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ORIGINAL ARTICLES

CASE REPORT

Original Article

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Vitamin D Level of Individuals Having Medical Service in a Tertiary Hospital

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ABSTRACT

OBJECTIVE: This study aimed to investigate the status of vitamin D level, rates of insufficiency and deficiency in individuals seeking medical services in a tertiary hospital.

METHODS: This retrospective study was conducted between October 1, 2022, and December 24, 2023. Inclusion criteria were individuals aged 18 years or older who had sought medical services in our hospital between August 2021 to May 2022. Information such as age, gender, ethnicity, and personal health data including vitamin D level, blood pressure, body mass index, and bone mineral density were collected. The percentages and risk features among the individuals with inadequate vitamin D levels were analyzed.

RESULTS: Among the 2,459 participants, the mean age was 52.0 years (interquartile range [IQR] 40, 67 years). Approximately one third had one or more health disorders (31.1%), with 29.7% being overweight, 10.8% being obese, and 57.3% having high blood pressure. Osteopenia or osteoporosis was found in 33.2%. The median vitamin D level was 26.3 ng/mL (IQR 19.4, 36.0 ng/mL), with 61.8% had inadequate vitamin D levels as insufficiency in 35.2% and deficiency in 26.6%. Univariable analysis revealed the following three features showing significant association with inadequate vitamin D levels: age younger than 60 years (odds ratio [OR] 2.3, p-value < 0.001), Thai ethnicity (OR 1.3, p-value = 0.014), and overweight/obesity (OR 1.3, p-value = 0.002). Multivariable analysis showed that all three features were independent risk factors for inadequate vitamin D levels.

CONCLUSION: This study showed a high percentage of inadequate vitamin D levels among the participants, with insufficiency in approximately one third and deficiency in one fourth. Older people, Thai people, and overweight/obese people were at risk of inadequate vitamin D levels.

KEYWORDS: calciferols, vitamin D, vitamin D deficiency, vitamin D insufficiency

INTRODUCTION

Vitamin D, or calciferol, exists as D2 and D3. It is obtained from sunlight, vitamin D-rich foods like fatty fish and fortified dairy, and supplements. Vitamin D is synthesized in the skin from 7-dehydrocholesterol upon UVB exposure, forming previtamin D3 and then active vitamin D3 (cholecalciferol). After binding to its receptor, vitamin D is transported to the liver, where it converts to calcifediol, and then to the active form, calcitriol, in the kidneys[1]. Calcitriol acts through the vitamin D receptor in various tissues to regulate physiological processes, including bone mineralization by managing calcium and

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phosphate levels, enhancing intestinal absorption, renal reabsorption, and osteoclast activity[2]. Additionally, vitamin D impacts the immune system, potentially affecting autoimmune diseases, infections, and cell growth, with implications for cancer prevention[3]. Inadequate vitamin D can reduce calcium absorption by 10%–15% and phosphorus absorption by 60%, causing bone issues like fractures, bone loss, muscle weakness, and falls. It can also lead to other health problems, including neurocognitive disorders, mental illness, cardiovascular disease, autoimmune diseases, infections, diabetes, cancer, infertility, and poor pregnancy outcomes[4,5].

The status of inadequate vitamin D, either insufficiency or deficiency, may be associated with many factors, such as environment or geographic location, socioeconomic status, season, culture, habitat, and individual features, including aging, dark skin color, gender, lifestyle, sunscreen usage, obesity, personal illness, and dietary intake of vitamin D-rich foods or supplements and medications that interfere with vitamin D metabolism[1,6-8].

Although the prevalence of vitamin D insufficiency slightly decreased from 2000–2010 to 2011–2022, it remained high among some populations, such as in the Eastern Mediterranean region and lower-middle income countries or living in high latitudes[9]. Several studies in Thailand conducted between 2009 and 2013 revealed the varying prevalences of 34%–78% for vitamin insufficiency[10-12] and 6% for vitamin D deficiency[13]. However, these previous studies were conducted in the community in non- or suburban areas of Thailand, which might not provide a situation in the hospital especially in urban area of the country. Our hospital, which is a tertiary hospital in a metropolitan city of the country, had many individuals who had medical services including vitamin D level determination. Data on the status of vitamin D and relevant information should be useful.

Our study aimed to investigate the status of vitamin D level, rates of insufficiency and deficiency in individuals seeking medical services in the hospital. The associated features, including the characteristics and their health status with vitamin D level, were also studied.

METHODS

This retrospective cross-sectional study received ethical approval from the Institutional Review Board of MedPark Hospital, Thailand (COA 001/2022 E) and was conducted between October 1, 2022, and December 24, 2023. Inclusion criteria were adult patients aged 18 years or older who had sought medical services in our hospital between August 2021 to May 2022. The participants must have had serum vitamin D level testing as a part of either health surveillance program or medical care for any abnormal health condition. Pregnant women and individuals whose basic characteristic data were unavailable were excluded. Based on a 65% prevalence of vitamin D insufficiency in central Thailand[8], the sample size of at least 350 participants was required with the significant level was 0.05, and the $Z_{\alpha/2} = 1.96$.

The electronic database of the hospital was used to collect basic characteristics of age, gender, ethnicity, serum 25(OH)D level, and personal health data, such as health history and findings when vitamin D level was determined, including blood pressure, body mass index (BMI), and bone mineral density (BMD). The first value of determined vitamin D in our hospital was used for the analysis.

Serum 25(OH)D levels were measured in the hospital laboratory by electrochemiluminescence immunoassay (Cobas e801 immunoassay analyzer; Roche Diagnostics, Basel, Switzerland) using Cobas e801 analyzer, and the results were correlated with those using the CDC Vitamin D Reference Laboratory by LC-MS/MS (r = 0.980). The detection limit of total 25(OH)D was 3–100 ng/mL or 7.5–250 nmol/L. Internal quality controls were performed daily, and external quality assessment was conducted monthly by Randox Laboratories Ltd., RIQAS program (ISO 17043: 2010).

All statistical analyses were conducted using IBM SPSS Statistics for Windows,

Version 28.0 (IBM Corporation, Armonk, NY, USA). Descriptive data were presented as frequencies and percentages for categorical variables and mean ± standard deviation or median and ranges for continuous data. For comparison, the data were grouped as follows: age (< or ≥ 60 years), gender (male vs. female), ethnicity (Thai vs. others), health status (normal vs. abnormal), BMI (normal vs. overweight/obese), BMD (normal, abnormal), and vitamin D status (sufficiency, insufficiency, and deficiency). Overweight/obesity was defined with BMI ≥ 25 kg/m^2. Abnormal BMD was defined as osteopenia and osteoporosis according to the BMD report. Vitamin D status was defined in accordance with the Institute of Medicine guidelines: deficiency with serum 25(OH)D level < 20 ng/mL (< 50 nmol/L), insufficiency with a level between 20 and 29.9 ng/mL (50–72.5 nmol/L), and sufficiency with a level > 30 ng/mL (75 nmol/L)[14]. Chi-square or Fisher's exact tests were employed to explore the associations between vitamin D status and findings of health outcomes of interest. Multivariable logistic regression analyses were conducted for factors that were significant as determined by univariable analysis. A p-value < 0.05 was considered statistically significant.

RESULTS

Among the 2,727 individuals who sought health services and underwent vitamin D measurement in our hospital, 268 were excluded because of having an age lower than 18 years old (n = 223) or lacking available data on their vitamin D levels (n = 45).

A total of 2,459 individuals who underwent vitamin D measurement and had available characteristic data were included in this study. The median age was 52.0 years (interquartile range [IQR] 40, 67 years), with females accounting for slightly more than half (55.6%). The majority was Asian (94.4%; being Thai in 82.9%). Table 1 shows the basic characteristics of the participants.

Table 2 shows the health history or findings of the participants. Nearly one third (31.1%) reported or were found to have one or more health disorders. Basic physical examination revealed that 29.7% were overweight and 10.8% were obese. More than half (57.3%) had high blood pressure. Among the 696 participants who underwent BMD examination, approximately one third (33.2%) showed abnormalities of either osteopenia or osteoporosis.

Table 1 Basic characteristic features of the participants (n = 2,459)

Basic characteristics features	n	%
Age, years		
< 40	570	23.2
≥ 40 to 49	530	21.6
≥ 50 to 59	398	16.2
≥ 60	961	39.1
Gender		
Male	1,093	44.4
Female	1,366	55.6
Ethnic		
Asian	2,320	94.4
Thai	2,038	82.9
Others*	282	11.5
Western	139	5.6

Abbreviation: n, number
*Other ethnics included people from 26 countries from the 5 different regions in Asia

Table 2 Health history or findings of the participants

Health features	n	%
Health history & finding* (n = 2,459)		
Normal	1,694	68.9
Abnormal*	765	31.1
Cardiovascular	135	15.1
Respiratory	286	31.9
Neurological	22	2.5
Gastrointestinal	51	5.7
Musculoskeletal	147	16.4
Skin	54	6.0
Endocrine	201	22.4
Blood pressure level (n = 1,922)		
Normal	821	42.7
High	1,101	57.3
Body mass index (n = 2,389)		
Underweight	157	6.6
Normal	1,264	52.9
Overweight	709	29.7
Obesity	259	10.8
Bone mineral density (n = 696)		
Normal	465	66.8
Abnormal	231	33.2

Abbreviation: n, number
*One may have one or more health disorders

Among the 2,459 participants, the median vitamin D level was 26.3 ng/mL (IQR 19.4, 36.0 ng/mL). We found that 61.8% had inadequate levels, with insufficiency in 35.2% and deficiency in 26.6%. Table 3 lists the vitamin D status and levels of the participants.

We also explored the association between vitamin D status and the characteristics and health features of the participants with inadequate vitamin D levels (table 4). Univariable analysis showed the following significant features associated with inadequate vitamin D levels: age younger than 60 years, Thai ethnicity, and overweight/obesity. Multivariable analysis revealed that all of these features were independent factors associated with inadequate vitamin D status. The frequency of inadequate vitamin D level was higher in individuals with a history of illnesses or abnormal health findings than in normal individuals (40.1% vs. 37.3%). However, the difference was not statistically different.

Vitamin D status was also studied among the 696 participants who had available data of BMD. A significantly higher rate of osteopenia/osteoporosis was found among the individuals who had adequate vitamin D levels (41.9%, 106 out of 253 participants) compared with those with inadequate vitamin D status (28.3%, 125 out of 442 participants).

Table 3 Vitamin D status and level (n = 2,459)

Vitamin D status	Vitamin D level, mean ± SD (ng/mL)	n	%
Sufficient	46.46 ± 19.10	939	38.2
Inadequate	20.70 ± 5.62	1,520	61.8
Insufficient	24.85 ± 2.80	867	35.2
Deficient	15.18 ± 3.13	653	26.6

Abbreviations: n, number; ng, nanograms; mL, milliliter; SD, standard deviation

Table 4 Features associated with inadequate vitamin D

Features	n	Vitamin D level		Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
		Inadequate	Sufficient				
Age (years), n = 2,459							
< 60	1,498	1,043 (69.6)	455 (30.4)	2.3 (1.97–2.75)	< 0.001	2.4 (2.02–2.85)	< 0.001
≥ 60	961	477 (49.6)	484 (50.4)				
Gender, n = 2,459							
Female	1,366	846 (61.9)	520 (38.1)	1.0 (0.86–1.19)	0.892	-	-
Male	1,093	674 (61.7)	419 (38.3)				
Ethnic, n = 2,459							
Thai	2,038	1,282 (62.9)	756 (37.1)	1.3 (1.05–1.61)	0.014	1.6 (1.28–2.00)	< 0.001
Others	421	238 (56.5)	183 (43.5)				
Health status, n = 2,459							
Abnormal	765	307 (40.1)	458 (59.9)	0.9 (0.75–1.06)	0.182	-	-
Normal	1,694	632 (37.3)	1,062 (62.7)				
Body mass index, n = 2,389							
Overweight/Obesity	968	635 (65.6)	333 (34.4)	1.3 (1.10–1.54)	0.002	1.4 (1.20–1.70)	< 0.001
Normal	1,421	845 (59.5)	576 (40.5)				

Abbreviations: CI, confidence interval; n, number; OR, odds ratio
*Abnormal health status referred to history of personal illnesses and/or presence of abnormal health findings

DISCUSSION

Our study found 61.8% of the individuals having medical service in the hospital had inadequate vitamin D level: insufficiency in 35.2% and deficiency in 26.6%. The prevalence of vitamin D status in various populations has been widely reported. The different prevalences of vitamin D insufficiency or deficiency across studies may lie on many factors, especially the characteristics of the participants in each study, e.g., ethnic or geographic area, altitude, age group, gender, and other personal features of BMI, skin color, living conditions or residence, use of sunscreen, and illnesses. The features of Thai ethnicity, age younger than 60 years, and overweight/obesity, which were found to be significantly associated with inadequate vitamin D in our study, had also been previously reported as influencing factors.

Regarding the ethnic or geographic impact on vitamin D status, people from areas with high sun exposure, such as Asia, should have a lower prevalence of inadequate vitamin D levels than those who live in Western countries[15]. However, the 35.2% of vitamin D insufficiency in our study, 34% to 39% in other reports from Thailand[10-12], and 36% to 43% in other reports from Asia[16,17] were not much different from 34% to 41% in the reports from the Western country (US)[9,18,19]. This was also the situation for vitamin D deficiency which was found 26.6% in our study compared to 21% to 23% in Asia[16,17], and 20% to 25% in the US reports[18,19]. The exceptions were high rates of vitamin D deficiency from one study which reported 58% among 982 Indian participants aged 45 years and over[20] and 68% from one systematic review including 65 studies from South Asia[21]. We wanted to focus on finding of vitamin D levels of Thai population in our study compared with the other ethnics altogether. Although not representing the overall population of the country, a higher rate of inadequate vitamin D among Thai was revealed compared to the other ethnic population in the study (adjusted OR 1.6). Many other factors, aside from ethnicity, may play certain roles in the relationship with vitamin D levels, e.g., characteristics of the participants, attitudes and lifestyle behaviors toward sun exposure, use of sunscreen, or diets[13,17,19,22].

Inconsistent data have been reported regarding the relationship between age and vitamin D status. Only a few investigations focused on this relationship, including one study from Thailand that found a positive association between older age and high vitamin D levels[18,19,23]. By contrast, one previous report[24] and our study found younger age was

a risk factor for low vitamin D (adjusted OR 2.4 in our study). Theoretically, the elderly should have low vitamin D levels due to the decreased production and metabolism of vitamin D caused by the physiological changes of aging. In addition, factors such as limited physical activity and concerns about skin cancer may lead to reduced sun exposure and subsequently low cutaneous vitamin D synthesis in older individuals[25]. However, older individuals, who are often more health-conscious than younger age groups, are highly likely to take vitamin D supplements, which could impact the findings.

Another risk factor for inadequate vitamin D levels identified in this study was overweight/obesity. This finding was consistent with previous reports that also found a direct association between overweight or high BMI and low or inadequate vitamin D levels[18,19,24,26]. The causes of poor vitamin D level in overweight/obese people are complex and may involve multiple pathways, e.g., reduced vitamin D synthesis in adipose tissues and the liver, gene expression dysregulation, parathyroid hormone modulation, and decreased 25-OH-vit D availability in most obese individuals, resulting in decreased levels of the active form, 1,25-dihydroxyvitamin D[27-29].

One contradictory finding of our study was the significant association between inadequate vitamin D levels and normal BMD, which was contradictory to the established understanding that low vitamin D levels or vitamin D deficiency have detrimental effects on bone health[17,30,31]. Considering that data on vitamin D supplementation were not available in this study, we simply proposed that individuals with abnormal bone conditions might have already taken vitamin D supplements, potentially explaining this unexpected result.

Although our study did not find a significant impact of gender or history of illnesses or abnormal health findings on vitamin D status, this relationship merited some discussion. Data regarding the impact of gender are inconsistent: some studies reported that males are associated with inadequate vitamin D levels[18], and others found that females have a high risk[19,23]. Various personal characteristics of the participants across studies may contribute to the different impacts of gender on vitamin D level. Regarding illnesses, many studies found a direct association between low vitamin D level or vitamin D deficiency and various illnesses[24,20,32-34]. Focusing on one hospital-based study, their study found lower vitamin D level in the patients with high blood pressure compared to those with normal pressure[34]. Although our study found a slightly higher percentage of inadequate vitamin D in individuals with who had abnormal health status than healthy individuals (table 4: 40.1% vs 37.3%), the difference was not statistically significant which may be attributed to a heterogeneous illness or findings, especially when most of them were not confirmed or had longitudinal follow-up data for verification.

Our study have some limitations. First, it was a retrospective design, so data which have an impact on vitamin D status such as prior vitamin D levels and diet or sun-exposure habits and/or vitamin D-containing supplements were not available. Second, some participants simply sought a health check-up without requiring specific medical care for health problems, so the health history and findings from various investigations were heterogeneous. With a lack of complete data and longitudinal follow-up, their association with vitamin D status should be interpreted cautiously. Third, the association between vitamin D status and bone health measured with BMD was against all odds. With the unavailability of data on vitamin supplements, this finding could not represent a genuine association. Lastly, this study was conducted as one hospital-based study which may not represent all Thai ethnic across the country.

Nevertheless, our study had some strengths. The number of individuals who underwent vitamin D measurement was quite large, and these people had various characteristics, including ethnicity. Our findings may be informative for specific groups with abnormal vitamin D levels. Further research should focus on these specific groups with vitamin D insufficiency or deficiency and associated illnesses. The genuine causal relationship between characteristic features and vitamin D inadequacy should be confirmed, especially among the Thai population. Moreover, longitudinal studies are necessary to gain additional insight into the direction of the associations.

CONCLUSION

This study demonstrated a high percentage of inadequate vitamin D levels, with insufficiency in approximately one third and deficiency in one fourth of the participants. Age younger than 60 years, Thai ethnicity, and overweight/obesity were found to be independent risk factors.

CONFLICT OF INTEREST

The authors have declared no competing interests exist.

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DATA AVAILABILITY STATEMENT

Please contact the corresponding author for data availability.

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Original Article

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A Randomized, Double-Blind Study on the Combined Effects of Low-Level Laser Therapy and Exercise on Pain, Functional Level, and Range of Motion in Patients with Chronic Non-Specific Low Back Pain

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ABSTRACT

OBJECTIVE: To investigate the combined effects of low-level laser therapy (LLLT) in conjunction with an exercise program (LLLT group) compared to exercise alone (control group) on pain, functional level, and range of motion in patients with chronic non-specific low back pain (CNLBP).

METHODS: Sixty participants with CNLBP were randomized and divided into 2 groups: laser group (30 participants) and control group (30 participants). Both groups were assigned the same homework exercises (once a day, 3 days a week for 4 weeks). The assessments were performed at baseline and 4 weeks after low-level laser therapy application 3 times per week for 4 weeks. Pain level (visual analogue scale), functional level (the Oswestry low back pain disability questionnaires Thai version) and range of motion (Schober's test) were evaluated.

RESULTS: The participants who completed the study totaled 60, with 30 in the LLLT group and 30 in the control group. Both groups showed statistically significant differences in improved pain level and functional level ($p < 0.001$) from baseline to the 4th week, with the exception of range of motion in the control group ($p = 0.644$). Outcome of mean difference across the intervention arm for group comparison analysis indicated statistically significant differences in favor of the experimental group across all measures ($p < 0.01$, $p < 0.03$ and $p < 0.01$, respectively).

CONCLUSION: Combining LLLT with exercise significantly reduced pain, improved functional ability, and increased lumbar range of motion, providing a more effective treatment for Thai patients with CNLBP compared to exercise alone. The Minimal Clinically Important Difference for Visual Analog Scale (0.211) and Oswestry Disability Index (0.216) confirmed that the improvements at week 4 were clinically significant beyond natural recovery.

KEYWORDS: chronic non-specific low back pain, low-level laser therapy, Oswestry, Schober's test

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INTRODUCTION

Chronic non-specific low back pain (CNLBP) is a prevalent health issue, with peak incidence occurring around age 30 and the prevalence increasing until ages 60-65, after which it gradually declines[1,2]. Individuals with back pain have a 24-80% chance of experiencing symptom recurrence within one year[1]. This condition not only limits patient function but also imposes a significant socio-economic burden due to lost productivity and increased healthcare costs[3].

The primary goals in treating CNLBP are pain reduction, functional improvement, and disease progression prevention. Surgery is typically reserved for patients with severe symptoms, such as weakness, bowel or bladder involvement, or those who do not respond to conservative treatments. Conservative management includes both nonpharmacologic and pharmacologic approaches, with clinical practice guidelines recommending nonpharmacologic methods as the first line of management to reduce pain and enhance daily function. These methods include physical therapy, exercise, acupuncture, cognitive behavioral therapy, and massage[4-7]. Nonpharmacologic treatments are preferred due to fewer side effects and better cost-effectiveness compared to the pharmacologic options[4].

Exercise is a recommended practice guideline to enhance mobility and prevent disease progression for treating patients with CNLBP[8,9]. However, studies have shown that combining exercise with physical therapy devices or other treatment methods can enhance treatment efficacy (synergistic effect). For example, the study by Vallone et al.[10] demonstrated that low-level laser therapy (LLLT) combined with exercise in patients with CNLBP significantly reduced pain levels. In contrast, the meta-analysis by Jang et al. found that some studies reported no significant difference in treatment outcomes between using LLLT combined with exercise and exercise alone[11]. Both LLLT and exercise are non-invasive, relatively safe, and widely used in clinical practice. Therefore, further research is needed to optimize treatment effectiveness.

In recent years, laser treatment has gained popularity for its ability to moderate pain and inflammation, including in CNLBP. It works by modulating cellular processes such as reducing pro-inflammatory cytokine release, promoting mitochondrial activity, and enhancing tissue repair[12]. Studies have demonstrated that LLLT is an effective treatment for pain without side effects. Various settings, such as laser type, mode, wavelength, energy intensity, and treatment duration, can be tailored to individual patient conditions[11,13,14]. This study aimed to evaluate the combined effects of LLLT and exercise on pain, functional level, and range of motion and confirm the synergistic effect of combining LLLT and exercise for Thai patients with CNLBP, with the goal of providing clinically relevant insights into the integration of laser therapy as a viable treatment option for this condition.

METHODS

The study described was a simple randomized controlled trial with a double-blind design in which both participants and therapists were blinded. Its purpose was to investigate the effects of LLLT combined with exercise on pain, functional level, and lumbar range of motion in patients aged more than 18 years old with CNLBP for more than 3 months[15]. The trial included 60 participants from the outpatient rehabilitation clinic at Ratchaphiphat Hospital who met specific inclusion criteria: no prior LLLT, no recent back trauma or surgery, and no contraindicating underlying diseases, such as lower extremity weakness, malignant melanoma, and epilepsy (Figure 1). Ethics approval was obtained from the Bangkok Metropolitan Administration Human Research Ethics Committee (Project ID: S004h/63). After volunteers agreed to participate in the research project, they received an information sheet explaining the details of the study.

If the participants consented and were willing to join the research, they had to sign an informed consent form in order to indicate their agreement to participate in the study. Sixty participants were randomly (by computer) divided into 2 groups: 30 participants in the LLLT group and 30 participants in the sham laser group, as shown in Figure 1. The experimental group received LLLT combined with exercise, while the control group received a sham laser treatment combined with the same exercise regimen. The first physical therapist administered the treatment, setting the laser machine as specified and activating the sample light to appear the same for both groups. The second physical therapist was responsible for turning the laser on, ensuring that it only worked for the LLLT group, and setting a timer to ensure equal treatment durations for both groups. Patients were only informed that the laser might alternate between different power levels and wavelengths. Both groups were taught the exercise program by the same physical therapist (first physical therapist). Outcome measurements for both groups were conducted by the same assessor, who was blinded to the group assignments. The gallium–aluminum–arsenide laser used in the study had a probe scan wavelength of 638 nm, continuous mode, and a maximum power wattage of 650 mW[16]. Calculated output power for low backs was 120-240 J/spot with a 4-8 J/cm^2 and 30 cm^2 treatment area; the calculated treatment time was 6.09-12.18 minutes. In this study, the laser accumulated power per area was 200 J/area and patient treatment time was 10.15 minutes. Laser treatment was administered 3 times a week for 4 weeks[17].

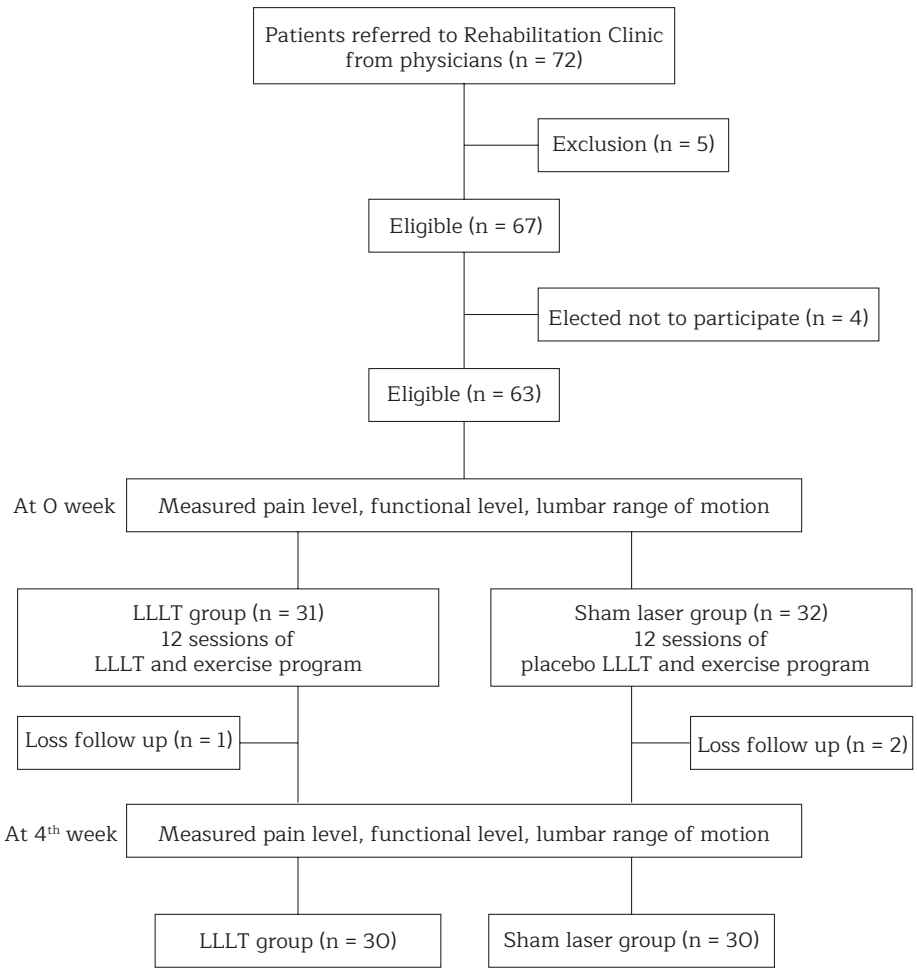

Figure 1 Consort diagram of patients eligible, recruited, numbers followed up and included in analysis

The primary outcomes measured included: the first was pain assessment by Visual Analog Scale (VAS)[18], ranging from 0 (no pain) to 10 (maximum pain). Secondary outcomes were functional assessment by Oswestry low back pain disability questionnaire (Thai version)[19], assessing disabilities across various activities: personal care, lifting, walking, sitting, standing, sleeping, sexual activity, participating in social activities, and traveling. For each section the possible disability score ranged from 0 to 5, and thus the total possible score was 50. The score was calculated as a percentage and sorted into 5 groups that were 0-20% for minimal disability, 21-40% for moderate disability, 41-60% for severe disability, 61-80% for crippling disability, and 81-100% for bed-bound disability. The other secondary outcomes were lumbar range of motion by Schober's test[20]. In order to conduct the test, the patient was barefoot and standing upright. A mark was made at the lumbosacral junction and then a second mark 10 cm. above it. The distance between the 2 marks on the lower back was then measured during maximum forward flexion. The mean of 3 measurements was used in the study.

Both groups followed a prescribed exercise regimen[21], which included pelvic tilt exercises, abdominal and back strengthening exercises, and hip flexor relaxation exercises, seen in Figure 2. All the exercises were performed once a day (5 repetitions per set for each exercise, 3 sets per day), 3 times a week for 4 weeks. Compliance with the exercise regimen was recorded in a logbook. Assessments were made prior to and 4 weeks post intervention. Dropout criteria were VAS increased more than 3 levels from baseline, and if a patient received the treatment fewer than 12 times during the intervention's 4 weeks of sessions. The participants were requested to stop other treatments and medications during the sessions.

The sample size used in this study was calculated by STATA 14.2 and referenced from a study of Vallone et al.[10] in 2014. The research design included type I error set to 0.05, type II error set to 0.8, delta at 1.64. The 60 subjects were calculated from a 50 subject minimal sample size with a dropout rate of 20 percent. The demographic characteristics were identified as mean with standard deviation and compared by independent T-test. Treatment outcomes, VAS[18], the Oswestry Low Back Pain Disability Questionnaire (Thai version)[19], and Schober's test,[20] were compared pre and post intervention by paired T-test. The differences between the 2 groups were compared by independent T-test. The statistical significance of the study was set at $p < 0.05$.

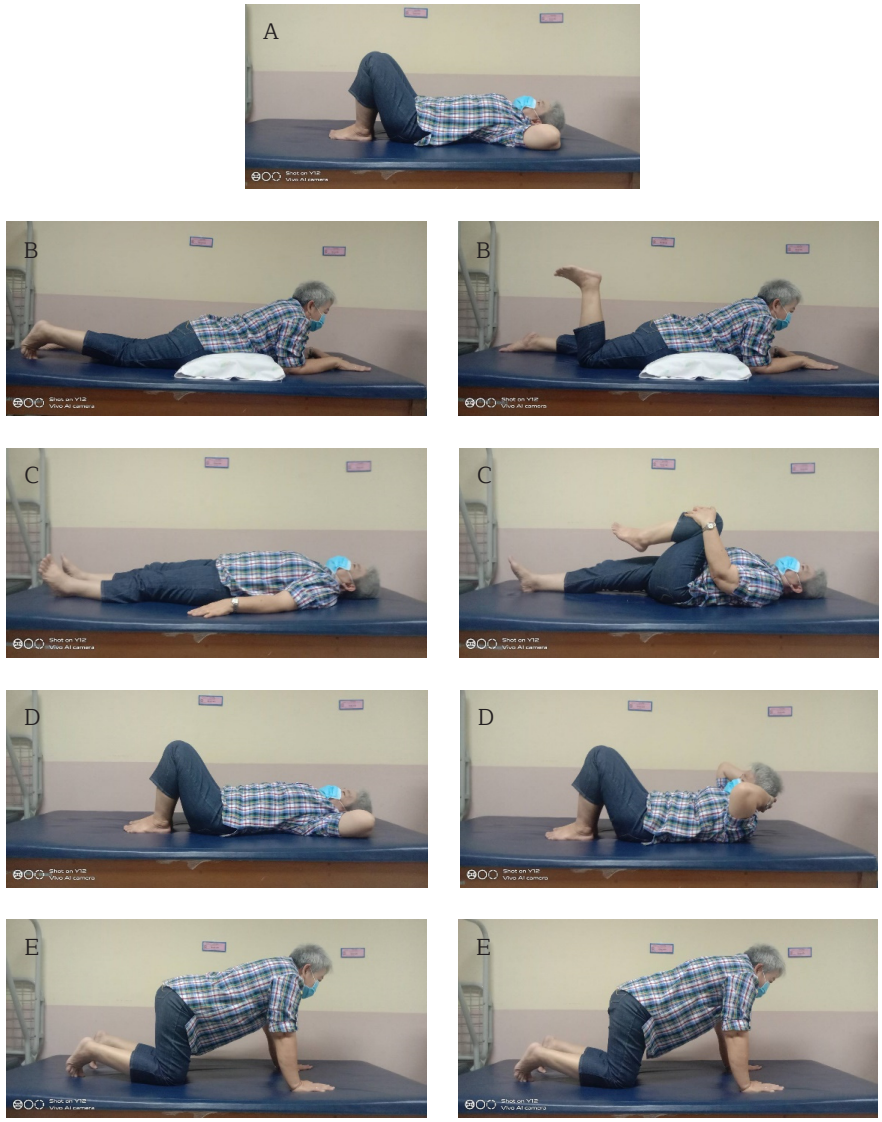

Figure 2 All exercises in exercise program were performed once a day (perform 5 repetitions per set for each exercise, 3 set per day), 3 times a week for 4 weeks.

(A) Posterior pelvic tilt exercise: Lie on your back on the bed with both hands clasped behind your head. Bend both knees with your feet flat on the bed. Inhale while tensing your abdominal and gluteal muscles. Hold for 20 seconds, then relax and exhale.

(B) Anterior pelvic tilt exercise: Lie face down with a pillow under your abdomen. Inhale while bending your dominant knee at a 90-degree angle. Hold for 20 seconds, then exhale as you lower your knee. Repeat on the other side.

(C) Relax hip flexor muscles exercise: Lie on your back with both legs straight. Bend your knee and hip on the dominant side, placing your foot flat on the bed. Then, bend the knee further to bring it toward your chest while extending the non-dominant leg straight. Hold for 20 seconds, then switch sides.

(D) Abdominal muscles exercise: Lie on your back on the bed with both hands clasped behind your head. Bend both knees with feet flat on the bed. Then, curl your body up to touch your forehead to your knees in a sit-up position.

(E) Exercise to control lumbar lordosis: Start on your hands and knees in a kneeling position with your back parallel to the floor. The physical therapist instructs you to contract your abdominal and gluteal muscles while arching and rounding your back, keeping it parallel to the floor. Hold for 20 seconds.

RESULTS

Sixty participants, 30 subjects in the control group and 30 subjects in the experimental group, completed the study (Figure 1). Demographic characteristics are shown in Table 1. There were no statistically significant differences between the 2 groups. Pre- and post-intervention outcomes in the 2 groups were compared. VAS[18], the Oswestry low back pain disability questionnaire (Thai version)[19] and Schober's test[20] showed statistically significant improvements after laser treatment ($p < 0.001$), except for Schober's test[20] in the control group ($p = 0.644$) (Table 2). Outcome of mean difference across the intervention arm for group comparison analysis indicated statistically significant differences in favor of the experimental group across all measures ($p < 0.01$, $p < 0.03$ and $p < 0.01$) in Table 2.

Table 1 Demographic characteristics

Demographic data	LLLT group (n = 30)	Sham laser group (n = 30)	P-value
Gender: n (percent)			0.584
Male	9 (30)	11 (37)	
Female	21 (70)	19 (63)	
Age (years)	54.93 ± 12.17	55.18 ± 13.15	0.938
Weight (kg)	68.11 ± 14.01	67.51 ± 15.90	0.878
Height (m)	1.58 ± 0.10	1.60 ± 0.12	0.312
BMI (kg/m^2)	27.31 ± 4.51	26.14 ± 5.15	0.359
Educational profile: n (percent)			0.524
Uneducation	0 (0)	1 (3)	
Elementary school	14 (47)	15 (50)	
Middle school	2 (7)	2 (7)	
High school	5 (17)	5 (17)	
Bachelor degree	9 (30)	5 (17)	
Master degree	0 (0)	2 (6)	
Alcohol history: n (percent)			1.000
Drink	3 (10)	3 (10)	
No drink	27 (90)	27 (90)	
Smoking history: n (percent)			0.506
Smoke	2 (7)	1 (3)	
No smoke	26 (87)	26 (87)	
Now smoking	1 (3)	0 (0)	
Use to smoke	1 (3)	3 (10)	

Abbreviations: kg, kilogram; kg/m^2, kilogram per square metre; LLLT, low-level laser therapy; m, meter; n, number

Table 2 Comparing VAS, Oswestry scale and Schober's test at week 0 and 4th and outcome of mean difference across the intervention arm for group comparison analysis

	Pretest At week 0	Posttest At week 4th	Difference 95%(CI)	P-value
VAS (mean ± SD)				
Sham laser	6.24 ± 1.33	3.79 ± 1.77	2.45 (1.80, 3.11)	< 0.001
LLLT	6.86 ± 1.34	2.67 ± 1.73	4.19 (3.58, 4.79)	< 0.001
VAS: Mean difference	-0.62 (-1.31, 0.70)	1.11 (0.21, 2.02)	P-value < 0.01	
Oswestry score (mean ± SD)				
Sham laser	14.60 ± 8.09	7.80 ± 5.36	6.80 (4.48, 9.12)	< 0.001
LLLT	18.27 ± 6.40	5.40 ± 2.63	12.87 (10.64, 15.09)	< 0.001
Oswestry score: Mean difference	-3.67 (-7.44, 0.10)	2.40 (0.21, 4.58)	P-value < 0.03	
Schober's test (mean ± SD)				
Sham laser	12.70 ± 2.13	12.50 ± 2.14	0.21 (-0.70, 1.11)	0.644
LLLT	11.66 ± 1.36	13.76 ± 1.87	-2.10 (-2.91, -1.29)	< 0.001
Schober's test: Mean difference	1.04 (0.12,1.97)	-1.26 (-2.30, -0.23)	P-value < 0.01	

Abbreviations: CI, confidence interval; LLLT, low-level laser therapy; SD, standard deviation; VAS, visual analog scale
Statistical significance, p-value < 0.05

DISCUSSION

LLLT has the potential to reduce pain, lower disability levels, and improve range of motion through several mechanisms. It modulates inflammation by inhibiting pro-inflammatory cytokines and promoting anti-inflammatory mediators, which helps relieve pain and enhance tissue healing. LLLT also stimulates angiogenesis and collagen synthesis, aiding in the regeneration of damaged tissues and improving functional outcomes. Additionally, LLLT can improve nerve function by reducing pain sensitivity and enhancing motor control. LLLT may have psychological benefits, such as reducing anxiety and improving mood. This can indirectly contribute to pain reduction and improved quality of life[12]. The superior outcomes of LLLT groups compared to control groups are due to several factors. LLLT directly targets inflammation, tissue damage, and nerve dysfunction. Additionally, LLLT can be combined with other therapies, such as exercise or physical therapy, in order to enhance the overall treatment effect (synergistic effect). Lastly, the placebo effect may be stronger due to the perceived technological advancement and the potential for non-invasive treatment[12,22].

Previous studies had shown positive results in treating musculoskeletal diseases with laser therapy[23,24]. CNLBP findings are consistent with prior studies that demonstrated LLLT's efficacy in pain reduction and functional improvement, such as those by Huang et al.[13], Hadi et al. [17], and Rubira et al.[25]. Abdelbasset et al.[15] noted that LLLT reduced pain, enhanced function (measured by the Oswestry Disability Index), and increased lumbar range of motion. In this study, the combination of LLLT and exercise resulted in significant outcomes for patients with CNLBP. Specifically, there was a marked reduction in pain intensity as measured by the VAS, an improvement in functional capacity assessed by the Oswestry Low Back Pain Disability Questionnaire (Thai version), and an enhancement in lumbar range of motion measured by Schober's test; there were no reported side effects during the research participation period. Comparisons of pre- and post-intervention outcomes between the LLLT and sham laser groups showed significant improvements in the VAS, Oswestry Questionnaire, and Schober's test after LLLT and exercise treatment ($p < 0.001$), except for Schober's test in the sham laser group ($p = 0.644$).

Additionally, mean difference of post-intervention results indicated statistically significant differences in favor of the LLLT group across all measures. These findings are consistent with studies by Vallone et al.[10], Djavid et al.[21], and Gur et al.[26], confirming the synergistic effect of combining LLLT and exercise for patients with CNLBP. This combination therapy not only enhances pain reduction and functional improvement but also increases lumbar range of motion, demonstrating its effectiveness as a comprehensive treatment approach for managing CNLBP in this population. The placebo effect from LLLT is likely due to patients' belief in the effectiveness of a novel, advanced treatment they had not previously experienced[27]. This is reflected in improvements in subjective measures, such as pain intensity (VAS) and functional ability (Oswestry Questionnaire), where patients assessed themselves. However, no significant improvements in lumbar range of motion (Schober's test) ($p = 0.644$), as shown in Table 2, which was objectively measured by professionals, were observed in the control group receiving sham laser treatment. LLLT may also offer psychological benefits, such as reducing anxiety and improving mood, indirectly contributing to pain relief and quality of life. The strong placebo effect may be driven by the perceived technological advancement and non-invasive nature of the treatment. These findings emphasize the importance of objective measures, like Schober's test, to accurately assess treatment efficacy, as subjective outcomes are more prone to placebo effects[22].

In this study, the Minimal Clinically Important Difference (MCID) was set at 0.211 for the VAS and 0.216 for the Oswestry Disability Index[28-30]. In Table 2, the primary outcome shows a VAS mean difference posttest at week 4 of 1.11, and the secondary outcome, the Oswestry score, shows a mean difference posttest at week 4 of 2.4. Both values exceed the MCID thresholds (0.211 for VAS and 0.216 for the Oswestry Disability Index), indicating that the posttest outcomes at week 4 reached a clinically significant level and were not due to natural recovery.

These were study limitations that the study did not collect data on the duration of CNLBP or the occupations of the participants in either group, which may have influenced the results. Future studies should evaluate the long-term effectiveness of LLLT in pain reduction while assessing the potential side effects from its prolonged use. Additionally, this study did not compare LLLT with other therapeutic modalities, such as high-level laser therapy, ultrasound diathermy, short-wave diathermy, or alternative treatments like acupuncture. Further research should address these gaps.

CONCLUSION

Combining LLLT with exercise significantly reduced pain, improved functional ability, and increased lumbar range of motion, providing a more effective treatment for Thai patients with CNLBP compared to exercise alone. The MCID for VAS (0.211) and Oswestry Disability Index (0.216) confirmed that the improvements at week 4 were clinically significant beyond natural recovery.

CONFLICT OF INTEREST

The authors report no conflict of interest for this article.

ACKNOWLEDGEMENTS

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DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article. Future enquiries can be directed to the corresponding author.

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Original Article

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Referral Time for Diabetic Retinopathy Screening and Impact on the Visual Acuity of Patients in a Tertiary Hospital

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ABSTRACT

OBJECTIVE: To evaluate the referral time for the screening of diabetic retinopathy (DR) from the initial diagnosis of diabetes to retinal examination. We also compared visual results among groups screened at various duration of referral time.

METHODS: This cross-sectional study was conducted retrospectively from medical records for the poorer-seeing eyes of 100 patients with type 2 diabetes from January 2021 to December 2022 at the tertiary eye center. Patients were classified based on the time duration from the initial diagnosis of diabetes to retinal examination or imaging. Visual acuity (VA) and DR stages categorized by the period of referral time were compared among each group.

RESULTS: Seventy-five patients (75%) took > 2 months from the first diagnosis of diabetes to DR screening performed by an ophthalmologist. Twenty-three patients (23%) were diagnosed with DR at the first ophthalmic visit; among these, 16 had a referral time of > 2 months. Twelve patients were diagnosed with vision-threatening DR; six of these had diabetic macular edema. The receiver operating characteristic (ROC) curve analysis indicates that patients receiving ophthalmic examination within 91 days from the diagnosis of diabetes likely maintain a best-corrected VA of ≥ 20/50. This recommended period of referral time yielded an area under the ROC curve, sensitivity, and specificity of 70.63%, 61.90%, and 83.33%, respectively.

CONCLUSION: In clinical practice, a prolonged period for the first DR screening is relatively common and may result in more patients with vision-threatening DR. Proactive and systematic work should be undertaken to create patient awareness on the importance of detection of asymptomatic and early-stage DR to prevent irreversible visual loss.

KEYWORDS: diabetic retinopathy screening, patient awareness, referral time

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood sugar levels, resulting from insufficient amounts of insulin or decreased insulin effectiveness. Type 2 diabetes is a significant global health concern[1,2], and the prevalence of diabetes is increasing continuously. According to the International Diabetes Federation, 537 million people (10.50% of the global population) were affected by diabetes in 2021, and this is projected to reach 783 million (12.20%) by 2045[3]. In 2021,

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approximately 2.30 million people died because of diabetes, with 48% of them dying before the age of 70[4]. In Thailand, approximately 4.40 million individuals have diabetes, ranking fourth in the Western Pacific region, following China, India, and Japan[5]

Diabetic retinopathy (DR) is a common ocular complication in individuals with diabetes, with a worldwide prevalence of up to 34.60%. The reports indicate a DR prevalence of 24%–31.40%[6] among individuals with type 2 diabetes. DR is a major cause of visual impairment globally, with the Vision Loss Expert Group reporting a 1.07% prevalence of vision impairment and a 1.25% prevalence of moderate to severe vision loss due to DR in 2015[7]. DR is the second leading cause of blindness after cataracts, contributing significantly to visual impairment[8]. The increasing prevalence of diabetes and the duration of diabetes contribute to the rising incidence of DR, which leads to vision loss[9]. Despite global efforts to reduce vision impairment, the early detection and timely treatment of DR remain crucial. Efficient screening, coupled with prompt referral for examination and treatment, can help prevent the development of DR-related visual impairments. However, challenges persist in implementing effective screening and timely interventions. The screening process aims to identify asymptomatic individuals, provide a diagnosis, and initiate treatment in the early stages, thereby preventing long-term complications[10]. DR, a serious complication of diabetes, can cause blindness if not promptly treated. Therefore, screening tests are essential for detecting asymptomatic individuals, allowing for early diagnosis and treatment. Nevertheless, general population screening for DR can be expensive and may not yield sufficient benefits if applied universally. By assessing risk factors such as age, body mass index (BMI), family history, and lifestyle choices, healthcare providers can pinpoint individuals who are at higher risk. This targeted approach not only optimizes resource allocation but also enhances early intervention opportunities for those most likely to benefit from screening. Additionally, it can help in implementing preventive measures for at-risk populations, ultimately reducing the overall incidence of diabetes, ultimately preventing long-term complications[11].

The guidelines for DR screening follow the 2023 Clinical Practice Guidelines for Diabetes. For patients with type 2 diabetes, eye examinations should be conducted promptly after diagnosis. Once the eye examination results are known, patients with DR should schedule an annual eye checkup. If DR is present, this is categorized as mild nonproliferative DR (NPDR), and patients should have eye examinations every 6 months. For moderate NPDR, eye examinations are recommended every 3–6 months while severe NPDR and proliferative DR (PDR) require follow up examinations by an ophthalmologist[12].

The outpatient clinic at the Ophthalmology Department in Vajira Hospital has implemented these guidelines, patients receiving services at the hospital who are diagnosed with diabetes will have their blood test results evaluated. They will then be referred from various units to the eye examination room for an initial assessment and schedule an appointment or meet with an ophthalmologist based on the screening criteria. Guidelines for diabetic retinopathy screening referral, categorizing patients into three groups as shown in the **Figure 1.**

The Faculty of Medicine Vajira Hospital, Navamindradhiraj University, has seen a continuous increase in the number of patients with diabetes in the outpatient department with 14,196, 16,972, and 18,051 patients in 2020, 2021, and 2022, respectively. This rise in patient numbers emphasizes the importance of maintaining high-quality screening procedures.

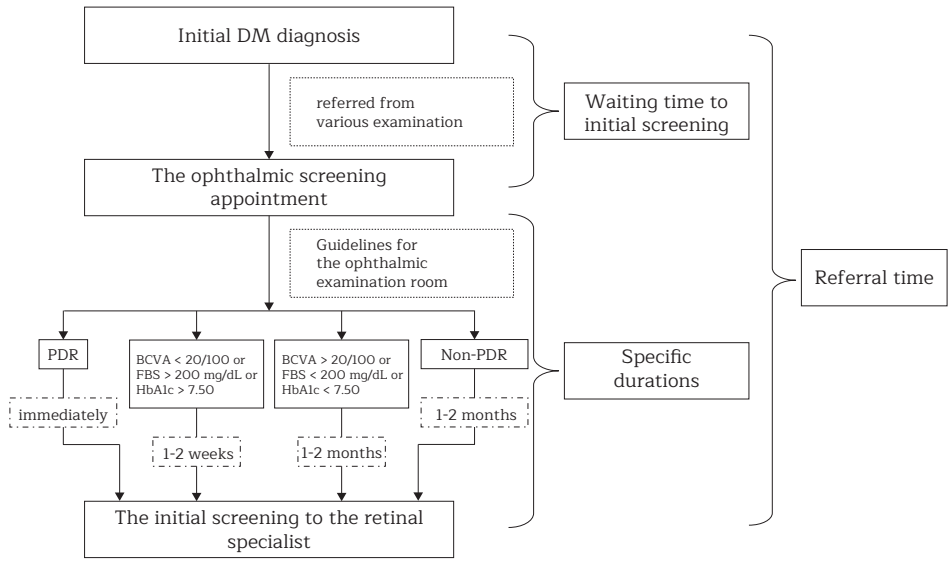

Figure 1 Guidelines for diabetic retinopathy screening referral

The various hospitals have made efforts to extensively screen patients with diabetes for DR[13]. This involved using tools to aid in screening for DR and educating technical staff and nurses in primary care units to assist with screening. However, despite these efforts, the number of ophthalmologists in Thailand (approximately 1,300) is inadequate when compared to the 4 million patients with diabetes in 2023[14]. State organizations have formulated guidelines for DR screening to benefit other units that can implement them[15]. Nevertheless, the number of screened patients remains insufficient. In countries such as the United States, the United Kingdom, Australia, and New Zealand[16,17], screening guidelines from the International Council of Ophthalmology and the American Diabetes Association are followed. According to international guidelines, patients without DR (NO DR) should have eye exams every 1–2 years. For those with DR, it is classified as mild NPDR, with exams recommended every 6–12 months. Moderate NPDR should be checked every 3–6 months, while severe NPDR and PDR require follow-ups every 1–3 months and less than a month, respectively. Patients with DR from moderate NPDR onward and with visual acuity over 20/40 should be referred to an ophthalmologist. However, despite the high prevalence of DR, only 62.30% of patients with diabetes receive screening examinations in the United States, and a study by Scanlon and colleagues established guidelines in medical practice for referring these for retinal screening. These guidelines included quality standards recommending that patients undergo retinal examinations within 3 months of diagnosis; additionally, patients who have not undergone screening before should receive retinal screening within 3 years from the time of diagnosis[18]. A review of literature both in Thailand and internationally shows that research is lacking on the delay in referring patients. Meanwhile, the number and severity of patients with diabetes and DR are increasing, particularly in younger age groups. Consequently, efficient and rapid assessments for DR screening are needed, as well as effective referral strategies. This study aims to study the duration of DR screening from the beginning of the first diagnosis of diabetes to the appointment in the ophthalmology clinic and examination by an ophthalmologist and examination of the results of visual acuity (VA) levels and compare them between groups screened at different time duration.

METHODS

This cross-sectional study was approved by the internal review board of the Faculty of Medicine Vajira Hospital, Navamindradhiraj University. The certificate of approval number in this project is 164/2023. All information had been evaluated for research ethics and was used for research purposes only. No personal information is disclosed. The study enrolled individuals diagnosed with type 2 diabetes, like a diagnosis from internal medicine/endocrinologist for type 2 who received diabetes and DR screening services at Vajira Hospital from January 1st, 2021, to December 31st, 2022. The sample size of 100 patients (one eye with visual impairment per participant) was determined using the infinite population mean formula an appendix.

Inclusion criteria were eligible patients who received diabetes screening services at Vajira Hospital. This included new patients referred from various units for the assessment of DR. Eligible patients had blood test results, including HbA1c (hemoglobin A1c) and FBS levels, and had undergone pupil dilation. Additionally, there were results from retinal examinations that can identify the severity level of the disease in the hospital's data recording system and the patients did not have any eye conditions such as cataracts or glaucoma.

Exclusion Criteria were patients who received diabetes screening and DR examinations at Vajira Hospital and had been diagnosed and referred from various centers. Due to the lack of information, clear data could not be obtained and those with a history of eye conditions that may have affected the assessment, such as glaucoma or other eye diseases.

The data collection instrument comprised two parts: Part 1) Personal and health status factors, including gender, age, BMI, HbA1c levels, and fasting blood sugar (FBS) levels. Part 2) Time and results of eye examination record 2.1) Record of the duration for patients from the initial diagnosis of diabetes to the ophthalmology appointment 2.2) Duration of diabetic patients who have an ophthalmology appointment and have undergone an eye examination by an ophthalmologist. 2.3) Results of the DR examination and VA measurements from the examination by an ophthalmologist. 2.4) Number of patients with diabetes referred from various screening units for DR screening.

Statistical analysis for general information was described by percentages. Comparison data of DR and NO DR were analyzed via mean, standard deviation, and paired t-test of variance considering p-values of < 0.05 as significant. Afterward, the independent-samples Mann–Whitney U test was used to compare best-corrected visual acuity (BCVA) (logMAR) data between the DR and NO DR groups across various screening duration and calculate the appropriate cutoff value for referring diabetic patients for DR screening using a BCVA (logMAR) of 0.4, using the Euclidean index method.

Statistical analyses for this study were performed using STATA (StataCorp, College Station, TX) software version 13 for data analysis. The analysis includes descriptive statistics (percentages, mean, median, percentiles, standard deviations, and interquartile ranges (IQR)) to summarize continuous variables. Frequency and percentages were used in summarizing categorical variables. Comparisons of the levels of visual impairment and the severity of DR based on the time duration during which patients were referred.

RESULTS

This study involved a review of medical records of 100 patients, of whom 50% were male. The average age was 60.43 (± 12.40) years, ranging from 19 to 86 years. The average BMI was 26.71 (± 12.40) kg/m^2 (17.26–43.60 kg/m^2) The average duration of diabetes was 3.30 (± 4.00) years (0–16.30 years). The mean HbA1c level was 7.83 (± 1.79) (5.20–13.50). The average FBS level was 153.32 (± 50.44) mg/dL

(85–307 mg/dL) (**Table 1**). In total, 27 cases of DR were found (27%). These were further categorized as follows: NPDR stage at 20%, with the majority being at the moderate NPDR stage at 13%, followed by mild NPDR stage at 5%, the least found being severe NPDR stage at 2% and found PDR stage at 7%. In addition to these stages, both NPDR and PDR cases were found to have diabetic macular edema (DME) in 6% with NPDR combined with DME at 3% and PDR combined with DME also at 3%. Furthermore, 12 cases (12%) were identified with vision-threatening diabetic retinopathy (VTDR) include stages severe NPDR, NPDR with DME and PDR with DME requiring immediate treatment (**Table 2**).

Table 1 Demographic and clinical characteristics of diabetic patients compared between those with and without DR (n = 100)

Parameters	DR (n = 27) n (%)	No DR (n = 73) n (%)	P-value
Gender			
Male	15 (55.56)	35 (47.95)	
Female	12 (44.44)	38 (52.05)	0.50
Age (years)			
< 60	15 (55.56)	28 (38.36)	0.14
Mean ± SD	48.29 ± 9.87	51.46 ± 3.62	
BMI (kg/m^2)			
≥ 23 (obesity)	17 (62.96)	60 (82.19)	0.07
Mean ± SD	28.17 ± 3.60	28.35 ± 4.40	
Duration of DM (years)			
≥ 10	1 (3.70)	7 (9.59)	0.25
Mean ± SD	10 ± 0	13.20 ± 1.76	
HbA1c (%)			
≥ 7	19 (70.37)	42 (57.53)	0.24
Mean ± SD	9.26 ± 1.83	8.51 ± 1.69	
Fasting Blood Sugar (mg/dL)			
≥ 130 not control	22 (81.48)	38 (52.05)	0.003*
Mean ± SD	190.50 ± 58.59	173.92 ± 58.59	

Abbreviations: BMI, body mass index; DM, diabetes mellitus; DR, diabetic retinopathy; HbA1c, hemoglobin A1c; kg/m^2, kilogram per square meter; mg/dL, milligrams per deciliter; n, number; SD, standard deviation
Data are presented as number (%), mean ± standard deviation or median.
*Statistically significant p-value < 0.05, pair t-test

Table 2 Prevalence, Type, and Severity of DR

Diabetic retinopathy	Total n = 100 n (%)
NO DR	73
DR	27
NPDR	20
Mild NPDR	5
Moderate NPDR	13
Severe NPDR	2
PDR	7
VTDR	12

Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; n, number; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy
Data are presented as number (%).

Patients with diabetes at initial diagnosis undergo evaluations to schedule ophthalmic screenings for DR. The findings indicated that 67% had a screening duration of > 6 months (67%), 25% had duration of < 2 months, and 8% had duration of 2–6 months among patients with DR, 50% had screening duration of 2–6 months, 44% had duration of < 2 months, and 17.91% had duration of > 6 months. The median duration was 27 months, with an IQR of 8.8 weeks to 6 years. The range was from 0 to 15.90 years. Patients with diabetes scheduled for screenings are examined by ophthalmologists for DR, 47% had screening duration > 8 weeks, 41% had duration of 2–8 weeks, and 12% had duration of < 2 weeks. Among patients with DR, 40% had duration of 2–8 weeks, 38.46% had duration of < 2 weeks, and 12.77% had duration of > 8 weeks. The median duration was 7 weeks, with an IQR of 3 weeks to 3 months. The range was from 0 to 15.70 weeks (**Table 3**).

The VA results are presented based on different screening time duration and are categorized into two groups. **Group 1**: Time duration from the initial diagnosis of diabetes to the scheduled ophthalmology appointment. To categorize patients into two groups, the median BCVA (logMAR) in the NO DR group with a duration ≥ 2 months, was 0.50 (0.30–0.60) and with a duration < 2 months was 0.50 (0.28–0.63). The median BCVA (logMAR) for the DR group with a duration ≥ 2 months was 0.50 (0.40–0.75) and with a duration < 2 months was 0.40 (0.30–0.60). **Group 2**: The time duration since the scheduled appointment of a patient with an ophthalmologist. In this group, two patient subgroups are identified in the NO DR group; the median BCVA (logMAR) was 0.50 (0.40–0.83) with a duration of 2 weeks, 0.45 (0.30–0.60), with a duration of 2–8 weeks, and 0.40 (0.30–0.60) with a duration of > 8 weeks. In the DR group, the median BCVA (logMAR) was 0.40 (0.35–1.10) with a duration of < 2 weeks, 0.45 (0.40–0.60) with a duration of 2–8 weeks, and 0.40 (0.08–0.68) with a duration of > 8 weeks (**Table 4**).

Table 3 Analyzes factors related to diabetic retinopathy based on the screening time duration (n = 100)

Parameters	Total (n = 100) n	DR (n = 27) n (%)
• The time duration for diabetic patients starts from the beginning of the first diagnosis of diabetes until they receive an appointment for an ophthalmology examination.		
> 2 months	25	11 (44)
2-6 months	8	4 (50)
< 6 months	67	12 (17.91)
• The time duration for diabetic patients who have scheduled appointments with an ophthalmologist and undergo examination by an ophthalmologist.		
> 2 weeks	13	5 (38.46)
2-8 weeks	40	16 (40)
< 8 weeks	47	6 (12.77)

Abbreviations: DR, diabetic retinopathy; n, number
Data are presented as number (%).

Table 4 Visual acuity of patients at different screening time duration (n = 100)

• The time duration for diabetic patients starts from the beginning of the first diagnosis of diabetes until they receive an appointment for an ophthalmology examination.					
Parameters	**n (%)**	**Duration**			
		< 2 months		**≥ 2 months**	**P-value**
NO DR	73 (73)	14		59	
Median (IQR) BCVA (logMAR)		0.50 (0.28-0.60)		0.50 (0.30-0.60)	0.93
DR	27 (27)	11		16	
Median (IQR) BCVA (logMAR)		0.40 (0.30-0.60)		0.50 (0.40-0.75)	0.42
• The time duration for diabetic patients who have scheduled appointments with an ophthalmologist and undergo examination by an ophthalmologist.					
Parameters	**n (%)**	**Duration**			
		< 2 weeks	**2-8 weeks**	**> 8 weeks**	**P-value**
NO DR	73 (73)	8	24	41	
Median (IQR) BCVA (logMAR)		0.50 (0.40-0.86)	0.45 (0.30-0.60)	0.4 (0.30-0.60)	0.64
DR	27 (27)	5	16	6	
Median (IQR) BCVA (logMAR)		0.40 (0.35-1.10)	0.45 (0.40-0.60)	0.4 (0.08-0.68)	0.69
• **Median (IQR) BCVA (logMAR)**				**P-value**	
Group 1 NO DR	73 (73)	0.50 (0.30-0.60)			
Group 2 DR	27 (27)	0.40 (0.40-0.60)		0.86	

Abbreviations: BCVA, best-corrected visual acuity; DR, diabetic retinopathy; IQR, interquartile range; n, number
Data are presented as number (%), mean ± standard deviation or median (interquartile range).
P-value by Independent-Samples Mann-Whitney U test

The Receiver Operating Characteristic (ROC) curve is analyzed to find an appropriate threshold using the ROC analysis method. The area under the ROC curve is calculated as 70.63% (**Figure 2**) When determining the suitable threshold for referring diabetic patients for DR screening with a specified BCVA (logMAR) of 0.40 using the duration from screening for DR after the initial diagnosis of diabetes until the eye examination with an ophthalmologist by the Euclidian's index, a cutoff point at 91 days is identified. This cutoff point has a sensitivity of 61.90% and a specificity of 83.33%.

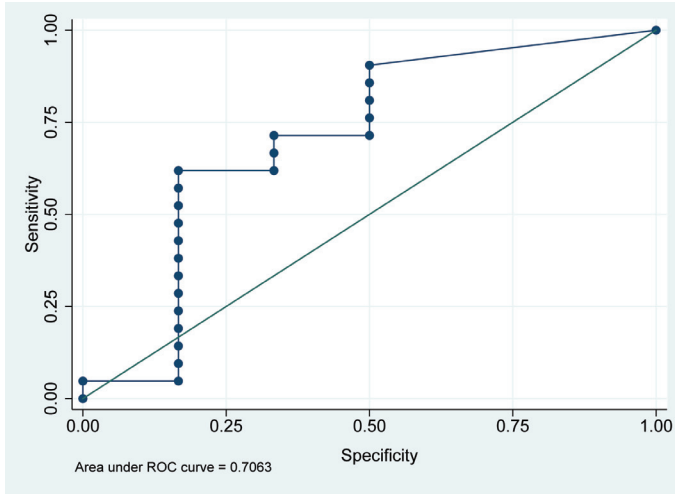

Figure 2 ROC curve

The units sending patients for diabetes for screening on eye monitors are categorized as follows: endocrinology, 62 cases (62%), general medicine, 23 cases (23%), primary care, 5 cases (5%), cardiology, 5 cases (5%), and nephrology, 4 cases (4%). According to statistics from the endocrine unit in 2021 and 2022, there were 3,235 and 3,221 patients, respectively, but only 1,154 and 1,005 patients, receiving assessments, before referring them to the Ophthalmology Department.

DISCUSSION

This study showed that in a clinical context, despite clear guidelines for patient referrals between departments in the hospital, patients still experience delays in receiving ophthalmic evaluations compared with the screening guidelines for DR set by the 2023 Diabetes Mellitus Clinical Practice Guidelines. According to these guidelines, patients with type 2 diabetes should undergo retinal examination promptly after diagnosis. However, approximately 67% of the patients experienced delays in receiving eye screenings beyond 6 months, and approximately 27% were found to have DR from their initial eye examination. These results align with the research conducted by Bresnick et al.[19] who reported a significant delay of up to 44% in patients receiving eye examinations even after being prescribed diabetes medications in primary care[20]. Each year, the eye clinic experiences an average of about 7,200 patients who miss their appointments, with diabetic patients making up one-third of the total. The lack of importance and awareness among diabetic patients can lead to delays in examinations and treatment, potentially resulting in severe DR. The reasons for missed appointments may include a lack of understanding of the importance of health screenings or issues related to accessing services, such as inconvenient times and travel distances. Additionally, patients may lack support from their families. The clinic should implement campaigns to raise awareness about the importance of appointments, provide clear information, and utilize new communication methods, such as sending reminder messages and making follow-up calls, encouraging patients to attend their scheduled appointments. Reducing the number of missed appointments requires continuous improvement in information sharing and supported from healthcare professionals. If both the clinic and patients cooperate, it will help reduce the incidence of severe DR and ensure timely treatment.

This study analyzed the relationship between the duration of diabetes screening and DR concerning BCVA. Patients were classified into two groups based on the screening interval. Both groups were further divided for comparison between those with NO DR and those with DR. In Group 1, where the screening duration was ≥ 2 and < 2 months, the BCVA values in both the NO DR and DR groups were not significantly different ($p < 0.05$). However, in the DR group with appointments scheduled with in < 2 months, a reduced BCVA was observed, with a BCVA (logMAR) of 0.6 or equivalent to 20/80, compared to a BCVA (logMAR) of 0.5 or equivalent to 20/63, for those with appointments ≥ 2 months who had better vision and no significant statistical correlation was present. This finding aligns with the theoretical framework that indicates that VTDR affecting VA often reaches an advanced stage or has progressed for some time. For instance, in the proliferative stage of DR, the development of new blood vessels typically occurs outside the macular area, leading to relatively normal vision. Neovascular complications only arise from these new blood vessels in the last stages and can result in vitreous hemorrhage, tractional retinal detachment, or central DME, which significantly affect vision. These complications begin to affect the outer retinal layers or ellipsoidal layers of the macula. The reason why VA is not directly correlated with the stage of DR described above can cause patients to

neglect regular eye examinations. Therefore, healthcare providers must emphasize the characteristics of the disease, highlighting the often-asymptomatic nature of DR until an advanced stage is reached. This awareness can encourage patients to undergo regular eye examinations before the disease progresses, preventing potential complications and preserving their vision.

This study revealed a 27% prevalence of diabetes with DR. This is a 27% prevalence of symptoms or conditions related to vision, such as DR. This finding can help assess the need for screenings and inform the management of care for patients with diabetes and a 12% prevalence of VTDR. Patients in this group underwent treatment, with 6% requiring immediate treatment. Most cases had moderate NPDR (13%), followed by PDR (7%). This study revealed that one-third of the patients developed DR, with VTDR occurring in 12% of cases. Of those with VTDR, half required immediate treatment. These findings closely resemble the prevalence of DR reported by Pawaranggoon with a DR prevalence of 27.72%[20] but less than that reported by Puangmee et al. with a DR prevalence of 46.40%[1] at a service tertiary care center. Studies by Kongtham, Boontakanon, and Bumrungsena reported a DR prevalence of 10.02%[8], 8.52%[6], and 6.91%[21] (in a service primary care center), respectively. The differences in the prevalence of DR arise from variations in the emphasis of services provided at different healthcare levels. Primary healthcare units focus on proactive services for both at-risk and general populations. Secondary care centers emphasize treating complex diseases and managing patients with complications. Tertiary care centers in hospitals, like Vajira Hospital, offer services and referrals to service recipients within the city area. Emphasizing proactive examination, assessment, and referral from primary to tertiary care can contribute to early detection and severe reduction in the incidence of DR.

The analysis of general data for its correlation with DR demonstrated that FBS levels significantly impact the incidence of DR ($p < 0.05$), which is consistent with results by Jindapet et al[22]. The average FBS level in the DR group was 190.50 ± 58.59 mg/dL, which was higher than the NO DR group with an average of 173.92 ± 58.59 mg/dL. Normal FBS levels typically range from 70 to 100 mg/dL, and elevated FBS levels increase the risk of developing DR. However, factors such as gender, age, BMI, duration of diabetes, and HbA1c did not show a significant relationship with the occurrence of DR, which is consistent with results in Rodchua et al[23].

Patients with faster screening experienced a reduction in VA, and in the DR and NO DR group, those with appointments < 2 weeks had worse BCVA than those with longer wait times. However, no significant relationship was present between these factors. This suggests that the screening criteria used by the ophthalmology department have established an appropriate screening guideline. If patients are found to have reduced VA, they will be referred to an ophthalmologist more quickly. Additionally, there are other eye conditions, such as cataracts, which not only reduce the patient's vision but also make it more difficult to detect DR because cataracts can obscure the retina during examination. This is also a factor that affects reporting results, making it a variable that needs to be excluded from the study. This study investigated the duration for DR screening referrals by calculating an appropriate cutoff point. A logMAR value of 0.4, or BCVA = 20/50, indicates a good level of eyesight. This criterion has been established as an outcome to determine the appropriate timeframe for patient referral. Determined by the receiver operating characteristic (ROC) analysis, was 91 days. If patients receive screening from other units and are scheduled for an ophthalmology appointment within 3 months, they can maintain a BCVA of 20/50, which is consistent with

results obtained by Scanlon et al[18]. The area under the ROC curve, which is considered relatively good, Patients with this level of vision will be considered for appropriate care and follow-up according to effective treatment guidelines for DR. This will help ensure that the management and treatment are more appropriate and effective the ophthalmology department has established clear screening criteria and has retinal specialists available for examination every day, allowing patients to receive treatment more quickly and these can be used as a criterion for referral guidelines to reduce vision loss. However, research is limited on the relationship between the level of visual impairment and the severity of DR. Upon examining the data, referrals from various screening units still suffer delays, potentially because of factors such as inadequate screening, patients presenting at a later stage of diabetes diagnosis, missed appointments, or patient neglect of examinations[24]. These factors contribute to delays in scheduling appointments. Emphasizing the importance of diabetic screening via eye exams will help patients with diabetes receive screenings with relatively good visual outcomes early on. These patients could maintain good VA because healthcare facilities have fast-track screening systems that offer quick turnaround times. Additionally, since most patients reside in Bangkok where transportation is convenient and costs are lower, they have increased opportunities for regular screenings than patients in rural or remote areas who face financial and transportation challenges and delays in appointment scheduling cause frustration and neglect, resulting in patients with diabetes missing eye screenings, leading to vision loss. Studying these factors further is crucial for developing future enhancements in service quality.

Additionally, a study was conducted on the units involved in the referral process for screening patients. It was found that the endocrine unit had the highest proportion in referring patients for screening, accounting for 62%. This highlights the importance of this unit in screening and assessing diabetic patients in various aspects, such as hands, feet, and eyes. According to statistics from the endocrine unit in 2021 and 2022, only 35.67% and 31.20% of patients, respectively, received assessments before being referred to the Ophthalmology Department. However, determining the number of diabetic patients receiving services in each unit is quite challenging due to data redundancy, which may hinder the accurate collection of the actual numbers. Following this, the general medicine unit, primary care unit, cardiology unit, and nephrology unit play significant roles in managing diabetic patients. However, the referral of patients to the ophthalmology department for eye health examinations still requires improvement in efficiency to ensure that patients receive appropriate and timely care. This study thus underscores the importance of establishing an effective system for tracking and referring patients to enhance the efficiency of screening and treatment for diabetes patients comprehensively.

CONCLUSION

Most patients with diabetes are undergoing eye examinations and DR assessments later than recommended in clinical practice guidelines. From the initial diagnosis of diabetes during screenings in various clinics to referrals and appointments for ophthalmic examinations with ophthalmologists, patients should be referred within three months to maintain good vision. However, it is still observed that more than 60% experience delays in referrals and assessments. This delay occurs from the initial diagnosis of diabetes during screenings in various clinics to subsequent referrals and appointments for ophthalmic examinations with ophthalmologists. Therefore, raising awareness among patients for early DR screenings could help reduce the incidence of severe DR. Moreover, receiving an early diagnosis of DR in the initial stages,

even when vision appears normal, can prevent permanent vision impairment from advanced stages of the disease. This emphasizes the importance of timely and proactive screening to prevent the progression of DR.

CONFLICT OF INTEREST

The authors report no conflict of interest for this article.

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DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article. Future enquiries can be directed to the corresponding author.

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Original Article

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Factors Associated with Malnutrition in Older Patients with Type 2 Diabetes in Bangkok, Thailand

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ABSTRACT

OBJECTIVE: Malnutrition is a widespread but often neglected concern among older individuals with type 2 diabetes mellitus (T2DM), especially in Thailand, where research on this issue is limited. In this group, malnutrition can result in adverse outcomes, such as diminished physical function, decreased quality of life, longer hospitalizations, and increased mortality. This study aimed to examine the prevalence of malnutrition and the factors contributing to it among older adults with T2DM.

METHODS: This cross-sectional study was conducted in August and September 2024 at our local hospital. Older patients diagnosed with T2DM for at least 6 months were included in the analysis. Nutritional status was evaluated using the Global Leadership Initiative on Malnutrition criteria, which assess weight loss, low body mass index, and reduced muscle mass. Logistic regression was used to determine predictors of malnutrition.

RESULTS: Of the 176 participants, 30.1% were malnourished. Factors significantly associated with malnutrition included being underweight (adjusted odds ratio [AOR]: 11.07, 95% CI: 2.69–45.53), being married (AOR: 5.29, 95% CI: 1.14–24.64), living with diabetes for more than 10 years (AOR: 2.99, 95% CI: 1.23–7.25), poor medication adherence (AOR: 2.55, 95% CI: 1.06–6.17), and having a glomerular filtration rate below 60 (AOR: 4.70, 95% CI: 1.95–11.34).

CONCLUSION: Malnutrition is prevalent in older individuals with T2DM. Routine nutritional evaluations should be implemented to reduce health risks and improve patient outcomes in this population.

KEYWORDS: cross-sectional study, malnutrition, older individuals, type 2 diabetes mellitus

INTRODUCTION

Globally, diabetes mellitus (DM) is a prevalent chronic, noncommunicable disease[1]. In 2021, it was estimated that approximately 2.4 million people in Thailand were living with diabetes[2]. This condition is common among the older population, many of whom are undiagnosed[3]. In the United States, approximately 40% of people over 65 years of age have diabetes, and nearly half of these cases are undetected[4,5]. Managing diabetes in older adults is challenging because of the complexity of the disease, heightened risk of additional health issues, and increased susceptibility to frailty[6,7]. Furthermore, malnutrition rates among the older population can vary widely, from 12.0% to 77.1%[8].

Malnutrition and type 2 DM (T2DM) are closely interconnected, particularly among older adults. Although diabetes is often associated with excessive caloric intake and obesity, malnutrition can paradoxically occur alongside these conditions, especially in older patients. With advancing age, the risks of malnutrition and T2DM increase because of various physiological and lifestyle changes.

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Older individuals often encounter challenges in maintaining proper nutrition, such as a reduced sense of hunger, difficulty in chewing or swallowing, and limited access to nutrient-rich foods, all of which increase the likelihood of malnutrition[9,10].

Malnutrition can occur in older individuals with T2DM, worsening diabetes management. Complications, such as peripheral neuropathy and gastroparesis, which are common in older patients with diabetes, can impair food intake by reducing appetite and causing digestive issues, including nausea, vomiting, and a sensation of early fullness[11]. Additionally, diabetes medications, especially insulin and oral hypoglycemics, can increase the risk of hypoglycemia. This results in patients limiting their food intake to avoid such episodes, thereby intensifying malnutrition[8,11]. This creates a harmful cycle in which poor nutrition negatively affects blood glucose control, leading to further complications and increased frailty.

Malnutrition can severely hinder diabetes management by exacerbating insulin resistance, increasing the risk of infection, and slowing wound healing[12]. It also contributes to sarcopenia and frailty and reduces the physical function and mobility of older patients with diabetes. Sarcopenia is closely linked to poor blood glucose control because reduced muscle mass leads to decreased glucose utilization[9]. These interactions between diabetes and malnutrition increase the risk of adverse outcomes, including falls, fractures and mortality rates[12].

Malnutrition in patients with diabetes often goes undiagnosed, further complicating treatment and worsening health outcomes[8]. Although the prevalence of diabetes in Thailand is well documented, malnutrition, particularly among older patients with diabetes and remains under-investigated. Early detection and appropriate nutritional interventions are essential to improve the care and outcomes of older patients with T2DM. This study aimed to assess the prevalence of and risk factors for malnutrition among older individuals with and without T2DM in Thailand, with the goal of developing strategies for managing malnutrition alongside diabetes[8,11-13].

METHODS

This cross-sectional study was conducted over 2 months at our local hospital from August to September 2024. The study population comprised patients with T2DM who had been receiving treatment at an outpatient clinic, diagnosed at least 6 months according to the Global Leadership Initiative on Malnutrition (GLIM) criteria[14].

Based on the study by Ahmed et al. (2022)[15], which reported a malnutrition prevalence of 10.6% among older adults with diabetes, the sample size for this study was calculated using a 95% confidence level and a 5% margin of error. The sample size formula used was $n = \frac{Z^2 \cdot p \cdot (1-p)}{d^2}$, where Z is the Z-score for a 95% confidence level (1.96), p is the prevalence (0.106), and d is the margin of error (0.05). Substituting these values, the calculated sample size was approximately 146 participants. To account for potential dropouts, the final sample size was adjusted to 176 participants, ensuring sufficient statistical power to assess malnutrition prevalence in this population accurately. The selection criteria for the diabetes group included patients who were 60 years or older, agreed to participate and did not have other chronic conditions, such as HIV, heart failure, thyroid disease, or chronic obstructive pulmonary disease.

Participants were recruited using a convenience sampling method from the Family Practice Outpatient Department at Phramongkutklao Hospital, which is located in an urban area of Bangkok, Thailand. This department primarily provides care to older adults with chronic conditions, including DM. The inclusion of patients from this setting allows the study to focus on a population representative of older adults receiving primary care for diabetes management.

Data collection and recording procedures were carried out in several stages. Initially, public relations efforts and volunteer recruitment took place, followed by obtaining written informed consent from all participants. The sample population consisted of individuals aged 60 years or above, diagnosed with T2DM, and able to communicate in Thai. Participants filled out personal information questionnaires and logged various factors while waiting for outpatient services. Nutritional status was assessed using the GLIM criteria. Additionally, participants' weight and height were measured to calculate their body mass index (BMI). Those at risk for malnutrition were referred to the clinical nutrition unit for muscle mass measurements using bioelectrical impedance analysis (BIA). Regarding data recording, all data were documented on a specially prepared data collection form. The confidentiality of participant data was rigorously maintained, stored separately from the consent forms, and all data was collected and recorded on a computer system exclusively by the researchers.

The assessment of malnutrition utilizes the GLIM criteria[14], which consist of five points divided into two groups to ensure comprehensive evaluation. The clinical characteristics assessed include unintentional weight loss, low BMI below the threshold, and decreased muscle mass, measured using standard methods. Additionally, the causes of malnutrition are evaluated, focusing on reduced food intake and malnutrition related to illness or inflammation. For the measurement of muscle mass, BIA is employed, which provides a detailed analysis of muscle mass critical for determining malnutrition severity and planning appropriate interventions.

Malnutrition, as assessed by the GLIM criteria, refers to a condition where the body lacks essential nutrients, which affects the growth and functioning of various systems[14]. Unintentional weight loss is defined as a reduction of more than 5% of body weight within six months or more than 10% beyond six months[16]. BMI below threshold is considered a BMI less than 18.5 for individuals under 70 years old, and less than 20 kg/m^2 for those over 70[17]. Decreased muscle mass involves a reduction measured by standard methods such as dual-energy X-ray absorptiometry or BIA[18]. Reduced food intake is defined as consuming less than 50% of the body's required intake for at least one week or reduced intake for more than two weeks[19]. Malnutrition related to illness or inflammation pertains to acute or chronic conditions associated with mild to moderate inflammation, such as cancer or other chronic diseases[20].

Anemia is defined by the World Health Organization as a packed cell volume of less than 36% in women and less than 39% in men[21]. Albuminuria is identified when the albumin-to-creatinine ratio exceeds 30 mg/g[22]. Kidney impairment is diagnosed when the estimated glomerular filtration rate falls below 60 mL/min/1.73 m^2 [23]. Good blood glucose control is characterized by a hemoglobin A1C level of less than 7.0%[24]. Regarding BMI, underweight is defined as a BMI of less than 18.5 kg/m^2, normal weight falls between 18.5 to 24.9 kg/m^2, overweight is a BMI from 25 to 29.9 kg/m^2, and obesity is considered a BMI of 30 kg/m^2 or higher[17].

This study was conducted in accordance with the principles of the Declaration of Helsinki and its later amendments. Ethical approval was obtained from the Research and Ethics Committee of the Office of the Subcommittee for Research Project Review, Royal Thai Army Medical Department (reference number IRBTA 1064/2567). Consent was obtained from all participants, and data confidentiality was ensured throughout the study.

The collected data were analyzed using the SPSS software version 26. Descriptive data were presented in a table format. Categorical variables in both groups were presented as proportions and percentages. Relationships between categorical variables were analyzed using the chi-square test. Logistic regression analysis was used to identify the predictors of malnutrition in older individuals with T2DM. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 176 older individuals with T2DM were included in the study: 61 (34.7%) were male. The average age of participants with T2DM was 70 ± 6 years. Seventy-three (41.5%) participants had an average monthly income below 10,000 Baht. Among the groups with and without malnutrition, a significantly higher proportion of those with malnutrition were older individuals with T2DM who were underweight [9 (17.3%) vs. 5 (4.1%)]; had low medical adherence [20 (37.7%) vs. 26 (21.1%)]; had a glomerular filtration rate (GFR) < 60 [20 (37.7%) vs. 23 (18.7%)] and had diabetes for more than 10 years [23 (43.4%) vs. 27 (22.0%)] ($p < 0.05$; Table 1).

Table 1 Basic characteristics of study participants

Variable (unit)		Non-malnutrition	Malnutrition	Total	P-value
No. participant		123 (69.9%)	53 (30.1%)	176	
Sex	male	39 (31.7%)	22 (41.5%)	61 (34.7%)	0.21
	female	84 (68.3%)	31 (58.5%)	115 (65.3%)	
Age (years)	< 70	71 (57.7%)	29 (54.7%)	100 (56.8%)	0.712
	≥ 70	52 (42.3%)	24 (45.3%)	76 (43.2%)	
BMI (kg/m^2)	underweight	5 (4.1%)	9 (17.3%)	14 (8.1%)	0.001*
	normal range	62 (51.2%)	33 (63.5%)	95 (54.9%)	
	overweight	40 (33.1%)	6 (11.5%)	46 (26.6%)	
	obesity	14 (11.6%)	4 (7.7%)	18 (10.4%)	
Status	single	20 (16.7%)	11 (22.0%)	31 (18.2%)	0.028*
	married	72 (60.0%)	36 (72.0%)	108 (63.5%)	
	divorced	28 (23.3%)	3 (6.0%)	31 (18.2%)	
Education	lower secondary education	66 (55.0%)	30 (60.0%)	96 (56.5%)	0.549
	secondary education and above	54 (45.0%)	20 (40.0%)	74 (43.5%)	
Living arrangement	alone	48 (40.0%)	14 (28.0%)	62 (36.5%)	0.139
	not alone	72 (60.0%)	36 (72.0%)	108 (63.5%)	
Income (Baht)	≤ 10,000	56 (44.2%)	17 (32.0%)	73 (41.5%)	0.141
	> 10,000	67 (54.5%)	36 (67.9%)	103 (58.5%)	
Glycemic control	good	21 (17.1%)	18 (34.0%)	39 (22.2%)	0.013*
	poor	102 (82.9%)	35 (66.0%)	137 (77.8%)	
Medical adherence	high	73 (59.3%)	22 (41.5%)	95 (54.0%)	0.047*
	medium	24 (19.5%)	11 (20.8%)	35 (19.9%)	
	low	26 (21.1%)	20 (37.7%)	46 (26.1%)	
Albuminuria	yes	59 (48.0%)	21 (39.6%)	80 (45.5%)	0.308
	no	64 (52.0%)	32 (60.4%)	96 (54.5%)	
GFR (mL/min/1.73 m^2)	≥ 60	100 (81.3%)	33 (62.3%)	133 (75.6%)	0.007*
	< 60	23 (18.7%)	20 (37.7%)	43 (24.4%)	
Duration of diabetes (years)	≥ 10	27 (22.0%)	23 (43.4%)	50 (28.4%)	0.004*
	< 10	96 (78.0%)	30 (56.6%)	126 (71.6%)	

Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; kg/m^2, kilogram per square meter; m, meter; min, minute; mL, milliliters
*P-value < 0.05

For the multivariate analysis, variables were selected based on their significance in the univariate analysis, with a threshold of p-value < 0.05. This criterion ensures that only statistically significant factors are considered, reducing the risk of including irrelevant variables. Factors such as BMI, marital status, duration of T2DM, medical adherence, and GFR were included in the adjusted model as they showed significant associations with malnutrition in the univariate analysis or were deemed clinically relevant based on prior literature.

From the logistic regression analysis, significant predictors of malnutrition in patients with T2DM included being underweight compared to the normal BMI range of 18.5–24.9 (adjusted odds ratio [AOR] 11.07; 95% confidence interval [95% CI] 2.69–45.53), being married relative to being single (AOR 5.29; 95% CI 1.14–24.64), having been diagnosed with T2DM for more than 10 years compared to less than 10 years (AOR 2.99; 95% CI 1.23–7.25), having low medical adherence relative to high adherence (AOR 2.55; 95% CI 1.06–6.17), and having a GFR < 60 compared to a GFR ≥ 60 (AOR 4.70; 95% CI 1.95–11.34) (p < 0.05; Table 2).

DISCUSSION

This investigation evaluated the prevalence of malnutrition among older people with and without T2DM using markers such as low albumin levels, BMI, and the Mini Nutritional Assessment Short-Form. We also identified the factors linked to malnutrition in older patients with T2DM. The findings revealed a significantly higher malnutrition rate in older individuals with T2DM than in their age- and sex-matched counterparts without diabetes. Being underweight was more common in older individuals with diabetes in the malnourished group (17.3%) than in the non-malnourished group (4.1%). The underweight prevalence in this study was nearly identical to the 4.8% reported by Adebusoye et al.[25] in an older population in Nigeria, suggesting a link between diabetes and malnutrition in these groups. Diabetic autonomic neuropathy, which manifests as gastroparesis, diarrhea, and intestinal diseases and is common in older adults with diabetes, may contribute to malnutrition. The prevalence of malnutrition in older patients with diabetes was 30.1% in this study, which is closely aligned with the 29% prevalence reported by Vural Keskinler et al.[26] in Turkey.

Table 2 Association between sociodemographic factors among older adults with type 2 diabetes

		Crude OR (95% CI)	P-value	Adjusted* OR (95% CI)	P-value
BMI (kg/m^2)	Normal range	Ref.		Ref.	
	Underweight	3.85 (1.38–10.77)	0.01*	11.07 (2.69–45.53)	0.001*
Status	single	Ref.		Ref.	
	married	5.13 (1.27–20.81)	0.022*	5.29 (1.14–24.64)	0.034*
Duration of diabetes (years)	< 10	Ref.		Ref.	
	≥ 10	2.73 (1.37–5.44)	0.004*	2.99 (1.23–7.25)	0.016*
Medical adherence	high	Ref.		Ref.	
	medium	1.52 (0.65–3.59)	0.338	0.63 (0.21–1.84)	0.394
	low	2.55 (1.20–5.42)	0.015*	2.55 (1.06–6.17)	0.038*
GFR (mL/min/1.73 m^2)	≥ 60	Ref.		Ref.	
	< 60	2.64 (1.29–5.40)	0.008*	4.70 (1.95–11.34)	0.001*

Abbreviations: BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; kg/m^2, kilogram per square meter; m, meter; min, minute; mL, milliliters; OR, odds ratio; Ref, reference
*P-value < 0.05

In patients with T2DM and a BMI in the underweight category, malnutrition risks are significantly elevated due to poor glycemic control. This condition accelerates the catabolic process, leading to the breakdown of muscle and fat stores to compensate for energy deficits. Over time, this metabolic imbalance results in reduced muscle mass, diminished physical strength, and increased frailty. Additionally, poor glycemic control can impair gastrointestinal function, leading to diminished nutrient absorption and exacerbating micronutrient deficiencies. Complications such as diabetic gastroparesis, which slows gastric emptying, further hinder nutrient intake by causing symptoms like nausea, early satiety, and vomiting, making it difficult for patients to maintain adequate nutritional levels. Furthermore, psychological factors, including anorexia and depression, are prevalent in underweight T2DM patients and can significantly reduce food consumption. These mental health conditions may be exacerbated by the chronic stress of managing diabetes, creating a vicious cycle that worsens both malnutrition and glycemic control[26,27]. Moreover, long-term malnutrition in this group can lead to sarcopenia, a condition characterized by severe muscle loss, which further diminishes glucose utilization and impairs insulin sensitivity. This interplay between malnutrition and T2DM complications highlights the critical need for early identification and intervention to address nutritional deficits and improve clinical outcomes[26,27].

Marital status changes, such as marriage, can affect the risk of malnutrition in T2DM due to various psychosocial factors. Marriage may introduce additional responsibilities, such as caregiving or managing household duties, which could interfere with the individual's ability to prioritize proper nutritional management, particularly if spousal support is inadequate or inconsistent. This aligns with findings from previous studies, which have demonstrated that the presence of a supportive spouse is associated with better adherence to dietary and medication regimens in chronic disease management, including diabetes[28,29]. Previous research also highlights that marital conflict or stress within the relationship may negatively impact nutritional intake. Emotional stress has been shown to affect meal patterns, often leading to reduced appetite or reliance on less nutritious, high-calorie convenience foods, further exacerbating malnutrition risks. However, supportive marital environments can buffer against these risks, fostering healthier eating habits and improved glycemic control, as documented in studies focusing on the psychosocial aspects of diabetes care[28,29]. These findings collectively emphasize the complex role of marital status and quality in shaping nutritional and health outcomes in patients with T2DM, suggesting that interventions aimed at improving spousal support and relationship quality could be beneficial in mitigating malnutrition risks in this population.

A duration of over 10 years with T2DM increases the risk of malnutrition, as long-term illness can lead to complications such as anorexia, muscle loss, and digestive system decline. Metabolic issues associated with chronic diabetes, such as chronic kidney disease (CKD) and peripheral neuropathy, can impair nutrient absorption and utilization. Thus, a long history of diabetes correlates with an increased risk of malnutrition[30].

Poor medical adherence dramatically increases the malnutrition risk in patients with T2DM. Inadequate adherence to dietary and medication guidelines can disrupt blood glucose balance, impacting metabolism and nutrient absorption. Neglecting regular health checks or failing to seek nutritional advice can result in poor dietary knowledge and unbalanced nutrient intake, increasing malnutrition risks[31].

A low GFR (below 60 mL/min/1.73 m^2) indicates possible CKD in patients with T2DM, greatly increasing malnutrition risks. Reduced kidney function disrupts waste removal and nutrient balance. CKD causes toxin buildup in the bloodstream, leading to symptoms such as appetite loss, nausea, and anorexia. It also reduces the capacity of the body to absorb and use proteins and essential nutrients, thereby promoting muscle loss and malnutrition. The dietary restrictions required to manage mineral levels in patients with CKD can also contribute to nutrient imbalances, increasing the risk of malnutrition[32,33].

This study has several limitations that should be acknowledged. First, the cross-sectional design limits the ability to establish causality between identified risk factors and malnutrition in older patients with T2DM. Longitudinal studies are needed to explore causal relationships and temporal associations. Second, the use of convenience sampling from a single urban hospital may limit the generalizability of the findings to rural or non-urban populations, as well as to healthcare settings with different patient demographics or resources. Third, while we relied on the GLIM criteria for malnutrition assessment, which include objective measures like BMI and BIA, subjective factors such as dietary intake and psychosocial influences were not comprehensively captured. Future studies should incorporate more detailed dietary assessments and psychosocial evaluations to provide a holistic understanding of malnutrition in this population. Lastly, although the study adjusted for several confounding variables, unmeasured confounders such as physical activity levels and comorbidities may have influenced the results. Addressing these limitations in future research could strengthen the understanding and management of malnutrition in older patients with T2DM.

CONCLUSION

This study assessed and compared the prevalence of malnutrition using the GLIM criteria among older patients with and without T2DM. It also identified risk factors associated with malnutrition in patients with T2DM. Significant risk factors included a BMI less than 18.5, a history of diabetes of more than 10 years, non-adherence to medical advice, low kidney filtration rates (GFR < 60 mL/min/1.73 m^2), and being married. This study highlights the importance of systematically assessing malnutrition in patients with T2DM to develop appropriate management strategies for malnutrition in this group.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data underlying this study are openly available in PubMed. For further correspondence, please contact kasidid.lawongsa@gmail.com

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Original Article

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Characterization of *Entercytozoon bieneusi* and Drug Resistance-Associated Mutations Using the β-Tubulin Gene

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ABSTRACT

OBJECTIVE: In the β-tubulin gene of *Enterocytozoon bieneusi*, five mutations: His_6, Phe_{167}, Glu_{198}, Phe_{200}, and Arg_{241} have been implicated in reducing albendazole efficacy, with Glu_{198} and Phe_{200} being particularly significant. The primary objective of this study was to characterize mutations in the β-tubulin gene of *E. bieneusi* associated with albendazole resistance. Specifically, the study focused on mutations at codons Glu_{198} and Phe_{200} using newly developed primers (EbBtu198/200) with DNA extracted from fecal samples of pigs and humans.

METHODS: A total of 38 stored DNA samples, comprising 30 from different pigs and eight from different humans, were analyzed to evaluate the sensitivity of the newly designed primers and optimize the polymerase chain reaction (PCR) conditions. The encoded amino acid sequences were examined to identify the mutations at codons 198 and 200 in the β-tubulin gene of *E. bieneusi*. Additionally, a phylogenetic analysis was performed using the β-tubulin nucleotide sequences to determine the genetic relationships among different isolates.

RESULTS: PCR-amplification of the β-tubulin gene yielded a 427 bp product, with a primer sensitivity rate of 94.74%. Sequencing of 18 gene products revealed that ten sequences from pigs corresponded to Haplotype A, while human samples showed four haplotypes: A, B, C, and D. Notably, a mutation resulting in the substitution of glutamic acid with glutamine at codon 198 (E198Q) was identified, uncovering a potential mechanism for albendazole resistance. Phylogenetic analysis applying the maximum likelihood method demonstrated that all β-tubulin sequences formed a monophyletic group, indicating low genetic diversity among the *E. bieneusi* isolates.

CONCLUSION: This study underscores the significance of mutations in the β-tubulin gene, particularly at codon Glu_{198}, as key factors potentially contributing to albendazole resistance in *E. bieneusi*. These findings may offer valuable insights for improving treatment strategies in patients harboring isolates with such mutations. Furthermore, the β-tubulin gene analysis revealed limited genetic diversity among *E. bieneusi* isolates, with distinct haplotypes detected in pig and human samples, suggesting possible host-specific adaptations or transmission patterns.

KEYWORDS: albendazole resistance, *Enterocytozoon bieneusi*, haplotype, β-tubulin gene

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INTRODUCTION

Enterocytozoon bieneusi, an obligate intracellular parasite from the group microsporidia, has been reported in various mammalian hosts, including humans globally. Intestinal infection of *E. bieneusi* can cause self-limiting watery diarrhea in immunocompetent persons and severe chronic diarrhea in immunocompromised persons, especially in HIV/AIDS patients. The drug of choice for the therapy of microsporidiosis caused by *Encephalitozoon* spp. is albendazole, a benzimidazole derivative, which inhibits microtubule assembly. This drug is also effective against diarrhea caused by *E. intestinalis* and disseminated infection by *E. hellem* and *E. cuniculi*[1,2]. However, it is ineffective against *E. bieneusi* which is possibly linked to mutations in the β-tubulin gene[3-6]. Several human- and animal-derived genotypes of *E. bieneusi* have been identified from the stools of infected humans proving the occurrence of cross-species transmission. The β-tubulin gene is one of the targets utilized for the detection and genotype characterization of albendazole resistance-associated mutations[2]. Mutations in β-tubulin in *E. bieneusi* at five amino acids, His_6, Phe_{167}, Glu_{198}, Phe_{200}, and Arg_{241}, have been reported to relatively reduce albendazole activity[1,2]. In particular, mutations at the codons of Glu_{198} and Phe_{200} are potentially implicated in albendazole resistance. Thus, this study investigated the characterization of β-tubulin gene of *E. bieneusi* using a polymerase chain reaction (PCR) assay, which provides beneficial for examining the genotype and can be employed to identify mutations associated with albendazole resistance. Albendazole resistance in *E. bieneusi* has been reported widely, but evidence in Thailand is still lacking. Several human and animal-derived genotypes of *E. bieneusi* have been discovered from the stools of infected humans, demonstrating the possibility of cross-species transmission. Furthermore, the findings of this study will provide valuable information on genotype characteristics and assist in identifying mutations linked to albendazole resistance, thereby contributing to a better understanding of the molecular epidemiology and transmission patterns of infections, which is essential for effective management and control.

METHODS

A total of 38 stored *E. bieneusi* DNA samples extracted from stool samples, were confirmed by PCR assay using the internal transcribed spacer (ITS) of the small subunit rRNA gene (ITS-PCR), following the protocol established by Thathaisong et al[7]. The DNA samples were obtained from the Department of Parasitology, Phramongkutklao College of Medicine, Thailand. Of them, 30 were from different pigs, and eight were from different humans.

Primers targeting the β-tubulin gene of *E. bieneusi*, specifically designed to amplify regions containing mutations at codons Glu_{198} and Phe_{200}, were designed using the Primer3 software (https://primer3.org/)[8]. The β-tubulin gene sequence of *E. bieneusi* (GenBank accession number: DQ242640) was used as a reference template. The forward primer (EbBtu198/200-F: 5′-TGGAAATAACTGGGCCAAAG-3′) spans 20 base pairs starting at position 817, while the reverse primer (EbBtu198/200-R: 5′-ACACGTTGTGATCCCACTCA-3′) spans 20 base pairs starting at position 1245. Both primers were optimized for an annealing temperature of 59°C and a GC content of 45–50%. The resulting PCR product is 427 base pairs in length, covering the target regions of interest. In addition, the specificity of the primers to the β-tubulin gene and the absence of off-target amplification were verified using NCBI Primer-BLAST, and the primers were synthesized by Macrogen, Seoul, Republic of Korea. The PCR assay optimal for amplifying the gene of interest was developed and evaluated for sensitivity and specificity. PCR amplifications were carried out in a reaction mix with a final volume of 50 µL,

consisting of the 2 µL of DNA template (~21ng/µL), 10 pmol of each primer, 200 µM dNTP, 2 mM of $MgCl_2$, 1X PCR buffer, and 1 unit of KAPA *Taq* HotStart DNA Polymerase (5 U/µL) (Merck, USA). The reactions were performed using a Mastercycler personal thermal cycler (Bio-Rad Laboratories, California, USA). The PCR program consisted of: denaturation at 94°C for 5 min; followed by 35 cycles at 94°C for 30 sec, 59°C for 30 sec, and 72°C for 30 sec; final extension at 72°C for 10 min; and a holding temperature of 12°C. The PCR products were run for 30 min on a 2% agarose gel in 1X Tris/borate/EDTA buffer at 100V. A 100 bp DNA ladder (Vivantis Technologies, Selangor Darul Ehsan, Malaysia) was used. DNA was stained with SYBR Safe DNA Gel (Invitrogen, USA) by mixing 1 µL with 10 mL of agarose gel. Finally, the PCR products were detected by the Molecular Imager® Gel Doc XR+ Imaging System (Bio-Rad Laboratories, CA, USA).

To evaluate the specificity of the Eb198/200 primers for amplifying the β-tubulin gene of *E. bieneusi*, DNA samples from a variety of common intestinal pathogens that may co-infect with *E. bieneusi* intestinal parasites were selected, including *Trichuris trichiura*, *Ascaris lumbricoides*, hookworm, *Blastocystis* sp., *Opisthorchis viverrini*, and *Haplorchis taichui*, allowing for a comprehensive assessment of primer specificity.

For DNA sequencing, 18 positive samples, accounting for 50% of the total, were randomly chosen from the pool of samples exhibiting high-intensity PCR-positive bands (28/34 samples). This method ensured a sufficient concentration of target DNA, minimizing the likelihood of sequencing errors or low-quality data and ensuring reliable and accurate results. The sequences were subjected to the Basic Local Alignment Search Tool (BLAST) to examine the similarity of nucleotide sequences and species identity. The DNA sequences were aligned utilizing the BioEdit software, version 7.0.9 and then translated to amino acid sequences using the nucleotide sequence translation tool Transeq (EMBOSS) (https://www.ebi.ac.uk/Tools/st/). The β-tubulin DNA and amino acid sequences have been submitted to the GenBank database for accession numbers.

For nucleotide sequence alignment, 18 nucleotide and amino acid sequences were analyzed using the ClustralW program (BioEdit software, version 7.0.9 and Seaview 5.0.5). The phylogenetic tree was constructed by applying the maximum likelihood tree based on the kimura-2 parameter method (+G+I) using Molecular Evolutionary Genetics Analysis Version X (MEGA version X)[9]. The clade stability of β-tubulin topologies was assessed with a bootstrap analysis consisting of 1,000 replicates of bootstrap values. The reference sequences of the β-tubulin gene of *E. bieneusi* (AB472273), *E. bieneusi* isolate M231 (DQ242640), *E. bieneusi isolate H206 (DQ242639), Encephalitozoon cuniculi* (KC513611), *Enterocytozoon hepatopenaei* (KY593130), *Encephalitozoon hellem* (L47271), *Nosema locustae* (AF190772), *Trachipleistophora hominis* (AF162081), and *Vittaforma corneae* (EU031749) as the outgroup, were retrieved from GenBank.

The research protocol was approved by the Institutional Review Board of the Faculty of Medicine at Vajira Hospital in Bangkok, Thailand. This approval confirms adherence to international standards for human research protection, such as the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). The study was approved under the approval number COE 008/2020.

RESULTS

PCR-amplification of the β-tubulin gene, using the Eb198/200 primers produced a single 427 bp DNA fragment (Figure 1). The left primer initiated amplification at position 817 bp, while the right primer extended to position 1,243 bp. The optimal annealing temperature for Eb198/200 primers, demonstrating nonspecific bands was 59°C. The PCR-based amplification of the β-tubulin gene of *E. bieneusi* took ~1 h and 45 min to complete 35 cycles. It showed a detection sensitivity of 94.74% (36/38) compared with the ITS-PCR assay. In addition, the Eb198/200 primers did not cross-react with other intestinal parasites, including *T. trichiura*, *A. lumbricoides*, hookworm, *Blastocystis* sp., *O. viverrini*, and *H. taichui* (Figure 2). The selected β-tubulin PCR products positive for *E. bieneusi*, ten from pigs and eight from humans were sequenced. The blast results of sequencing ten nucleotide sequences: isolates ChB36, ChB79, ChB83, ChB92, ChB95, ChB199, ChB200, ChB215, ChB239, and ChB243 (accession number: ON939626-ON939635) obtained from pigs revealed 100% identity to the β-tubulin gene of *E. bieneusi*, genotype K isolated from a cat (accession number: AB472273), 99.8% with *E. bieneusi* isolate M231, obtained from a rhesus macaque, (accession number: DQ242640) and 99.2% with *E. bieneusi* isolate H206, isolated from a patient (accession number: DQ242639). Moreover, eight nucleotide sequences (isolates NMU1–NMU8; accession number: ON939636-ON939643) of the *E. bieneusi* β-tubulin were isolated from humans; four sequences (isolates NMU1–NMU4,) were 100% identical to *E. bieneusi* genotype K (AB472273), 99.8% to *E. bieneusi* isolate M231 (DQ242640), and 99.2% to *E. bieneusi* isolate H206 (DQ242639). In addition, the other four sequences (isolates NMU5–NMU8) were 99.8% similar to the *E. bieneusi* genotype K (AB472273), 99.5% to the *E. bieneusi* isolate M231 (DQ242640), and 99.1% to the *E. bieneusi* isolate H206 (DQ242639). All 18 β-tubulin nucleotide sequences of *E. bieneusi*, accession numbers ON939626–ON939643, were submitted to GenBank.

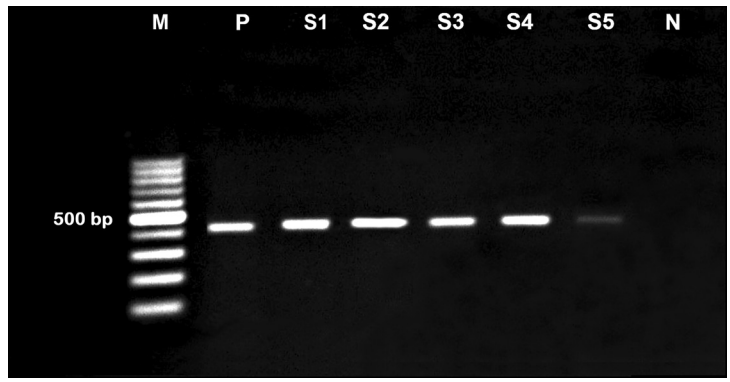

Figure 1 The PCR products of the β-tubulin gene in *Enterocytozoon bieneusi*
Note: Amplicons of β-tubulin products of *E. bieneusi* are 427 bp.
Lane M: a 100 bp DNA ladder; Lane P: positive control; Lanes S1 to S5 refer to five separate fecal samples obtained from individual pigs; Lane N: negative control
Abbreviations: bp, base pairs; PCR, polymerase chain reaction

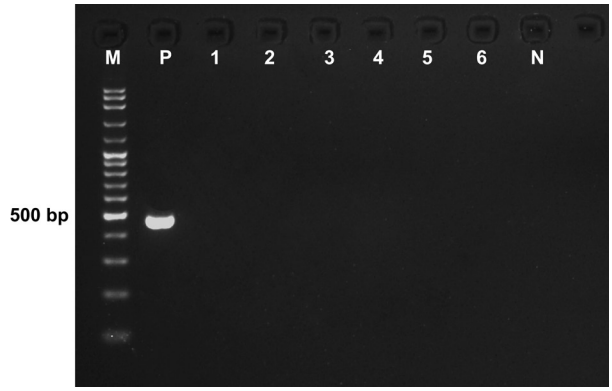

Figure 2 Agarose gel electrophoresis of DNA samples from intestinal parasites were used to evaluate the specificity of primers designed for the detection of *Enterocytozoon bieneusi*.
Lane M: a 100 bp DNA ladder; Lane P: positive control for *E. bieneusi* (427 bp); Lane 1: *T. trichiura* DNA, Lane 2: *A. lumbricoides* DNA, Lane 3: Hookworm DNA, Lane 4: *Blastocystis* sp. DNA, Lane 5: *O. viverrini* DNA, and Lane 6: *H. taichui* DNA and Lane N: negative control.
Abbreviation: bp, base pairs

The alignment of 18 nucleotide sequences of β-tubulin of *E. bieneusi* were classified into Haplotypes A, B, C, and D. All ten isolates: ChB36, ChB79, ChB83, ChB92, ChB95, ChB199, ChB200, ChB215, ChB239, and ChB243 from pigs were identified as Haplotype A. The eight isolates, NMU1–NMU8 from humans were recognized as Haplotypes A, B, C, and D.

A comparison of the β-tubulin nucleotide sequence of *E. bieneusi* from Haplotype A with the *E. bieneusi* isolate M231 (DQ242640), revealed a single nucleotide polymorphism (SNP) at the 1,066 bp position. Moreover, when comparing Haplotype A with the *E. bieneusi* isolate H206 (DQ242639) or the human isolate, three heterogenous SNPs were also detected at positions 1,036, 1,051, and 1,108 bp[2]. Haplotypes B (isolates NMU5 and NMU6) and D (NMU8) revealed four heterogenous SNPs at positions 850, 1,036, 1,051, and 1,108 bp compared with the *E. bieneusi* isolate H206 (DQ242639). Haplotype C showed five heterogenous SNPs at positions 850, 852, 1,036, 1,051, and 1,108 compared with H206 (DQ242639). The 18 β-tubulin nucleotide sequences of *E. bieneusi* were translated to 139 amino acids which revealed 100% identity to reference isolates (*E. bieneusi* genotype K: isolates M231 and H206) with no polymorphisms[2]. Interestingly, 18 amino acid sequences, including three reference sequences (genotype K: isolates M231 and H206), revealed a point mutation at codon Glu_{198} to glutamine (E198Q); however, no mutation at codon Phe_{200} was found (Figure 3).

The maximum likelihood tree was constructed with 1,000 bootstrapping values using an alignment of 416 β-tubulin nucleotide sequences with no gaps based on the kimura-2 parameter method (+G+I). The accession number lists display the selected β-tubulin reference sequences. The topologies of ten β-tubulin *E.bieneusi* isolates in the tree showed a monophyletic group with references including *E.bieneusi* accession numbers: DQ242640, DQ242639, and AB472273, with a bootstrapping value of 99% and was paraphyletic with *E. hepatopenaei* (KY593130); bootstrapping values of 100% (Figure 4). In addition, ten isolates of *E. bieneusi* obtained in this study diverged from the *E. cuniculi*, *E. hellem*, *N. locustae*, and *T. hominis* clade, with bootstrapping values at 97%.

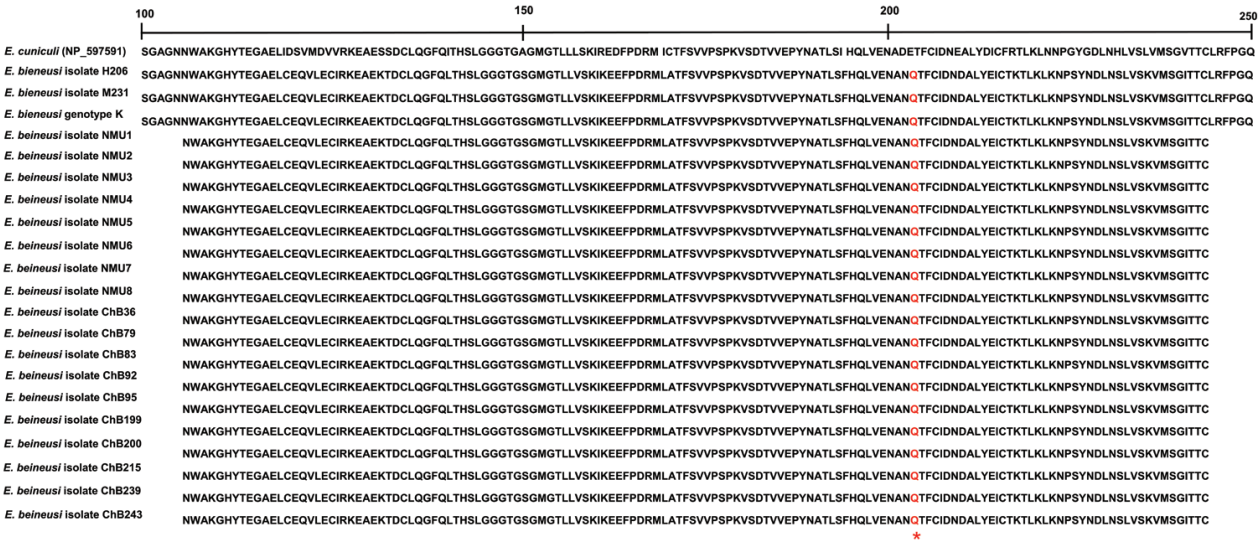

Figure 3 Amino acid alignment of β-tubulin in *Enterocytozoon bieneusi*

Note: The amino acid sequences consist of three reference sequences (isolates H206, M231, and genotype K), eight sequences from humans (isolates NMU1 to NMU8), and ten sequences from pigs (ChB36, ChB79, ChB83, ChB92, ChB95, ChB199, ChB200, ChB215, ChB239, and ChB243), demonstrating a length of 139 amino acids.

*a point mutation at the codon Glu_{198} with glutamine (E198Q). Numbering is based on *Saccharomyces cerevisiae* (Akiyoshi et al, 2007).

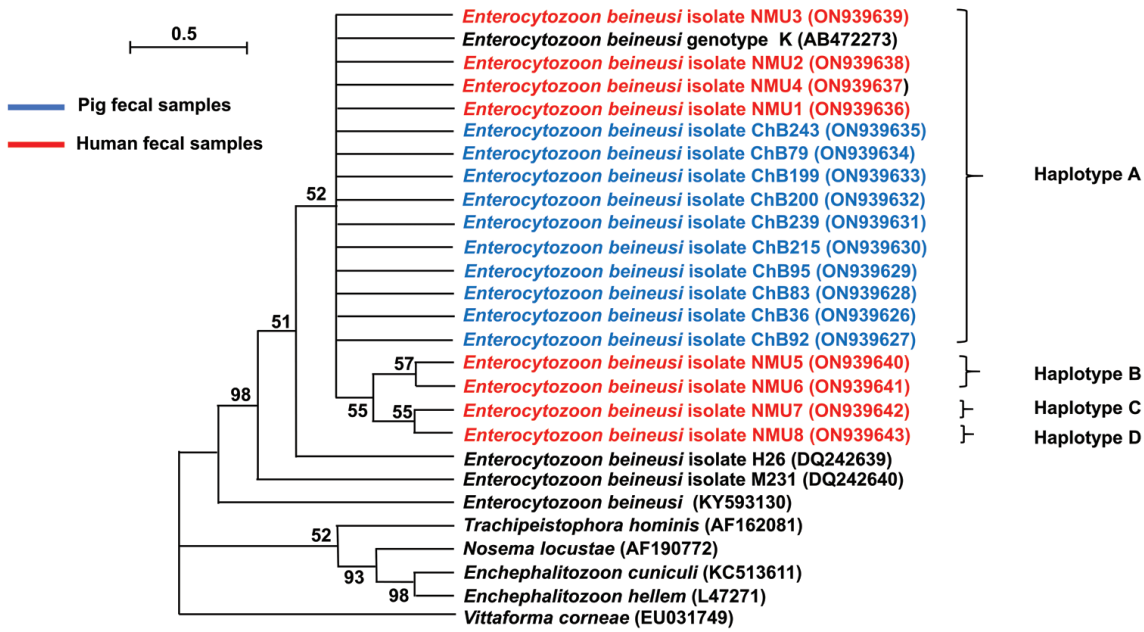

Figure 4 Maximum likelihood tree based on kimura-2 parameter method (+G+I) model of microsporidia species using β-tubulin gene sequences

A maximum likelihood tree was performed on an alignment of 401 nucleotide sequences with no gaps, and 27 β-tubulin sequences from microsporidia species were subjected to tree construction. The GenBank accession numbers are shown in this order after the species name, and the percentage of trees in which the associated taxa clustered together is shown next to the branches. Bootstrap values of > 50 are shown, and the branch lengths are measured in the number of substitutions per site.

DISCUSSION

This study developed a highly sensitive β-tubulin-PCR assay to amplify 427 bp that could be an alternative method for *E. bieneusi* characterization. The sensitivity of the primers EbBtu198/200 was 94.74% compared with the ITS-PCR assay for detecting *E. bieneusi* and did not cross-react with other intestinal parasites detected Haplotypes A, B, C, and D from pig and human stool samples. Furthermore, the specificity of PCR assay for accurately detecting mutations in the β-tubulin gene associated with albendazole resistance in *E. bieneusi* can be significantly enhanced through the use of advanced techniques such as nested PCR, real-time PCR, and allele-specific PCR. Haplotype A was identified in both pigs and humans; thus, it might be potentially zoonotic with cross-species transmission. Moreover, β-tubulin in *E. bieneusi* from this study showed a few SNPs, indicating a highly conserved region.

Transmission of *E. bieneusi* can occur from person to person or from animals to humans via a fecal-oral route by ingesting food and water contaminated with infective spores. Previous genotype identification of *E. bieneusi* from infected humans revealed that they were animal-derived, implying potential zoonotic and cross-species transmission[10,11]. In addition, asymptomatic persons infected with *E. bieneusi* possibly highlight the epidemiological relevance and play a role in the transmission of this organism. In a previous study, *E. bieneusi* genotype PigEBITS was identified in HIV patients in Thailand, supporting potential zoonotic transmission to humans. The PigEBITS genotype exists in many hosts that could infect both humans and pigs[10]. Moreover, other animal-derived genotypes were also identified in infected persons[12]. Pigs are considered one of the main reservoir hosts of *E. bieneusi*. Due to the ability to infect multiple host species, this finding supports the zoonotic transmission of *E. bieneusi* Haplotype A (genotype K in cats) from pigs to humans. Additionally, Haplotypes A, B, C, and D containing albendazole resistance-associated mutations were first described in Thailand. The β-tubulin nucleotide sequences of these haplotypes obtained from humans demonstrated a few SNPs compared with those of a rhesus macaque (DQ242639) and an HIV patient (DQ242640), suggesting high conservation of the β-tubulin gene among different host species. Using phylogenetic analysis, *E. bieneusi* isolated from ten individual pigs, one human, one rhesus macaque, and one cat were clustered into the same clade (99% of bootstrap values) or were monophyletic, demonstrating non-significant genetic variation of the β-tubulin gene among *E. bieneusi* genotypes.

Akiyoshi et al. analyzed the amino acids encoded at six codons of the β-tubulin gene, His_{6}, Ala_{165}, Phe_{167}, Glu_{198}, Phe_{200}, and Arg_{241}, to study the sensitivity or resistance of *E. bieneusi* to albendazole[2]. Of these six amino acids, those highly associated with albendazole sensitivity were substituted with either Glu_{198} or Phe_{200}, and changes in one or both of these resulted in albendazole resistance. While fungi and helminths carrying Glu_{198} and Phe_{200} genotypes are albendazole susceptible[1,2,13]. Albendazole has been widely used to treat microsporidiosis caused by *Encephalitozoon* spp. However, albendazole is quite ineffective in the treatment of *E. bieneusi* infection[6]. Mutations in the β-tubulin gene, particularly at key codons like Glu_{198} and Phe_{200}, pose a significant challenge in the treatment of *E. bieneusi* infections. These mutations reduce the binding affinity of albendazole to β-tubulin, its primary molecular target[14-17], which in turn diminishes its ability to inhibit microtubule assembly. As microtubule assembly is essential for parasite growth, survival, and reproduction, this reduction in albendazole's effectiveness leads to decreased therapeutic efficacy and an increased risk of treatment failure.

In those for whom albendazole derivative treatment failed, nitazoxanide has been successfully employed to treat *E. bieneusi*

infection in two case reports[18,19]. Although the exact mechanism of action of nitazoxanide remains unclear, it is believed to interfere with anaerobic energy production by inhibiting the enzyme pyruvate-ferredoxin oxidoreductase (PFOR)[20,21], with another target in microsporidia being protein disulfide isomerase[22]. Recently, nitazoxanide has shown effectiveness against various parasitic infections, including *G. intestinalis* and *Entamoeba histolytica*, positioning it as a promising alternative when albendazole is ineffective due to resistance[23]. Another alternative medicine for *E. bieneusi* infection is fumagillin, which inhibits methionine aminopeptidase type 2 (MetAP2) an essential enzyme for protein synthesis in *E. bieneusi* [24,25]. Fumagillin has completely eradicated *E. bieneusi* infections at 60 mg/day for two weeks[26]. However, more trials using nitazoxanide and fumagillin are required to evaluate their possible role in treating *E. bieneusi* infection.

Analysis of ten β-tubulin nucleotide sequences obtained from individual pigs revealed 100% identity with previously reported amino acid sequences (accession numbers BAH02265, ABB72136, and ABB72137), with no polymorphisms detected. Interestingly, a mutation at codon Glu_{198} to glutamine (E198Q) of the β-tubulin gene of *E. bieneusi* was observed among the ten amino acid sequences from ten different pigs that could be related to albendazole resistance. However, the limitation of the study includes the type of samples employed, as they lacked demographic data for a more comprehensive analysis and interpretation. Furthermore, alternative treatments for *E. bieneusi* infection need to be evaluated and validated to ensure proper management in patients with albendazole-resistant genotypes. A previous study demonstrated a point mutation of the β-tubulin gene of *G. intestinalis* at the codon Glu_{198} to lysine (E198K), showing albendazole resistance[27]. Additionally, a study in Sudan reported that *Haemonchus contortus*, a goat nematode, showed resistance to albendazole due to β-tubulin polymorphism at codon 198[14]. The eight amino acid sequences encoded by the *E. bieneusi* β-tubulin gene isolated from humans was also 100% identical to the reference amino acid sequences: BAH02265, ABB72136, and ABB72136, as well as the pig isolates that had the E198Q mutation in the β-tubulin gene. Other organisms also showed that mutations of one or both amino acids at codons 198 and 200 are potentially related to resistance to albendazole and its derivatives. These included the fungi: *Fusarium graminearum*, *Penicillium digitatum*, *Aspergillus* spp., *Saccharomyces cerevisiae*, *V. corneae*, *E. hellem*, *E. cuniculi*, *E. bieneusi* [1,28-31]; protozoa: *G. intestinalis*, *Acanthamoeba polyphaga*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Leishmania major* [1]; and nematodes: *A. lumbricoides*, *T. trichiura*, *H. contortus*, *Necator americanus*, and *Teladorsagia circumcincta* [32-36].

To circumvent drug resistance against *E. bieneusi*, information obtained from this study can be used in the rational design of new microsporidia therapeutic targets such as tubulin, type 2 methionine aminopeptidase polyamines, chitin synthases, topoisomerase IV, triosephosphate isomerase, and lipase or enzymes involved in spore germination and invasion processes[37]. Novel inhibitors and specific targets of *E. bieneusi* can be searched by screening compound libraries, and computational molecular modeling techniques, such as molecular dynamics and docking. The application of these computational methods can identify the action of different ligands on unique therapeutic targets, which can economize the drug discovery process[38]. Studies on therapeutic targets of these drugs in microsporidia are needed to further their development as an effective treatment against *E. bieneusi*.

CONCLUSION

In conclusion, a new PCR assay for the detection of β-tubulin in *E. bieneusi* was developed which showed 94.74% sensitivity. An analysis of the nucleotide sequence of

the β-tubulin in *E. bieneusi* provided one haplotype from pig stool samples and four haplotypes from human samples, revealing a few SNPs among host types. An analysis of the β-tubulin nucleotide sequence in *E. bieneusi* revealed one haplotype in pig feces samples, four haplotypes in human samples, and a few SNPs among host types. Examination of the amino acid sequences revealed mutations at codon Glu_{198} (E198Q); thus, *E. bieneusi* isolated in this study might have been involved in resistance to benzimidazole and its derivatives. Thus, further epidemiological investigation of β-tubulin in *E. bieneusi* in local animals and farms is required, which may play a significant role in disseminating benzimidazole resistance. The findings of this study emphasize the significance of genetic epidemiology, prevention, control, and dynamic transmission, particularly animal–human transmission.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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DATA AVAILABILITY STATEMENT

All data are available in the GenBank database with the following accession numbers, β-tubulin sequences: ON939626-ON939643, AB472273, DQ242640, DQ242639, L47271, AF190772, AF162081, EU031749, KY593130, KC513611 and NP_597591.

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Case Report

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Concomitant *Streptococcus Suis* Septic Arthritis and Gouty Arthritis: A Case Report

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ABSTRACT

Septic arthritis, when occurring together with crystal-induced arthritis such as gout, can make the diagnosis more difficult and increase the risk of complications compared with septic arthritis without concomitant gout. *Streptococcus suis*, a common pathogen in swine, can be transmitted from animals to humans. Human infections are rare. Patients often have a history of contact with or consumption of undercooked pork. Most patients present with meningitis, septicemia, endocarditis, and septic arthritis. A 71-year-old man presented with bilateral knee pain, swelling, and fever. Synovial fluid aspiration from the knee revealed intracellular urate crystals and *Streptococcus suis* on the culture. He was diagnosed with septic arthritis due to *Streptococcus suis* with concomitant gouty arthritis. The patient was treated with intravenous ceftriaxone and bilateral knee arthrotomy. After clinical improvement, he was switched to oral amoxicillin and completed a total of 4 months of antibiotics. At the 1-year follow-up, his function was near pre-infection levels. This is the first reported case of septic arthritis due to *Streptococcus suis*, an uncommon pathogen, in a patient with gouty arthritis. Concurrent septic arthritis and gouty arthritis can make the diagnosis more difficult. A high index of suspicion for septic arthritis with gouty arthritis is important for the accurate diagnosis and appropriate treatment in order to minimize complications.

KEYWORDS: gouty arthritis, septic arthritis, *Streptococcus suis*

INTRODUCTION

Septic arthritis is a serious medical condition characterized by infection and inflammation within a joint, typically resulting from the invasion of the joint by microorganisms. Prompt diagnosis and treatment are essential, as delayed identification can lead to severe complications. Although septic arthritis is not typically conducive to crystal formation in the joint, it can, on rare occasions, occur alongside crystal-induced arthritis, such as gouty arthritis. Gouty arthritis occurs when monosodium urate (MSU) crystals accumulate in the joint, where they are recognized and phagocytosed by neutrophils. This process initiates and modulates an inflammatory cascade, leading to an intense inflammatory response within the affected joint or tissue. Diagnosing concomitant septic arthritis and gouty arthritis is challenging, and there is limited literature reporting on the incidence of these conditions occurring together, with only scattered case reports. A high index of suspicion is necessary for accurate diagnosis, as untreated cases of combined septic and gouty arthritis are associated with an increase in intensive care unit (ICU) admissions, longer hospital stays, and a higher risk of limb amputation compared to septic arthritis alone[1,2].

Streptococcus suis is a gram-positive cocci bacterium commonly found in swine, which can

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be transmitted to humans. It causes a range of significant human diseases, including meningitis, sepsis, septic shock, infective endocarditis, and septic arthritis. Although most human infections manifest as meningitis and sepsis, with hearing loss being a frequent complication, septic arthritis, due to *Streptococcus suis,* accounts for only 2.9% of all infections. The mortality rate for these infections is 9.5-19.5%[3]. The first documented human case occurred in Denmark in 1968[4], and the first cases in Thailand were reported in 1987, both involving patients with meningitis[3]. *Streptococcus suis* is primarily found in Southeast Asia, where pig farming is widespread, and Thailand has the second-highest incidence of these infections globally, following China[5,6]. Risk factors for infection include occupations involving direct contact with pigs or pork products and the consumption of undercooked pork[3]. However, isolating *Streptococcus suis* from synovial fluid cultures remains exceedingly rare[5].

CASE REPORT

This case report was published after receiving ethical approval (COA No. SO21hc/67_NA). A 71-year-old Thai male presented after falling down the stairs 10 days prior to hospital admission. He experienced bilateral knee pain, inability to bear weight, and pain with knee flexion and extension. He had occasional acute knee and foot pain but did not seek treatment. His symptoms did not improve, and 2 days before admission, his knee pain worsened, causing him to seek medical attention. The patient denied any underlying medical conditions, alcohol consumption, or smoking.

The initial physical examination revealed: body temperature: 38.2°C, blood pressure: 137/60 mmHg, pulse rate: 79 beats/min, respiratory rate: 20 breaths/min, both knees were swollen, warm, and tender with positive joint effusion, particularly more in the right knee. No tophi were observed at the elbows or feet. Investigations showed complete blood count: white blood cell (WBC) 13,090 cells/μL (neutrophils 79.7%), uric acid: 4.4 mg/dL, high-sensitivity C-reactive protein (hsCRP): 324.66 mg/L, blood culture: pending, synovial fluid analysis (right knee): yellow-turbid fluid 20 ml, WBC 54,481 cells/mm^3 (polymorphonuclear cell (PMN) 95%), Gram stain: negative, crystals: not found, synovial fluid culture: pending, plain radiograph of both knees: moderate degenerative changes.

The patient was treated with intravenous fluids and antibiotics (ceftriaxone 2 g IV once daily). On the second day, left knee arthrocentesis was performed, revealing yellow-turbid fluid (10 ml), WBC 43,402 cells/mm^3 (PMN 94%), Gram stain negative, and intracellular urate crystals were observed, marking the first diagnosis of gout in this patient. He was started on colchicine (0.6 mg) once daily after meals and naproxen (250 mg) twice daily after meals. Synovial fluid culture from the left knee grew *Streptococcus suis*, confirmed by molecular identification. The patient denied risk factors for *Streptococcus suis* infection such as working with pigs or pork products or consuming undercooked pork. The organism was sensitive to ampicillin, cefotaxime, ceftriaxone, vancomycin, and levofloxacin. After IV antibiotics therapy, his pain decreased but he had a low-grade fever. He exhibited no neurological symptoms, hearing loss, or cardiac problems. Blood cultures showed no organism growth. On the ninth day of hospitalization, he underwent bilateral knee arthrotomy and lavage. Post-surgery, his fever subsided; he began physical therapy and was discharged after a total hospital stay of 16 days. Upon discharge, he was prescribed oral amoxicillin (500 mg), 2 tablets twice daily, colchicine (0.6 mg) once daily, and naproxen (250 mg) as needed for pain.

The patient's condition and hsCRP levels were monitored monthly. His joint pain decreased, mobility improved, and he was able to walk more. The total duration of antibiotic therapy was 4 months (discontinued once hsCRP levels normalized for 2 consecutive measurements). After stopping antibiotics, hsCRP levels remained normal, and he was able to walk with minimal pain.

At the 1-year follow-up, he had occasional mild pain and his functionality was near pre-infection levels.

This case report presents the key clinical findings of a patient who presented with bilateral knee pain and fever. On examination, both knees were swollen, warm, and tender with positive joint effusion. Synovial fluid analysis revealed a positive Gram stain, and culture identified *Streptococcus suis*. Intracellular urate crystals were also observed. The patient was diagnosed with *Streptococcus suis* septic arthritis along with gouty arthritis. Treatment included colchicine, naproxen, intravenous ceftriaxone, and bilateral knee arthrotomy. After one year of treatment, the patient's knee function was close to pre-infection levels.

DISCUSSION

Currently, there are few studies on concomitant septic arthritis with crystal-induced arthritis. In this case, the patient had gouty arthritis along with *Streptococcus suis* septic arthritis, which is an uncommon pathogen. To the author's knowledge, this is the first reported case of *Streptococcus suis* septic arthritis occurring concurrently with gouty arthritis. Septic arthritis is not typically conducive to crystal formation within the joint. In this case, the clinical presentation of acute gouty arthritis was atypical, as the patient had no clear prior history of gout, which further complicated the diagnostic process. Alternatively, it is possible that monosodium urate crystals were found concurrently with septic arthritis, rather than being the primary cause of joint inflammation from acute gouty arthritis.

Prior-Español et al. reported a retrospective study that collected data from 1985 to 2015 on 25 patients with coexisting septic and crystal-induced arthritis, with a mean age of 67 years. The most commonly involved joint was the knee, with the most frequent crystals being monosodium urate (68%), calcium pyrophosphate dihydrate (20%), and hydroxyapatite (12%). The most common pathogens were methicillin-sensitive Staphylococcus aureus (48%), methicillin-resistant Staphylococcus aureus (12%), and Mycobacterium tuberculosis (12%). Arthrotomy was performed in 36% of cases. Complications included infected wounds (8%), septic shock (8%), mortality (8%), and no complications (36%)[2]. In comparison, the patient in this case report underwent arthrotomy on the ninth day after admission due to the difficulty of diagnosing septic arthritis initially, pending synovial fluid culture results, negative Gram stain, and the patient's initial refusal of surgery.

Yu et al. conducted a retrospective study that collected data from 1987 to 2001 on 30 patient with concomitant septic arthritis and gouty arthritis. The mean age was 52.8 years, and one-third of the patients did not have a fever. Normal WBC counts were seen in 30% of patients, and 10% had a synovial fluid WBC count of less than 6,000/mm^3. The knee was the most commonly affected joint, followed by the ankle, shoulder, and wrist. Staphylococcus aureus was the most common pathogen. Fourteen patients underwent surgical debridement, with 2 requiring arthrodesis and 1 undergoing above-knee amputation. Two patients died. The study by Yu et al. found that the knee is the most common site for concomitant septic and gouty arthritis, consistent with the current case[1].

Hong et al. reported a retrospective study from 2011 to 2016 on 13 patients with concurrent septic arthritis and gouty arthritis. The mean age was 60.8 years. Ankle involvement was more common in concomitant septic arthritis and gout compared to septic arthritis without gout, in which the knee joint was more commonly involved. Patients with concomitant septic arthritis and gouty arthritis had higher risks of ICU admission, longer hospital stays, and limb amputation than those with septic arthritis alone. Hong et al.'s study underscores the need for a high index of suspicion for concomitant septic arthritis and gout due to the higher risk of severe symptoms and complications[7].

A study in Thailand by Wangsomboonsiri et al. in Nakhon Sawan Province found that among 66 patients with *Streptococcus suis* infection, most were diagnosed with meningitis (52%), sepsis (27%), septic shock (12%), and endocarditis (8%), with septic arthritis occurring in only 1%[8]. However, in the present case report, the patient had *Streptococcus suis* infection confined to the joint without systemic involvement.

Evidence from a Thai study by Wangkaew et al. shows that *Streptococcus suis* is sensitive to penicillin[9], differing from Nakaranurack et al. who reported that *Streptococcus suis* in 6 out of 11 Thai patients was not sensitive to penicillin, but was sensitive to cefotaxime and vancomycin[10]. Nonetheless, the study by Kerdsin found that β-lactam antibiotics are still widely used globally and that *Streptococcus suis* remains largely sensitive to this class of antibiotics[3]. In the present case report, the patient was initially treated with ceftriaxone, due to its broad-spectrum coverage before culture results were available. After confirming the sensitivity of *Streptococcus suis* to penicillin, the patient was switched to oral amoxicillin before discharge.

CONCLUSION

Concomitant septic arthritis and gouty arthritis are rare. The present case report is the first to document septic arthritis caused by *Streptococcus suis* alongside gouty arthritis. The coexistence of septic arthritis and gouty arthritis can complicate the diagnosis, emphasizing the need for a high index of suspicion and awareness of the potential for both conditions to occur together. Early recognition of concurrent infections is essential for accurate diagnosis and effective treatment, which can help prevent any serious complications and improve outcomes in arthritis cases, particularly in regions where zoonotic infections like *Streptococcus suis* are prevalent, in order to ensure comprehensive patient management.

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