divided into 2 groups: an early-stage group and an advanced stage group for quality-of-life comparisons.

Questionnaires

A Thai version of the European Organization for Research and Treatment of Cancer quality of life questionnaire (EORTC QLQ-C30) version 3.0 which has demonstrated high validity and reliability was used with the cancer patients (10). The questionnaire includes 30 items consisting of multi-item scale, single item, and a global health status scales encompassing five functional scales (Physical, Role, Cognitive, Emotional, and Social), three symptom scales (Fatigue, Pain, and Nausea and Vomiting), six symptom items (Dyspnea, Loss of Appetite, Sleep Disturbance, Constipation, and Diarrhea), plus a self-assessment of overall health status and the financial impact of the disease itself and of the treatment. A Likert scale, ranging

from 1-4 (1 = "Not at all" and 4 = "Very much", was used to score each item and to determine an average score or "raw score". The raw score was then converted to a scale of 0-100 using the Linear transformation equation. A high score on the functional scale and the global health status scale represents a healthy level of functioning and a good QoL. A high score on the symptom scale and the multi-item scale indicates a high level of problems (11).

The Chiang Mai University Endometrial Cancer Quality of Life Questionnaire (CMU Endometrial Cancer QoL), which was developed by our institute, includes 35 items in the quality-of-life questionnaire (12) which are divided into 4 functional systems: (1) gastrointestinal system (9 items), (2) urinary system (7 items), (3) lymphatic system (4 items), and (4) Reproductive and hormonal system (14 items). Each item is assessed on a scale of 1 to 5, with 1 indicating no symptoms and 5 indicating the most severe symptoms. The scores for each item in each system were analyzed and are shown as means and percentages. If a participant had a high percentage in a system, they were likely to have poor symptoms in that system.

Data analysis

Statistical analysis was conducted using the SPSS program with statistical significance set at a 95% confidence level. Data analyzes were divided into two parts: general patient data and patient quality of life data. General information about the patient included quantitative data such as age, weight, height, monthly income as well as important details of the endometrial cancer in each patient which are shown as mean, standard deviation, median, and interquartile range. Qualitative information such as occupation, marital status, comorbidities, current illnesses, history of treatment for endometrial cancer and concerns that the disease will come back are show as frequencies and percentages.

For correlations between factors affecting the quality of life of the endometrial cancer patients, the quality-of-life score was divided into 2 parts: quality-of-life scores and symptom scores for cancer patients. The formula is based on the EORTC QLQ-C30 version 3.0 analysis. The range of quality-of-life scores and symptom scores was from 0 to 100. Having a high functional score indicates good quality-of-life, but a high symptom score reflects a poor quality of life. The quality-of-life scoring system for endometrial cancer patients, which was developed by the Faculty of Medicine, Chiang Mai University, computes symptom scores then analyzes the distribution of the quality-of-life scores using the One-Sample Kolmogorov-Smirnov test. If the data is normally distributed, parametric statistics were used; however, for not normally distributed data, the statistical pattern was non-parametric. For data analysis, the patients were divided into two groups according to the stage of endometrial cancer (early stage and advanced stage). The quality-of-life scores were compared between the two groups using the Mann-Whitney U test for skewed distributions. In the comparison of categorical outcome variables, odds ratios were used.

For analysis of the relationship between the factors used to study the quality of life of the study groups, a sample of patients with low quality of life scores was used with linear regression to analyze each factor.

RESULTS

A total of 103 women participated in the study, with 65 in the early-stage group and 38 in the advanced stage group. Table 1 summarizes the demographic, surgical, histological characteristics, and treatment data of the participants with comparisons between the two groups. Age, BMI and parity were equal between the 2 groups. The level of income in the advanced stage group was slightly higher than in the other group. The advanced stage group also had a higher rate of chemotherapy and radiotherapy. The two groups were equally concerned about the recurrence of the disease. In terms of treatment status, the majority of patients in advanced group were currently in treatment and completed treatment in less than 2 years while the early-stage group was in completed treatment, especially in less than 2 years.

Comparison between the two groups by EORTC QLQ-C30 scores, shown in Table 2, found no statistically significant difference in global health status/QoL scale, while participants in the early-stage group showed statistically significantly higher scores only in cognitive functioning on the functional scales. There was no significant difference

Table 1. Basic characteristics of participants (n=103)

Characteristics	Overall	Stage		p-value
		Early (n=65)	Advanced (n=38)	
Age (years)	59.7±9.1	59.4±9.3	60.1±8.9	0.701
Age at diagnosis (years)	56.7±9.4	56.2±10.0	57.5±8.3	0.494
BMI	24.6 (21.9-28.5)	24.5 (21.9-28.3)	24.6 (21.9-28.5)	0.896
Parity				0.836
0	33 (32.3)	20 (31.7)	13 (33.3)	
1	13 (12.1)	10 (14.3)	3 (8.3)	
2	38 (38.4)	23 (36.5)	15 (41.7)	
≥3	19 (17.2)	12 (17.5)	7 (16.7)	
Median (IQR)	2 (0-2)	2 (0-2)	2 (0-2)	0.942
Occupation				0.274
None	33 (32.0)	18 (27.7)	15 (39.5)	
Government officer/retired	27 (26.2)	20 (30.8)	7 (18.4)	
Company employee	2 (1.9)	2 (3.1)	0 (0.0)	
Self-employed	19 (18.4)	14 (21.5)	5 (13.2)	
General employee	9 (8.7)	4 (6.2)	5 (13.2)	
Others	13 (12.6)	7 (10.8)	6 (15.8)	
Income group (Baht)				0.182
None	9 (12.3)	3 (6.8)	6 (20.7)	
< 10,000	27 (37.0)	15 (34.1)	12 (41.4)	
10,001-20,000	14 (19.2)	9 (20.5)	5 (17.2)	
>20,000	23 (31.5)	17 (38.6)	6 (20.7)	
Median (IQR)	12,000 (2,000-30,000)	15,000 (3,250-32,500)	8,000 (500-20,000)	0.120
Marital status				0.078
Married	62 (60.2)	43 (66.2)	19 (50.0)	
Single	21 (20.4)	13 (20.0)	8 (21.1)	
Divorced	6 (5.8)	1 (1.5)	5 (13.2)	
Widowed	14 (13.6)	8 (12.3)	6 (15.8)	
Prior hormonal Replacement therapy				0.0006*
No	96 (93.2)	64 (98.5)	32 (84.2)	
Yes	7 (6.8)	1 (1.5)	6 (15.8)	
Surgery				0.786
TAH	1 (1.0)	1 (1.6)	0 (0.0)	
TAH & BSO	91 (88.0)	56 (87.5)	35 (92.1)	
TAH c Lt. SO	1 (1.0)	1 (1.6)	0 (0.0)	
TLH & BSO	8 (8.7)	5 (6.3)	3 (8.3)	
TVH c BSO	1 (1.0)	1 (1.6)	0 (0.0)	
TVH c laparoscopic BSO	1 (1.0)	1 (1.6)	0 (0.0)	
LN dissection				0.903
No	39 (38.6)	25 (39.1)	14 (37.8)	
Yes	64 (61.4)	40 (60.9)	24 (62.2)	
Histopathology				<0.009*
Type 1	53 (51.0)	39 (60.0)	14 (35.1)	
Type 2	31 (30.4)	13 (20.0)	18 (48.6)	
Others	19 (18.6)	13 (20.0)	6 (16.2)	
Metastases				<0.001*
No	59 (58.1)	53 (82.0)	6 (15.7)	
Yes	44 (41.9)	12 (18.0)	32 (84.2)	

Table 1. Basic characteristics of participants (n=103) (continued)

Characteristics	Overall	Stage		p-value
		Early (n=65)	Advanced (n=38)	
Chemotherapy				<0.001*
No	48 (46.6)	45 (69.2)	3 (7.9)	
Yes	55 (53.4)	20 (30.8)	35 (92.1)	
Number of chemotherapies	2 (0-6)	0 (0-2)	6 (5.5-6)	<0.001*
Radiotherapy				0.009*
No	47 (45.6)	36 (55.4)	11 (28.9)	
Yes	56 (54.4)	29 (44.6)	27 (71.1)	
Concerned about recurrence				0.751
No	65 (63.6)	41 (62.5)	24 (63.1)	
Yes	38 (36.4)	24 (37.5)	14 (36.8)	
Treatment status				0.071
Current treatment	16 (15.5)	7 (10.7)	9 (23.6)	
Complete treatment <2 year	51 (49.5)	30 (46.1)	21(55.2)	
Complete treatment ≥2 year	36 (35.0)	28 (43.1)	8 (21.2)	

Data is expressed as frequency (percentage); SD, standard deviation; IQR, interquartile range; *p*-value was computed using the Chi-square test for categorical variables, T-test and Mann-Whitney U test for continuous variables with and without normal distribution respectively;
*Statistically significant $p < 0.05$.

Table 2. Characteristics of EORTC QLQ-C3- scores in women with early versus advanced endometrial cancer (n=103)

Questionnaire scores	Overall	Stage		p-value
		Early (n=65)	Advanced (n=38)	
Global health status/QoL	83.3 (75-91.7)	83.3 (75-91.7)	83.3 (66.7-91.7)	0.052
Functional scales				
Physical functioning	93.3 (80.0-100.0)	93.3 (80.0-100.0)	93.3 (80.0-100.0)	0.477
Role functioning	100.0 (83.3-100.0)	100.0 (100.0-100.0)	100.0 (83.3-100.0)	0.181
Emotional functioning	100.0 (83.3-100.0)	100.0 (83.3-100.0)	100.0 (83.3-100.0)	0.808
Cognitive functioning	100.0 (83.3-100.0)	100.0 (83.3-100.0)	83.3 (83.3-100.0)	0.007*
Social functioning	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (83.3-100.0)	0.194
Symptom scales/items				
Fatigue	0.0 (0.0-22.2)	0.0 (0.0-22.2)	0.0 (0.0-22.2)	0.868
Nausea and vomiting	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.364
Pain	0.0 (0.0-16.7)	0.0 (0-16.7)	16.7 (0-16.7)	0.094
Dyspnea	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.649
Insomnia	0.0 (0.0-33.3)	0.0 (0.0-33.3)	0.0 (0.0-33.3)	0.661
Appetite loss	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.640
Constipation	0.0 (0.0-33.3)	0.0 (0-33.3)	0.0 (0.0-0.0)	0.728
Diarrhea	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.963
Financial difficulties	0.0 (0.0-33.3)	0.0 (0.0-0.0)	0.0 (0.0-33.3)	0.051
QLQ-C30 summary score	93.7 (86.3-98.5)	95.5 (88.5-99.5)	91 (81.6-97.4)	0.036*

Data was expressed by median (interquartile range); *p*-value was performed using Mann-Whitney U test;
*Statistically significant $p < 0.05$.

between the two groups in any of the nine symptom scales/items. The QLQ-C30 summary score was significantly higher in the early-stage group than in the advanced stage group.

CMU endometrial cancer QoL scores were also compared between the early and the advanced stage groups. Participants with advanced disease had significantly higher (worse) scores in

the sexual/hormonal domain (total domain score and two of fourteen item scores) than those with early disease. The scores for the other domains were similar, with no statistically significant differences between the two groups (Table 3).

Multivariable analysis (Table 4) showed that recurrence had a negative impact on the QLQ-C30 summary score (adjusted β, 95% confidence interval (CI): -10.04, -14.49 to -5.59) and on domain I (adjusted β, 95% CI: 5.00, 1.40 to 8.60) of the CMU Endometrial Cancer QoL scores. Current treatment was associated with lower CMU Endometrial Cancer QoL scores in domain I (adjusted β, 95% CI 6.37, 1.63 to 11.12) and domain IV (adjusted β,

Table 3. Comparison of CMU Endometrial Cancer QoL scores between Early stage and Advanced stage group (n=103)

Questionnaire scores	Overall	Stage		*p*-value
		Early (n=65)	Advanced (n=38)	
Domain I (gastrointestinal) score	27.8 (25-33.3)	27.8 (25-33.3)	27.8 (25-36.1)	0.652
I1 I have felt a loss of appetite	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	0.256
I2 I have felt nauseous/vomited	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.543
I3 I have had abdominal cramping while resting	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.970
I4 I have had anal cramping while resting	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.331
I5 I have been constipated	1.0 (1.0-1.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.566
I6 I have had diarrhea	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.446
I7 I have had anal pain while passing stool	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.375
I8 I have had blood in stool	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.897
I9 I have had difficulty in holding stool	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.402
Domain II (urinary) score	28.6 (25-35.7)	28.6 (25-32.1)	28.6 (25-35.7)	0.857
II1 I have had difficulty in passing urine	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.213
II2 I have had difficulty in completely emptying bladder	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.983
II3 I have had to urinate more frequently	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.813
II4 I have had a burning sensation while passing urine	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.459
II5 I have had blood in urine	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.878
II6 I have had leaking of urine while coughing, sneezing, laughing, or lifting heavy objects	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	0.387
II7 I have had a sudden urge to urinate and was unable to hold it	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	0.100
Domain III (lymphatic) score				
III1 I have felt heavy or weighed down leg	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	0.173
III2 I have had leg swelling	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.269
III3 I have had genital swelling	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.611
III4 I have had abdominal wall swelling	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.133
Domain IV (sexual/hormonal) score				
IV1 I have had bleeding from vagina	34.5 (30.0-40.0)	30.2 (28.4-36.3)	37.5 (30.0-45.0)	0.020*
IV2 I have had abnormal discharge or foul smell from vagina	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.600
	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.728
IV3 I have had soreness at vagina while resting.	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.126
IV4 I have had hot flushes or sweating.	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.621
IV5 I have had numbness at my fingers or toes.	2.0 (1.0-3.0)	1.0 (1.0-2.0)	3.0 (1.0-4.0)	0.002*
IV6 Frequency of sexual intercourse.	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.5 (1.0-2.0)	0.854
IV7 I have been afraid of having sexual intercourse	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.482
IV8 I have felt that I am physically not attractive	3.0 (2.0-4.0)	3.0 (1.0-4.0)	2.5 (2.0-3.0)	0.879
IV9 I have had a loss of desire for sexual activity	2.0 (1.0-4.0)	2.0 (1.0-4.0)	2.5 (1.0-4.0)	0.874
IV10 I have felt dry vagina while having sexual intercourse	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.706
IV11 I have felt disappointed concerning orgasm	1.0 (1.0-2.0)	1.0 (1.0-2.0)	4.0 (4.0-4.0)	0.010*
IV16 I have felt short/tight vagina	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.580
IV17 I have felt inflexible vagina	1.0 (1.0-2.0)	1.0 (1.0-2.0)	2.0 (1.0-3.0)	0.614
IV14 I have had pain in vagina during sexual intercourse	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.715

Data was expressed by median (interquartile range); *p*-value was performed using Mann-Whitney U test;
*Statistically significant $p < 0.05$.

Table 4. Factors associated with EORTC QLQ-C30 scores, and CMU Endometrial Cancer QoL scores

Outcome	Multivariable analysis		
	predictors	adjusted β (95% CI)	*p*-value
QLQ-C30 summary score	Number of chemotherapy	-0.79 (-1.47 to -0.10)	0.03
	Concerning of recurrence	-10.04 (-14.49 to -5.59)	<0.01
Domain I	Concerning of recurrence	5.00 (1.40 to 8.60)	<0.01
	Current treatment	6.37 (1.63 to 11.12)	<0.01
Domain II	Histopathology		0.03
	Type 1	Ref	
	Type 2	0.90 (-3.24 to 5.05)	
	Others	-5.93 (-10.83 to -1.03)	
Domain III	Metastasis	7.95 (2.13 to 13.77)	<0.01
	Concerning of recurrence	7.26 (1.35 to 13.18)	0.02
Domain IV	Histopathology		0.01
	Type 1	Ref	
	Type 2	0.88 (-3.94 to 5.69)	
	Others	-7.92 (-13.70 to -2.15)	
	Current treatment	9.99 (4.48 to 15.50)	<0.01

The factors associated with outcome were analyzed using univariable linear regression. Factors with $p \leq 0.10$ were used in the multivariable logistic regression model. Backward elimination was used for considering associated factors with $p \leq 0.05$

95% CI 9.99 (4.48 to 15.50). Metastasis was associated with lower Domain III scores on the CMU Endometrial Cancer QoL (adjusted β, 95% CI 7.95 (1.35 to 13.18)). However, histopathology and the number of chemotherapy regimens were independent predictive factors for quality of life outcomes.

DISCUSSION

According to the study, endometrial cancer patients had a good quality of life, as assessed by the EORTC QLQ-C30 questionnaires. The survivors of endometrial cancer had a median score of 83.3 out of 100 on the EORTC global health status/QoL scale. There was no difference in functional scale scores between the early and advanced stage groups. For the symptom scales, the patients in the advanced-stage group had more pain problems (16.7 from 100) but the difference was not statistically significant. The advanced stage group had a statistically significantly worse quality of life compared to the early-stage group, as shown by the QLQ-C30 summary score. The CMU Endometrial Cancer QoL scores found that the advanced stage group had more problems related to domain 4 (sexual/hormonal), which included numbness of hands and feet and disappointment in orgasm, than the early-stage group. The quality of life prediction factors from the EORTC QLQ-C30 scores were the number of chemotherapy sessions and the patient's level of concern about recurrence. In the domains of the CMU Endometrial Cancer QoL scores, factors which could predict scores in each of the domains were concern about recurrence (domains 1 and 3), current treatment (domains 1 and 4), metastasis (domain 3), and histology (domains 2 and 4).

The most frequent adjuvant therapies in the advanced stage group following comprehensive surgery were radiotherapy, chemotherapy, or both. Patients receiving chemotherapy may experience neuropathy which can include numbness and tingling in the fingers and toes. However, late complications are rare with radiotherapy (13, 14). This study found significantly lower EORTC QLQ-C30 cognitive functioning scale scores in the advanced stage group which could have been a result of chemotherapy-induced cognitive impairment. Jeffrey et al. reported on potential mechanisms of neurologic injury caused by chemotherapy, including direct injury to both cerebral gray and white matter, as well as microvascular injury, which can lead to cognitive impairment (15).

Current treatment, i.e., chemotherapy or radiation therapy, significantly worsens the quality of life of endometrial cancer patients in the gastrointestinal and sexual/hormonal domains. Their side effects, e.g., nausea/vomiting, constipation,

loss of hair, and mucositis, might cause patients to be uncomfortable and impair their self-image (16).

Most of the patients in the study had had a lymphadenectomy during comprehensive surgical staging, especially those with advanced stage and type 2 histology which caused lymphedema with different levels of severity of swelling. In cases of metastasis of cancer, lymph nodes are one of the most frequent locations of recurrence where lymphedema would worsen. The patient might suffer from leg swelling, have to wear compression stockings, worry about their symptoms, and have a greater number of medical appointments, all of which can affect the patients' quality of life (17).

The histopathology of endometrial cancer is also associated with the quality of life of the patients. Patients with high grade or type 2 endometrial cancer had a worse quality of life in terms of socioeconomic, psychological, and physical factors (18). Type 2 endometrial cancer has an aggressive manner which is associated with higher stages and the need for more adjuvant chemotherapy and radiotherapy, resulting in some adverse consequences.

CONCLUSIONS

Women with endometrial cancer generally have a good quality of life. Survivors in advanced stages may have a quality of life similar to those in the early stage, but with more frequent gastrointestinal, sexual, and hormonal complications.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ADDITIONAL INFORMATION

Author contributions

Conceptualization, C.M., N.S., N.P., W.T., C.S., C.T., and D.J.; proposal development, C.M., N.S., N.P., W.T., and C.S.; study design, C.M., N.S., N.P., W.T., C.T., and D.J.; data collection, C.M., N.S., N.P., and W.T.; data validation, C.S., C.T., and D.J.; formal analysis, C.T., and D.J.; manuscript writing, C.M., N.S., N.P., and W.T.; manuscript editing, C.S., C.T., and D.J.; supervision, C.S., C.T., and D.J. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Occupational Characteristics and Working Hours of Stroke and Myocardial Infarction Patients at a Hospital in Bangkok: A Descriptive Study

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ABSTRACT

OBJECTIVE The aim of this study was to investigate occupational risk factors, particularly focusing on working hours, in the patients with cerebrovascular diseases and cardiovascular diseases to help reduce the incidence of those diseases in the future.

METHODS This descriptive study was conducted retrospectively. Patients age 18 to 65 with a first diagnosis of acute stroke and acute myocardial infarction in the years 2019 to 2022 were included. Demographic data were obtained from in-patient records. Occupational data, including working hours and other missing data, were obtained through telephone interviews with the patients.

RESULTS After excluding individuals who were unemployed at the time of the disease incident, deceased, unwilling or unreachable to participate, a total of 146 patients were included in this study. Of those, 115 participants (78.8%) were first diagnosed with acute stroke and 31 participants (21.2%) were first diagnosed with acute myocardial infarction during the study period. The median and interquartile range of weekly working hours of all participants, participants with acute stroke, and participants with acute myocardial infarction were 56 (25), 56 (35), and 59 (27) hours per week, respectively. The range of working hours per week was from 4 to 112 hours. The majority of participants reported not engaging in dangerous work as defined by Thailand's Ministry of Labour, reported no significant occupational hazards, stated they had minimal stress and that they had control over their own job.

CONCLUSIONS The results indicate that the majority of stroke and myocardial infarction patients from this center worked ≥ 55 hours per week. Although the majority reported having no significant occupational factors, recognizing and incorporating a history of occupational factors could be beneficial in disease diagnosis, disease prevention, prevention of recurrence, and aiding the return-to-work process of the patients.

KEYWORDS overwork, cerebrovascular disease, cardiovascular disease, myocardial infarction, stroke, occupational disease, long working hours

INTRODUCTION

Acute stroke and acute myocardial infarction are the two leading causes of death of both men and women in Thailand (1). These two diseases also rank at the top of causes of morbidity and disability-adjusted life years (DALYs) lost (1). Based on

available statistical information, it is evident that both acute stroke and myocardial infarction are very severe diseases that can profoundly impact patients, their families, and the entire country in the long term. It is well known that the primary method of preventing both stroke and myocardial infarction is changing modifiable risk factors, e.g., obesity, controlling underlying diseases, changing smoking habits and alcohol drinking habits, and engaging in physical activity (2-4).

Work and health are two subjects that are inescapably intertwined. Hazards in the workplace can lead to negative effects on health as well as poor health outcomes. Poor health can also impact ability to work, work effectiveness, workers' income, and productivity. A wide spectrum of occupational factors are considered risk factors for several diseases, including coronary heart disease and stroke. A comprehensive review of occupational risk factors for stroke found that job stress, working in extreme temperatures, long working hours, and shift work can increase the risk for stroke (5). A cross-sectional study in Poland showed a significant association between stress, noise, fine particulate dust and the incidence of myocardial infarction (6). The incidence of Karoshi, also known as death from overwork, with cerebrovascular diseases or cardiovascular diseases as the cause of death, has brought work stress and working hours into focus in Japan (7). A longitudinal study conducted in Sweden found that job strain and low job control increases risk for coronary heart disease (8). A case-control study conducted in Korea in 2002-2004 reported an increased risk for hemorrhagic stroke in individuals working ≥ 9 hours/day (9). A systematic review and meta-analysis of data from the USA, Europe, and Australia similarly found an increased risk for stroke and coronary heart disease in individuals working ≥ 55 hours/week compared to those who worked 35-40 hours/week (10). The World Health Organization's (WHO) systematic review of the effects of long working hours showed that working ≥ 48 hours per week is associated with stroke, and working ≥ 55 hours per week is associated with both stroke and ischemic heart disease (11,12). Working long hours has also been found to lead to an increase in adverse health behaviors, such as smoking, alcohol drinking, sleep deprivation, and lack of exercise. Additionally, it can cause psychological stress leading to an increased release of stress hormones which, in turn, result in an increase of autonomic nervous system activity (11).

While numerous cases of work-related cerebrovascular diseases and cardiovascular diseases have been identified in Japan, Taiwan, and Korea (13), there have been no such cases reported in Thailand yet. Occupational factors are typically not included in the questions doctors or nurses ask when taking patients' history in Thailand. Recognizing occupational factors can significantly aid other specialists in providing better prevention and care for patients. In order to identify and provide appropriate compensation for patients with work-related cerebrovascular diseases and cardiovascular diseases, diagnostic criteria have been developed in Japan, Taiwan, and Korea. These criteria primarily emphasize three key aspects: psychological effects, short-term overwork in terms of work hours and overtime, and long-term overwork, which also takes into account work hours and overtime (13). The objective of this study was to examine the working hours and work characteristics of patients with acute stroke and myocardial infarction in Thailand and to identify potential occupational factors, including working hours, with the goal of reducing the incidence of work-related cerebrovascular and cardiovascular diseases in the future.

METHODS

This cross-sectional descriptive study was conducted retrospectively. Basic patient information was obtained through in-patient records. Information about health behaviors and occupational factors was obtained through telephone interviews with the patients conducted by a single interviewer. Ethical approval was granted by Nopparat Rajathanee Hospital (COA 44/2566).

Subjects

This census-based study was conducted among patients with a first diagnosis of acute myocardial infarction, including both ST segment elevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (non-STEMI), as well as acute stroke, including both ischemic and hemorrhagic cases, from 2019 through 2022. From the International Classification of Diseases (ICD-10), I60, I61, I63, and I64 were selected for acute ischemic stroke and acute hemorrhagic

stroke, while I21 and I22 were chosen for acute myocardial infarction. The diagnosis was confirmed to be the initial incidence of the disease through a review of in-patient records and interviews with the patients. The inclusion criteria were the ability to communicate in Thai, age 18 to 65 years (selected as representative of the working population in Thailand), being employed either during or before the occurrence of the disease, and willingness to participate in the study. Exclusion criteria include missing information and a lack of patient's willingness to participate in the study. Out of 2,398 patients first diagnosed with either acute stroke or myocardial infarction, records of 571 in-patient were retrieved for an initial review. After excluding 28 deceased patients, 81 who were unemployed at the time of diagnosis, 21 who were unwilling to participate, and 295 who couldn't be reached, a total of 146 patients remained. All 146 patients were interviewed and included in the study.

Interview

The questions in the interview were developed based on a literature review, and their content validity was assessed by three experts using the Index of Item-Object Congruence (IOC). All telephone interviews were conducted by a single interviewer. The interview questions were divided into two parts: the first part focused on general background information and health behaviors, while the second part addressed participants' occupational information. The general demographics section included questions about education level, underlying diseases, minutes of exercise per week, hours of sleep per day, smoking, and drinking. Underlying diseases were identified as being present or absent at the time of the disease occurrence. Smoking and drinking were defined as yes or no at the time of disease occurrence.

The occupational factors section included questions about occupation, monthly income, job description, work intensity, weekly working hours, job control, stress, workplace hazards, history of work-related accidents, changes in work conditions during the 3 to 6 months prior to the disease occurrence, and abnormal situations at work during the 3 to 6 months before the disease occurrence. Weekly working hours are hours patient spent working, regardless of the work location or whether the working time falls within official work hours. Patients were asked about the time they started their workday and their overtime working hours. Job description included categories of routine work, use of creativity, management, or self-owned business. The definition of dangerous work was classified according to Thailand's Ministry of Labour law in the Labour Protection Act of 2019. Dangerous work included jobs in confined spaces, working with radiation, welding, transporting hazardous materials, working with hazardous chemicals, jobs involving exposure to vibration, and jobs in extreme temperature conditions. Work intensity was graded as low, moderate, or heavy according to Thailand's Ministry of Labour Occupational Safety, Health, and Environmental Act of 2011. Stress was reported as yes or no and included whether the stress was related to work, income, health, and/or family. Job control was defined according to Karasek's model (14) to include a person's ability to influence what happens in their work environment, and classified as yes if the individual had decision-making authority over their own work. Questions for assessing job control were 'Do you have control over your work?' and 'Can you manage both time and work quantity by yourself?'. Abnormal situations and changes were defined following Japanese, Taiwanese, and Korean diagnostic criteria for work-related cardiovascular disease. Abnormal situations included unusual events that trigger extreme psychological tension, excitement, fear, stress, taking on an extreme physical workload, or a dramatic change in the working environment. Changes in work conditions refers to alterations in conditions that lead to mental and physical exhaustion. These changes can include an irregular work schedule, increased workload, heightened job responsibility, frequent travel, shift work, or exposure to extreme temperatures or noise. After obtaining informed consent from the patient via telephone, information from in-patient records was confirmed, and any missing information was collected.

Analysis

The data was analyzed using STATA SE version 18.0 software (STATA Corp. LLC, College Station, TX, USA). Descriptive analysis was used. Categorical data are presented as numbers and percentages,

while numerical data are presented as median and interquartile range. The median and interquartile range of each work characteristic are also presented.

RESULTS

This study included a total of 146 patients. Table 1 presents demographic data and working hours for each group of participants. Out of the 146 patients, 115 (78.8%) were first diagnosed with acute stroke and 31 (21.2%) were first diagnosed with acute myocardial infarction. The majority of participants were male (67.8%). Participants' ages ranged from 28 to 65 years, with a median of 53 and an interquartile range of 12. The majority fell into the group of 45 years old and above (78.77%). Within the group under 45 years old, there were 22 males and 9 females. A BMI equal to or greater than 23 was considered as indicative of overweight, accounting for 78.1% of the total. In terms of health behavior, over 60% of the participants

Table 1. Demographic characteristics and working hours of each group in the study participants

Variables	Frequency, n (%)	Working hours, median hours/week (IQR)
Gender		
Male	99 (67.8)	56.0 (25.0)
Female	47 (32.2)	56.0 (28.0)
Age (years)		
<45	31 (21.2)	55.0 (25.0)
≥45	115 (78.8)	57.0 (27.0)
Age (years), median (IQR)	53 (12.0)	
Education		
None	2 (1.3)	56.0 (28.0)
Primary school	61 (41.8)	63.0 (28.0)
High school	47 (32.2)	54.0 (25.0)
University	36 (24.7)	55.0 (26.5)
BMI (kg/m^2)		
< 23	32 (21.9)	57.5 (22.0)
≥ 23	114 (78.1)	56.0 (27.0)
BMI (kg/m^2), median (IQR)	25.59 (5.5)	
Sleep duration per day (hours)		
Less than 8 hours/day	97 (66.4)	60.0 (32.0)
More than 8 hours/day	49 (33.6)	55.0 (18.0)
Sleep duration per day (hours), median (IQR)	6.75 (3.0)	
Exercise duration per week (mins)		
No exercise	111 (76.0)	57.0 (25.0)
Exercise less than 150 mins per week	20 (13.7)	63.0 (40.5)
Exercise more than 150 mins per week	15 (10.3)	54.0 (29.0)
Exercise duration per week (mins), median (IQR)	140 (220)	
Smoking		
No	77 (52.7)	56.0 (25.0)
Yes	69 (47.3)	56.0 (25.0)
Alcohol		
No	66 (45.2)	54.0 (21.0)
Yes	80 (54.8)	60.0 (31.0)
Underlying disease		
No underlying disease	57 (39.0)	57.0 (24.0)
Hypertension	71 (48.6)	54.0 (28.0)
Diabetes Mellitus	39 (26.7)	59.0 (36.0)
Dyslipidemia	33 (22.6)	54.0 (26.0)
Heart diseases	5 (3.4)	70.0 (30.0)
Others	21 (14.4)	57.0 (23.0)
Diagnosis		
Stroke	115 (78.8)	56.0 (25.0)
Myocardial Infarction	31 (21.2)	59.0 (27.0)

typically slept less than 8 hours per day. The majority of the participants didn't exercise (76%). About 47% of the participants were smokers, and 55% were alcohol drinkers at the time of the disease occurrence. Fifty-seven participants (39%) were free from any underlying disease at the time of the disease occurrence. Participants with hypertension, diabetes mellitus, and dyslipidemia at the time of the disease occurrence numbered 71 (48.6%), 39 (26.7%), and 33 (22.6%), respectively. The number of stroke participants with heart diseases, defined as arrhythmia, valvular heart disease, or old ischemic heart disease, was 5 (3.42%).

Details of occupational characteristics and working hours of each group are presented in Table 2. The overall median working hours of the participants was 56 hours per week, with an interquartile range of 25. The range of work duration per week ranged from 4 to 112 hours. Patients with the highest median working hours, 70 hours per week (interquartile range 30) were in the food and beverage services occupation group. The majority of participants were doing routine work (58.9%) and 36.3% of the participants owned their

Table 2. Occupational characteristics and working hours in each group of study participants

Variables	Frequency, n (%)	Working hours, median hours/week (IQR)
Working duration per week (hours)		
Less than 55 hours	68 (46.6)	
More than or equal to 55 hours	78 (53.4)	
Working duration per week (hours), median (IQR)	56 (25.0)	
Income per month (Bath), median (IQR)	15,000 (20,000)	
Dangerous work		
No	110 (75.3)	56.0 (27.0)
Yes	36 (24.7)	56.5 (22.5)
Job		
Routine work	86 (58.9)	56.5 (22.0)
Use of creativity	3 (2.1)	45.0 (14.0)
Management	4 (2.7)	59.5 (18.0)
Owning a business	53 (36.3)	60.0 (35.0)
Work intensity		
Light	21 (14.4)	45.0 (19.0)
Moderate	104 (71.2)	60.0 (29.0)
Heavy	21 (14.4)	57.0 (23.0)
Job control		
No	63 (43.2)	57.0 (22.0)
Yes	83 (56.8)	56.0 (30.0)
Stress in work/income matters		
No	95 (65.1)	54.0 (27.0)
Yes	51 (34.9)	65.0 (30.0)
Stress in personal matters		
No	111 (76.0)	55.0 (25.0)
Yes	35 (24.0)	57.0 (29.0)
Occupational hazards		
No occupational hazards	83 (56.8)	54.0 (28.0)
Physical hazards	13 (8.9)	60.0 (22.0)
Chemical hazards	43 (29.5)	56.0 (15.0)
Psychological hazards	19 (13.0)	72.0 (36.0)
History of work accident		
No	123 (84.2)	56.0 (25.0)
Yes	23 (15.8)	57.0 (30.0)
History of changes in work during the past 3 to 6 months		
No	123 (84.2)	55.0 (25.0)
Yes	23 (15.8)	63.0 (32.0)
History of abnormal situation in work during the past 3 to 6 months		
No	144 (98.6)	56.0 (26.0)
Yes	2 (1.4)	61.0 (8.0)

own business. Participants' occupations were grouped into categories: food and beverage workers, drivers, office workers, mechanics, builders, wholesalers, delivery personnel, general employees, security personnel, farmers, cleaners, programmers, healthcare workers, lawyers, tailors, and teachers. Table 3 presents the groups of occupations along with their median and interquartile range of working hours. The top three occupational groups were food and beverage services (18.49%), drivers (12.33%), and office workers (11.64%). About 71% of the participants were classified in the moderate intensity group. Thirty-six participants worked a dangerous work, with the majority involving welding and/or working with hazardous chemicals. Eighty-three participants (56.8%) stated that they had control over their jobs, 51 participants (34.9%) experienced stress related to work or income, and 35 participants (24.0%) experienced stress related to personal matters, e.g., health status or family issues. After evaluation of the initial telephone interview, 63 participants (43.2%) were found to have had occupational hazards, with majority experiencing chemical hazards (29.5%). In this study, 23 participants (15.8%) experienced changes in work and 2 (1.4%) experienced an abnormal situation at work around the time of the disease occurrence. Reported changes in work included change of workplace, adjustments of work schedules, change of occupation, increased workload, and heightened workplace hazards. Reported abnormal situations at work included conflicts with one's superior and issues with one's own business.

DISCUSSION

The demographic data in this study are consistent with prior studies, which have reported that male gender, increased age, higher body mass index, low physical activity, alcohol consumption are risk factors for both stroke and myocardial infarction. The only factor in this study that differs from previous reports is smoking. The proportion of individuals who were smokers and nonsmokers at the time of the disease occurrence, unlike earlier studies, was approximately equal in the present study. This could be due to a general reduction in the number of smokers in the Thai population. As for underlying diseases, the largest group had hypertension, followed by diabetes mellitus and dyslipidemia, which is consistent with prior knowledge that these are risk factors for stroke and myocardial infarction.

A total of 16 participants (10.96%) were under the age of 45 with no underlying diseases. This group had a median of 55.5 working hours per week (IQR 23.5). Further exploration of this group revealed that 4 participants (2.74%) were under the age of 45, had no underlying diseases, denied smoking, and denied alcohol consumption. In this subgroup, the median working hours was 61.5 hours per week (IQR 35.5).

Table 3. Occupational groups and working hours

Occupational group	Frequency, n (%)	Working hours, median hours/week (IQR)
Food and beverage services	27 (18.49)	70 (30.0)
Office workers	18 (12.33)	54 (20.0)
Drivers	17 (11.64)	66 (22.0)
Builders	14 (9.59)	57 (16.0)
Wholesales	13 (8.90)	54 (31.0)
Mechanics	12 (8.22)	60 (18.5)
General employees	10 (6.85)	38 (18.0)
Delivery	8 (5.48)	55 (18.0)
Security personnel	8 (5.48)	84 (8.0)
Farmers	5 (3.42)	44 (13.0)
Cleaners	4 (2.74)	57 (7.5)
Programmers	3 (2.05)	45 (14.0)
Healthcare workers	3 (2.05)	40 (108.0)
Lawyers	2 (1.37)	37 (34.0)
Tailor	1 (0.68)	63*
Teacher	1 (0.68)	42*

*Data on only one patient available

The median working hours was 56 hours per week for all participants, 56 hours per week for participants with stroke, and 59 hours per week for participants with myocardial infarction. A study in Japan reported that over half of workers compensated for occupational cerebrovascular and cardiovascular diseases were confirmed to have overtime working hours ranging from 80 to 119 hours per month (15). The mechanism by which long working hours can cause stroke and myocardial infarction are divided into two major mechanisms: unhealthy behavior and chronic psychological stress (5). A recent WHO/ILO meta-analysis study reported that working 55 hours or more per week increases the risk of both stroke and myocardial infarction (11,12). One of the differences between the present study and the WHO/ILO study is the age of the participants. The WHO/ILO study included participants aged 15 years and above, considering them as paid workers. According to Thailand's Ministry of Labour, individuals aged 15 to 17 years old are considered children, while those aged 18 and above are considered adults. Thus, with the aim of focusing on the working population in Thailand, the age range of 18 to 65 years old was selected for this study. According to the Thai Ministry of Labour's Labour Protection Act of 2019, employees' working hours are not allowed to exceed 9 hours per day and 48 hours per week in the case of non-dangerous work, and 7 hours per day and 42 hours per week for dangerous work. Employees are not allowed to work overtime more than 36 hours per week, including work on weekends and holidays. The maximum working hours permitted by Thailand's law is 84 hours per week for non-dangerous work and 78 hours per week for dangerous work. However, it is not against the law to extend working hours as needed in cases where cessation of workflow would disrupt the entire process or in emergency conditions, provided there is an agreement with the employees. In this study, 16 participants (10.96%) reported working more than 84 hours per week. Two participants worked 112 hours per week, but this was due to having more than one job. In terms of occupational categories, security personnel had the highest median working hours, followed by workers in the food and beverage sector and drivers. A 2020 study in Korea reported that individuals who were self-employed or employers in the accommodation and food service industry had the highest working hours per week, while employees in the transportation industry had the highest working hours per week (16). A study of overwork-related cerebrovascular or cardiovascular diseases in Japan found that 30.7% of compensated cases worked in transport and postal activities, followed by wholesale and manufacturing (15).

In terms of chronic psychological stress, having no job control can lead to job stress, which can activate a neuroendocrine stress response, and, the same as long working hours, can lead to unhealthy behaviors (5). The majority of participants who reported having job control were in the 'owning a business' category, while the majority of participants who reported having no job control were in the 'routine work' category. About 43% of the participants (n=63) reported having no job control, and 26 participants, or about 41% of those who had no job control, had stress related to work or income matters. In this group, the median working hours was 61.5 with an IQR of 30. Three of the participants were aged under 45, had no underlying disease, but had no job control and were stressed by factors related to work or income matters. These three participants had a median of 57 working hours with an IQR of 23.

Shift work, considered to be psychological or organizational hazard, is an occupational factor that was found to reduce melatonin secretion leading to increased pressure and thrombus formation. Reduced duration of sleep due to shift work also can lead to altered body physiological processes. Shift work has been linked to a higher risk of stroke compared to day workers (17, 18). In this study 17 participants (11.64%) were shift workers and had been diagnosed with stroke. Their median weekly working hours was 72, with an IQR of 24.

In terms of physical hazards, heat and noise both had a possible association with cardiovascular diseases. Previous studies have tried to identify the relationship of noise and cardiovascular diseases (19-21). A recent WHO/ILO study about the effects of occupational exposure to noise on ischemic heart disease, stroke and hypertension, however, stated that there is still only limited evidence of harmfulness in humans (22). In the present study, 5 participants (3.42%) reported

being exposed to noise. Four were in the building category, and one was in the mechanics category. Only 2 participants reported being exposed to heat, one in the construction category and the other in the food and beverage service category. Four participants (2.74%) reported being exposed to physical hazards, having no job control, and having stress related to work or income matters. Those 4 participants had a median of 68 working hours with an IQR of 21.5.

Carbon monoxide, methylene chloride, nitroglycerin, solvents, pesticides, fluorocarbons, hydrocarbons, and heavy metals are chemical hazards associated with the development of cardiovascular disease (5,23). In this study, the majority of participants who reported being exposed to chemical hazards were in the mechanics category. Ten participants (6.85%) reported being exposed to chemical hazards, having no job control, and having stress related to work or income matters. This group of participants had a median of 58.5 working hours and an IQR of 6.

Examination of the criteria for diagnosing work-related cardiovascular disease in Japan, Korea, and Taiwan, which consider three key aspects of occupational factors, psychological, short-term overwork, and long-term overwork, suggests that adapting these criteria in Thailand would be a significant challenge. Obtaining records of working hours from formal workers is already a challenging task; obtaining objective evidence of working hours from informal workers would be an even greater challenge. History taking related to occupational factors could be a starting point for the development of a diagnostic procedure for Thailand and the establishment of Thailand-specific criteria in the future.

Occupational factors can be likened to small but crucial missing pieces of a jigsaw puzzle. These factors can aid in diagnosing diseases and in determining whether a patient is suffering from a work-related or occupational disease. After a diagnosis is made, adjustments to the workplace or other occupational factors could be implemented to prevent the disease from occurring in other workers. Accommodations in the workplace can greatly assist patients undergoing treatment upon their return to work and can also help prevent recurrence. It is evident that long working hours are associated with stroke and myocardial infarction (11,12). Previous studies have also found that working ≥55 hours per week is associated with the risk of recurrent coronary heart disease, with a HR of 1.67 and a 95% CI of 1.10 to 2.53 (24). Other occupational factors may also be associated with disease recurrence, indicating the need for further study. For future study and diagnosis of work-related and occupational diseases, recognition of occupational factors is crucial.

There are several limitations in this study. Some degree of recall bias is inevitable in a retrospective study such as this. A telephone interview was chosen with the aim of obtaining as much detail as possible regarding working hours and other occupational factors in order to mitigate recall bias. Obtaining official or company records of working hours and other occupational factors presents a significant challenge. The second limitation of this study is the relatively low number of participants. Although this is a census-based study, the number of participants was still limited. A larger number of participants could provide a clearer image and offer more insights for future studies in Thailand. The third limitation of this study pertains to generalization. Since data collection in this study was from a single center, a multi-center study in the future might provide a better understanding of occupational factors in Thailand.

CONCLUSIONS

The number of working hours of the majority of stroke and myocardial infarction patients in this study did not exceed the limits set by Thailand's Ministry of Labour law, but did exceed those outlined in the WHO/ILO study. Most participants were employed in the food and beverage service industry and were not engaged in dangerous work. The majority reported they had control in their work, experienced minimal stress, and encountered no significant occupational hazards at their workplace. However, a small proportion did report experiencing stress and encountering occupational hazards. Recognizing and documenting the history of occupational factors could serve as a starting point for future studies, including disease diagnosis and disease prevention efforts. Furthermore, acknowledging of occupational factors during the return-to-work process of patients is crucial for facilitating work accommodations and preventing disease recurrence.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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Original Article

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High-throughput Virtual Screening- and Molecular Docking-based Prediction for Acetylcholinesterase Inhibitors and Exploring its Mechanisms against Alzheimer's Disease based on Network Pharmacology

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ABSTRACT

OBJECTIVE This study aimed to identify promising ligands for inhibiting acetylcholinesterase (AChE) activity using virtual screening (VS).

METHODS VS was used to identify potential AChE inhibitors from the PubChem database. Ligands with favorable binding pocket interactions were selected. SwissADME and pkCSM tools were used to assess drug-likeness and pharmacokinetic properties. Molecular dynamic (MD) simulations provided insights into binding interactions. Network pharmacology was used to explore interactions between the target molecule and AD-related genes to determine its mechanism of action.

RESULTS VS identified promising AChE inhibitor candidates with acridone, carbazole, and xanthone scaffolds. Docking simulations showed strong binding with AChE. These ligands displayed favorable drug-likeness and ADMET properties, with one (M5) lacking predicted hepatotoxicity. MD simulations suggested stable binding of M5 to AChE, potentially affecting both catalytic and peripheral sites, hinting at dual inhibition. M5's interactions, especially near His440, appeared more favorable than donepezil. Network analysis implicated M5 in targeting multiple pathways in AD, with potential focus on neuroinflammation.

CONCLUSIONS This study identified promising AChE inhibitor candidates through virtual screening. Ligand M5 emerged as particularly promising due to its favorable binding characteristics, lack of predicted hepatotoxicity, and potential for targeting multiple pathways in AD. However, further *in vitro* and *in vivo* validation is essential for clinical development.

KEYWORDS virtual screening, acetylcholinesterase inhibitors, PubChem database, molecular dynamics simulation, gene ontology, KEGG pathways

INTRODUCTION

Alzheimer's disease is a progressive neurological disorder characterized by cognitive decline. It is an age-related disease and the most common cause of dementia among the elderly (1). The AD neuropathological hallmark is the accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles. However, some studies have reported

pathophysiological changes during the AD progression involving the depletion of acetylcholine (ACh) neurotransmitter as well as oxidative stress and neuroinflammation. ACh is regulated by the cholinergic system, playing a crucial role in memory and learning processes. Its activity is modulated by acetylcholinesterase (AChE), which also terminates its function (2). The loss of cholinergic neurotransmission is responsible for deterioration of cognitive function observed in the AD brain (3). Treatment with AChE inhibitors (AChEIs) has been shown to be associated with a slower cognitive decline (4), and it is currently used as a main approach for AD treatment in mild and moderate stages (5). There are still challenges in AD drug discovery because no novel anti-AD drugs have been approved for years. Although aducanumab received accelerated approval from the USFDA in June 2021, experts continue to discuss arguments in favor of aducanumab due to insufficiency of evidence supporting its ability to prevent or reverse the AD symptoms (6).

Virtual screening (VS), a pivotal initial step in rational drug design, involves computationally screening large libraries of chemical compounds to identify potential drug candidates that could interact with a specific target of interest (7, 8). It is possible to interpret and screen millions of ligands in a matter of minutes, resulting in the identification of novel chemical structures with great potential for pharmacological activity (9). Additionally, computational approaches can predict physical, chemical, and pharmacokinetic properties to assess the potential drug candidate based on the structure of a ligand (10). Molecular dynamics (MD) simulations complement virtual screening by providing insights into the dynamic behavior of biomolecular systems at the atomic level, elucidating protein-ligand interactions and conformational changes over time (11). Integrated with network pharmacology, it has the capacity to comprehensively analyze complex interactions between drugs, targets, and biological pathways within a network framework. Together, these techniques offer a powerful synergistic approach to drug discovery, facilitating the rational design of novel therapeutics and the exploration of their pharmacological mechanisms of action.

To overcome the challenges of AD, one of the approaches to developing ligands based on tacrine-scaffold with anti-AChE activity is particularly fascinating because the presence of condensed aromatic cores in tacrine contributes to high ligand efficiency. In this study, we utilized VS to screen planar aromatic ligands from PubChem that could potentially act as AChEIs. These ligands were then visually inspected, and their binding poses in the AChE binding pocket were analyzed. Promising ligands were subjected to further screening for drug likeness and ADMET properties using SwissADME (12) and pkCSM (13) online webservers. In combination with MD, we studied the binding interactions between molecules to provide insights into the biomolecular processes and function. Additionally, network pharmacology has been utilized to study the complex interactions between M5 and gene targets related to AD with the objective of understanding its predicted mechanism of action.

METHODS

Enzyme template validation

Over 2500 x-ray AChE crystal structures were obtained from PDB. The AChE templates were superimposed one by one to confirm the position of the active site. All ligands in each template were extracted and their energies minimized before performing molecular docking. Water was deleted from each template, hydrogen atoms were added, and charges were assigned for template preparation. Re- and cross-docking were performed as a validation procedure and the results were analyzed using root mean squared deviation (RMSD) between the docked pose and the former crystal conformer.

Molecular docking and post-docking analysis

High-throughput docking-based virtual screening was performed using the GOLD program version 5.3.0. The semi-empirical ChemPLP scoring function was used to validate the ligand binding modes and obtain the relative binding energy. The active site was identified following treatment with donepezil which is one of the first-line AChEIs for AD treatment. Ten docking runs per ligand were conducted for pilot virtual screening. In the post-docking process, Autodock 4.2.6 was used to observe the binding interaction of the top-ranked ligands. To elucidate the binding interaction, hits were docked in the AChE active site using Lamarckian generic algorithms. A grid map (70 Å

x 70 Å x 70 Å) with 0.375 Å of grid spacing, and a default box size of x=4.753 Å, y=66.521 Å, z= 64.857 Å was generated to cover the active site. Discovery studio 2020 was used for depth analysis of the ligand-AChE complexes.

Physiochemical properties and pharmacokinetic prediction

SwissADME and pKCSM were utilized in this study to evaluate the physicochemical properties and the pharmacokinetics of potential drug candidates. The absorption factor was evaluated based on intestinal absorption, while the volume of distribution (VDss) was used to determine whether the drug was primarily distributed in the plasma or tissues. In addition, the blood-brain barrier (BBB) permeability (logBB) and central nervous system (CNS) permeability were used to assess brain distribution. The CYP450 inhibition model was used to predict metabolism, while renal OCT2 substrate was used to predict excretion. Finally, the hepatotoxicity was predicted to evaluate drug toxicity.

Molecular dynamics (MD) simulation

The molecular dynamics (MD) simulation was carried out with OpenMM using Google Compute Engine backend (GPU) Python 3; the GPU nodes assigned are NVIDIA-SMI 525.85.12, Driver Version 525.85.12, and CUDA Version 12.0 (14). ff19SB and GAFF2 force fields from AMBER tools were used to minimize the bond stretching energy of protein and ligand, respectively (15). The TIP3P water model produced and solvated apo AChE (unbound), the AChE-ligand complex, and the AChE-donepezil complex before neutralizing them with NaCl ions. Before running the MD simulation, its concentration was set to 0.15 M. The 50 ns MD simulations were performed under constant temperature (310 K) and pressure (1 bar) after reaching equilibrium. The frequency of trajectory file was written and set to 10 ps. Structural stability, flexibility, and compactness were analyzed from root mean square deviation (RMSD), root mean square fluctuation (RMSF), and the radius of gyration (RoG), respectively.

The protein-protein interaction (PPI) network, clustering and functional enrichment analysis

SwissTargetPrediction and GeneCards were used to identify predicted target genes of hit and AD, respectively. The common target prediction between hit and AD was generated by Venn diagram. To establish the protein-protein interaction (PPI) network, STRING database was used and the minimum interaction threshold was set as medium confidence (>0.4) with "*Homo sapiens*" species. The shared target of hit and AD were imported into ShinyGO V0.77 to analyze Gene Ontology (GO) enrichment and KEGG pathways. The top 10 targets were constructed and were then clustered using kmeans from the String global scores.

RESULTS

AChE template preparation and validation

From 2,500 templates, 24 TcAChE structures with similar folds were identified. Resolution ranged from 2.05 to 2.85 Å, with 2CEK (2.01 Å) being the most favorable. Re-docking and cross-docking validations (Figure 1) suggested 2CEK's superior ability to accommodate diverse ligands with good accuracy (low RMSD), making it ideal for docking simulations. Therefore, 2CEK was chosen for further docking studies.

High-throughput virtual screening for AChE inhibitors

Following the workflow outlined in Figure 2A, a high-throughput virtual screening based on docking simulations was conducted to identify potential AChE inhibitors. Seven ligand classes (17,330 ligands) were selected for analysis, including phenothiazine, phenoxazine, phenazine, acridone, acridine, xanthone, and carbazole (Figure 2B). These ligands were virtually screened and docked within the binding pocket of AChE using the GOLD program. Ligands exhibiting a docking score superior to donepezil (93.01 kcal/mol) were predicted to bind potently with AChE. The remaining 388 ligands displayed scores ranging from 93.02 to 124.04 kcal/mol. Lipinski's rule (16) was applied to assess the drug-likeness of these ligands, considering their potential as oral drug candidates. This analysis identified 32 ligands for further investigation of their in-silico blood-brain barrier (BBB) permeability using SwissADME. Ultimately, eight ligands were identified as capable of crossing the BBB. These promising candidates included three acridones, one acridine, two carbazoles, and two xanthones.

PDB ID / Ligand	1H22_E20	1UT6_A6N	2CEK_N8T	2CMF_F11	2ACK_EDR	3I6M_G3X	3I6Z_G6X	1E3Q_EBW	1EVE_E20	1E66_HUX	2W6C_BM4	1DX6_GNT	1W6R_GNT	1GPK_HUP	Mean RMSD
1H22	**1.82**	4.41	3.34	2.15	3.28	2.4	2.63	2.66	1.61	6.91	2.93	0.37	0.55	0.71	2.56
1H23	1.97	4.39	3.38	1.99	3.05	4.71	2.62	2.81	1.7	7.53	1.7	0.57	0.54	0.77	2.70
1ODC	2.75	2.22	2.84	1.34	5.12	5.38	3.04	2.99	2.43	1.15	1.78	0.68	0.98	1.81	2.47
1UT6	1.5	**1.27**	3.51	0.99	3.68	4.77	2.84	2.77	4.73	1.03	1.68	0.62	0.98	0.76	2.22
1ZGB	2.69	1.84	3.03	1.85	5.45	4.7	3.66	2.85	1.82	0.93	1.81	0.57	0.66	0.63	2.32
2CEK	0.88	1.45	**1.37**	2.07	5.57	1.02	3.6	2.1	3.02	0.99	1.13	0.6	0.73	3.59	2.01
2CKM	2.87	2.13	2.64	0.97	4.55	4.66	3.03	2.84	3.8	0.7	1.23	0.36	0.55	1.78	2.29
2CMF	3.77	1.26	2.9	**0.65**	6.91	4.68	4.04	2.69	2.71	0.59	5.36	3.87	3.54	2.02	3.21
2ACK	2.42	5.15	3.25	2.27	**0.77**	4.91	3.07	2.27	3.24	3.68	5.36	1.09	1.01	1.98	2.89
3I6M	2.72	4.08	2.97	2.54	4.16	**0.62**	1.71	3.33	1.74	7.1	3.1	0.73	0.81	1.05	2.62
3I6Z	1.98	4.22	3.17	2.66	4.05	0.9	**1.59**	2.47	1.6	4.02	3.31	0.65	0.85	4.16	2.55
1VOT	1.89	3.75	2.58	2.01	3.56	4.96	2.91	2.67	2.14	3.72	4.1	6.48	3.22	1.9	3.28
1E3Q	2.33	4.07	3.75	2.12	3.97	2.74	3.71	**1.53**	2.29	3.55	3.67	3.45	4.67	2.56	3.17
1OCE	2.72	4.16	3.64	2.63	4.03	2.43	3.09	2.77	2.81	4.02	1.98	0.85	4.13	3.43	3.05
1EVE	2.49	4.03	3.25	3.25	4.27	1.85	1.69	2.91	**0.88**	4.05	1.99	1	1.31	1.17	2.44
1E66	1.32	2.98	2.68	1.57	5.52	4.84	3.3	2.71	2.68	**0.35**	5.33	3.86	3.58	0.9	2.97
1W4L	2.12	4.07	3.63	2.29	3.74	0.82	1.7	2.72	1.63	4.03	3.66	0.63	0.86	0.86	2.34
2W6C	1.62	4.3	3.74	2.99	4.3	0.64	1.57	3.01	1.55	3.5	**1.28**	1.2	1.29	2.07	2.36
1DX6	3.66	3.79	3.55	2.19	4.09	0.84	1.68	2.97	1.16	4.05	3.01	**0.76**	0.9	3.98	2.62
1W6R	2.72	4.11	3.35	2.5	1.15	0.97	1.71	2.75	1.92	3.84	1.73	0.27	**0.54**	1.89	2.10
1GPK	2.7	4.14	3.95	2.57	3.29	4.72	2.96	2.9	4.42	3.91	5.77	2.9	3.17	**1.72**	3.51
2VQ6	2.54	7.43	4.04	1.8	3.41	4.64	3.53	3.2	3.47	10.82	5.13	6.69	6.7	10.22	5.26
3ZV7	3.27	4.27	3.62	2.22	4.12	4.28	1.55	2.89	1.73	4.13	1.62	0.68	0.81	0.83	2.57
1HBJ	2.14	4.66	3	2.29	4.16	5.79	2.91	2.73	2.11	2.51	3.5	4.12	3.24	2.36	3.25
Mean RMSD	2.37	3.67	3.22	2.08	4.01	3.26	2.67	2.73	2.38	3.63	3.01	1.79	1.90	2.21	2.78

Figure 1. The validation of the docking results with RMSD values

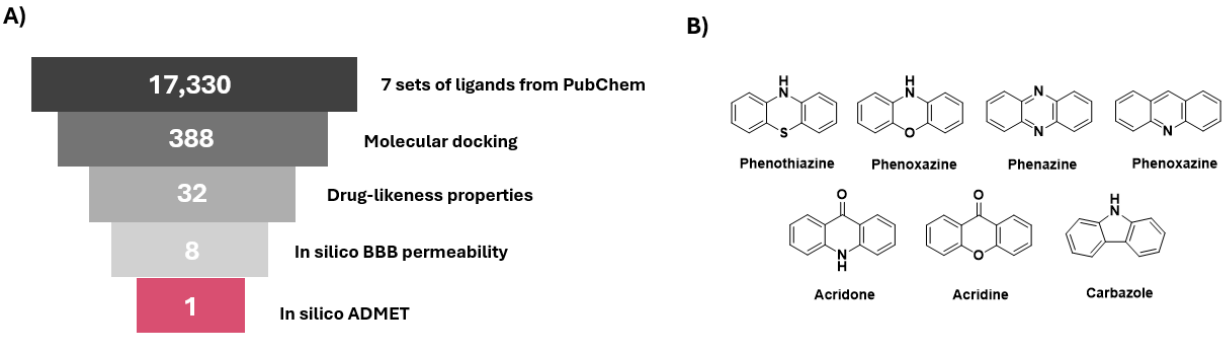


Figure 2. VS workflow (A) and core structure of ligands (B)

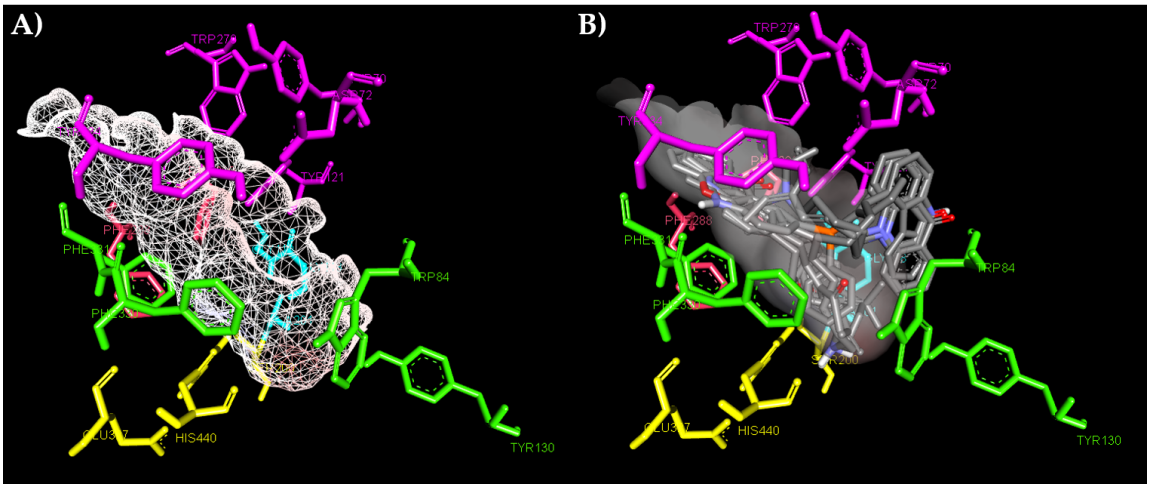


Figure 3. Binding groove of AChE (A) and binding orientation of hits docked into AChE (B). The binding sites are color-coded (catalytic triad; yellow, anionic site; green, peripheral site; purple)

Binding modes of the hit ligands

The active site of AChE is highly specific for ACh, playing a critical role in regulating neurotransmission. It comprises three key subsites: the catalytic site (CAS), the anionic site (AS), and the peripheral site (PAS). Eight promising ligands were docked into the active site of AChE using Autodock 4.2.6 software to understand their binding interactions. The AChE binding groove and orientations of these docked ligands within AChE are illustrated in Figure 3. The specific interactions between these ligands and AChE residues are presented in Table 1. When the binding modes were visualized, ligands were in the AChE

Table 1. Structures of Hits, their binding interactions, and binding energies

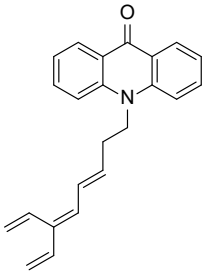
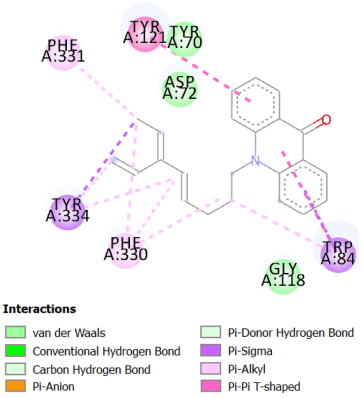
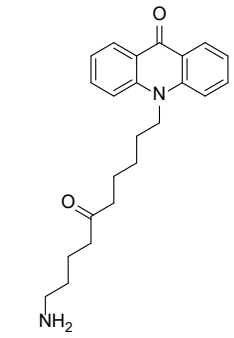
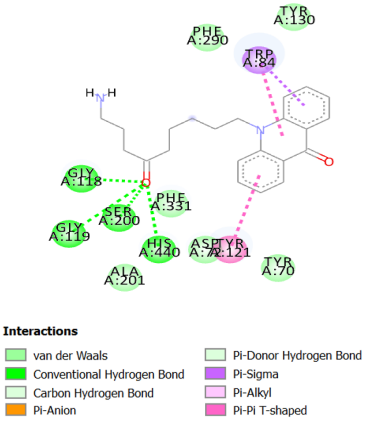
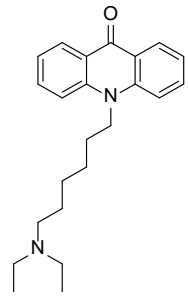
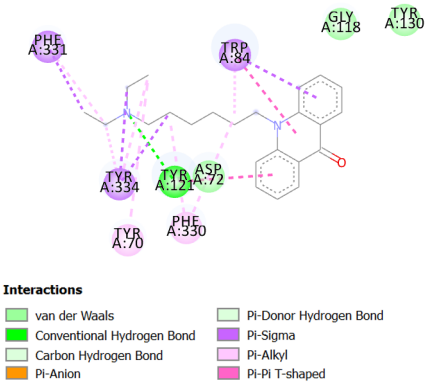
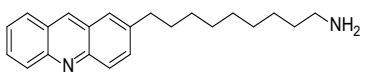
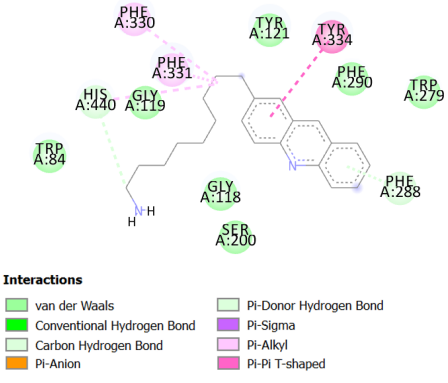
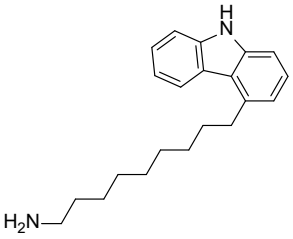
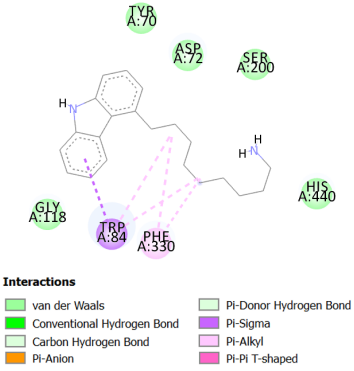
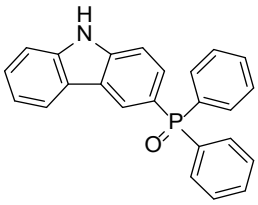
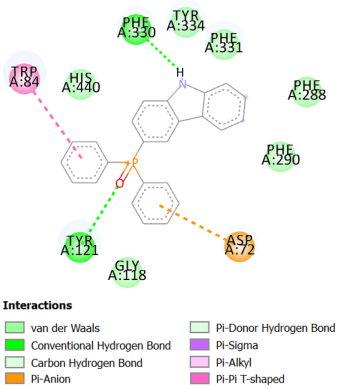
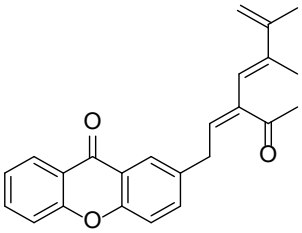
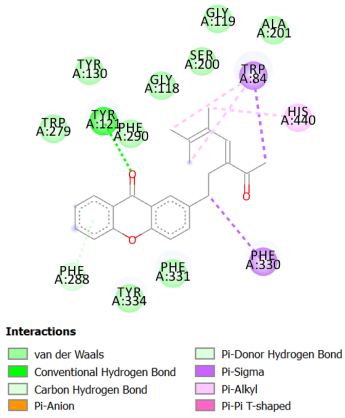
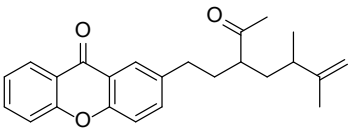
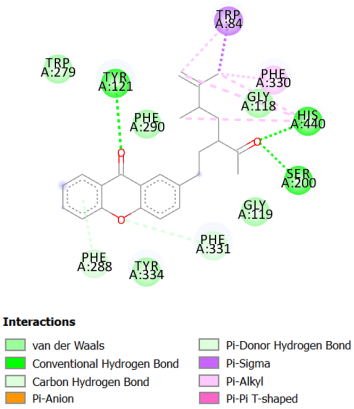
Name/ Structure	Binding interaction	Binding energy (kcal/mol)
(E)-10-(6-vinylocta-3,5,7-trien-1-yl) acridin-9 (10H)-one (M1) 		-10.12
10-(10-amino-6-oxodecyl) acridin-9 (10H)-one (M2) 		-11.71
10-(6-(diethylamino) hexyl) acridin-9 (10H)-one (M3) 		-9.62
9-(acridin-2-yl)nonan-1-amine (M4) 		-11.51

Table 1. Structures of Hits, their binding interactions, and binding energies (continue)

Name/ Structure	Binding interaction	Binding energy (kcal/mol)
9-(9H-carbazol-4-yl) nonan-1-amine (M5) 		-11.52
(9H-carbazol-3-yl) diphenylphosphine oxide (M6) 		-11.31
2-((2Z,4E)-3-acetyl-5,6-dimethylhepta-2,4,6-trien-1-yl)-9H-xanthen-9-one (M7) 		-10.84
2-(3-acetyl-5,6-dimethylhept-6-en-1-yl)-9H-xanthen-9-one (M8) 		-11.15

mid-gorge. The ligands were divided into two groups based on their pose position. The first group consisted of acridones with an aliphatic linker chain at the 10-N position. M6 carbazole was also included in this group due to its binding position. While acridone core structure had a π-π interaction with Trp84 located in AS with their 9-O formed the H-bond with Gly123, its aliphatic linker chain was in CAS. The length of their linker chain at the 10-N position varies with the number of carbons (from 8 to 10). Due to the flexibility of the linker chain, a ketone group in the middle of an M2 linker chain can interact with two amino acids (Ser200 and His440) of the catalytic triad via H-bonds, resulting in its highest binding energy at -11.71 kcal/mol. The linker chains of M1 and M3 were anticipated to interact with Tyr334, Tyr70, and Tyr121 in PAS. Consequently, they were expected to disrupt the acceleration of Aβ aggregation by AChE. M6 was the only structure with a bulky linker chain. It had the H-bond interaction between 9-NH and Phe330, and two aromatic rings on the linker chain bound to Trp84 and Asp72. The oxygen atom connecting to phosphorus can interact with Tyr121 via the H-bond. The second group belonged to acridine and xanthone, whose core structures were substituted with an aliphatic linker chain at C-7, similar to the first group. M5 carbazole was also in this group because of its binding pose. The acridine binding interaction revealed that the core structure was located near the PAS and bound to Tyr334 via a π-π interaction. The M5 core structure, carbazole, is in the same position as acridine. The NH at C-9 formed interaction with Gln69, and its hydrogen atoms on the linker chain bound to Glu199 through H-bonds. However, one aromatic ring of carbazole can form a π-sigma bond with Trp84. Both ketones at C-9 of xanthone formed an H-bond with Tyr121 at PAS, and their linker chains formed π-sigma interactions with Trp84. The difference between their linker chains was that M7 had a double bond in the middle of the chain, which may reduce its flexibility. Thus, both the oxygen atoms on the linker chain showed different interactions because M8 had more flexibility, supporting that the oxygen atom can form H-bonds with His440 and Ser200.

Drug-likeness and pharmacokinetic properties

To assess the drug-likeness of the eight most promising candidates, their key molecular descriptors were calculated and summarized in Table 2 using the SwissADME. Additionally, the pharmacokinetic properties of the selected ligands were investigated using pKCSM, and the results are presented in Table 3. Ligands have a molecular weight in the range of 308-367 which might easily be transported, diffused, and absorbed. The number of hydrogen bond acceptors and donors were both determined following Lipinski's rule and ranged between 1-2 and 1-3, respectively. Lipophilicity (log P) and topological polar surface area (TPSA) values are two important parameters for predicting oral drug bioavailability. Analysis of the data revealed log P values ranging from 2.41 to 4.29, indicating good cell membrane permeability for these ligands. TPSA is a useful tool both for predicting the oral bioavailability of drug candidates and for optimizing their chemical structures to improve their intestinal absorption. Drugs with TPSA values above 140 Å^2 tend to be more polar and less likely to cross cell membranes, including the intestinal wall, which can limit their absorption into the bloodstream. As our ligands had ≤10 rotatable bonds and TPSA of ≤140 Å, they were more likely to have good intestinal absorption, in the range of 95-100%. Additionally, all these ligands showed good distribution values of more than -2, indicating their potential to penetrate the CNS. Moreover, M1 and M3 had values greater than 0.3, indicating their ability to cross the BBB and that the remaining ligands could also pass through it. In metabolism, some hits are susceptible to biotransformation by the CYP450 enzyme system. However, M8 was found to be a substrate of P-glycoprotein, which effluxes drugs and other substances for further metabolism and clearance. However, none of the ligands were found to be OCT2 substrates, which can induce nephrotoxicity by changing drug accumulation in the kidney. Only M5 was found to be unlikely to cause hepatotoxicity.

Molecular dynamics simulations

Molecular dynamics simulations were used to assess the structural stability, flexibility, and compactness of the M5-AChE complex. The M5-AChE complex was compared to a native enzyme (apo) and donepezil-AChE complex (donepezil) in terms of various parameters including RMSD, RMSF, and RoG analysis. Our results showed that RMSD between apo, standard, and M5 are in

Table 2. Physicochemical properties of hits predicted by the SwissADME program.

Molecule	MW	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	MLOGP
M1	327.42	6	1	0	22.00	4.14
M2	350.45	9	3	1	65.09	2.41
M3	350.50	9	2	0	25.24	3.55
M4	320.47	9	2	1	38.91	4.29
M5	308.46	9	1	2	41.81	4.11
M6	367.38	3	1	1	42.67	4.25
M7	358.43	5	3	0	47.28	3.47
M8	362.46	7	3	0	47.28	3.61

MW, molecular weight; TPSA, Topological polar surface area

Table 3. Pharmacokinetic properties of hits predicted by pKCSM program.

ADMET	Parameters	Acridone			Acridine	Carbazole		Xanthone	
		M1	M2	M3	M4	M5	M6	M7	M8
Absorption	Caco2 permeability	1.054	0.53	1.029	1.225	1.399	0.894	1.079	1.119
Absorption	Intestinal absorption (human)	97.622	98.1	95.352	94.412	91.142	100	98.36	98.488
Distribution	VDss (human)	0.738	1.021	1.667	1.376	1.208	-1.123	0.176	0.418
Distribution	BBB permeability	0.703	-0.425	0.786	-0.083	-0.164	-0.027	-0.241	-0.294
Distribution	CNS permeability	-1.263	-2.448	-1.536	-1.639	-1.957	-1.275	-1.338	-1.453
Metabolism	CYP2D6 substrate	No	No	Yes	No	No	No	No	No
Metabolism	CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Metabolism	CYP1A2 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Metabolism	CYP2C19 inhibitor	Yes	No	Yes	No	Yes	Yes	Yes	Yes
Metabolism	CYP2C9 inhibitor	Yes	No	No	No	No	No	Yes	Yes
Metabolism	CYP2D6 inhibitor	No	No	Yes	Yes	Yes	Yes	No	No
Metabolism	CYP3A4 inhibitor	No	Yes	Yes	No	No	No	No	No
Excretion	Renal OCT2 substrate	No	No	No	No	No	No	No	No
Toxicity	Hepatotoxicity	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

ADMET stands for absorption, distribution, metabolism, elimination, and toxicity

the range of 0.59 to 1.77 Å throughout the 50 ns which is within the acceptable range (1–3 Å) (Figure 4A). The average RMSD of apo, donepezil, and M5 were 1.31±0.17, 1.34±0.14, and 1.39±0.16 Å, respectively. However, the flexibility pattern of M5 was similar to apo and donepezil, except regions 70-80 and 420-450 (Figure 4B). The mean RMSF value of AChE–M5 complex was determined to be 0.74 Å. The average RoG of apo, donepezil and M5 were 22.79, 22.86 and 22.83 Å, respectively (Figure 4C). There was no significant deviation observed and compactness was maintained throughout the trajectory.

Identification of potential anti-AD targets and protein-protein interaction (PPI) network analysis

SwissTargetPrediction identified 107 potential targets of M5. Subsequently, 13,564 targets associated with AD were retrieved from GeneCards.

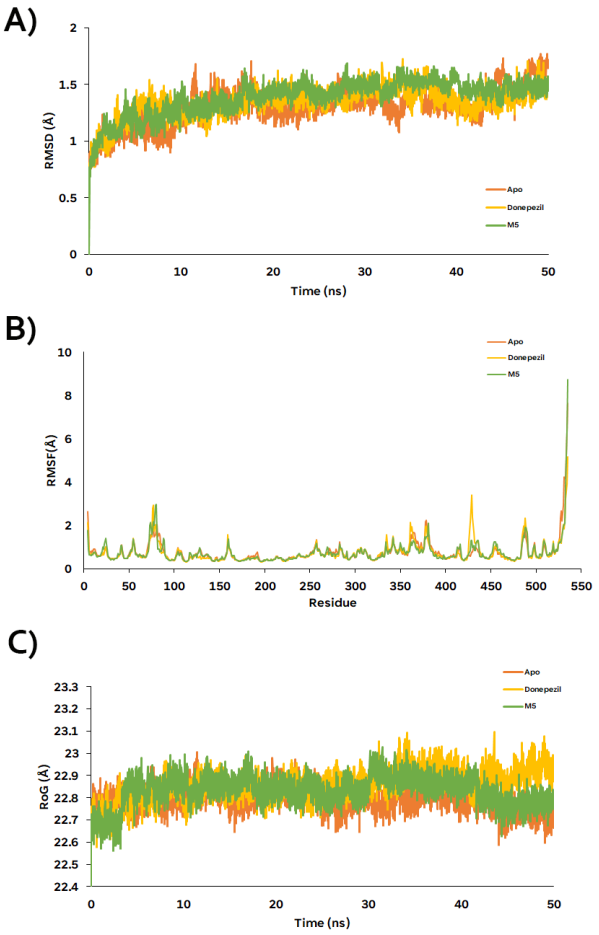


Figure 4. Molecular dynamics analysis: RMSD (A), RMSF (B), RoG (C)

A Venn diagram (Figure 5A) was created to visualize the overlap between these M5-related and AD-related genes, resulting in the identification of 94 potential anti-AD target genes of M5. To further explore the interactions between these 94 targets, a PPI network was constructed using the STRING database (Figure 5B). This network provides a comprehensive view of the molecular associations relevant to M5 and AD.

Functional enrichment analysis of M5 targets

GO and KEGG pathway enrichment analyses were performed to understand the functions and pathways associated with M5 targets. The targets were significantly enriched ($p < 0.05$) in 665 GO terms, with a breakdown of 527 for biological processes (BP), 55 for cellular components (CC), and 83 for molecular functions (MF). The top 10 most enriched GO terms are visualized in Figure 5C. The response to the chemical (GO:0042221) in BP is the process which resulted in a change in cell activity via chemical stimulation. CC is prominently engaged as an integral component of plasma membrane (GO:0005886) and neuron projection (GO:0043005). The MF analysis highlighted involvement in neurotransmitter receptor activity (GO:0030594) and signaling receptor activity (GO:0038023).

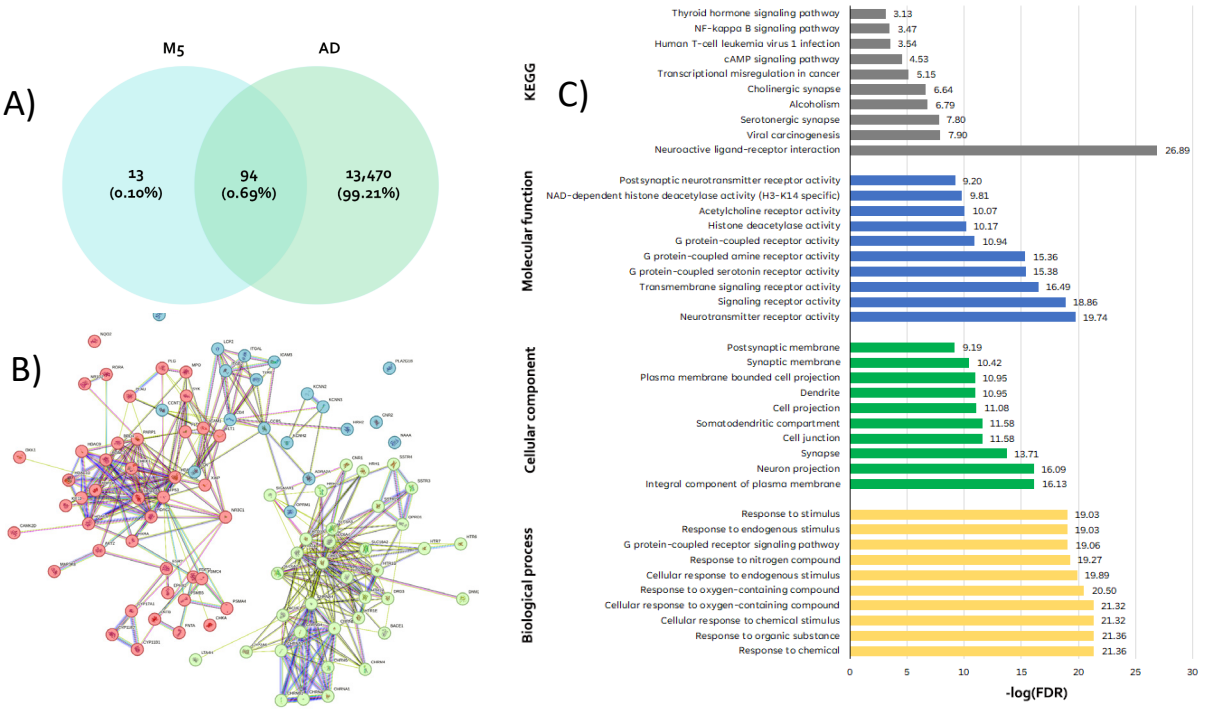


Figure 5. Shared targets represented in a Venn diagram (A), the top three cluster subnetworks (B), GO and KEGG function analysis (C)

KEGG pathway analysis

To further validate that these enriched biological processes are relevant to AD, KEGG pathway analysis was used to identify significantly enriched pathways ($p < 0.05$) associated with the target genes (Figure 5C). KEGG pathway analysis constitutes a valuable tool for augmenting comprehension of the biological functions attributed to genes. The investigation of M5 targets through the KEGG pathway unveiled noteworthy enrichment within neuroactive ligand-receptor interaction (hsa04080).

Analysis of gene clusters for key genes

To clarify the effects of M5 on AD, we identified the top three clusters with high scores and closely interconnected regions from the PPI network (Figure 5B). These clusters, containing 43 (red), 36 (green), and 19 (blue) genes (Table 4), represent potential key players and their interactions in the context of M5 and AD. Analyzing these gene clusters can aid in identifying key genes and their interactions. The clusters retrieved from the String-db and kmeans clustering algorithm was applied to the gene correlation. The major pathways of the clusters were selected and are represented in Figure 6. Cluster 1 demonstrated a predominant involvement in neutrophil extracellular trap (NET) formation and viral carcinogenesis. Cluster 2 was associated with neuroactive ligand-receptor interaction, and Cluster 3 was engaged in natural killer (NK) cell-mediated cytotoxicity and the modulation of cell adhesion molecules.

DISCUSSION

This study employed a virtual screening (VS) approach to identify potential drug candidates for Alzheimer's disease (AD). We focused on targeting acetylcholinesterase (AChE), a key enzyme implicated in AD pathogenesis. Following virtual screening, *in silico* docking simulations were used to predict and confirm binding interactions of identified hits with AChE. Finally, network pharmacology was utilized to explore the potential mechanisms by which these promising candidates might exert their effects on AD.

Public databases like PubChem offer a wealth of chemical structures, but raw data can be incomplete and poorly understood, leading to unstructured data concerns (17–19). Tacrine, a simple AChEI fragment, suffers from hepatotoxicity, highlighting the need for alternative scaffolds (20). AChE binding pocket is known to comprise a hydrophobic pocket, which is ideally suited for planar aromatic molecules. A total of seven classes of such ligands were chosen for VS and docking simulations. Donepezil's high selectivity for AChE supports the cholinergic hypothesis, and ligands scoring higher were considered promising candidates (21). Drug-likeness and blood-brain barrier (BBB) permeability filters were applied to prioritize suitable chemical candidates. Orally active drugs reaching phase II clinical trials often adhere to Lipinski's rule. Additionally, BBB permeability is crucial for CNS drugs due to restricted passage: only lipophilic molecules with low molecular weight (typically below 400-500 Da) can pass through the BBB (22).

Table 4. The clusters of the most interlinked regions

Bubble	Cluster	Gene count	Protein names
Red	1	43	AKT2, BRD4, CAMK2D, CHEK1, CHKA, CYP11B1, CYP11B2, CYP17A1, DKK1, EPHX2, ESR2, FDFT1, FLT1, FLT3, FNTA, FNTB, HDAC1, HDAC10, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC9, ICAM1, KIF11, MAP3K8, MPO, NQO2, NR1D2, NR3C1, PARP1, PLAU, PLG, PSMA4, PSMB5, PSMC4, RORA, RXRA, SYK, TOP2A, TP53, XIAP
Green	2	36	ACHE, BACE1, CHRM4, CHRM5, CHRNA1, CHRNA3, CHRNA4, CHRNA7, CHRNB1, CHRNB2, CHRNB4, CNR1, CYP2A6, DNM1, DRD2, DRD3, HRH1, HRH3, HTR1A, HTR1B, HTR1D, HTR1E, HTR2A, HTR2C, HTR6, HTR7, LTA4H, OPRD1, SIGMAR1, SLC18A2, SLC6A2, SLC6A3, SLC6A4, SSTR2, SSTR3, SSTR4
Blue	3	19	ADRA2A, CCNT1, CCR5, CD4, CNR2, HRH2, ICAM3, ITGAL, ITGB2, KCNH2, KCNN2, KCNN3, LCK, LCP2, NAAA, OPRM1, PIM2, PLA2G1B, TLR8

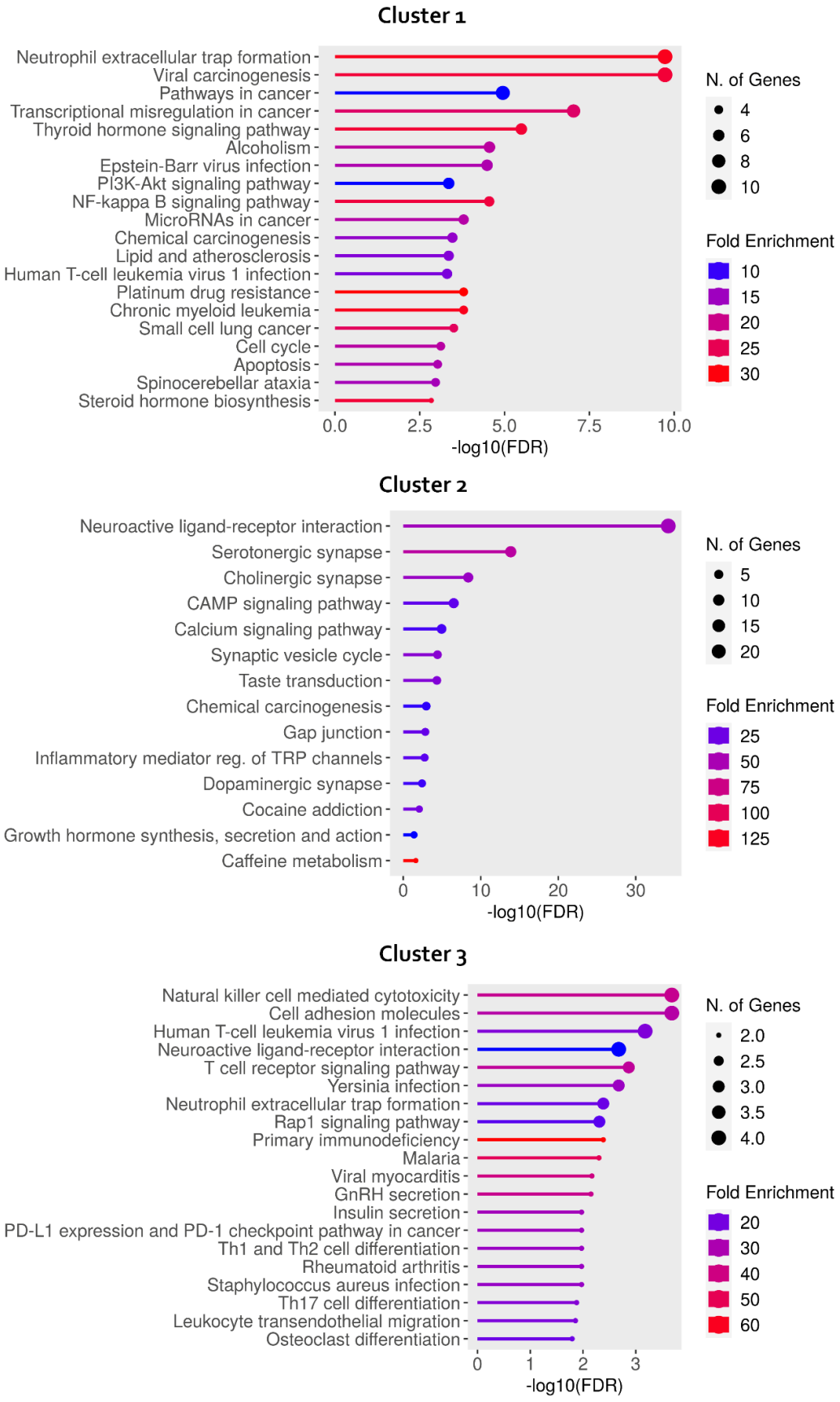


Figure 6. KEGG pathway enrichment analysis of key targets after clustering

Our analysis categorized the identified hits into two distinct groups based on their binding pose within the AChE active site. In group 1 (acridones), these ligands primarily reside near the anionic site. They interact with Trp84, a critical amino acid responsible for binding the quaternary ammonium group of ACh and ensuring its proper positioning for hydrolysis (23). This interaction suggests group 1 ligands may act as acetylcholinesterase inhibitors by competitively blocking or interfering with this crucial step. Additionally, their binding to Gly118 and Gly119 in the oxyanion hole near CAS could significantly impact ACh hydrolysis by destabilizing the transition state, likely leading to potent inhibition (23). For group 2 (acridine, xanthone, and carbazole (M5)), the binding orientation of this group suggests potential interaction with both the PAS and the CAS. While they may establish hydrogen bonds within the CAS, potentially influencing ACh hydrolysis, their primary location near the PAS suggests a possible role in interfering with Aβ aggregation.

Poor pharmacokinetics and pharmacodynamics features are the main causes of the clinical failure and limitations of many drug candidates. The permeability of the CNS refers to a substance's ability to cross the membranes that surround and protect the brain, spinal cord, and cerebrospinal fluid (24). The BBB permeability is critical in determining the efficacy and safety of drugs targeting the CNS. The BBB acts as a specialized barrier that regulates the entry of drugs and other substances into the brain (24). CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 are the significant isoforms of the cytochrome P450 (CYP450) family which is involved in drug metabolism and clearance (25). The disturbance of CYP450 metabolism can lead to altered pharmacokinetics, such as reduced bioavailability or increased clearance, which may affect the efficacy of the drug. Furthermore, drug-drug interactions can occur when a drug candidate alters the activity of CYP450 enzymes, resulting in changes in the metabolism of co-administered drugs and potentially leading to adverse effects (26, 27). All candidates showed favorable pharmacokinetic profiles with the exception of hepatotoxicity. This might be because of their structures as the pkCSM webserver was built using the liver-associated side effects of compounds observed in humans in their database. It is important to note that hepatotoxicity caused by drugs is rare and unpredictable. Nevertheless, *in vitro* and *in vivo* studies are required to observe their effects further.

Through the VS approach, M5 was identified as a promising ligand, exhibiting lower binding energy compared to a current standard AD drug donepezil, along with having favorable predicted pharmacokinetic profiles. Moreover, it showed a similar pharmacophore feature, containing one hydrogen bond acceptor, two hydrogen bond donors, one aromatic ring and one hydrophobic center, which is consistent with a hit in a previous study (28).

MD simulations were used to further explore the interaction and stability of M5 with AChE. The RMSD was used to assess the fluctuation in system stability and to determine the time point at which the system achieved equilibrium. After 20 ns, the complex reached equilibrium with slight fluctuations, exhibiting stable binding between ligand and enzyme. On the other hand, the RMSF was calculated to study the protein flexibility with the ligand binding, providing insight into changes in conformation. High fluctuations occurred at regions 70-80 and 420-450 which correspond to the PAS and CAS regions. There was a significant fluctuation observed near His440 which indicates that M5 had more stable interaction than donepezil, a current benchmark treatment. This supports that M5 can bind with both CAS and PAS regions.

Network pharmacology was employed to delineate its underlying mechanisms in AD. Through this analysis, ninety-four overlapping genes were identified, and subsequently an interaction network was constructed to explore the core regulatory genes. GO enrichment analysis is instrumental in providing information for understanding the diverse roles that genes play in various biological contexts (29). BP is particularly relevant to the changes in the biological functions, and it can provide insights into the specific activities or events involving genes that contribute to the overall functioning of a cell. CC aspect focuses on identifying the specific parts of a cell or its extracellular environment where a gene product is located while MF describes the specific biochemical activity performed by the gene product. Our findings regarding the functional characteristics of M5 indicate it might bind to the target protein and

exert a sophisticated network of interactions. The chemical signals, recognized by receptors in the integral component of the plasma membrane, initiate signaling cascades that influence neuron projections and neurotransmitter receptor activity (30-32). Understanding this response is crucial for unraveling the complex mechanisms underlying neural communication and cellular function. KEGG analysis was used to better explain the mechanism of M5 at the pathway level. The neuroactive ligand-receptor interaction pathway serves as a critical framework for understanding the complexities of synaptic transmission, neuromodulation, and the control of neuronal excitability. That pathway includes various neuroactive ligands, such as neurotransmitters, hormones, and other signaling molecules, that play crucial roles in neuronal signaling (33). AD involves complex molecular mechanisms which might be associated with the neuroactive ligand-receptor interaction pathway. This pathway is interconnected with several pathways, such as those related to cholinergic deficiency, glutamate dysregulation, inflammation, oxidative stress, and cell death. The crosstalk between these pathways contributes to the multifaceted nature of AD.

Clustering represents the links between genes, corresponding to gene interaction relationships. Three clusters were speculated to play roles in M5 treated AD. Cluster 1 is enriched for genes involved in neutrophil extracellular trap (NET) formation. NET formation has been linked to chronic neuroinflammation, a hallmark of AD pathology. Additionally, activated neutrophils contribute to inflammation by triggering MMP-9, an enzyme associated with BBB disruption (34-39). This suggests that M5 might target NET formation and BBB integrity, potentially reducing neuroinflammation. Cluster 2 focused on neuroactive ligand-receptor interactions. These interactions influence neuronal function and gene expression. Genetic disruptions in these pathways are known to impair memory function (40). M5's influence on this cluster suggests a potential role in memory preservation. Cluster 3 is associated with natural killer (NK) cell activity and cell adhesion. NK cells eliminate abnormal cells, including those linked to inflammation (41). Cell adhesion molecules facilitate communication between immune cells which is crucial for effective immune responses (42). Dysfunctional NK cells and dysregulated immune responses are observed in AD (43-45). M5's impact on this cluster indicates a potential role in enhancing NK cell function and immune response regulation. These findings suggest M5 might exert anti-AD effects through multiple molecular targets beyond just AChE inhibition. The clustering analysis points towards neuroinflammation as a potentially key target for M5's action against AD. However, it is important to acknowledge the limitations of this study: the proposed mechanisms are theoretically supported, but further research is needed for confirmation.

Overall, this virtual screening study identified M5 as a promising lead compound for Alzheimer's disease with a predicted binding affinity stronger than donepezil, a current standard treatment. M5 exhibited favorable pharmacokinetic properties and interacted stably with both the catalytic site and peripheral anionic site of acetylcholinesterase in molecular docking simulations. Network pharmacology analysis suggested M5 might target multiple pathways implicated in Alzheimer's disease, potentially through its interaction with neuroactive ligand-receptor interactions and modulation of inflammatory responses. While this study provides a promising starting point for drug development, further validation is needed. *In vitro* and *in vivo* experiments are required to confirm the identified hits and their mechanisms of action. Additionally, the potential hepatotoxicity of M5 needs investigation. Addressing these limitations could potentially translate these *in silico* findings into a robust pre-clinical development plan for M5 as a potential therapeutic candidate for AD.

CONCLUSIONS

Our VS identified promising ligands predominantly from three scaffolds: acridone, carbazole, and xanthone. Docking simulations demonstrated their strong binding affinity and stability within the active site of enzymes. These ligands, characterized by an aromatic core structure, interacted effectively with key amino acids, potentially disrupting ACh hydrolysis. These ligands were in an active site, preventing ACh from accessing the CAS, thus potentially inhibiting AChE activity more effectively. To optimize binding, extending the linker chain to enhance hydrophobic interac-

tions is crucial. Incorporating an electron-withdrawing group, such as a ketone, in the linker chain can further enhance binding affinity. The *in silico* pharmacokinetic properties were investigated, and ligands were found to exhibit several favorable pharmacokinetic properties. M5 was the only ligand that did not exhibit hepatotoxicity, suggesting it may present a lower risk of causing liver damage compared to others. MD simulations explored the interaction and stability of M5 with AChE, comparing it with native complexes and the donepezil-AChE complex. Results showed that M5 bound stably, potentially engaging both CAS and PAS regions, suggesting it might act as dual-binding AChE inhibitor. Compared to donepezil, M5 showed potentially more favorable interactions, particularly near His440, indicating unique binding characteristics. In addition, network analysis found that M5 might exert anti-AD effects through multiple molecular targets, extending beyond AChE inhibition. Clustering analysis hinted at a potential focus on neuroinflammation in the M5 mechanism of action against AD. Additional experimental validation *in vitro* and *in vivo* is necessary for advancing M5 towards clinical application.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ADDITIONAL INFORMATION

Author contributions

Conceptualization, C.B., P.W., Y.C. and C.Ba.; methodology, P.T., C.B., P.W.; software, P.T., K.C., C.B.; formal analysis, P.T., C.B., P.W.; investigation, P.T., C.B., P.W. P.A.; resources, C.B.; writing—original draft preparation, P.T., and C.B.; writing—review and editing, C.B., and P.W.; project administration, C.B. All authors have read and agreed to the published version of the manuscript.

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Case Report Open Access

Coinherited Hemoglobin Lansing-Ramathibodi/Southeast Asian Deletional α^0-thalassemia and Hemoglobin E Causing Falsely Low Oxygen Saturation on Pulse Oximetry

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ABSTRACT

Hemoglobin (Hb) variants resulting from mutations in globin genes and leading to qualitative abnormalities of globin proteins can result in a spectrum of clinical presentations. Herein we describe a case of a 10-year-old boy with an Hb variant, Hb Lansing-Ramathibodi (*HBA1*: c.264C>G, codon 87 His>Gln), who was incidentally found to have low pulse oximetry oxygen saturation readings (low SpO_2). Laboratory investigations revealed mild microcytic anemia. Arterial blood gas showed normal partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SpO_2) values, indicating an oxygen saturation gap. Subsequent Hb analysis identified an abnormal peak, prompting further molecular studies which confirmed the coinheritance of Hb Lansing-Ramathibodi, Southeast Asian deletional α^0-thalassemia and the Hb E trait. The patient's mother, carrying the Hb Lansing-Ramathibodi and Hb E traits without Southeast Asian deletional α^0-thalassemia, was asymptomatic. This case underscores the importance of identifying and characterizing rare Hb variants presenting with an oxygen saturation gap for informed clinical management and genetic counseling.

KEYWORDS alpha-thalassemia, coinheritance, hemoglobin Lansing-Ramathibodi, low SpO_2, oxygen saturation

INTRODUCTION

Hemoglobin (Hb), a tetrameric molecule composed of hemes and globin proteins, plays an important role in oxygen transportation from the lungs to tissues. Hb A, the predominant Hb in both children and adults, is composed of two α-globin and two β-globin chains. Hb variants arising from mutations in globin genes can lead to qualitative abnormalities in globin proteins, and can result in a spectrum of clinical presentations, including alterations in oxygen affinity. These variants may affect oxygen affinity through mechanisms such as R and T configuration, 2,3-BPG affinity, globin contact zone, heme pocket and length of globin chain (1, 2). These Hb variants, categorized into high and low oxygen affinity types, exhibit distinctive clinical features.

High oxygen affinity Hbs typically present as erythrocytosis due to elevated erythropoietin levels secondary to reduced tissue oxygen delivery, potentially leading to hyperviscosity, thrombotic events, and hemolysis (3-8). Conversely, low oxygen affinity Hbs are characterized by central cyanosis, low oxygen saturation and anemia (9-13). Additionally, several Hb variants associated with falsely low oxygen saturation measured using a

pulse oximeter (SpO_2) without actual hypoxemia have been reported (13).

Monitoring oxygen saturation is crucial in clinical diagnosis and patient management. Oxygen saturation can be assessed using a pulse oximeter which provide SpO_2 values, or through arterial blood gas measurements yielding partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SaO_2) values. Certain Hb variants can result in an oxygen saturation gap, characterized by low SpO_2 measurements but normal SaO_2 and PaO_2 levels. Pulse oximetry relies on the principle of differential light absorption by oxygenated and deoxygenated Hbs to determine blood oxygen saturation levels. It employs two specific wavelengths of light, typically red (640-660 nm) and infrared (880-940 nm), which are absorbed differently by these Hb forms (14). The SpO_2 results may inaccurately appear lower due to changes in the light absorbance characteristics of the Hb variant (13).

In Thailand, Hb Lansing-Ramathibodi (*HBA1*:c.264C>G, codon 87 His>Gln) has been reported in association with falsely low SpO_2 values in two families (15, 16). The diagnostic process for Hb Lansing-Ramathibodi poses challenges due to its rarity (15-17). This report presents a case of an individual harboring a compound heterozygosity for Hb Lansing-Ramathibodi, α^0-thalassemia and Hb E (*HBB*:c.79G>A, codon 26 Glu>Lys), which provides insight into the clinical characteristics of this uncommon Hb variant.

CASE REPORT

A 10-year-old Thai boy was referred to our hospital due to low SpO_2 values detected by pulse oximetry. Approximately one month prior to the admission, he had been hospitalized for food poisoning, during which an SpO_2 of 74% was recorded. Arterial blood gas while in room air showed pH 7.515, $PaCO_2$ 29.7 mmHg, PaO_2 111.5 mmHg, HCO_3 23.8 mmol/L, and SaO_2 98.5%. Administration of supplemental oxygen via a heated humidified high flow nasal cannula (HHHFNC) with FiO_2 of 0.6 resulted in an increase in SpO_2 to 84-85%. Arterial blood gas while on HHHFNC showed PaO_2 221.7 mmHg and SaO_2 99.7%. Notably, the patient remained asymptomatic, with no complaints of dyspnea or chest pain, and had been in overall good health.

The patient was born at term, with a gestational age of 40 weeks, and had been delivered by Cesarean section due to fetal distress. The Apgar score was 9 at 1 and 5 minutes. At birth, his SpO_2 in room air was 82%, with a slight improvement to 89-90% following the administration of supplemental oxygen. At that time, the patient was diagnosed with mild persistent pulmonary hypertension of the newborn (PPHN).

He had no history of other medical conditions. He was the only child of the family. Both parents were healthy. Antenatal thalassemia screening indicated that the parents were not at risk of having a fetus with severe thalassemia. Specifically, the mother was identified as a carrier of Hb E, while the father carried Southeast Asian deletional α^0-thalassemia. There was no familial history of low oxygen saturation or hematologic disorders. The SpO_2 levels in room air for both parents were 98-99%.

At our hospital, physical examination revealed normal vital signs, with SpO_2 ranging from 75% to 77% in room air. He weighed 41.1 kg (75^{th} percentile) and measured 143 cm in height (25^{th}–50^{th} percentile), showing no cyanosis, pallor, or jaundice. Additionally, the examination revealed normal cardiac and respiratory profiles, absence of hepatosplenomegaly, and no digital clubbing.

Results of laboratory investigations of the patient and his mother are shown in Table 1. Hb analysis was done by high-pressure liquid column chromatography (HPLC) using the Variant II HPLC system (Bio-Rad Laboratories, Hercules, CA, USA). An abnormal Hb peak was detected during the analysis, as illustrated in Figure 1. PCR for Southeast Asian deletional α^0-thalassemia was performed using a method with modifications from a previous study (18). β-thalassemia mutations were searched for using the PCR-high resolution melting analysis method as previously described (19). Subsequent Sanger DNA sequencing of α-globin genes *HBA2* and *HBA1* (Figure 2) identified heterozygous mutations causing Hb Lansing-Ramathibodi (*HBA1*:c.264C>G, codon 87 His>Gln) in both the patient and the mother. The patient was found to have Hb Lansing-Ramathibodi, Southeast Asian deletional α^0-thalassemia and Hb E, while his mother had Hb Lansing-Ramathibodi and Hb E trait without Southeast Asian deletional α^0-thalassemia.

Table 1. Results of laboratory investigations of the patient and his mother

	Patient	Mother
Hb (g/dL)	10.6	13.6
Hct (%)	34.0	41.4
RBC count (x10^6/mm^3)	5.94	5.63
MCV (fL)	57.2	73.5
MCH (pg)	17.8	24.2
MCHC (g/dL)	31.2	32.9
Reticulocyte (%)	1.14	1.68
WBC (/mm^3)	6,720	8,210
Platelet (/mm^3)	292,000	350,000
Hb analysis (by HPLC)	Hb A 62.0%, Hb A_2/E 15.9%, Hb F 1.2%, abnormal peak at retention time 1.92 min 17.5%	Hb A 63.2%, Hb A_2/E 27.9%, Hb F 1.2%
α-globin genes (*HBA2* and *HBA1*) analysis	Heterozygous Hb Lansing-Ramathibodi Heterozygous Southeast Asian deletional α^0-thalassemia	Heterozygous Hb Lansing-Ramathibodi
β-globin gene (*HBB*) analysis	Heterozygous Hb E	Heterozygous Hb E

Hb, hemoglobin; Hct, hematocrit; HPLC, high-pressure liquid column chromatography; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell

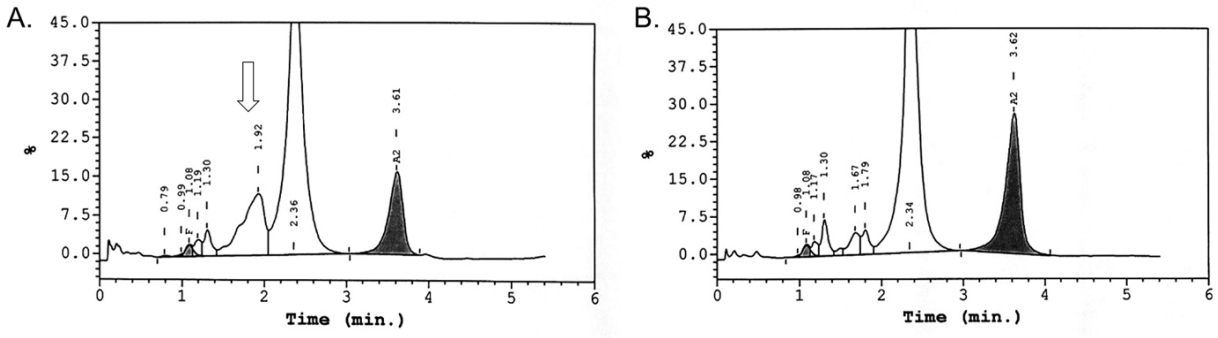

Figure 1. Hemoglobin analysis results using the high-pressure liquid column chromatography (HPLC) method from (A) the patient and (B) his mother. An abnormal hemoglobin peak at a retention time of 1.92 min (arrow) was detected in the patient.

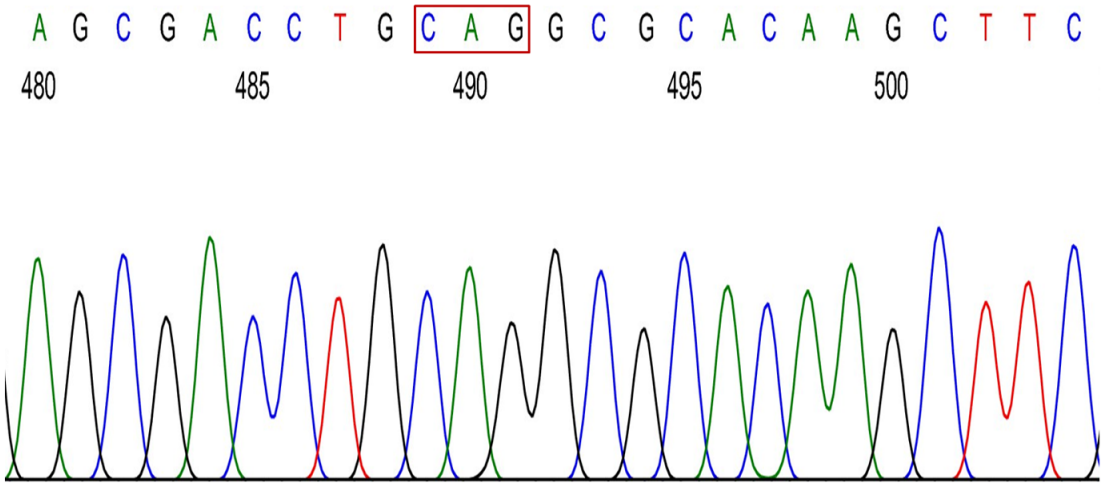

Figure 2. Sanger DNA sequencing of the *HBA1* gene from the patient identified a missense mutation causing hemoglobin Lansing-Ramathibodi (*HBA1*:c.264C>G). The mutation was co-inherited with Southeast Asian deletional α^0-thalassemia.

Other laboratory investigations revealed normal results, with a methemoglobin level of 1.2% (<2%), G6PD level of 18.6 U/gHb (normal range for males >4.0 U/gHb), serum iron level of 82 μg/dL (33-193 μg/dL) and total iron binding capacity of 251 μg/dL (228-428 μg/dL).

DISCUSSION

This case report illustrates the clinical presentation of low SpO_2 measurements with normal PaO_2 and SaO_2 and mild microcytic anemia in a patient with coinheritance of Hb Lansing-Ramathibodi, Southeast Asian deletional α^0-thalassemia and the Hb E trait. The impact of the heterozygous mutation causing Hb Lansing-Ramathibodi without co-inherited α-thalassemia appeared to be minimal as evidenced by the asymptomatic status of the mother who harbored only the heterozygous Hb Lansing-Ramathibodi mutation. Hb analysis of the mother did not reveal an Hb Lansing-Ramathibodi peak. Additionally, the coinheritance of Hb E also appeared to exert minimal effect.

In a systematic review conducted by Verhovsek et al., eleven Hb variants with low SpO_2 and discordantly normal SaO_2 were identified (13). All of these variants are caused by missense mutations, comprising six α-globin Hb variants: Hb Lansing (*HBA2*:c.264C>G, codon 87 His>Gln), Hb Titusville (HBA2 or *HBA1*:c.283G>A, codon 94 Asp>Asn), Hb Bonn (*HBA1*:c.262C>G, codon 87 His>Asp), Hb Delaware (*HBA2*: codon 91 Leu>Val), Hb M-Iwate (HBA2 or *HBA1*:c.262C>T, codon 87 His>Tyr), and a novel Hb (*HBA2* or *HBA1*: codon 62 Val>Ala), along with five β-globin Hb variants: Hb Hammersmith (*HBB*:c.128T>C, codon 42 Phe>Ser), Hb Cheverly (*HBB*:c.137T>C, codon 45 Phe>Ser), Hb Okazaki (*HBB*:c.280T>C, codon 93 Cys>Arg), Hb Regina (*HBB*:c.289C>G, codon 96 Leu>Val), and Hb Köln (*HBB*:c.295G>A, codon 98 Val>Met). Studies examining the absorption spectra of Hb Bonn, Hb Cheverly and Hb Köln have revealed alterations in the light absorbance characteristics of oxyhemoglobin and/or deoxyhemoglobin (13). These changes can potentially contribute to erroneous measurements observed on pulse oximetry. Other reported Hb variants with low SpO_2 and normal SpO_2 are Hb Hirosaki (*HBA2*:c.132C>G, codon 43 Phe>Leu), Hb Santa Ana (*HBB*:c.266T>C, Leu>Pro) and Hb Grifton (*HBA1*:c.263A>C, codon 87 His>Pro) (20-22).

Hb Lansing (*HBA2*:c.264C>G, codon 87 His>Gln) is among the Hb variants linked with unexpectedly low SpO_2 levels (13). Initially observed in a family of Hispanic background by Sarikonda et al. in 2009, subsequent cases have been reported spanning different ethnic backgrounds in individuals of Japanese, Turkish (Hb Lansing A) and Omani descent (23-26). Recently, a case of coinherited Hb Lansing, Hb S and α^+-thalassemia was reported with falsely low SpO_2 levels and hemolytic anemia (27). Hb Lansing-Ramathibodi (*HBA1*:c.264C>G, codon 87 His>Gln) results from the same missense mutation at codon 87, but on the *HBA1* gene. This variant was first identified in four members of a Thai family by Trakulsrichai et al. in 2016 and later reported in a Thai newborn by Prachukthum et al. in 2017 (15, 16). Recently, a case of a 2-year-old boy with Hb Lansing-Ramathibodi who presented with similarly low SpO_2 readings was reported (17).

The clinical findings from the three previously reported families with Hb Lansing-Ramathibodi are summarized in Table 2 (15-17). All cases presented with incidental findings of low SpO_2 levels. Hb levels and mean corpuscular volume (MCV) in cases with heterozygous Hb Lansing-Ramathibodi were normal or borderline low. In our case with the Hb Lansing-Ramathibodi, α^0-thalassemia and Hb E trait, both Hb levels and MCV were low. In terms of Hb analysis, a distinct, measurable abnormal Hb peak at a retention time of 1.7-2.0 minutes, as analyzed by HPLC method, was observed only in cases with co-inherited α-globin gene mutations (Hb Pakse, *HBA2*:c.429A>T, or Southeast Asian deletional α^0-thalassemia). Using the capillary electrophoresis (CE) method, a non-measurable peak was located between Hb A and Hb F. Notably, in the present report, the mother, who had the Hb Lansing-Ramathibodi trait and also harbored the Hb E mutation, exhibited normal SpO_2 levels, whereas two cases from the initial report of the Hb Lansing-Ramathibodi trait presented with SpO_2 levels of 88-90% in room air. The potential impact of co-inherited Hb E on SpO_2 levels warrants further investigation.

This case report underscores the clinical presentation of low SpO_2 measurements with normal PaO_2 and SpO_2 and mild microcytic anemia in a patient with co-inherited Hb Lansing-Ramathibodi, Southeast Asian deletional α^0-thalassemia,

Table 2. Summary of case reports with Hb Lansing-Ramathibodi

	Case number	Age (y)	Gender	Genotype	OxyHb (%) by co-oximetry	SpO_2 (%)	PaO_2 (mmHg)	SaO_2 (%)	Hb (g/dL)	MCV (fL)	Hb analysis
Trakulsrichai et al. 2016 (16)	1	40	Female	$\alpha^{Pakse}\alpha/\alpha\alpha^{L-R}$	69.1	84 in room air 89 on O_2 canula 5 L/min	385 on O_2 mask 10 L/min	100 on O_2 mask 10 L/min	11	81.8	Hb A 80.6%, Hb A_2 2.3%, Hb F 0.6%, abnormal peak at retention time 2.03 min (HPLC) A peak with a shoulder between Hb A and Hb F window (CE)
	2	48	Male	$\alpha\alpha/\alpha\alpha^{L-R}$	69.3	88	-	-	15.5	84.7	Hb A 74.8%, Hb A_2 2%, Hb F 0.8%, abnormal peak at retention time 1.70 min (HPLC) No peak seen by CE
	3	44	Male	$\alpha^{Pakse}\alpha/$	64.1	71	-	-	13.3	77.9	Hb A 76%, Hb A_2 1.7%, Hb F 2.1%
	4	17	Male	$\alpha\alpha^{L-R}$	-	90	-	-	14.5	78.7	-
Prachukthum et al. 2017 (15)	1	Term newborn (GA 38 weeks)	Male	$\alpha\alpha/\alpha\alpha^{L-R}$ $^{--SEA}/\alpha\alpha^{L-R}$	-	84 90 in O_2 hood, 90 on HHHFNC 5 L/min, FiO_2 1.0	389.5 on HHHFNC 5 L/min, FiO_2 1.0, 85.1 in room air	100 on HHHFNC 5 L/min, FiO_2 1.0, 97 in room air	16	-	Hb A 14.7%, Hb F 69.1%, Hb A_2 0.3%, Hb Bart's 15.9% by CE
Quinn and Klouda 2024 (17)	1	2	Male	Hb L-R mutation	-	92 in room air, not improved with O_2 supplement	normal	-	-	-	-
This report	1	10	Male	$^{--SEA}/\alpha\alpha^{L-R}$ β/β^{E}	-	75-77 in room air	221.7 on HHHFNC, FiO_2 0.6, 111.5 in room air	99.7 on HHHFNC, FiO_2 0.6, 98.5 in room air	10.6	57.2	Hb A 62.0%, Hb A_2/E 15.9%, Hb F 1.2%, abnormal peak at retention time 1.92 min 17.5%
	2	38	Female	$\alpha\alpha/\alpha\alpha^{L-R}$ α/α^{E}	-	99 in room air	-	-	13.6	73.5	Hb A 63.2%, Hb A_2/E 27.9%, Hb F 1.2%

CE, capillary electrophoresis; Hb, hemoglobin; HHHFNC, heated humidified high flow nasal cannula; HPLC, high-pressure liquid column chromatography; L-R, Lansing-Ramathibodi; MCV, mean corpuscular volume; PaO_2, partial pressure of oxygen; SaO_2, arterial oxygen saturation; SpO_2, oxygen saturation readings on pulse oximetry

and Hb E trait. The minimal impact of the heterozygous Hb Lansing-Ramathibodi mutation without co-inherited β-thalassemia is evidenced by the asymptomatic status of the mother harboring solely the heterozygous Hb Lansing-Ramathibodi mutation, with no detectable Hb Lansing-Ramathibodi peak in her Hb analysis. This case adds to the understanding of the rare Hb variant and its clinical implications for accurate diagnosis and management.

Research ethics approval

The research protocol complies with the research with exemption category. It has been certified as exempt from ethical review by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (No. 0487/2023).

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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