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CONTENTS

Original Articles

Review Article

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Original Article **Open Access**

Radiological and Functional Outcomes of Conservatively Managed Osteoporotic Vertebral Fractures at the Thoracolumbar Junction: A Cross Sectional Study

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ABSTRACT

OBJECTIVE This study examined how osteoporotic vertebral fracture (OVF) patients treated conservatively at a multi-specialty hospital were progressing clinically and radiologically.

METHODS This cross sectional, observational study was conducted at Sir Ganga Ram Hospital, New Delhi, India during the period of July 2021 to October 2021. Cases with at least a 6-month follow-up were evaluated. VAS pain score, Oswestry Disability Index (ODI), and local kyphotic angle (COBB's angle) at the time of fracture and at the latest follow-up were recorded and analyzed.

RESULTS There were 30 patients (female: male = 2.75:1) with a mean age of 67.37 years (45-85). The average VAS score at the time of fracture was 8 (6 to 10) and at the time of final follow-up was 2 (1 to 6) (p = 0.001). The average ODI score at the time of fracture was 44 (35 to 62) and at the time of final follow up was 5 (4 to 40) (p = 0.001). The average Cobb's angle at the time of fracture was 14.31° and at the final follow up was 15.66° (p = 0.011).

CONCLUSIONS Conservative management of OVF can lead to an increase in the local kyphotic angle. The fact that the patients experienced significant decreases in VAS pain scores and ODI scores by the final follow-up leads to the conclusion that patients can have a good quality of life even with conservative management of osteoporotic vertebral fractures.

KEYWORDS conservative management, osteoporotic, thoracolumbar, vertebral fracture

INTRODUCTION

The incidence of osteoporotic vertebral fracture (OVF) is increasing every year as the average life expectancy increases. This type of fracture results in a huge burden to the health system as approximately 200 million people are affected worldwide each year (1). Among the various approaches used to manage OVF, conservative treatment has long been a traditional line of management (2). Sir Ganga Ram Hospital has been providing conservative management for all osteoporotic fractures of the spine with the exception of cases with neurological involvement, excruciating pain or a local

kyphotic angle of more than 35 degrees. Although it has been used since long time, little is known regarding the natural course following this management practice. Of the available studies, one reported that 20% of the conservatively managed group eventually develop chronic back pain (3). In addition to back pain, spinal deformity (kyphosis, kyphoscoliosis and loss of vertebral height), and impaired function leading to diminished quality of life are known sequelae of conservatively managed OVF.

These types of fractures occur because of compression failure of the anterior and middle vertebral columns which have decreased bone mineral density. The fracture can occur even under physiological axial load or minimal stress following trivial injury. The thoracolumbar (TL) junction is the mechanical transition zone between the rigid, kyphotic thoracic spine and the flexible, lordotic lumbar spine (4). The absence of stabilizing articulations with the ribs at the thoracolumbar junction, a straighter spine and more sagittal oriented facet joints increase the chances of fracture at this level (5).

Numerous classification systems have been proposed to guide the management of OVF. The Spine Section of the German Society for Orthopedics and Trauma (DGOU) classification (6) suggested type I and II for conservative management, whereas the Sugita classification (7) suggested that concave types and dented types have a good prognosis with conservative management. Type III, IV and V fractures in the DGOU classification, including the swollen front type, bow-shaped type and projecting type, need to be managed operatively or with minimally invasive techniques such as vertebroplasty or kyphoplasty (7,8). The choice of management is based on three principles: restoring biomechanical stability to promote healing with an acceptable alignment, neurological optimization, and long-term preservation of maximum painless mobility (8). These measures have been used extensively. What has yet to be examined, however, is the natural course following the conservative management of OVF.

This study aimed to assess the natural course of conservatively managed osteoporotic TL junctional fractures. We assessed the change in the local kyphotic angle of the fractured vertebrae between the time of the fracture and the last follow-up, and the functional status of the patient in terms of Visual Analogue Scale (VAS) pain score (9) and Oswestry Disability Index (ODI) score (10).

METHODS

This single-center cross-sectional observational study was conducted at the Department of Ortho-Spine Surgery in Sir Ganga Ram Hospital, New Delhi, India. All patients with OVF at the thoracolumbar junction who were managed conservatively since 2015 and who were willing to participate in the research were invited to be included in the study. All patients included in the study had been managed by the same senior consultant of the department and had followed the same conservative management protocol. However, depending upon improvement in pain and compliance with medications, there were some individual variations in the duration of anti-osteoporotic medications, bed rest, physiotherapy and application of braces. Patients who had previously undergone vertebroplasty or kyphoplasty for an osteoporotic vertebral fracture, those with concomitant fractures of other regions of the spine or fractures around the hip, those with follow-up of less than 6 months or who had lost follow-up, and those with a pathological fracture secondary to malignancy or infection were excluded from the study.

As outlined in the flowchart below, a total of 49 patients who had been managed conservatively for OVF between 2015 and 2021 were identified through the hospital electronic record system and OPD records. Of those patients, two had a multiple level fracture, one had an L4 fracture, one had an L5 fracture, two had a follow-up of less than six months, one had undergone vertebroplasty one month after conservative treatment, and 12 had no subsequent follow up records. Hence, only 30 cases were finally included in the study.

The data of most of the participants were collected at the time of the OPD visit. A questionnaire was administered to assess general information and associated comorbid conditions, and the risk of osteoporotic fracture. The

patient's functional status was evaluated by VAS pain score and ODI score at the time of the fracture and at the latest follow-up. Records of dual energy X-ray absorptiometry (DEXA) scans, if done at the time of the fracture were obtained and the latest DEXA scans were advised to evaluate improvement following the treatment. The lists of all anti-osteoporotic medications prescribed for each patient were recorded. X-ray and/or MRI images at the time of diagnosis and the final X-ray or MRI images at the latest follow-up were used to assess the progression of segmental kyphosis. Fractures were classified using the DGOU classification and Sujita's morphological classification (7). In cases where a patient failed to visit OPD, a telephonic interview was conducted to acquire the required information for the study.

Statistical analysis was done using SPSS version 25.0. Mean and standard deviation were calculated for continuous and numeric variables. The average VAS and ODI scores at the time of the fracture and at the latest follow up were compared using the Wilcoxon Signed Rank Test. P values less than 0.05 were considered to be statistically significant.

RESULTS

Among the 30 patients, 22 (73.3%) were female and eight (26.7%) were male (F:M = 2.75:1). The average age of the patients was 67.37±10.95 years (45-85). Three males were public service holders, four males were retired officers, one male was a businessman, and all 22 females were housewives. Seventeen patients (56.7%) had the fracture because of a slip injury, eight (26.7%) fell on level ground, three (10%) fell from a bed, and two (6.7%) had the fracture while changing posture. Thirteen patients (43.3%) had a DGOU type II fracture, 11 (36.7%) had a type III fracture and six (20%) had a type IV fracture. The average follow-up time was 20 months, with a range of 6 to 72 months.

Classifying fractures according to Sujita's morphological classification was not feasible for three patients (10%). According to this classification system, 17 (56.7%) had concave type, seven (23.3%) had dented type, and three (10%) had bow type. Among the bow type patients, one had a two degree increment in the segmental kyphotic angle at the 11 month follow-up. Another had a five degree increment in the segmental kyphotic angle at the 57 month follow-up and the third case had no change in segmental kyphotic angle at the 34 month follow-up.

One patient was a long-term steroid user as she had undergone renal transplant surgery thrice in her life. One patient had recovered from a breast carcinoma and one patient had undergone open heart surgery. The most common co-morbid condition was hypertension (n = 18, 60%), followed by type II diabetes (n = 10, 33.3%).

The average VAS score at the time of fracture was 8 (6 to 10) and at the time of final follow-up was 2 (1 to 6) (p = 0.001). The average ODI score at the time of fracture was 44 (35 to

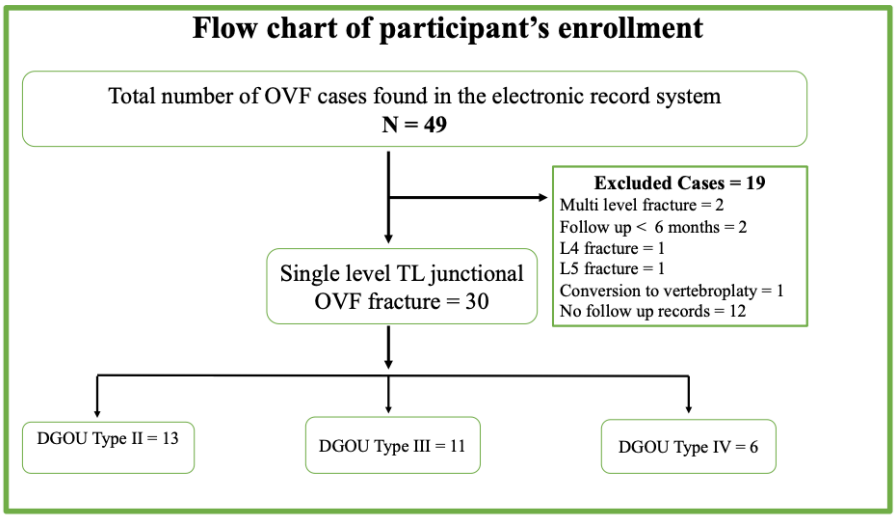

Figure 1. Flow chart of the participants' enrollment

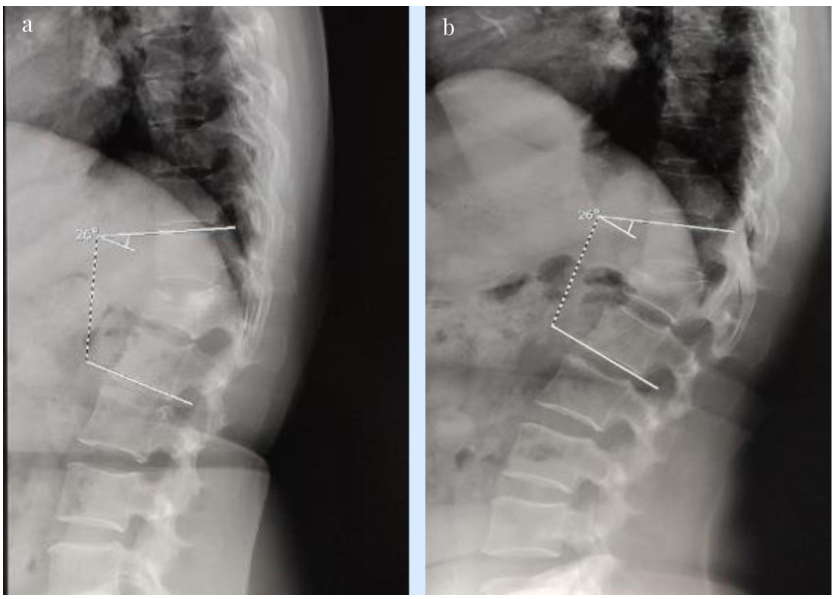

Figure 2. 65 years female with D12 fracture OF type III, with Cobb angle of 26 degree at the time of fracture (a) and similar Cobb angle of 26 degree (b) at 10 months follow up period

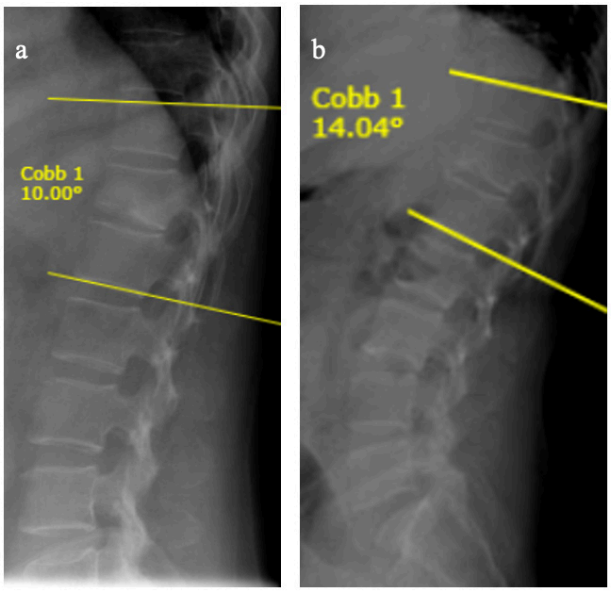

Figure 3. 58 years female with D12 fracture OF type III, with Cobb angle of 10 degree at the time of fracture (a) and Cobb angle of 14 degree at 34 months follow up period

62) and at the time of final follow-up was 5 (4 to 40) (p = 0.001). The average Cobb's angle at the time of the fracture was 14.310 and at the final follow up was 15.66 o (p = 0.011). Representative cases are shown in figure 2 and figure 3. We could only trace the bone density scores of six patients during the final follow up visit, so an appropriate analysis with regard to the DEXA scan was not feasible.

All patients received calcium and vitamin D as a part of their medical treatment. As a part of their anti-osteoporotic medications, 10 (33.3%) received oral alendronate whereas 20 (66.7%) received injectable alendronate, i.e., zoledronic acid, 21 (70%) received calcitonin nasal spray, 28 (93.3%) received teriparatide injections, and only two patients (6.7%) received denosumab injections.

Complete bed rest was advised for 21 patients (70%). Bed rest ranged from two weeks to three months, the most common being two weeks (40%). Twenty-four patients (80%) used a Knight Taylor brace. The duration of the brace application ranged from two weeks to three months. Sixteen patients (53.33%) received physiotherapy for periods ranging from four to six weeks.

DISCUSSION

This study found a significant increase in the local kyphotic angle after conservative management of the OVF. Similarly, there was a significant improvement in VAS pain scores and ODI scores following conservative management of OVF.

This study found a higher incidence of OVF among female patients than males with a ratio of 2.75:1. This result is similar to several other studies which have also reported a preponderance of female osteoporotic vertebral fracture patients (11-13). This difference is due to an acceleration of bone loss in the first decade after the onset of menopause in females and is referred to as type I osteoporosis (11). Within the first decade after the onset of menopause, the rate of bone loss affecting the lumbar spine nearly triples (12, 13).

The most common mode of injury was trivial slip injury at home (56.67%) followed by falls on level ground (26.67%). This distribution could be explained by the fact that most of the patients in this study (63.33%) were above 65 years old. Additionally, all 22 females were housewives which suggests a high risk of trivial injuries around the home.

Most of the patients in our study were advised to take two weeks of bed rest. However, they were allowed to be mobile for basic daily activities and for brief ambulation around the bed with a support brace which is comparable to previous studies. For example, Shah, et al. mentioned a short period of bed rest, analgesic medications, antiosteoporosis pharmacotherapy, and bracing support for the fracture along with guided physical therapy and postural correction aid for lasting alleviation of pain (11). Similarly, Park, et al. recommended confinement to bed for two to three days, accompanied by the use of analgesics, hot packs, massage, and lumbar orthosis (14).

Francis, et al. stated that management of patients with acute vertebral fractures should include measures to reduce pain and improve mobility as well as treatment for osteoporosis (2). Similar recommendations were found in the prescriptions given to the participants we studied. The main goals of the treatment were to allow pain-free mobilization as early as possible, and to prevent further collapse of the vertebrae through the use of anti-osteoporotic medications, and to stimulate bone formation using injections of teriparatide.

For the management of pain, all patients in this study were given oral analgesics. Anti-osteoporotic medication was found to have been started from the very beginning of treatment. All patients received alendronate, calcium and vitamin D supplementation. Twenty-eight patients (93.3%) in our study received injectable teriparatide, while only two patients for whom teriparatide was contra-indicated received injections of denosumab. This resembles the reports of meta-analyses which described the use of teriparatide for pain management in patients with acute fractures (15, 16). Seventy percent of the patients received calcitonin nasal spray. Calcitonin, administered either by subcutaneous or intranasal routes, can be beneficial in reducing pain from acute vertebral fractures (17). A recent systematic review and meta- analysis of the use of calcitonin for patients with a painful osteoporotic fracture (n = 246) concluded that calcitonin is an effective analgesic for acute pain in recent osteoporotic fractures (18). Alendronate, popularly used in the management of osteoporosis, has also been used for management of pain. In a randomized, double-blinded, controlled trial on the efficacy of intravenous pamidronate, it was observed that pamidronate provided rapid and sustained pain relief in patients with an acute osteoporotic fracture compared with a placebo (19).

For the management of chronic pain, the back muscles should be strengthened through manual therapies and exercise intervention. Physical rehabilitation has a beneficial effect on bone metabolism, bone turnover, and bone mineral content (11). All the patients with OVF in this study were advised to engage in physical rehabilitation and active ambulation as soon as they were pain free; however, only sixteen (53.3%) were found to have actively participated in a physiotherapy rehabilitation program. A number of systematic reviews and meta-analyses, similar to our study, have reported positive effects of exercise on bone mineral density, muscle strength, and quality of life in both men and women with osteoporosis or low BMD (20).

Spinal orthoses can lessen pain by reducing mobility, decreasing postural flexion and providing axial support in patients with muscle fatigue and spasms (11). All the patients in our study were advised to use a Knight Taylor brace for a short period of time so that it would be much easier for the patients to facilitate ambulation. However, only 80% (24 patients) were found to have actually used the brace as recommended. The remaining 20% (7 patients) had suffered more discomfort during the application of the brace. The degree of vertebral body compression in patients who used the brace as advised was quite low, mainly in DGOU type II fracture and they had a rapid reduction in pain after initiation of anti-osteoporotic medications.

There was a significant decrease in the height of the anterior column in the patients leading to a significant increase in the local kyphotic angle after conservative management

of OVF at the thoracolumbar junction. The change in the height of anterior column could be explained by the fact that 80% of the body weight is transferred to the anterior column, creating a constant compression force through the anterior column, leading to decreased anterior column height and a significant increase in the local kyphotic angle (p = 0.011). However, the change in local kyphotic angle was not found to have hampered activities of daily living, as our patients had significantly decreased VAS pain scores and ODI scores by the end of their treatment (p = 0.001).

This study has some limitations. There could have been some recall bias as the patients had to report their VAS score and ODI score at the time of the fracture based on memory. A few patients had only six months of follow-up, which may be too short period to arrive at conclusions regarding the final vertebral height of the fractured vertebrae as the height and local kyphotic angle could continue to change with time. Our inability to get access to 12 patients who were treated conservatively for OVF might also have affected the outcome of our analysis. However, as this was a cross-sectional study, we analyzed only the available cases in the specified time frame. Additionally, there are various potentially confounding factors that could have affected the VAS pain scores and ODI scores of the patients both at the time of the fracture and at the latest follow up. In this study, however, we considered only the pain and disability induced by the vertebral fracture as the final functional outcome. We would recommend a prospective cohort study which includes records of all essential parameters as well as potential confounding factors in order to obtain more robust results.

CONCLUSION

Conservative management of osteoporotic vertebral fractures, based on the perceptions and experience of patients, can be concluded to have importance even in recent times. Conservative management can lead to an increase in the local kyphotic angle. That the patients experienced a significant decrease in VAS pain scores and ODI scores by the end of the treatment leads to the conclusion that the patients can have a good quality of life with conservative management of osteoporotic vertebral fractures.

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CONFLICTS OF INTEREST

None

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ADDITIONAL INFORMATION

Data availability statement

The data in this study are available from the corresponding author upon reasonable request.

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Original Article **Open Access**

Evaluation and Comparison of Daily Total Energy Intake and Macronutrient Proportion Consumption Between Poor vs Good Glycemic Control Type 2 Diabetes Mellitus Patients: A cross-sectional study at Family Medicine OPD, Maharaj Nakorn Chiang Mai Hospital, Thailand

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ABSTRACT

OBJECTIVE To evaluate and compare daily total energy intake and macronutrient proportion consumption between poor and good glycemic control type 2 diabetic patients.

METHODS A cross-sectional study was conducted from December 2021 to March 2022 at Maharaj Nakorn Chiang Mai Hospital. Patient data was collected using a questionnaire. Dietary intake data was collected using 24-hour dietary recall and was analyzed by a dietitian. Factors and their association with poor glycemic control were analyzed.

RESULTS Of the 127 participants, 40.16% had poor glycemic control. The mean HbA1c level in the poor and the good glycemic control group was 7.67±0.61% and 6.39±0.44% respectively ($p < 0.001$). Among all patients, the mean total energy intake was 1,640.21±495.92 kcal/day, with mean proportions of 51.25% for carbohydrate, 16.56% for protein, and 32.12% for fat. There were no significant differences between the poor and good glycemic control groups in total energy intake (1702.63 ±503.48 kcal/day vs. 1598.32±489.65 kcal/day, $p = 0.247$), carbohydrate intake (222.78±89.98 g/day vs. 203.72±79.36 g/day, $p = 0.211$), protein intake (70.12±21.50 g/day vs. 65.44±21.38 g/day, $p = 0.230$), or fat intake (58.94±19.26 g/day vs. 57.86±24.33 g/day, $p = 0.790$).

CONCLUSIONS The poor glycemic control group was more likely to consume more total energy and a higher proportion of carbohydrate and fat, which suggests that proper individualized dietary energy intake and diet proportions may enhance nutritional status and glycemic control.

KEYWORDS DM, nutrition, diet, calories, glycemic control, consumption

INTRODUCTION

Type 2 diabetes mellitus (DM) is one of the most common non-communicable diseases worldwide. It can lead to various health problems and can have negative consequences, including heart disease, kidney damage, nerve damage, an increased mortality rate, rising healthcare costs, and a diminished quality of life (1). The incidence of diabetes is a major public health concern worldwide, and is anticipated to increase to 700 million by 2045 (2). Thailand ranks fifth among Western Pacific

nations in the number of DM cases (3), with approximately 64.4% of DM cases experiencing poor glycemic control (4).

Effective treatment of diabetes involves several key components, including healthy diet, regular physical activity, and often the use of pharmacotherapy. The American Diabetes Association (ADA) suggests that daily energy intake balance has an impact on glycemic control and recommends reducing energy intake while maintaining a healthy eating pattern (5). A previous study in Korea found that individuals with high total energy intake were more likely to have poor glycemic control (6). Moreover, dietary proportions can also affect the outcome of diabetes treatment (7).

The proportion of dietary consumption is an important aspect of a healthy diet, as it can affect overall nutrient intake, energy balance, and blood sugar control. Macronutrients are essential nutrients that provide energy required to maintain body functions and daily activities. Macronutrients consist of carbohydrate, fat, and protein, each of which contribute energy at different rates: 4 kcal/g for carbohydrate, 9 kcal/g for fat, and 4 kcal/g for protein (8). Macronutrients not only provide energy but also have an impact on blood sugar levels. Carbohydrate directly and quickly impacts blood sugar levels by breaking down into glucose, which raises blood sugar levels (8). Fat and protein have a lesser effect on blood sugar levels compared to carbohydrate, but they can still impact glucose metabolism (8, 9). They can also be converted into glucose through gluconeogenesis when carbohydrate sources are insufficient (9). For individuals with diabetes, monitoring food proportions can be particularly important in managing blood sugar levels and preventing diabetic complications. Many published papers have reported that meal planning strategies that emphasize portion control, such as using measuring cups and portion plates, can be effective in improving blood sugar control and weight management in individuals with type 2 diabetes (10, 11), with the carbohydrates playing a significant role. A study in Iran found an association between carbohydrate intake and hyperglycemia (12). To ensure proper carbohydrate consumption, the Food and Nutrition Board of the National Academy of Sciences recommends a daily carbohydrate intake of 45-65% of total daily energy intake and a minimum of 130 g/day as essential to providing adequate glucose to the brain (13). Additionally, a study in Japan showed that eating a low-carbohydrate diet of 130 g/day can help reduce HbA1c in diabetic patients with poor glycemic control (14). However, the optimal amount of fat and protein intake for diabetic patients is still uncertain due to the limited evidence available (5). In general, dietary reference intakes (DRIs) recommend a daily protein intake to fulfill the needs of most individuals of 1-1.5 g/kg/day or 15-20% of daily total energy intake (15). For fat consumption, the National Academy of Medicine recommends a daily fat intake in the range of 20-35% of daily total energy intake (16). Additionally, the United States National Cholesterol Education Program (NCEP) suggests that fat intake should not exceed 30% of total daily energy intake (15). Thus, a proper proportion of macronutrient consumption is important to the outcome of diabetic treatment. Healthcare providers should regularly work with patients to evaluate and provide information regarding management of dietary intake tailored to each patient.

Nutrition, both daily total energy intake and dietary proportions, plays an important role in maintaining glycemic control. However, there has been only limited research on the effects of dietary intake on glycemic control in Thailand (17), particularly regarding dietary proportions and including dietitian-conducted dietary assessments. Thus, the purpose of this study is to evaluate and compare daily total energy intake and the proportions of macronutrient consumption by type 2 diabetic patients with poor and good glycemic control.

METHODS

A cross-sectional descriptive study was conducted from December 2021 to March 2022 at the Outpatient Clinic of the Family Medicine Department, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (FAM-2564-08500). The study

included individuals who met the following criteria: 1) known cases of type 2 diabetes with scheduled appointments and HbA1c testing during the study period, 2) at least 18 years of age, 3) able to complete the questionnaire, and 4) voluntarily willing to participate. Written informed consent was obtained from all participants following an explanation of the study's purpose. Individuals who were not willing to participate were excluded from the study.

The study recruited a total of 127 patients who met the inclusion criteria using consecutive sampling. The sample size was calculated using the estimating an infinite population proportion formula (18), and was based on an estimated 50.2% of patients having poor glycemic control type 2 diabetes (19), error(d) = 0.10, alpha = 0.05, Z(0.975) = 1.96. The calculated sample size was 96 patients. An additional 20% was added to minimize potential data collection errors.

$$n = \frac{z^2_{1-\frac{\alpha}{2}}\, p(1-p)}{d^2}$$

Data collection was conducted using a questionnaire consisting of three parts including 1) the questionnaire regarding personal and behavioral characteristics obtained during interviews with one research data collection assistant. Information included age, sex, marital status, education level, occupation, income, health insurance, comorbidities, duration of type 2 diabetes, smoking, alcohol consumption, cognitive screening results, and physical activity. Cognitive screening was performed using the Thai version of the Mini-Cog©, a brief cognitive screening test (Cronbach's α = 0.80) (20), with a score ≤ 3 indicating cognitive impairment. Physical activity data were collected using the Thai version of the Global Physical Activity Questionnaires (GPAQ) (Cronbach's α = 0.82) (21) which collected physical activity data in three categories: work-related activities, leisure-time activities, and transportation. The data were then converted into metabolic equivalents (METs) (21, 22), and categorized into different levels of physical activity (PAL): sedentary, light active, moderately active, very active, and extra active (23, 24). The study also evaluated total daily energy expenditure (TDEE), which represents the calories an individual burns in a day as well as their resting metabolism and physical activity. Estimated TDEE can be calculated using the formula TDEE = REE x PAL (23, 24). Resting Energy Expenditure (REE) represents the energy the body needs at rest. This study used the Mifflin St-Jeor equation to calculate REE which is widely used and considered accurate for estimating REE (25). This equation calculates REE based on weight, height, and age (26). PAL refers to physical activity level (24). 2) Medical characteristics data were obtained from medical records, including weight, height, waist circumference, blood pressure, fasting blood sugar (FBS), glycated hemoglobin (HbA1c) and diabetes treatment modalities. Anthropometric data (weight, height, and waist circumference) and blood pressure were measured by a clinic nursing assistant using the hospital's regularly calibrated standard tools. Blood pressure was taken with standard digital sphygmomanometers, while standing height and body weight were measured using a portable stadiometer and an electronic scale. Waist circumference was measured in centimeters at the midpoint between the lowest rib and the iliac crest using a standard non-elastic measuring tape. Abdominal obesity was defined as a waist circumference exceeding 90 cm in men and 80 cm in women (27). Glycemic control was determined according to ADA Guideline 2023 with a target HbA1c < 7%, while HbA1c ≥ 7% was considered to indicate poor glycemic control (28). 3) Dietary assessment was collected using a 24-hour dietary recall. The research assistant instructed participants to provide details about the name, type, and quantity of foods and beverages consumed within the previous 24 hours. To aid in this process, standard food exchange models and sample household containers were utilized, e.g., a 9-inch diameter plate or bowl, a 240 mL glass, measuring cup, teaspoon, tablespoon, etc. (29).

A certified dietitian analyzed the estimated daily total energy intake in kcal and interpreted the intake of macronutrients (carbohydrate, fat, and protein) in grams, converting them into calories and percentages using Microsoft Excel

(1 gram of carbohydrate and protein equals 4 kcal, and 1 gram of fat equals 9 kcal). The nutrition analysis was based on the Thai Food Composition Tables 2015 (30). There was only one dietitian in this study, so to prevent bias the dietitian was kept blinded to other data.

Statistical analysis was performed using Stata version 16.0 software (StataCorp, College Station, Texas, USA). For descriptive statistics, categorical data are presented as frequencies and percentages, while numerical data are presented as mean ± SD or median (IQR) depending on the underlying distribution. Factors and their association with poor glycemic control type 2 diabetes were analyzed using the independent t-test, Fisher's exact test, and the Mann-Whitney U test. A p-value of <0.05 was considered statistically significant.

RESULTS

In this study, 127 type 2 diabetic patients, with mean age of 66.23±7.34 years, were enrolled of whom 65.35% were aged 65 or older and 51.97% were female. Most were married (73.21%). The median personal monthly income was 18,000 Thai baht, and 44.88% had a bachelor's degree or higher. Furthermore, 87.40% of patients had more than one additional underlying disease, including hypertension (83.46%), dyslipidemia (96.85%), chronic kidney disease (14.17%), and osteoarthritis (7.87%). However, all patients had a good functional status, were not dependent, and did not have severe diseases or conditions limiting life expectancy. Among the patients, 94.49% showed no cognitive impairment on the Mini-Cog© test. The mean waist circumference was 87.68±9.79 cm and 61.42% had abdominal obesity. Few patients were currently alcohol drinkers (25.98%) or smokers (4.72%). The median duration of diabetes was 8 years, with a mean HbA1c level of 6.91±0.88%, and 40.16% of the patients had poor glycemic control. Most patients (88.19%) were currently taking medication for diabetes.

Table 1 presents a comparison of baseline characteristics between type 2 diabetic patients with poor and with good glycemic control. The mean HbA1c levels were 7.67 ± 0.61% and 6.39 ± 0.44% in the poor and good glycemic control groups, respectively ($p < 0.001$). Demographi-

Table 1. Comparison of general characteristics between poor and good glycemic control type 2 diabetic patients (n=127)

Factor	n (%)	DM treatment outcome		*p*-value
		Poor glycemic control (n=51) n (%)	Good glycemic control (n=76) n (%)	
Personal characteristics				
Age (years) (mean±SD)	66.23±7.34	65.55±7.21	66.68±7.44	0.395[a]
Age < 65 years old	44 (34.65)	20 (39.22)	24 (31.58)	
Age ≥ 65 years old	83 (65.35)	31 (60.78)	52 (68.42)	0.448[c]
Sex				
Male	61 (48.03)	22 (43.14)	39 (51.32)	
Female	66 (51.97)	29 (56.86)	37 (48.68)	0.469[c]
Marital status				
Married	93 (73.21)	36 (70.59)	57 (75.00)	
Single, widow, divorce	34 (26.77)	15 (29.41)	19 (25.00)	0.683[c]
Education level				
Lower than bachelor's degree	70 (55.12)	31 (60.78)	39 (51.32)	
Bachelor's degree or higher	57 (44.88)	20 (39.22)	37 (48.68)	0.363[c]
Occupation				
Have a job	48 (37.80)	23 (45.10)	25 (32.89)	
No job/Pensioner	79 (62.20)	28 (54.90)	51 (67.11)	0.193[c]
Personal monthly income (Thai baht) (median (IQR))	18,000 (10000,32000)	20,000 (10000,30000)	17,883 (10,000,32,000)	0.819[b]
Health Insurance				
Government	121 (95.28)	50 (98.04)	71 (93.42)	
Non-government	6 (4.72)	1 (1.96)	5 (6.58)	0.400[c]

Table 1. Comparison of general characteristics between poor and good glycemic control type 2 diabetic patients (n=127) (continued)

Factor	n (%)	DM treatment outcome		*p*-value
		Poor glycemic control (n=51) n (%)	Good glycemic control (n=76) n (%)	
Medical characteristics				
Comorbidities				
Hypertension	106 (83.46)	39 (76.47)	67 (88.18)	0.093[c]
Dyslipidemia	123 (96.85)	49 (96.08)	74 (97.37)	1.000[c]
Chronic kidney disease	18 (14.17)	8 (15.69)	10 (13.16)	0.797[c]
Myocardial infarction	2 (1.57)	2 (3.92)	0 (0.00)	0.159[c]
Osteoarthritis	10 (7.87)	6 (11.76)	4 (5.26)	0.199[c]
Other (e.g., allergic rhinitis, dyspepsia, gout, benign prostate hyperplasia, etc.)	46 (36.22)	19 (37.25)	27 (35.53)	0.853[c]
> 1 other underlying disease	111 (87.40)	42 (82.35)	69 (90.79)	0.181[c]
Body weight (kg) (mean±SD)	64.14±11.89	64.76±11.01	63.72±12.49	0.628[a]
Height (cm) (mean±SD)	158.91±8.47	157.14±8.22	160.09±8.48	0.053[a]
Waist circumference (cm) (mean±SD)	87.68±9.79	87.41±10.91	87.03±10.25	0.840[a]
Abdominal obesity (Waist circumference ≥ 90 cm in male, ≥ 80 cm in female)	78 (61.42)	31 (60.78)	47 (61.84)	1.000[c]
Blood pressure (BP)				
BP < 140/90 mmHg	64 (50.39)	22 (43.14)	42 (55.26)	
BP ≥ 140/90 mmHg	63 (49.61)	29 (56.86)	34 (44.74)	0.208[c]
Cognitive screening by mini-Cog				
No cognitive impairment (mini-Cog > 3)	120 (94.49)	48 (94.12)	72 (94.74)	
Cognitive impairment (mini-Cog ≤ 3)	7 (5.51)	3 (5.88)	4 (5.26)	1.000[c]
Behavioral characteristics				
Current smoking	6 (4.72)	4 (7.84)	2 (2.63)	0.218[c]
Current alcohol drinking	33 (25.98)	15 (29.41)	18 (23.68)	0.538[c]
Physical activity (MET score) (median (IQR))	1120 (600,2520)	1120 (600,2880)	1120 (660,2060)	0.779[b]
Diabetes characteristics				
FBS (mg/dL)(mean±SD)	132.49±27.58	149.39±31.99	121.14±16.47	<0.001[a]
HbA1c (%)(mean±SD)	6.91±0.88	7.67±0.61	6.39±0.44	<0.001[a]
Duration of DM (years) (median (IQR))	8 (3,11)	9 (2,10)	8 (3,11)	0.816[b]
Diabetes treatment				
Lifestyle modification without any medication	15 (11.81)	6 (11.76)	9 (11.84)	
Medical treatment with lifestyle modification	112 (88.19)	45 (88.24)	67 (88.16)	1.000[c]
Type of medication treatment (single or combination regimen)				
Biguanide	108 (85.04)	44 (86.27)	64 (84.21)	0.805[c]
Sulfonylureas	34 (26.77)	17 (33.33)	17 (22.37)	0.220[c]
Thiazolidinedione	5 (3.94)	3 (5.88)	2 (2.63)	0.390[c]
DPP-4 inhibitor	48 (37.80)	20 (39.22)	28 (36.84)	0.853[c]
SGLT-2 inhibitor	4 (3.15)	2 (3.92)	2 (2.63)	1.000[c]
Insulin	1 (0.79)	0 (0.0)	1 (1.32)	1.000[c]

[a]Independent t-test, b Mann-Whitney U test, c Fisher's exact test

cally, there were no significant differences in age, sex, marital status, education level, occupation, personal monthly income, or health insurance between the two groups. Behaviorally, there were no significant differences in smoking, alcohol consumption, or physical activity. Additionally, no significant differences were found between the two groups in comorbidities, body weight, height, waist circumference, blood pressure, cognitive screening test results, duration of diabetes or type of diabetes treatment.

Table 2 presents a comparison of total energy intake and dietary composition. The mean daily energy intake was 1,640.21±495.92 kcal, with macronutrient intakes of 211.38±83.96 g/day of carbohydrate, 67.32±21.47 g/day of protein, and 58.29±22.36 g/day of fat, accounting for 51.25%, 16.56%, and 32.12%, respectively, of total consumption. More than half (51.97%) of the patients exceeded the recommended carbohydrate intake (carbohydrate intake > 50% total energy intake), 42.52% exceeded the recommended fat intake (fat intake > 30% total energy intake), and 47.27% had inadequate protein intake (protein intake < 1 g/kg/day). Regarding the association between dietary intake and glycemic control, the poor glycemic control group had a higher daily total energy intake and consumed a greater amount of carbohydrate and fat than the good glycemic control group. However, there were no significant differences in total energy intake (1702.63±503.48 kcal/day vs. 1598.32±489.65 kcal/day, ***p*** = 0.247), carbohydrate intake (222.78±89.98 g/day vs. 203.72±79.36 g/day, *p* = 0.211), protein intake (70.12±21.50 g/day vs. 65.44±21.38 g/day, *p* = 0.230), or fat intake (58.94±19.26 g/day vs. 57.86±24.33 g/day, *p* = 0.639) between the two groups.

DISCUSSION

This study aimed to evaluate and compare the daily total energy intake and macronutrient proportion consumption between type 2 diabetic patients with poor and good glycemic control in the OPD of Family Medicine at Maharaj Nakorn Chiang Mai Hospital, Thailand. The mean daily energy intake was 1,640.21±495.92 kcal, with 35.43% consuming above their TDEE. The mean proportion of consumption of each macronutrient that provides energy to the body included carbohydrate 51.25%, protein 16.56%, and fat 32.12%. Among the patients, 51.97%

Table 2. Comparison of daily total energy intake and macronutrient composition between poor and good glycemic control type 2 diabetic patients (n=127)

	n (%)	DM treatment outcome		*p*-value
		Poor glycemic control (n=51) n (%)	Good glycemic control (n=76) n (%)	
Total daily energy intake (TDEE) (kcal) (mean±SD)	1640.21±495.92	1702.63±503.48	1598.32±489.65	0.247[a]
Total energy intake ≤ TDEE	82 (64.57)	31 (60.78)	51 (67.11)	
Total energy intake > TDEE	45 (35.43)	20 (39.22)	25 (32.89)	0.571[c]
Carbohydrate (kcal) (mean±SD)	845.51± 335.85	891.14±359.93	814.89±317.44	0.211[a]
Carbohydrate (gm/day) (mean±SD)	211.38±83.96	222.78±89.98	203.72±79.36	0.211[a]
Carbohydrate intake < 130 g/day	15 (11.81)	3 (5.88)	12 (15.79)	
Carbohydrate intake ≥ 130 g/day	112 (88.19)	48 (94.12)	64 (84.21)	0.102[c]
Protein (kcal) (mean±SD)	269.26±85.87	280.47±85.99	261.74±85.54	0.230[a]
Protein (gm/day) (mean±SD)	67.32±21.47	70.12±21.50	65.44±21.38	0.230[a]
Protein intake ≥ 1 g/kg/day	67 (52.76)	30 (58.82)	37 (46.68)	
Protein intake < 1 g/kg/day	60 (47.27)	21 (41.18)	39 (51.32)	0.288[c]
Fat (kcal) (mean±SD)	524.63±201.24	530.47±173.33	520.71±219.01	0.790[a]
Fat (gm/day) (mean±SD)	58.29±22.36	58.94±19.26	57.86±24.33	0.790[a]
% Carbohydrate (mean±SD)	51.25±10.39	51.67±10.76	50.97±10.20	0.711[a]
≤ 50% total energy intake	61 (48.03)	22 (43.14)	39 (51.32)	
> 50% total energy intake	66 (51.97)	29 (56.86)	37 (48.68)	0.469[c]
% Protein (mean±SD)	16.56±2.91	16.65±2.97	16.50±2.88	0.774[a]
≥ 20% total energy intake	15 (11.81)	9 (17.65)	6 (7.89)	
< 20% total energy intake	112 (88.19)	42 (82.35)	70 (92.11)	0.159[c]
%Fat (mean±SD)	32.12±9.13	31.65±9.22	32.43±9.13	0.639[a]
≤ 30% total energy intake	73 (57.48)	26 (50.98)	47 (61.84)	
> 30% total energy intake	54 (42.52)	25 (49.02)	29 (38.16)	0.273[c]

[a]Independent t-test, [b]Mann-Whitney U test, [c]Fisher's exact test

consumed excess carbohydrates (> 50% total energy intake), 42.45% consumed excess fat (> 30% total energy intake), and 47.27% had inadequate protein intake (protein intake < 1 g/kg/day).

Energy intake is crucial for glycemic control. Reducing energy intake has been linked to improved glycemic control, either through direct restriction or by balancing energy (31). A high energy intake is related to poor glycemic control (6). This study found a mean energy intake of 1,640.21±495.92 kcal/day, similar to an Italian study (1,725±497 kcal/day) (7). Notably, 35.43% of patients in this study consumed more energy than they expended daily, which can lead to weight gain and increased blood glucose (6, 32). Even though it was not statistically significant, those with poor glycemic control had a tendency to consume more energy than those with good glycemic control, a finding which is consistent with a previous study (33). The lack of statistical significance in this study might be due to the small sample size. A larger sample size might enhance the ability to detect significant differences. Counseling and strategies for caloric restriction should be promoted for better glycemic management.

High carbohydrate intake substantially affects post-meal glucose levels which is correlated with hyperglycemia due to its conversion into glucose which elevates blood glucose levels, especially in diabetics (34). Although fat does not directly affect glucose levels, excessive fat intake can lead to weight gain and insulin resistance and can result in higher blood sugar levels (35). Although there is evidence suggesting that macronutrient intake (carbohydrate, fat, and protein) can affect glycemic control, studies in this area are still inconclusive (6, 36). A systematic review of previous studies found that carbohydrate-restricted diets result in short-term reductions in HbA1c levels (3-6 months), but that their effectiveness tends to decline over the longer term (12-24 months) (37). One study reported an association between high carbohydrate intake and poor glycemic control (17). However, the present study did not find a significant association: it only found that the group with poor glycemic control was more likely to consume higher amounts of carbohydrates and fats. This may be due to the small sample size. A larger sample size might enhance the ability to detect significant differences. This discrepancy with previous studies may be attributed to differences in sociodemographic factors and healthcare settings. In this study, the patients had higher levels of education and personal monthly income compared to those in previous studies. Regarding the healthcare setting, our Family Medicine OPD is located in a university hospital with comprehensive facilities and a multidisciplinary team. These factors may contribute to a more comprehensive and holistic approach to care and may more effectively encourage healthy dietary choices than in other primary care settings. Although protein intake has a minimal effect on blood glucose levels in healthy individuals, excessive protein consumption can lead to hyperglycemia in diabetic patients (9). One study reported that adequate protein and reduced carbohydrate intake are associated with weight loss and improved glycemic out come (38). Conversely, insufficient protein intake in diabetic patients, particularly in elderly individuals, can affect kidney function (GFR) (39) and can lead to sarcopenia (4). Sarcopenia, a gradual loss of muscle mass and strength which can increase the risk of fractures, is associated with frailty, disability, and death (40). However, similar to a previous study (6), this study did not find a significant association between protein intake and glycemic control. For that reason, it is necessary to evaluate and plan personalized dietary interventions for each diabetic patient, especially total energy intake and appropriate food proportions.

A strength of this study is that it included detailed information about dietary intake, including total caloric intake, carbohydrates, proteins, and fats, data which has been lacking in previous studies in Thailand. Another strength is that having only a single assistant to collect data helped to reduce inconsistencies in data collection and potential bias. Additionally, the expertise of a certified dietitian helped ensure the quality of dietary assessment results. However, there are also some limitations to this study. First, due to the cross-sectional design, the study was not able to confirm a causal rela-

tionship between dietary intake and glycemic control. Future research should use a cohort-based design to confirm causality. Second, there may have been some recall bias due to the high proportion of elderly patients, although that bias is likely to have been minimal as most of the patients had no cognitive impairment. The lack of statistical significance in some areas of this study may be due to the small sample size and the fact that the study population was restricted to patients at the OPD of Family Medicine, Maharaj Nakorn Chiang Mai Hospital, so the findings of the study may have limited generalizability. Future studies should include a larger and more diverse population to improve the generalizability.

CONCLUSION

A large proportion of the patients in this study consumed an excessive amount of food. Additionally, the group with poor glycemic control was more likely to consume a higher total energy intake as well as a greater proportion of carbohydrates and fat. Appropriate dietary control and management tailored to each patient's needs may be able to play an important role in improving nutritional status and glycemic control in diabetic patients.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Original Article **Open Access**

Reference Values of Nerve Cross-sectional Area Obtained by Ultrasound in the Upper Extremity Correlated with Electrodiagnosis in Thai Adults

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ABSTRACT

OBJECTIVE To evaluate the ultrasonography cross-sectional area (CSA) reference values of nerves in the upper extremity correlated with electrodiagnosis in healthy Thai adults.

METHODS A cross-sectional study was performed. Ninety participants were recruited and their CSA at 10 sites on the median, ulnar, and radial nerves were measured bilaterally. A nerve conduction study (NCS) was conducted and the correlations between the nerve CSA and age, sex, height, weight, body mass index (BMI), and NCS parameters were studied.

RESULTS The mean CSA ranged from 5.8±1.4 to 9.5±1.5 mm^2 along the median nerve and 4.5±0.8 to 7.7±1.7 mm^2 along the ulnar nerve. The mean CSAs of the radial nerve at the elbow and spiral groove were 5.0±0.9 and 4.6±0.8 mm^2, respectively. The CSA of the median nerve at the wrist and the CSA of the radial nerve at the spiral groove were positively correlated with weight and BMI, whereas the CSA of the median nerve at the elbow was positively correlated only with weight. There was an association between CSA values and electrodiagnosis parameters as the nerve CSA increased, as the latency was prolonged, and as the amplitude decreased.

CONCLUSIONS The reference values of nerve CSA in the upper extremity at multiple sites can be helpful in the evaluation of peripheral nerve disorders in the Thai population.

KEYWORDS cross-sectional area, ultrasonography, peripheral nerves, electrodiagnosis

INTRODUCTION

High-resolution ultrasound (HRU) is an emerging technology for evaluation of the peripheral nervous system because it is a non-invasive investigation which provides real-time, high-quality images. Peripheral nerves and their surroundings can be described morphologically using HRU. The cross-sectional area (CSA) obtained by HRU provides valuable information about nerve size and potential pathological changes. It has become clear that several conditions can result in an increase in nerve CSA. These conditions include entrapment, hereditary neuropathies, acquired neuropathies, trauma, and nerve tumors (1-5). Patients with amyotrophic lateral sclerosis, however, have significantly lower median and ulnar nerve CSAs than healthy controls (6). The nerve CSA reference values and their correlation with demographics have been reported

in several studies (7-11). Hsieh et al. examined reference values for CSA of peripheral nerves in the Taiwanese population, reporting that the nerve CSAs of Taiwanese individuals were smaller than those of Caucasians. Additionally, multiple sites exhibited a positive correlation between the nerve CSA and both weight and body mass index (BMI) (11). Other studies have reported that the nerve CSA varies significantly among ethnic groups (12, 13).

Electrodiagnostic study is an important component of the evaluation of patients with suspected peripheral nerve disorders. Previous studies have shown electrodiagnostic study and ultrasound to be similar with regard to sensitivity and specificity for diagnosis of carpal tunnel syndrome (14). Bathala et al. correlated CSA reference values for the ulnar nerves with electrophysiological parameters in Asian subjects. The results showed that CSAs increased with age, and that men had larger CSAs. The correlation between distal ulnar motor latency and CSA at the wrist in that study was statistically significant (15). Another study similarly evaluated CSA reference values for median nerves and their correlation with electrophysiological parameters in Asian subjects. The results found no correlation between electrophysiological parameters and height, weight, BMI, or median nerve CSA (16).

The nerve CSAs in the upper extremity in healthy Thai adults have never been studied. The aim of this study is to evaluate the ultrasonography CSA references values of nerves in the upper extremity correlated with electrodiagnosis in healthy Thai adults.

METHODS

Study design

This cross-sectional study was conducted at Lerdsin Hospital in Bangkok, Thailand from May 2022 to May 2023. The trial protocol was approved by the Lerdsin Hospital Ethics Committee (Number LH651011) and was registered in the Thai Clinical Trials Registry (TCTR 20220204006).

Participants

Asymptomatic, healthy Thai volunteers age 19-80 years were recruited. Exclusion criteria were signs and symptoms of neurological problems, alcoholism, diabetes mellitus, chronic kidney disease, liver disease, thyroid disease, autoimmune disease, immunodeficiency disorder, malnutrition, previous surgery with metallic implants on the upper extremities, pregnancy, patients with an implanted pacemaker, and patients with abnormal nerve conduction study (NCS) results based on the American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) reference values (17). All participants provided written informed consent. Age, sex, dominant hand, height,-weight, and calculated BMI were recorded for each participant.

Sample size

The sample size estimation was based on a study by Tan et al. (13). The sample size was determined using the estimation of an infinite population mean formula. We assumed $\alpha = 0.05$ and a margin of error of 0.06, and represented the mean CSA and the standard deviation (SD) of the median, ulnar, and radial nerves of each site. The maximum calculated sample size (radial nerve) of 80 participants was used. With an estimated drop-out rate of 10%, the target sample size was 88 participants. We attempted to recruit an equal number of males and females with 30 in each of three age groups: 18 to 30 years, 31 to 50 years, and 51-80 years. The final sample size was 90 participants.

Intervention

Electrodiagnosis studies

Two experienced, board-certified physiatrists performed a standardized NCS on each subject using a standard electrodiagnostic machine (Nicolet Synergy, Natus Medical Inc., San Carlos, California, USA) in accordance with AANEM recommendations (17). Motor and sensory NCS of bilateral upper and lower extremities was performed to screen for peripheral neuropathies, including median compound motor action potential (CMAP), median sensory nerve action potential (SNAP), ulnar CMAP, ulnar SNAP, sural SNAP, tibial CMAP, and fibular CMAP. Throughout the procedure skin temperature was maintained at between 32 and 34 degrees Celsius. NCS parameters of the median and

ulnar nerves were used to evaluate correlations with the CSA.

Median CMAP: the active electrode was placed on the abductor pollicis brevis motor point, and the stimulation sites were at the wrist (8 cm proximal to the active electrode) and the elbow (medial to the brachial pulse).

Ulnar CMAP: the active electrode was placed on the hypothenar eminence, and the stimulation sites were at the wrist (8 cm proximal to the active electrode) and the olecranon fossa.

Tibial CMAP: the active electrode was placed on the medial foot, and the stimulation sites were at the ankle (posterior to the medial malleolus and 8 cm proximal to the active electrode) and mid-popliteal fossa.

The fibular CMAP: the active electrode was placed on the midpoint of the extensor digitorum brevis, and the stimulation sites were at the ankle (lateral to the tibialis anterior tendon, 8 cm proximal to the active electrode) and below fibular head.

Median SNAP: the active electrode was placed on the index finger, and the stimulation site was at the wrist, 14 cm proximal to the active electrode.

Ulnar SNAP: the active electrode was placed on the little finger, and the stimulation site was at the wrist, 14 cm proximal to the active electrode.

Sural SNAP: the active electrode was placed posterior to the lateral malleolus, and the stimulation site was at the calf, 14 cm proximal to the active electrode.

Ultrasound examinations

All ultrasound examinations were conducted bilaterally by a single board-certified physiatrist with 6 years of experience in musculoskeletal ultrasound using 4- to 18- megahertz (MHz) linear array transducers (SONIMAGE HS1, Konica Minolta Inc., Tokyo, Japan) in B mode. The transducer was positioned perpendicular to the nerve to obtain the minimal cross-sectional image. Using the ultrasound machine's elliptical function, the CSA was measured at the inner border of the nerve's hyperechoic rim from the distal to the proximal arm. The color Doppler was used to distinguish between nerves and blood vessels. Based on the distance between the skin and the target area, the focus and depth were adjusted as necessary. The CSA was measured three times for each site for intra-rater reliability, and the average value was used for analysis. The inter-rater reliability evaluation was performed one week after the participant's first visit by a board-certified physiatrist with 1 year of experience in neuromuscular ultrasound who was blinded from the results. Thirty participants were chosen randomly. The average value of the three measurements was analyzed. Both investigators underwent specialized training for this investigation prior to commencing data collection. Anatomical landmarks or clinically significant points were used to select the measured sites of each nerve (Figure 1).

Median nerve: Participants were supine with 45 degrees shoulder abduction and the forearm supinated. Four locations along the median nerve were assessed: (1) wrist (proximal margin of the flexor retinaculum), (2) mid-forearm (halfway between the distal wrist crease and the elbow crease where the nerve is located between the flexor digitorum superficialis and flexor digitorum profundus muscles), (3) elbow (antecubital fossa), and (4) mid-humerus (halfway between the elbow crease and the axilla).

Ulnar nerve: Participants were supine with 45 degrees of shoulder abduction and external rotation, with the forearm supinated and the elbow flexed to 90 degrees. Four locations along the ulnar nerve were assessed: (1) wrist (between the pisiform bone and the ulnar artery at the distal wrist crease), (2) forearm (2 cm distal to the tip of the medial epicondyle), (3) elbow (the tip of medial epicondyle), and (4) mid-humerus (halfway between the elbow crease and the axilla).

Radial nerve: Participants were supine with the forearm pronated and the elbow fully extended. Two locations of the radial nerve were assessed: (1) elbow (antecubital fossa at elbow crease, where the nerve is located between the brachialis and brachioradialis muscles) and (2) spiral groove at mid-humerus.

Outcome measurements

The nerve CSA of each measured site was recorded. The wrist-to-forearm median nerve CSA ratio (WFR) was calculated by dividing the median nerve CSA at the wrist by the median

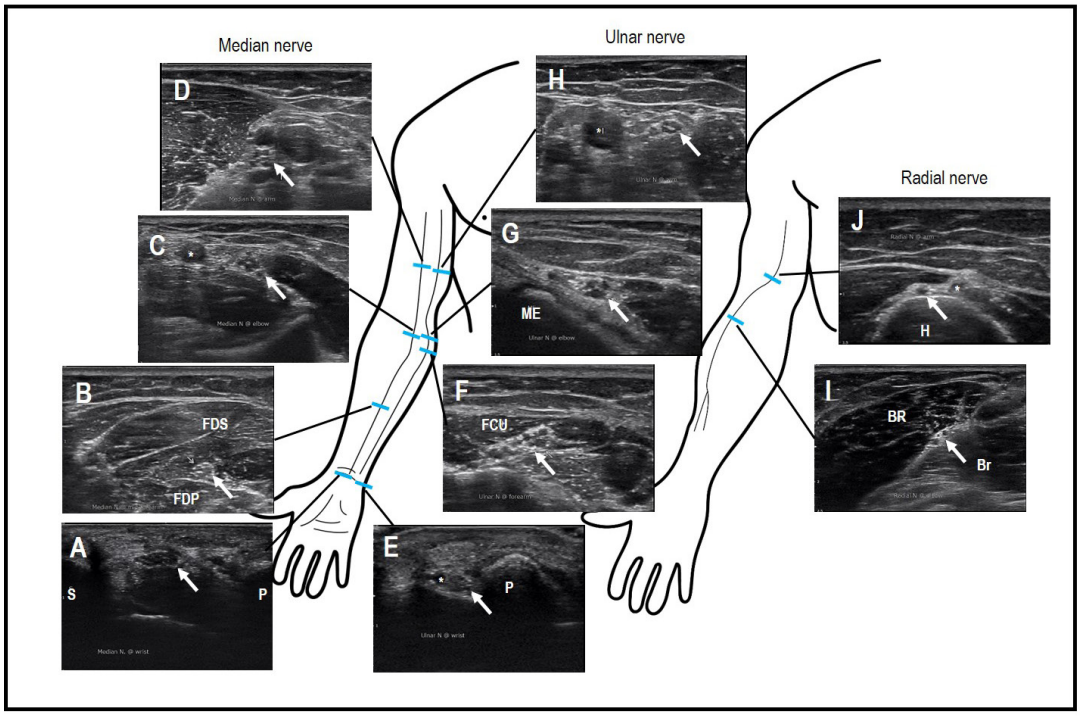

Figure 1. Ultrasonography of the nerve cross-sectional area (CSA) at each measured site. (A) median nerve at wrist, (B) median nerve at mid-forearm, (C) median nerve at elbow, (D) median nerve at mid-humerus, (E) ulnar nerve at wrist, (F) ulnar nerve at forearm, (G) ulnar nerve at elbow, (H) ulnar nerve at mid-humerus, (I) radial nerve at elbow, (J) radial nerve at spiral groove. Br - brachialis; BR - brachioradialis; FCU - flexor carpi ulnaris; FDP - flexor digitorum profundus; FDS - flexor digitorum superficialis; H - humerus bone; ME - medial epicondyle; P - pisiform bone; S - scaphoid bone. Arrows show the CSA of the nerves. The vessels are indicated by an asterisk (*).

nerve CSA at the mid-forearm. The NCS parameters were recorded, including SNAP latency, SNAP amplitude, CMAP distal latency, CMAP distal amplitude, CMAP distal area under the curve, CMAP proximal latency, CMAP proximal amplitude, CMAP proximal area under the curve, and nerve conduction velocity (NCV) of the median and ulnar nerves.

Statistical methods

Continuous data are presented as mean and SD and categorical data are presented as frequencies and percentages. The reference range for the nerve CSA was determined as the mean± 2SD. The upper limit reference values for the side-to-side difference were calculated by determining the mean of the absolute difference between the measurements on the right and left sides of each site plus 2SD. The Pearson's correlation coefficient (r) was used to determine the correlation between the nerve CSA and age, weight, height, BMI, and NCS parameters. Male and female nerve CSAs were compared using the unpaired t-test. The paired t-test was used to compare differences of the nerve CSA between two sides, while one-way ANOVA was used to compare differences among age groups. Statistical significance was set at a p-value of less than 0.05. The data were analyzed using the PASW Statistics version 18.0 program (SPSS Inc., Chicago, IL., USA).

RESULTS

A total of 90 healthy participants, 30 each in the groups 18-30 years, 31-50 years, and 51-80 years, with an equal number of males and females in each group were recruited for the study. The mean age was 41.4±14.5 years (range 19-72 years). The mean weight, height, and BMI were 64.1±13.8 kg, 163.2±8.5 cm, and 25±4.3 kg/m^2, respectively. Of the participants, 95.6% were right-handed (Table 1).

The nerve CSAs were obtained bilaterally. There was no significant difference in the CSA between the right and left sides, with the exception of the median nerve at the wrist (right: 9.4±2.1 vs. left 9.1±2.0 mm^2, p = 0.03). A bifid median nerve at the wrist was observed in 8

Table 1. Demographic data of the participants

Parameters[a]	Total (n=90) (SD) [range]	Male (n=45) (SD) [range]	Female (n=45) (SD) [range]
Age (yr)	41.4 (14.5) [19-72]	40.6 (14.4) [19-72]	42.2 (14.8) [23-70]
Weight (kg)	64.1 (13.8) [42-100]	69.4 (12.5) [45-100]	58.9 (13.1) [42-90]
Height (cm)	163.2 (8.5) [144-189]	169.2 (6.4) [157-189]	157.3 (5.8) [144-168]
BMI (kg/m^2)	25.0 (4.3) [15.2-34.1]	24.2 (4.1) [15.2-33.9]	23.7(4.5) [17.5-34.1]

[a]Mean (SD)
BMI, body mass index; kg, kilogram; cm, centimeter; m, meter; n, number; SD, standard deviation

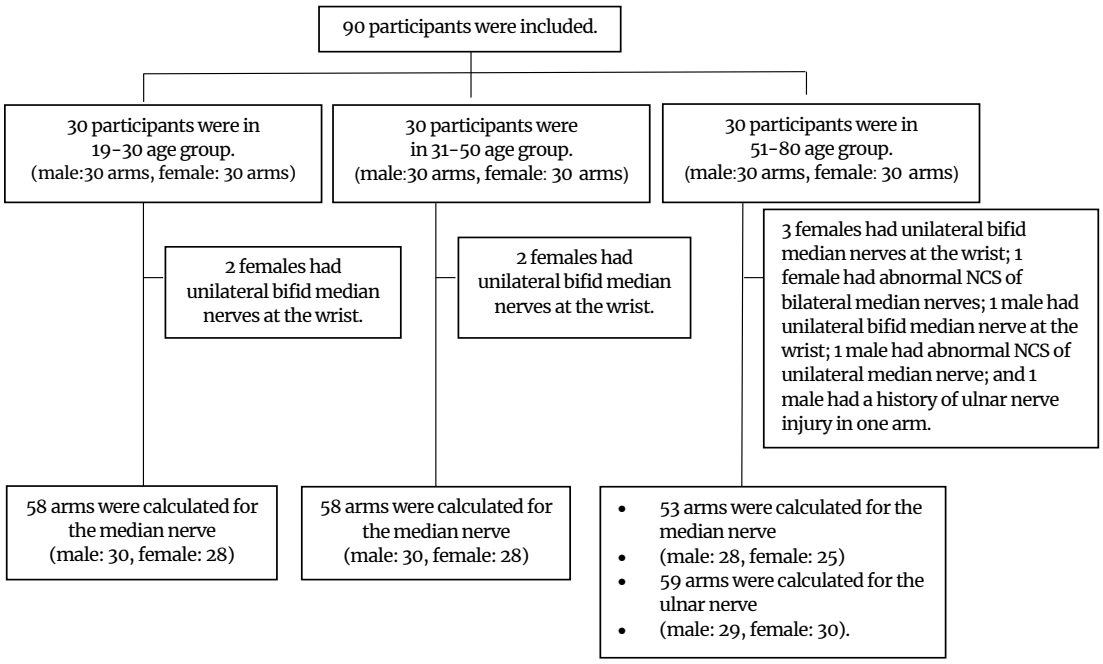

Figure 2. Participant flow chart

arms, and 3 arms had abnormal median NCS based on the AANEM reference values, so we included the CSA values of the median nerve in only 169 arms. The ulnar nerve was excluded in 1 arm because of a history of ulnar nerve injury, so 179 arms were used to calculate the CSA value of the ulnar nerve (Figure 2). During elbow flexion greater than 90°, ulnar nerve dislocation was observed in 16 of 169 arms (9.5%). The mean CSA and reference range for each measured site, the mean WFR, as well as the side-to-side difference upper limit, are shown in Table 2.

Table 3 shows the mean CSA values at each site and WFR for the different age groups (18-30 years, 31-50 years, and 51-80 years) and sex. The CSA at most sites was significantly larger in the older age groups except for the two measured sites of the radial nerve (Table 3). There were no significant differences in CSA between males and females except for the median nerve at the elbow, where males tended to have a larger CSA than females (Table 3). The correlation between age, weight, height, and BMI with the nerve CSA at each site and WFR is shown in Table 4. Except for the radial nerve at the spiral groove, the CSA at most sites increased significantly with age. The CSA of the median nerve at the wrist and that of the radial nerve at the spiral groove were positively correlated with weight and BMI, whereas the CSA of the median nerve at the elbow was positively correlated only with weight. The WFR was not significantly correlated with age, sex, weight, or height, but it was significantly correlated with BMI (Table 4).

We found a moderate correlation between CSA values and electrodiagnosis parameters between the median nerve CSA at the wrist and median SNAP latency, median SNAP amplitude, and median CMAP distal latency, and

Table 2. Nerve CSA (mm^2) reference values, WFR reference values, and side-to-side difference upper limit (mm^2)

Nerve	Sites (n)	Mean	SD	Min, Max	Reference range	Side-to-side difference upper limit
Median	Wrist (169)	9.2	2.0	6, 17	5.3-13.1	2.3
	Mid-forearm (169)	5.8	1.4	3.7, 15.2	3.0-8.5	1.8
	Elbow (169)	9.3	1.8	5.2, 15.5	5.8-12.9	3.3
	Mid-humerus (169)	9.5	1.5	6.0, 14.8	6.6-12.4	2.6
	WFR (169)	1.6	0.3	0.9, 2.7	1.0-2.3	0.7
Ulnar	Wrist (179)	4.5	0.8	3.0, 7.5	2.8-6.2	1.5
	Forearm (179)	7.2	1.4	4.5, 11.2	4.4-9.9	2.4
	Elbow (179)	7.7	1.7	5.2, 13.8	4.3-11.1	2.6
	Mid-humerus (179)	6.5	1.2	4.0, 10.3	4.2-8.9	2.7
Radial	Elbow (180)	5.0	0.9	3.5, 9.8	3.2-6.7	1.5
	Spiral groove (180)	4.6	0.8	3.2, 6.7	3.0-6.2	1.6

CSA, cross-sectional area; mm^2, square millimeter; WFR, wrist-to-forearm median nerve cross-sectional area ratio; n, number; SD, standard deviation; Min, minimum; Max, maximum
The reference range was determined as the mean±2SD. The side-to-side difference upper limit is the mean plus 2SD for the absolute value of the side-to-side difference in each site.

Table 3. The nerve CSA (mm^2) classified by age group and sex and its correlation

Nerve	Site	Age[a]				Sex[b]		
		18-30	31-50	51-80	*p*-value	Male	Female	*p*-value
		Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Median	Wrist	8.6 (1.7)	8.5 (1.4)	10.5 (2.2)	< 0.001*	9.4 (1.8)	8.9 (2.1)	0.215
	Mid-forearm	5.4 (0.7)	5.6 (1.0)	6.3 (2.0)	0.032*	5.8 (0.9)	5.7 (1.8)	0.845
	Elbow	8.8 (1.7)	9.3 (1.4)	10.0 (2.0)	0.034*	10.1 (1.5)	8.5 (1.7)	< 0.001*
	Mid-humerus	9.0 (1.3)	9.5 (1.3)	10.0 (1.6)	0.020*	9.8 (1.3)	9.2 (1.6)	0.053
	WFR	1.6 (0.3)	1.6 (0.3)	1.7 (0.3)	0.130	1.7 (0.3)	1.6 (0.3)	0.545
Ulnar	Wrist	4.3 (0.8)	4.4 (0.8)	4.9 (0.8)	0.013*	4.6 (0.9)	4.4 (0.8)	0.127
	Forearm	6.6 (1.2)	7.0 (1.3)	7.9 (1.3)	< 0.001*	7.4 (1.3)	6.9 (1.4)	0.064
	Elbow	7.1 (1.2)	7.3 (1.4)	8.6 (2.0)	< 0.001*	8.0 (1.6)	7.4 (1.8)	0.088
	Mid-humerus	5.9 (0.8)	6.6 (1.2)	7.2 (1.2)	< 0.001*	6.7 (1.0)	6.3 (1.3)	0.119
Radial	Elbow	4.8 (0.5)	5.0 (0.8)	5.2 (1.1)	0.186	5.0 (0.7)	4.9 (1.0)	0.533
	Spiral groove	4.5 (0.7)	4.6 (0.6)	4.7 (1.0)	0.592	4.7 (0.8)	4.4 (0.8)	0.070

[a]One-way ANOVA test, [b]Unpaired t-test, *statistically significant ($p < 0.05$)
CSA, cross-sectional area; mm^2, square millimeter; SD, standard deviation; WFR, wrist-to-forearm median nerve cross-sectional area ratio

between the median nerve CSA at the elbow and median CMAP proximal latency. As the nerve size increased, the latency was prolonged, and the amplitude decreased (Table 5). There was a weak correlation between the ulnar nerve CSA at the wrist and ulnar SNAP amplitude and ulnar CMAP distal amplitude, as well as between the ulnar nerve CSA at the elbow and ulnar CMAP proximal latency, ulnar CMAP proximal amplitude, and ulnar CMAP proximal area under the curve (Table 6). There was a significant correlation between the median nerve CSA at the wrist and the median NCV. As the CSA increased, the NCV slowed (Table 7). The intra-rater reliability was moderate to excellent (intraclass correlation coefficient (ICC) = 0.735-0.945), as was the inter-rater reliability (ICC = 0.735-0.984).

DISCUSSION

This is the first study to report reference values for the CSA of upper extremity nerves at multiple sites in healthy Thai adults. The CSA values observed in our study are comparable to those reported in studies of other Asian populations (7, 9, 18). In our study, we found the CSA of median nerve at wrist (9.2±2.0 mm^2), median nerve at elbow (9.3±1.8 mm^2), median nerve at mid-humerus (9.5±1.5 mm^2), ulnar

Table 4. Correlation between the nerve CSA and age, weight, height, and BMI

Nerve	Site	Correlation coefficient (*p*-value)[a]			
		Age	Weight	Height	BMI
Median	Wrist	0.461 (< 0.001*)	0.240 (0.024*)	-0.066 (0.539)	0.226 (0.033*)
	Mid-forearm	0.310 (0.003*)	0.034 (0.755)	-0.003 (0.981)	-0.008 (0.939)
	Elbow	0.307 (0.003*)	0.281 (0.008*)	0.008 (0.942)	0.157 (0.141)
	Mid-humerus	0.317 (0.002*)	0.138 (0.196)	-0.112 (0.297)	0.125 (0.242)
	WFR	1.183 (0.086)	0.205 (0.054)	-0.092 (0.390)	0.265 (0.012*)
Ulnar	Wrist	0.312 (0.003*)	0.145 (0.173)	-0.017 (0.876)	0.098 (0.359)
	Forearm	0.431 (< 0.001*)	0.200 (0.058)	-0.030 (0.776)	0.138 (0.194)
	Elbow	0.435 (< 0.001*)	0.129 (0.227)	-0.070 (0.514)	0.088 (0.407)
	Mid-humerus	0.398 (< 0.001*)	0.178 (0.094)	-0.082 (0.440)	0.167 (0.116)
Radial	Elbow	0.264 (0.012*)	0.172 (0.105)	-0.071 (0.509)	0.142 (0.182)
	Spiral groove	0.150 (0.158)	0.289 (0.006*)	-0.112 (0.295)	0.226 (0.032*)

[a]Pearson's correlation coefficient (r); *statistically significant ($p < 0.05$)
CSA, cross-sectional area; BMI, body mass index; WFR, wrist-to-forearm median nerve cross-sectional area ratio

Table 5. Correlation between the median nerve CSA and NCS parameters

	CSA of median nerve at wrist[a]		CSA of median nerve at elbow[a]	
	r	*p*-value	r	*p*-value
Median SNAP lat	0.534	<0.001*	-	-
Median SNAP amp	-0.486	<0.001*	-	-
Median CMAP D lat	0.459	<0.001*	-	-
Median CMAP D amp	-0.170	0.112	-	-
Median CMAP D area	-0.067	0.533	-	-
Median CMAP P lat	-	-	0.384	< 0.001*
Median CMAP P amp	-	-	-0.110	0.307
Median CMAP P area	-	-	-0.044	0.685

[a]Pearson's correlation coefficient (r); *statistically significant ($p < 0.05$)
CSA, cross-sectional area; NCS, nerve conduction study; SNAP, sensory nerve action potential; CMAP, compound motor action potential; lat, latency; amp, amplitude; D, distal; P, proximal

Table 6. Correlation between the ulnar nerve CSA and NCS parameters

	CSA of the ulnar nerve at wrist[a]		CSA of the ulnar nerve at elbow[a]	
	r	*p*-value	r	*p*-value
Ulnar SNAP lat	-0.027	0.798	-	-
Ulnar SNAP amp	-0.308	0.003*	-	-
Ulnar CMAP D lat	0.067	0.531	-	-
Ulnar CMAP D amp	-0.240	0.023*	-	-
Ulnar CMAP D area	-0.140	0.187	-	-
Ulnar CMAP P lat	-	-	0.275	0.009*
Ulnar CMAP P amp	-	-	-0.292	0.009*
Ulnar CMAP P area	-	-	-0.223	0.035*

[a]Pearson's correlation coefficient (r); *statistically significant ($p < 0.05$)
CSA, cross-sectional area; NCS, nerve conduction study; SNAP, sensory nerve action potential; CMAP, compound motor action potential; lat, latency; amp, amplitude; D, distal; P, proximal

Table 7. Correlation between the CSA and NCV of the median and ulnar nerves

CSA	NCV of the median nerve[a]		NCV of the ulnar nerve[a]	
	r	*p*-value	r	*p*-value
Median nerve at wrist	-0.319	0.002*	-	-
Median nerve at mid-forearm	-0.058	0.589	-	-
Median nerve at elbow	-0.158	0.139	-	-
Ulnar nerve at wrist	-	-	-0.159	0.136
Ulnar nerve at forearm	-	-	-0.135	0.206
Ulnar nerve at elbow	-	-	-0.181	0.088

[a]Pearson's correlation coefficient (r); *statistically significant ($p < 0.05$)
CSA, cross-sectional area; NCV, nerve conduction velocity

nerve at elbow (7.7±1.7 mm^2), and ulnar nerve at mid-humerus (6.5±1.2 mm^2) were close to the values reported by Bae et al. (9.33±1.55 mm^2, 8.96±2.41 mm^2, 9.34±2.38 mm^2, 7.31±1.69 mm^2, and 6.37±1.56 mm^2 respectively) (9). Although the CSA values of the radial nerve in our study were smaller than those obtained by Bae et al. (elbow, 7.26±1.7 mm^2; spiral groove, 6.81±1.75 mm^2), they are comparable to those reported by Hsieh et al. (elbow, 4±1.4 mm^2; spiral groove, 5.1±1.6 mm^2) (11). Despite the similarities, the CSA values obtained in our study are greater than some previously published values for Asian participants (8, 13, 15, 16). These findings support a previous study conducted by Tan et al., which showed that nerve CSA was significantly different among Asian ethnic groups (13). In our study, the median nerve CSA at the wrist (9.2±2.0 mm^2) was larger than in a previous study that assessed the mean CSA of the median nerve at the wrist in healthy Thai adults (6.83±0.98 mm^2) (19). This difference might be due to the fact that our study included more participants (90 vs 44) and a wider age range (19-72 vs 30-57 years) than the Wanitwattanarumlug study. A number of studies have investigated the cut-off value of the CSA of the median nerve at the wrist for diagnosing carpal tunnel syndrome (CTS). Reported values range from 9 to 14 mm^2 (20). In contrast to our finding that the median nerve CSA at the wrist was 9.2±2.0 mm^2, a previous study found that using a cut-off value of 9 mm^2 provided high sensitivity and specificity in diagnosing CTS in the Thai population (21). This might be due to the use of different cut-off values for diagnosing CTS. Another possible reason is that the participants in the previous study did not include individuals older than 60 years (30-53 years in the control group and 32-59 years in the CTS group). A future study should investigate the cut-off value of each age group separately.

Demographic variables, including sex, age, height, weight, and BMI, were found to correlate with nerve CSA. In a number of previous studies, male nerve CSA was significantly greater than female nerve CSA (8, 9, 15, 16). In our study, males had a significantly larger CSA than females at only one of the ten sites (the median nerve at the elbow). This trend was also observed at the other measured sites, but without statistical significance. Previous studies have reported a positive correlation between nerve CSA and age (8, 15, 16, 22). In our study, we found a similar correlation: older participants had a significantly larger CSA than younger participants at most measured sites. In this study, weight was found to have a significant positive correlation with nerve CSA at 3 of 10 sites, while a positive correlation with BMI was found at only 2 sites. These results are consistent with previous research findings reporting that weight and BMI are correlated with nerve CSA (7, 9, 11, 13). In contrast, height had a very weak negative correlation with nerve CSA which was not statistically significant. These findings were similar to those of Niu et al., Tan et al., and Qrimli et al., which found that height had no significant correlation with nerve CSA (8, 13, 23). Based on these results, weight and BMI should be considered while assessing the nerve CSA for patients who are either overweight or underweight. The correlation between WFR and demographic factors in previous studies has shown varied results. Bae et al. reported that WFR was correlated with sex differences (9),

whereas Won et al. found no correlation between WFR and any demographic variables (7). Sugimoto et al. studied the CSA ratio of the median nerve between the distal wrist crease and the distal forearm and found the ratio was correlated with gender, age, height, and wrist circumference (18). In our study, we found that WFR is significantly correlated with BMI. The cut-off values of WFR for the diagnosis of CTS varied, ranging from 1.34 to 2.4, although a WFR of 1.3 and 1.4 showed a high sensitivity (20). Based on the finding of our study that the mean WFR in healthy Thai adults is 1.6±0.3, future studies should investigate the cut-off values of WFR for the diagnosis of CTS in the Thai population. There was no statistically significant difference in the CSA observed between the right and left sides at most measured sites, which is comparable to several previous studies (13, 15, 24). This finding suggests that the side-to-side difference could be used to detect unilateral nerve pathology.

There was a correlation between the CSA values and electrodiagnosis parameters as the nerve CSA increased, as the latency was prolonged, as the amplitude decreased, and as the area under the curve decreased, which is similar to a study by Bathala et al. which found that the ulnar nerve CSA at the wrist had a positive correlation with distal motor latency across the wrist (15). The CMAP amplitude indicates the number of responding motor nerve fibers, and the SNAP amplitude indicates the number of depolarized sensory axons (25). The area under the curve is an alternative way to estimate the quantity of depolarized muscle fibers and axons (26). Previous research has reported that the tibial nerve's histology exhibits a larger CSA as the nerve fascicle increases (27). This is contrary to our finding that in a healthy population as the CSA increases, the NCS amplitude decreases suggesting that the morphology of a nerve may not always correspond with its physiological properties (i.e., the number of nerve fascicles may not represent the number of functioning axons). The nerve CSA in a normal population could demonstrate an inverse relationship with the NCS amplitude. Nerve CSA should be considered for use in the interpretation of nerve pathology because several focal neuropathy conditions can also result in an increase in nerve CSA as well as a decrease in NCS amplitude (4, 5, 28). Although the correlations between the median and the ulnar nerve CSAs and the NCS parameters were not significant for all parameters in this study, the correlations were in the same direction for almost all parameters. The results of this study provide information for future studies use in further investigation of the correlation between the nerve CSA and the NCS parameters as a primary outcome. A study with a larger sample size might show greater statistical significance. A significant correlation was observed between the median nerve CSA at the wrist and the median NCV. As the CSA increased, the NCV slowed, which could be due to the nerve being slightly thicker at the entrapment site (8, 12, 16). Even routine activities like using a computer keyboard and mouse put the wrist in a posture that increases carpal tunnel pressure, compromising blood flow to nerves and putting users at risk for median nerve damage (29). There is a possibility of the existence of some degree of asymptomatic nerve damage with the NCS still showing normal values as the median nerve at the wrist tends to be larger and have a slower NCV.

Limitation

Our study has several limitations. First, we used elliptical function to measure the nerve CSA, which might not have included some nerve fascicles, especially nerves that have an irregular circumference. Second, the more proximal parts, such as the median and ulnar nerves at the axilla, were not studied. A future study should include the more proximal part of the nerves. Third, although the sample size of 90 participants was sufficient to evaluate the primary outcome, i.e., the nerve CSA, this sample size might not be large enough to evaluate the secondary outcomes (correlation between the nerve CSA and the NCS parameters) and the correlation between the nerve CSA and demographic data. Future studies investigating the correlation between the nerve CSA and the NCS parameters and the correlation between the nerve CSA and subgroup demographic data as a primary outcome are suggested. Fourth,

repeat reliability was not performed in this study. In future studies, repeat reliability of the same rater should be accomplished to help assure the accuracy of the results. Lastly, to investigate deeper structures, e.g., in an extremely obese participant, results would be more accurate using high-frequency ultrasound (24-70 MHz). There were also some positive aspects to this study. First, NCS of all extremities was performed to ensure that the participants did not have peripheral nerve disorders. Second, a single experienced physician was responsible for all ultrasonography. Finally, there was moderate to excellent inter-rater reliability in our study, which indicates that the measures had good accuracy.

CONCLUSIONS

Our study was the first investigation of reference values of the nerve CSA obtained by ultrasound in the upper extremities at multiple sites in healthy Thai adults. The mean CSA of the median nerve at the wrist was 9.2±2.0 mm^2, and the mean CSA of the ulnar nerve at the elbow was 7.7±1.7 mm^2. This should be helpful in the evaluation of peripheral nerve disorders in the Thai population. There was an association between the CSA values and electrodiagnosis parameters as the nerve CSA increased, the latency was prolonged, and the amplitude decreased.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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Original Article **Open Access**

Multi-Target Actions of Flavonoid Derivatives from *Mesua ferrea* Linn Flower against Alzheimer's disease Pathogenesis

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ABSTRACT

OBJECTIVE Kaempferol-3-*O*-rhamnoside (compound 1) and quercetin-3-*O*-rhamnoside (compound 2), two flavonoids isolated from *Mesua ferrea* L. flowers, were examined for their activities related Alzheimer's disease (AD) pathogenesis including antioxidant, acetylcholinesterase (AChE) inhibition, anti-beta amyloid (Aβ) aggregation and neuroprotection.

METHODS The two flavonoids were isolated from *M. ferrea* L. flowers using the column chromatography technique. Both compounds were evaluated for their effects on AD pathogenesis, including antioxidant action by ABTS assay, AChE inhibition by Ellman's method, and anti-Aβ aggregation by thioflavin T (ThT) assay and neuroprotection by cell base assay. To explain the mechanism of AChE inhibition and anti-Aβ aggregation, binding interactions between the test compounds and AChE and Aβ were studied *in-silico*.

RESULTS Compounds 1 and 2 showed an ability to scavenge ABTS radicals, with IC_{50} values of 424.57±2.97 and 308.67±9.90 µM, respectively, and to inhibit AChE function with IC_{50} values of 769.23±6.23 and 520.64±5.94, respectively. ThT assay indicated that both compounds inhibited Aβ aggregation with IC_{50} values of 406.43±9.95 and 300.69 ±1.18 µM, respectively. The neuroprotection study revealed that the two flavonoids could reduce human neuroblastoma (SH-SY5Y) cell death induced by H_2O_2. The *in-silico* study showed that both compounds bound AChE at catalytic anionic and peripheral anionic sites. In addition, the test compounds prevented Aβ aggregation by interacting at the central hydrophobic core, the C-terminal hydrophobic region, and the important residues of Ile41.

CONCLUSIONS Together, the results showed that kaempferol-3-*O*-rhamnoside and quercetin-3-*O*- rhamnoside exhibit multiple mechanisms of action that are involved in the pathogenesis of AD including antioxidant, AChE inhibition, anti-Aβ aggregation, and neuroprotection.

KEYWORDS flavonoid rhamnosides, Alzheimer's disease, oxidation, beta amyloid, acetylcholinesterase, molecular docking

INTRODUCTION

The most common kind of dementia is Alzheimer's disease (AD), also known as senile dementia. The prevalence of AD gradually increases with age, reaching a maximum incidence rate of 50% in people over the age of 85 (1). The World Health Organization reported that AD affected more than 58 million individuals worldwide in 2021. According to estimates, there will be 88 million AD patients worldwide in 2050. The cost of treating and caring for AD patients worldwide was $957.56 billion in 2015, and it is estimated to rise to $2.54 trillion in 2030 and $9.12 trillion in 2050 (2, 3). The pathogenesis of AD has been found to be associated with many pathways, including aggregation of Aβ (4), formation of neurofibrillary tangles (5), lack of cholinergic neurotransmission (6), neuroinflammation (7), and oxidative stress (8). Currently, only five drugs (rivastigmine, galantamine, donepezil, memantine, and Namzaric®) have been approved by United States Food and Drug Administration (FDA) for the treatment of AD (9). However, these drugs, which are single target drugs, only help with palliative care; they have no effect on curing or preventing AD. Thus, compounds that can hit multiple targets linked to AD are still required.

Flavonoids, a major class of hydroxylated polyphenolic compounds found in vascular plants, have numerous benefits as food and medication. Flavonoids have the basic structure C6-C3-C6. Based on the position of ring B that ring C is connected to and the degree of ring C unsaturation, flavonoids can be classified into several subclasses such as flavanols, flavanones, flavones, flavanonols, isoflavones, and anthocyanidins (10). Currently, over 9,000 compounds with a flavonoid skeleton have been identified, some of which are coupled with sugars like glucose, xylose, arabinose, glucuronic acid and rhamnoside (11, 12). Flavonoids have been found to exhibit numerous biological activities and anti-AD effects including antioxidant, anti-AChE, anti-Aβ and anti-inflammatory (13).

A plant of the Calophyllaceae family called *Mesua ferrea* L. is widely scattered in tropical region like India, Thailand, and China. Several studies revealed that the extract of *M. ferrea* L. flower showed biological activities related to AD such as anti-inflammation (14), antioxidant (15), anti-Aβ aggregation, and AChE inhibitory action (16). A review of the literature found that kaempferol-3-*O*-rhamnoside, and quercetin-3-*O*-rhamnoside are the major compounds isolated from the flower of *M. ferrea* L. (17). Both kaempferol-3-*O*-rhamnoside, and quercetin-3-*O*-rhamnoside have various biological activities, e.g., anti-cancer (18), anti-diabetic (19), and antiviral (20). However, there are no reports of flavonol rhamnoside as being anti-AD. The objectives of this study were to isolate the flavonoid rhamnosides from *M. ferrea* L. flower and to assess their activities associated with AD including antioxidant, AChE function, Aβ aggregation, and neuroprotection. To clarify the mechanism of AChE inhibition and anti-Aβ aggregation, binding interactions between the flavonoid rhamnosides and AChE or Aβ were also studied *in-silico*.

METHODS

Materials

The powder of *M. ferrea* L. flower was obtained from Chao Phya Abhaibhubejhr Hospital, Prachinburi Province, Thailand. It was identified by Benjawan Leenin, leader of the Traditional Knowledge Center, Chao Phya Abhaibhubejhr Hospital Foundation. The herbarium voucher specimen of *M. ferrea* L. was deposited at the museum of Chao Phya Abhaibhubejhr Hospital with voucher number YPJ013. The reference compounds, i.e. trolox, tacrine, curcumin, N-acetyl cysteine (NAC) and other chemicals like 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), acetylcholinesterase from *Electrophorus electricus* (electric eel), and Dulbecco's modified Eagle medium nutrient mixture F-12 (DMEM/F12), were purchased from Sigma-Aldrich (SM Chemical supplies Co., Ltd., Bangkok, Thailand). $A\beta_{1-42}$ was ordered from Abcam (Prima Scientific Co., Ltd., Bangkok, Thailand).

Extraction and isolation of flavonoids from *M. ferrea* L. flower extract

The powder of *M. ferrea* L. flower (3 kg) was extracted successively using hexane (3 × 4 L), EtOAc (3 × 4 L) and MeOH (3 × 4 L) at room

temperature. All the extracts were then evaporated using a rotary evaporator at 40-50 °C to obtain crude hexane (103 g), EtOAc (162 g), and MeOH (178 g) extracts. The MeOH extract was subjected to silica gel column chromatography (CC) and eluted with CH_2Cl_2 and MeOH using a gradient system to provide fractions F_1-F_9. Fraction F_6 (15 g) was purified by silica gel CC, eluted with a gradient system of CH_2Cl_2: MeOH (85:15→0:100, v/v) to give eight fractions, $F_{6.1}$-$F_{6.8}$. Fraction $F_{6.5}$ (3.2 g) was separated by CC using CH_2Cl_2: MeOH (95:5→0:100, v/v) to obtain nine subfractions, $F_{6.5.1}$-$F_{6.5.9}$. Subfraction $F_{6.5.8}$ was subjected to preparative thin-layer chromatography (PTLC) using CH_2Cl_2: MeOH (85:15) as the developing solvent to obtain compound 1 (229.1 mg). Fraction F_7 (13.3 g) was isolated by CC using silica gel, eluted with CH_2Cl_2: MeOH (85:15→0:100, v/v) to obtain eight fractions, $F_{7.1}$-$_{7.8}$. Fraction $F_{7.5}$ was subjected to reversed phase silica gel CC, eluted with a gradient system of H_2O_2, i.e., MeOH (2:1→1:1→0:1), to obtain five fractions, $F_{7.1}$-$F_{7.5}$. Fraction $F_{7.3}$ was subjected to Sephadex LH-20 CC and was eluted with 100% MeOH to obtain compound 2 (292.5 mg).

Identification of isolated compounds

For chemical structure elucidation, IR, 1H NMR, ^{13}C NMR, and MS, which are based on spectroscopic data, were used. IR spectra of the compounds 1 and 2 were recorded as KBr disks, using a Perkin Elmer Spectrum One FT-IR spectrophotometer. The 1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury plus spectrometer (Bruker, Model: Ascend-400) operating at 400 MHz and 100 MHz, respectively, using TMS as an internal standard. Liquid chromatography-high resolution electrospray ionization mass spectrometry (LC-HRESIMS) was used to accurately determine the mass of the isolated compounds.

***In vitro* analysis**

Antioxidant activity by ABTS assay

The effect of flavonoid rhamnosides on antioxidant activity was investigated using ABTS assay as described by Rajurkar et al. (21). Radical ABTS ($ABTS^{\cdot+}$) was generated by the oxidation of ABTS with potassium persulfate ($K_2S_2O_8$). A mixture of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ (1:1; v/v) was incubated in the dark for 12-16 h and kept at room temperature. A total of 150 µL of reaction mixture containing 100 µL of $ABTS^{\cdot+}$ and 50 µL of a sample was added to a 96-well plate. The plate was incubated for 30 minutes at room temperature in the dark and the absorbance of the samples was detected at a wavelength of 700 nm. The results are reported as IC_{50} values. Trolox was used as the reference standard.

Acetylcholinesterase activity by Ellman's method

The Ellman's method was used to measure AChE activity using a microplate reader. The AChE enzyme hydrolyses the substrate acetylthiocholine (ATCI) resulting in the product thiocholine. Thiocholine interacts with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to provide 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate, which can be measured at 405 nm. For the experiment, 25 µL of 1 mM ATCI, 125 µL of 1 mM DTNB, 25µL of the sample, and 50 µL of 0.2 U/mL AChE from an electric eel (type VI-S) were added to the 96-wells. The absorbance was measured at 405 nm every 30 s for 5 minutes using a microplate reader. The results are expressed as IC_{50} values (22). Tacrine was used as the reference compound.

Aβ aggregation activity by Thioflavin T assay

The Thioflavin T (ThT) assay was used to evaluate the Aβ aggregates. $Aβ_{1-42}$ was dis-solved in pH 7.4 phosphate-buffered saline (PBS) and kept at -20 °C until use. To evaluate the effects on Aβ aggregation, 2 µL of the sample at various concentrations dissolved in DMSO was incubated with 18 µL of 10 µM $Aβ_{1-42}$ at 37 °C for 48 h in the dark. After 48 h, 180 µL of 5 µM ThT in a glycine/NaOH solution (pH 8) were added to the plate. Then, the fluorescence was measured after 5 minutes using a microplate reader with excitation at 446 nm and emission at 490 nm (23). Curcumin was used as a reference standard.

Neuroprotective analysis

The SH-SY5Y cells were cultured in a medium of DMEM/F12 supplemented with 10% FBS in a humidified 5% CO_2 incubator at 37 °C. To differentiate neural cells, the cells were cultured in a 75 cm^2 cell culture flask for 24 h. Then the cells were differentiated with 10 µM retinoic acid (RA) in 1% FBS in a culture medium for 6

days. Every three days, the media that contained RA were replaced (24). Before testing, the differentiated SH-SY5Y cells were cultured in 96-well plates at a concentration of 5 x 10^5 cells/mL and incubated for 48 h. After 48 h, the cells were pretreated either with the samples or the reference compound (n-acetylcysteine; NAC) for 2 h. For oxidative stress induction, cells were treated with 100 µl of 250 µM H_2O_2 for 2 h. Then the cells were stained with 100 µL of 0.5 mg/mL MTT for 2 h and identified using microplate reader at a wavelength of 550 nm (25).

In-silico binding interaction studies

The interaction between the targets and flavonoid rhamnosides was determined using molecular docking studies. AChE structure was obtained from the protein data bank (PDB: code: 2CEK). For the 3D optimization of the test compounds, Chem3D 15.1 was used. The AutoDock 4.2.6 program was used for the docking study. After the docking process, BIOVIA Dis covery Studio 2017 was used to analyze the interactions. For the Aβ fibril study, X-ray crystallography with PDB code 2BEG was used to create the template. The Autodock 4.2.6 program was used to perform the docking which was repeated for 100 runs using the Lamarckian genetic algorithm. The maximum number of generations and the energy evaluation of determination were set at 27,000 and 1,000,000, respectively. BIOVIA Discovery Studio 2017 was used to analyze the interactions (26).

Statistical analyses

For *in vitro*, the data are reported as means ±SD (n = 3-5). Statistical significance was determined by one-way analysis of variance (ANOVA). A p <0.05 was considered as statistically significant. The data were analyzed using IBM SPSS statistics 19.0 software.

RESULTS

Characterization of isolated compounds from *M. ferrea* L.

The MeOH extract of *M. ferrea* L. flower was isolated by column chromatography to obtain two flavonol rhamnoside compounds, kaempferol 3-*O*-rhamnoside (compound 1) and quercetin 3-*O*-rhamnoside (compound 2). For chemical structure elucidation, the spectrum data of compounds 1 and 2 were obtained from IR, ^{1}H NMR, ^{13}C NMR, and HRESIMS.

Compound 1. Kaempferol 3-*O*-rhamnoside, a yellow powder; IR $_{Vmax}$ 3391, 2978, 1656, 1361, 1177, and 839 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz, δ_H) δ 7.76 (2H, dd, *J* = 8.8 Hz, H-2′ and H-6′), 6.92 (2H, dd, *J* = 8.4 Hz, H-3′ and H-5′), 6.34 (1H, d, *J* = 1.6 Hz, H-8), 6.17 (1H, d, *J* = 1.6 Hz, H-6), 5.36 (1H, d, *J* = 1.6 Hz, H-1″), 4.21 (1H, d, *J* = 1.2 Hz, H-2″), 3.70 (1H, dd, *J* = 3.2, 8.8 Hz, H-3″), 3.30 (2H, m, H-4″ and H-5″), and 0.91 (3H, d, *J* = 5.6 Hz, CH_3-6″). ^{13}C NMR (CD_3OD, 100 MHz, δ_C) 178.06 (C-4), 165.71 (C-7), 161.77 (C-5), 160.18 (C-2), 157.72 (C-4′), 157.25 (C-9), 134.70 (C-3), 130.42 (C-2′ and C-6′), 121.18 (C-1′), 115.13 (C-3′ and C-5′), 104.17 (C-10), 102.07 (C-1″), 98.77 (C-6), 93.62 (C-8), 71.78 (C-4″), 70.70 (C- 2″), 70.60 (C-3″), 70.50 (C-5″), 16.10 (CH3-6″). HRESIMS: m/z 455.0951 $[M+Na]^+$.

Compound 2. Quercetin 3-*O*-rhamnoside, a yellow powder; IR $_{Vmax}$ 3391, 2932, 1655, 1359, 1173, 814 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz, δ_H) δ 7.32 (^{1}H, d, *J* = 2.0 Hz, H-2′), 7.30 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.91 (1H, d, *J* = 8.4 Hz, H-5′), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 1.8 Hz, H-6), 5.35 (1H, d, *J* = 1.6 Hz, H-1″), 4.22 (1H, dd, *J* = 3.2, 1.6 Hz, H-2″), 3.75 (1H, dd, *J* = 9.2, 3.2 Hz, H-3″), 3.43 (1H, dd, *J* = 5.9, 9.6 Hz, H-5″), 3.34 (1H, dd, *J* = 5.9, 9.6 Hz, H-4″), 0.94 (3H, d, *J* = 5.6 Hz, CH3-6″). ^{13}C NMR (CD_3OD, 100 MHz, δ_C) 178.20 (C-4), 164.62 (C-7), 161.82 (C-5), 157.91 (C-2), 157.12 (C-9), 148.39 (C-4′), 145.04 (C-3′), 134.84 (C-3), 121.56 (C-1′), 121.44 (C-6′), 115.51 (C-5′), 114.95 (C-2′), 104.47 (C-10), 102.09 (C-1″), 98.44 (C-6), 93.32 (C-8), 71.85 (C-4″), 70.71 (C-2″), 70.62 (C-3″), 70.50 (C-5″), 16.10 (C-6″). HRESIMS: m/z 449.1079 $[M+H]^+$.

Compound 1 was a yellow amorphous powder. The IR spectrum showed the presence of the OH and carbonyl functional groups at 3,391 cm^{-1} and at 1656 cm^{-1}, respectively. C-H stretching vibrations appeared at 2,978 cm^{-1}, C=C stretching at 1,361 cm^{-1}, asymmetric C-O-C stretching at 1,171 cm^{-1}, and C-H bending at 839 cm^{-1}. The protons at positions C-6 and C-8 of ring A of the flavonol skeleton were predicted by the two aromatic hydrogen signals with 'meta coupling' at δ 6.34 ppm (1H, d, *J* = 1.6 Hz) and 6.17 (1H, d, *J* = 1.6 Hz) which appeared in

the 1H NMR spectra. Compound 1 was predicted to contain OH groups at C-5 and C-7 of ring A. The protons at positions C-2′, C-3′, C-5′, and C-6′ were assumed to be responsible for two signals with "ortho coupling" in ring B at δ 7.76 ppm (2H, dd, *J* = 8.8 Hz) and 6.92 ppm (2H, dd, *J* = 8.4 Hz). The compound was anticipated to be a flavonol rhamnoside based on the existence of an anomeric proton signal at δ 5.36 ppm (1H, d, *J* = 1.6 Hz) and the absence of a specific signal for olefinic hydrogen at C-3. A sugar moiety was present as evidenced by the formation of an anomeric carbon signal at 102.07 ppm in the ^{13}C NMR spectrum. The location of the sugar moiety was determined to be in the C-3 OH group based on the correlation between the anomeric proton signal (5.37 ppm) and the anomeric carbon signal (102.07 ppm) which were identified by analyzing the HMBC spectral data. The sugar moiety was identified as rhamnose by the methyl signal seen at 0.91 ppm (3H, s) in the 1H NMR spectrum and at 16.10 ppm in the ^{13}C NMR spectrum. The peak at m/z 455.0951 $[M+Na]^+$ was identified by the HRESIMS data as having the chemical formula $C_{21}H_{20}O_{10}$ (calculated for $C_{21}H_{20}O_{10}Na$, 455.0954 m/z). From the data, the isolated compound was identified as kaempferol-3-*O*-rhamnoside (Figure 1). We confirmed compound 1 to be kaempferol 3-*O*-rhamnoside by comparing the spectrum data of the isolated chemical with prior studies (27).

Compound 2 appeared as a yellow, amorphous powder. The spectrum data of compound 2 were similar to that of compound 1. The IR data showed that the absorption at 3,391, 2,932, 1,655, 1,359, 1,173, and 814 cm^{-1} were vibrations of the OH group, C-H stretching, the carbonyl functional group, the C=C olefin ring, C-O-C stretching, and C-H bending, respectively. Five aromatic proton signals at δ 7.32 ppm (1H, d, *J* = 2.0 Hz), 7.30 ppm (1H, dd, *J* = 8.0, 2.0 Hz), 6.91 ppm (1H, d, *J* = 8.4 Hz), 6.38 ppm (1H, d, *J* = 2.0 Hz), and 6.20 ppm (1H, d, *J* = 1.8 Hz) were observed in the 1H NMR spectra and were anticipated to be from the protons at C6, C8, C-2′, C-5′, and C6′. In ring A of the flavonol skeleton, two meta-coupled aromatic protons at δ 6.20 and 6.38 ppm (*J* = 2.0 Hz) indicated proton substitutions. Two aromatic proton signals with meta-coupling appeared at δ 7.32 ppm (d, *J* = 2.0 Hz) and 7.30 ppm (dd, *J* = 2.0 Hz), confirming three proton substitutions in ring B. The ortho coupling between the proton at δ 6.91 ppm and the proton at δ 7.30 ppm had a coupling constant of *J* = 8.0 Hz. The ^{13}C NMR spectrum contained 21 carbons, including 6 rhamnosyl carbon signals, (16.10, 102.09, 70.71, 70.62, 71.85, and 70.50 ppm). From the peak at m/z 449.1079 $[M+H]^+$ (calculated for $C_{21}H_{20}O_{11}H$, 449.1083 m/z), the chemical formula was determined to be $C_{21}H_{20}O_{11}$. We identified compound 2 as quercetin-3-*O*-rhamnoside (Figure 1) by comparing the spectrum data of the isolated compound with relevant literature (27).

In vitro analysis

Antioxidant activity of the test compounds was measured by ABTS assay. The results indicated that both compounds 1 and 2 showed an ability to scavenge ABTS radicals with IC_{50} values of 424.57±2.97 and 308.67±9.90 µM, respectively. Compound 2 scavenged ABTS radicals better than compound 1. For AChE

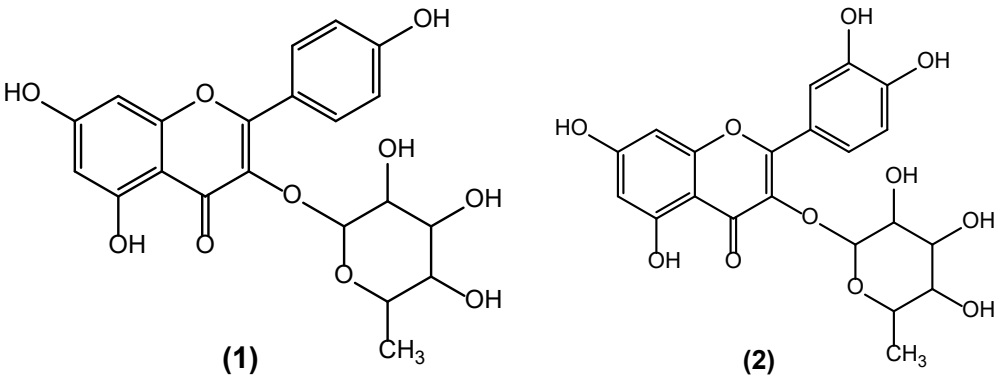

Figure 1. Structures of flavonoid rhamnosides: kaempferol-3-*O*- rhamnoside (compound 1) and quercetin-3-*O*-rhamnoside (compound 2).

inhibition, compound 1 showed an ability to inhibit AChE activity with IC_{50} 769.23±6.23 µM, while compound 2 could inhibit AChE function with an IC_{50} value of 520.64 ±5.94 µM. The investigation of the effect on Aβ aggregation showed that both compounds have an ability to inhibit Aβ aggregation with an IC_{50} values of 406.43±9.95 µM (compound 1) and 300.69 ±1.18 µM (compound 2). In summary, *in vitro* examination found that compound 2 showed better antioxidant activity as well as greater AChE and Aβ aggregation inhibition than compound 1. The results are presented in Table 1.

Flavonol compounds protect SH-SY5Y cells against H_2O_2-induced neurotoxicity

The neuroprotective effect against H_2O_2 toxicity of the flavonol compounds was determined in SH-SY5Y neuroblastoma cells. Before performing the neuroprotective assay, compounds 1 and 2 were tested for cytotoxicity against SHSY-5Y cells. The cells were treated with the test compounds at concentrations of 0.1, 1, 10, and 100 µM for 2 hours. The results showed that both test compounds were non-toxic to SHSY-5Y cells at concentrations up to 100 µM. Compounds 1 and 2 at the non-toxic concentrations of 0.1-100 µM were used in further neuroprotective effect investigation. In the neuroprotective assay, both compounds 1 and 2 showed neuroprotective effects against H_2O_2-induced neurotoxicity (Figure 2). Pretreatment of the cells with compound 1 at a concentration of 100 µM significantly reduced the cell viability loss induced by H_2O_2. Compound 2 greatly decreased the loss of cell viability induced by H_2O_2 at doses of 10 and 100 µM. The results obtained from the antioxidant assay indicate that both compounds exhibit antioxidant activity, indicating that the protective action against the H_2O_2 of compounds 1 and 2 might be enhanced by the antioxidant action.

Table 1. The activities related to AD of the flavonol compounds.

Test compounds	IC_{50} (µM)		
	Antioxidant	AChE inhibition	Anti-Aβ aggregation
Compound 1	424.57±2.97[a]	769.23±6.23[a]	406.43±9.95[a]
Compound 2	308.67±9.90[b]	520.64±5.94[b]	300.69±1.18[b]
Trolox	64.83±0.77[c]	ND	ND
Tacrine	ND	0.40±1.3[c]	ND
Curcumin	ND	ND	5.00±2.35[c]

Values expressed as mean ± SD (n = 3); [a,b,c] different letters in the same column are significantly different ($p < 0.05$).

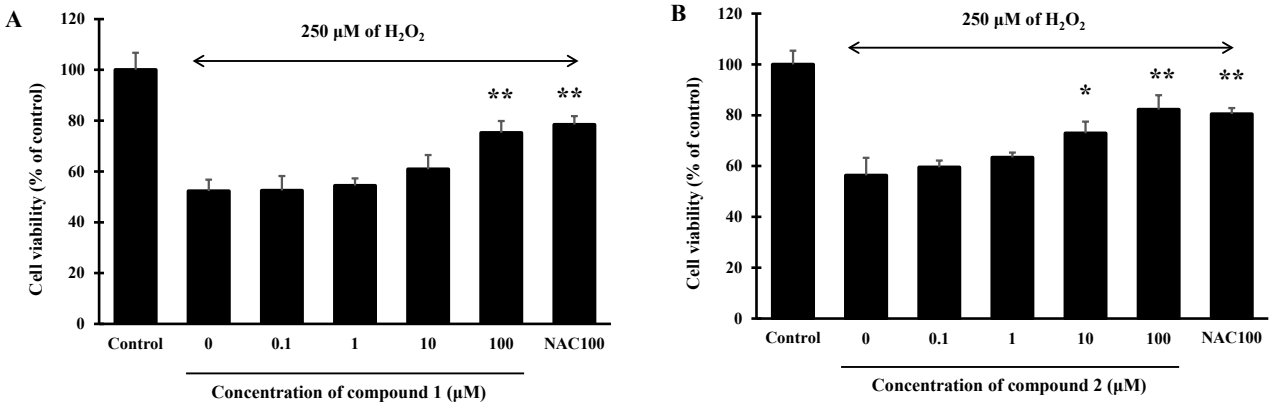

Figure 2. Effect of the flavonol compounds on H_2O_2-induced cell damage in SH-SY5Y cells: (A) compound 1; (B) compound 2. Data are means±SD (n=3) and $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the H_2O_2-treated group

In-silico binding interaction studies

Molecular docking studies of AChE inhibition

The binding interactions between flavonol rhamnosides and AChE were examined to further define the underlying mechanism utilizing the Autodock 4.2.6 and Discovery studio programs. Compounds 1 and 2 bound to AChE with binding energy values of -11.65 and -14.40 KJ/mol, respectively. The compound 1 occupied in middle gorge of the AChE active site by locating ring B in the peripheral anionic site (PAS) region and the rhamnoside structure in the catalytic active site (CAS). Rings A and C, which are the core structure of flavonoids, interacted with Trp84 via π−π stacking. Ring B formed a π−π stacking interaction with Tyr334 in the PAS regions. The OH group at position 3 of rhamnoside interacted with Ser220 and His440 in the CAS via hydrogen bonding. The OH group at position 5 of ring A interacted with His440 in the CAS via a hydrogen bond. The binding modes and interaction diagrams of compound 1 bound to AChE are presented in Figure 3A. The orientation of compound 2 is the same as compound 1, with ring A located in CAS and ring B in regions of the AChE site. The benzene rings (A) and a heterocycle pyrene ring (C) formed hydrophobic π−π stacking interaction with Trp84 of PAS residue. The hydroxy group at position 3 of rhamnoside interacted with amino acid residues Ser220 and His440 via hydrogen bonding inside the active pocket of AChE. In addition, His440 showed hydrogen bonding with the hydroxy group of ring A at position 5. The OH group at position 3 of ring B exhibited hydrogen bonding interaction with Ser122. Figure 3B shows the binding interactions that occurred between compound 2 and surrounding amino acid residues that are located at the active sites.

Molecular docking studies of Aβ inhibition

Two flavonol rhamnosides were investigated for binding interactions between flavonol rhamnoside and $Aβ_{1-42}$. The interactions were determined using the Autodock 4.2.6 program. Compounds 1 and 2 bound to $Aβ_{1-42}$ with binding energy values of -6.54 and -8.36 KJ/mol, respectively. The core structure of compound 1 interacted with amino acid residue Val40 via π-stacked by hydrophobic interaction in the C-terminal hydrophobic region. In addition, the OH group at position 4 of ring B formed a hydrogen interaction with Ile41. Substitution with OH groups at positions 5 and 7 of ring B interacted with amino acid residue Leu17 in the central hydrophobic region. Thus, the core structure of compound 1 is located to the central hydrophobic core region and the C-terminal hydrophobic region of $Aβ_{1-42}$, which is responsible for promoting fibril formation and

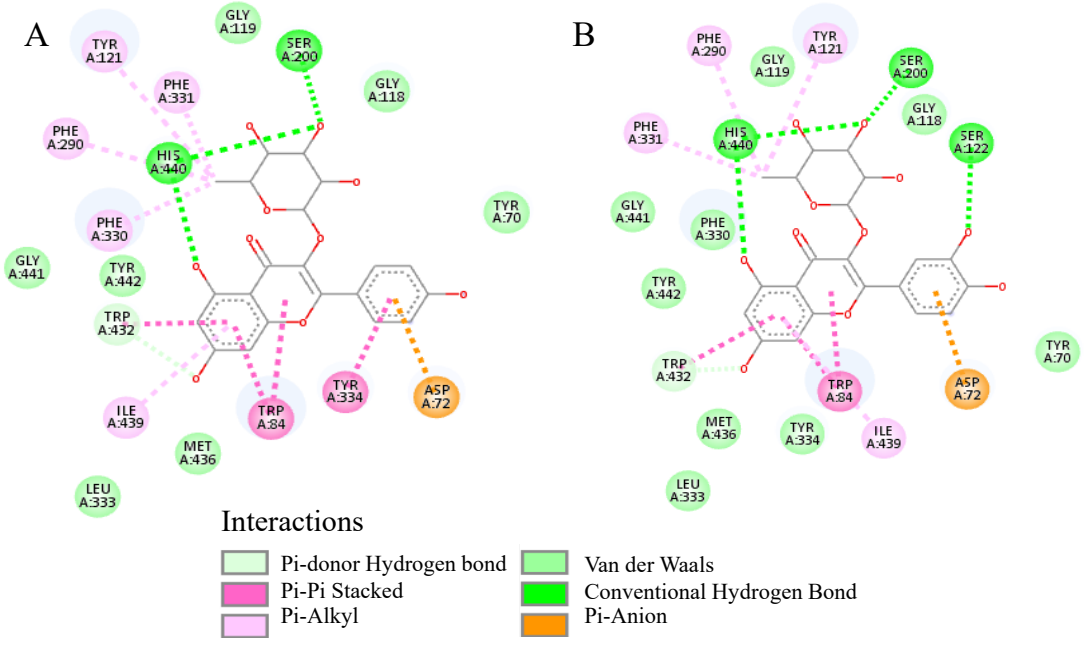

Figure 3. Binding interaction diagram of flavonol compounds: compound 1 (A), compound 2 (B) bound to AChE

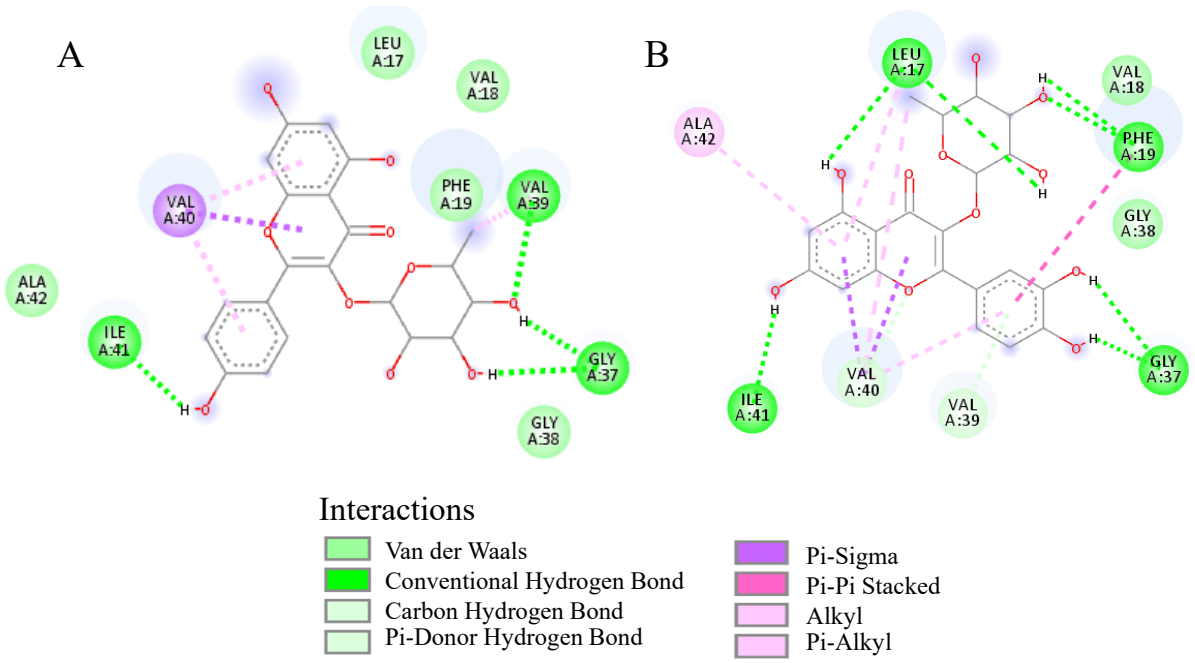

Figure 4. Binding interaction diagram of flavonol compounds: compound 1 (A), compound 2 (B) bound to $A\beta_{1-42}$

initiating nucleation. In addition, substitution with OH groups at positions 3 and 4 of rhamnoside interacted with amino acid residue Gly37 and Val39 via hydrogen bonding (Figure 4A). The binding orientation of compound 2 was the same as compound 1. The benzene rings (A) and a heterocycle pyrene ring (C) formed a hydrophobic π- sigma interaction with Val40 of the C-terminal hydrophobic region. The OH group at positions 5 and 7 of ring A interacted with Leu17 and Ile41 via hydrogen bonding. Ring B formed a π−π stacking interaction with Phe19. Amino acid residue Gly37 showed hydrogen bonding with the hydroxy group at positions 3 and 4 of ring B. The OH group at positions 2 and 3 of rhamnoside formed with Leu17 and Phe19, respectively, via hydrogen bonding in the central hydrophobic region (Figure 4B). These results indicate that these amino acids play a significant role in the networks of intra- and inter-molecular contacts that preserve the stability of fibrils through hydrogen bonding and hydrophobic interactions.

DISCUSSION

One of the direct causes of AD is oxidative stress. Overwhelming evidence suggests that oxidative stress is a factor in the development of AD and that it affects the brain tissue of patients. It is believed that oxidative stress, which is characterized by an imbalance in the creation of radical reactive oxygen species (ROS) and antioxidative defense, has a significant impact on age-related neurodegeneration and cognitive decline (28). Therefore, antioxidants might be useful for the prevention of oxidative stress in the AD brain. In this study, both flavonol compounds showed antioxidant activity in ABTS assay. The ability of compound 2 to scavenge ABTS radicals was higher than compound 1. These results revealed the importance of the OH group on position 3 of ring B of the flavonol skeleton as being important sites for scavenging free radicals. Normally, free hydroxyls on the flavonoid nucleus donate electrons, which results in the creation of less reactive aroxyl radicals (29). For this reason, it has been suggested that compound 2, which has two OH on ring B, is a stronger antioxidant than compound 1. Thus, it could be anticipated that the quantity of OH groups on ring B of the flavonol skeleton would affect the free radical scavenging ability. This is in accordance with previous research that found associations between the amounts of hydroxy groups on aromatic rings and their capacity to scavenge free radicals (30).

AD has been found to be associated with a decrease in the amount of acetylcholine in the brain, resulting in a cholinergic deficit. Acetylcholine is rapidly hydrolyzed by AChE at the cholinergic synapses, resulting in stopping

the transmission of nerve signals (31). Thus, it appears that inhibiting AChE function is an effective treatment approach to lessen, at least temporarily, the cognitive loss in AD. In the present study, the inhibitory activities of flavonol compounds against AChE were investigated by *in vitro* and molecular docking studies. In the *in vitro* study, both compounds showed an ability to inhibit acetylcholinesterase function. The degree of inhibition activity was found to vary depending on whether the structures of compound 2 provided stronger inhibition than compound 1. We confirmed that the OH group at the C-3 position in the structure of compound 2 increased AChE inhibitory activity and that it was essential for the occurrence of that activity. Similar results have been reported for quercetin and luteolin, with additional OH groups on the C-3 position of ring B showing activity higher than kaempferol and apigenin, respectively (32). According to the docking study, both flavonol rhamnosides concurrently occupy CAS and PAS of AChE. Interacting with His400 and Ser200 in CAS, the OH group of the rhamnoside structure plays a crucial role in the suppression of AChE activity. As a result, the flavonol rhamnoside attaches to the CAS of AChE and prevents ACh from being hydrolyzed. Moreover, the core structure of flavonoid could bind to the PAS via $\pi-\pi$ stacking with Tyr334 and Trp84, initiating the Aβ aggregation process.

The accumulation of toxic Aβ plaques in the brain is the key hallmark of AD pathogenesis. Therefore, the primary goal of numerous treatment approaches that are being developed or are undergoing clinical trials is to prevent or reduce the formation of Aβ plaques. $A\beta_{1-40}$ and $A\beta_{1-42}$ are the major elements of senile plaques (33, 34). According to several studies, $A\beta_{1-42}$ plays a significant role in the genesis of AD. According to previous research on transgenic mice and *in vitro* studies, $A\beta_{1-40}$ may produce amyloid plaques more slowly than $A\beta_{1-42}$ (35). Therefore, in order to evaluate the Aβ aggregation, we used $A\beta_{1-42}$. The cross-β structure of $A\beta_{1-42}$ fibrils consists of unstructured amino acid residues 1-17 at the N-termini, whereas amino acid residues 18-42 form the β-turn-β fold. There are two parallel β-turn regions, amino acid residues 17-21 and 29-35. These two β-strands are linked by a loop region where a salt bridge between Asp23 and Lys28 is created, supporting turn formation. The β-sheet structure is stabilized by two molecular interactions including that Phe19 packs against Gly38 and that Ala42 contacts the side chain of Met35. It has previously been determined that amino acid residues 17-21 in the central hydrophobic region and amino acid residues 39-42 in the C-terminal hydrophobic segment serve as the nucleation sites of Aβ aggregation. The steric zipper effect of these regions causes dimer formation and eventually greater aggregation. Interchain interactions are observed along with hydrogen bonds between the backbone of amino acid residues Val18-Val39, Asp23-Leu34, Lys28-Val36, Glu22-Met35, Val36-Ile41, and Phe20-Gly37. Additionally, it has been demonstrated that the addition of the amino acids Ile41 and Ala42 has a major impact on the development of AD. The production of paranuclei depends on amino acid Ile41, whereas the assembly of bigger oligomers requires Ala42 (36, 37). The central hydrophobic segment, the hydrophobic C-terminal, and the turn segment are vital areas of $A\beta_{1-42}$ that may enhance conformational shift, initiate nucleation, and encourage fibril formation. Compounds that have a potential to interact with these binding regions may be potent inhibitors of Aβ aggregation. In this study, both *in vitro* and *in-silico* methods were used to investigate the regulatory impact and mechanism of action of flavonol rhamnoside on $A\beta_{1-42}$ aggregation. In the *in vitro* study, we investigated the effects of two flavonol rhamnosides on the inhibition of Aβ aggregation using the ThT assay. The results showed that compound 2 could inhibit the aggregation of $A\beta_{1-42}$ more potently than compound 1. Compound 2 has many hydroxyls OH groups, which are crucial for inhibiting fibril growth by establishing hydrogen bonds through hydrophobic interactions between β-sheet structures and aromatic rings. By increasing the electron density in the aromatic rings, the hydroxyl OH group may improve the binding of compound 2 to the amino acid residue of β-sheet structures. The anti-amyloidogenic activity of the molecule increases with the number of OH groups

present in the structure (38). The results of the *in-silico* study showed the core structure of both compounds could form hydrophobic interactions with Val40 via π-stacked in the C-terminal hydrophobic region. For compound 1, substitution with OH group at position 4 of ring B formed a hydrogen bond only with Ile41, while compound 2 substitution with OH groups at positions 5 and 7 of ring A and at positions 3 and 4 of ring B enhanced hydrogen bonding with Leu17, Gly37 and Ile41. These amino acids play a significant role in the networks of intra- and intermolecular contacts that preserve the stability of fibrils through hydrogen bonding and hydrophobic interactions. The *in-silico* results indicate that the critical regions responsible for Aβ fibrillation and nucleation, the central hydrophobic segment and C-terminal hydrophobic area, as well as the residues at positions 41, may interact with flavonol rhamnoside to disrupt fibril stability.

Oxidative stress has been linked to AD, a neurodegenerative illness. An increase in the oxidation of brain lipids, carbohydrates, proteins, and DNA is characteristic of neurodegenerative disorders. Since the brain is a metabolically active organ, its levels of ROS are higher than those of other organs. The cell types most susceptible to free radical damage are neurons (39). Overproduction of ROS can result in cell death by damaging oxidative macromolecules. Oxidative stress has been linked to the onset and progression of neurodegenerative diseases, especially Alzheimer's disease (40). Therefore, reducing oxidative stress is one of the best ways to treat these diseases. Hydrogen peroxide (H_2O_2) acts as an inducer of oxidative stress damage by raising ROS levels and inducing cell death. H_2O_2 can also penetrate cell membranes and generate oxygen-derived free radicals. Thus, the Fenton reaction allows H_2O_2 to be transformed into hydroxyl radicals ($^{\cdot}OH$) in the presence of ferrous ions (Fe^{2+}) (41). For that reason, H_2O_2 was used to induce cell damage via oxidative stress. In this study, pretreatment of SH-SY5Y cells with compounds 1 and 2 greatly improved the survival of cells exposed to H_2O_2. The antioxidant abilities of both compounds could possibly explain their neuroprotective effects.

Overall, the results showed that two flavonol rhamnosides isolated from *M. ferrea* L. flower, kaempferol 3-*O*-rhamnoside and quercetin 3-O-rhamnoside, have multimode action related to the AD pathogenesis cascade, including antioxidants, anti-acetylcholinesterase, and anti-Aβ aggregation. In addition, both flavonol rhamnosides showed an ability to protect against neuronal cell damage induced by oxidative stress.

CONCLUSION

Two flavonoids isolated from *M. ferrea* L. flower, namely kaempferol-3-*O*-rhamnoside (1), quercetin-3-*O*-rhamnoside (2), were investigated for their activities involved with AD pathogenesis including antioxidant activity, AChE inhibition, anti-Aβ aggregation, and neuroprotection. Both compounds showed multifunctional activities targeting oxidation, AChE function and Aβ aggregation. The binding interactions between flavonol rhamnosides and AChE or $Aβ_{1-42}$ peptide were also examined in-silico to clarify the mechanism of action. The results demonstrated that both compounds could bind to AChE at both the CAS and the PAS, thus preventing the hydrolysis of ACh and Aβ aggregation. *In-silico* results revealed that both compounds might inhibit $Aβ_{1-42}$ aggregation by interacting with the residues of Ile41, the central hydrophobic core, and the C-terminal hydrophobic region of $Aβ_{1-42}$which are the important regions responsible for Aβ nucleation and fibrillation. Flavonoid rhamnosides were found to be multi-target agents that have the potential to prevent AD pathogenesis. Further studies to elucidate the mechanisms of action and the investigation in animal AD models, an important step in the search for new drug candidates, should be undertaken.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ADDITIONAL INFORMATION

Author contributions

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

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Knowledge, Attitudes, Practices, and Acceptance of Gynecologic Cancer Patients Toward Covid-19 Vaccine in Thailand: A Multicenter Cross-sectional Study

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ABSTRACT

OBJECTIVE To evaluate the knowledge, attitudes, practices (KAP), and acceptance of gynecologic cancer patients from the 4 regions of Thailand related to the COVID-19 vaccine.

METHODS Gynecologic cancer patients from Chiang Mai University Hospital, Khon Kaen University Hospital, Khon Kaen Hospital, Prince of Songkhla University Hospital, and the National Cancer Institute (NCI) in Bangkok were surveyed using a WHO survey instrument.

RESULTS Between February and September 2022, 1,263 patients participated in this project of whom 1,084 (85.8%) had received the COVID-19 vaccine. The highest rate of vaccination was the NCI followed by Khon Kaen, Chiang Mai, and Songkhla. Of the participants, 28.2% were infected with COVID-19 and 12.9% of the infected participants were unvaccinated. Regarding KAP, the average scale level of overall participants reported ease in obtaining health literacy, a moderate probability of having severe COVID-19 infection, knowledge and adoption of proper appropriate behavior for the prevention of COVID infection, little stress regarding possible COVID-19 infection, and quite a lot of a significant level of trust in healthcare workers. Most participants generally agreed with the lifting of some regular rules to reduce the risk of infection and often many expressed a feeling of general well-being. The significantly different levels of rating scale by unvaccinated and vaccinated participants in the key areas were as follows: (Patients rated each of the areas investigated on a scale of 0 to 6, with 0 indicating lowest level of agreement/acceptance and 6 indicating the highest level) health ministry recommendations (3.92 vs. 4.16), ease of getting the COVID vaccine (3.6 vs. 3.9), "no need to receive the vaccine due to the disease being rare" (2.6 vs. 2.2), "stress made me not want to get vaccinated" (2.6 vs. 2.1), "if everyone is vaccinated, no need for me to vaccinate" (2.5 vs. 1.9), and the importance of COVID-19 vaccines (3.7 vs. 4.2).

CONCLUSIONS Most gynecologic cancer patients from the 4 regions of Thailand had received the COVID-19 vaccine and exhibited good knowledge, attitudes, and practices related to this pandemic.

KEYWORDS COVID-19-vaccine, gynecologic cancer patients, attitude, practice

INTRODUCTION

Although worldwide the outbreak of COVID-19 nowadays was not as severe as in 2020, occasional COVID cases still did occur. According to the WHO Coronavirus (COVID-19) dashboard, 768,187,096 confirmed cases of COVID-19, including 6,645,714 deaths were reported in June 2023. In Thailand from 3 January 2020 to 21 June 2023 there were 4,749,910 confirmed cases of COVID-19 with 34,292 deaths reported, with the peak incidence between June 2021 and June 2022 (1). The COVID-19 pandemic in Thailand spread in five waves. In the first wave (March-May 2020), transmission was linked to boxing events and entertainment venues in Bangkok. The second wave (December 2020-January 2021) was triggered by the spread among migrants working at the Central Shrimp Market, Samut Sakhon Province. In the third wave (April-June 2021), the increase of COVID-19 cases was linked to an entertainment venue in the Thonglor District of Bangkok. That wave was severe and was driven by the highly transmissible Alpha type, resulting in more hospitalizations and an increased strain on healthcare services. During this third wave, many vaccination campaigns were rolled out on 7 June 2021. Two vaccine types (Sinovac and Astra Zeneca) were available at that time. The fourth wave (July-December 2021) was caused by the highly contagious Delta variant which was transmitted faster than the alpha type. The fifth wave (January-March 2022) was caused by the Omicron variant which increased the risk of infection but with a less severe clinical presentation (2, 3). At that time, more vaccine options, e.g., the m-RNA vaccine, were available.

To reduce the incidence of COVID-19 infection and the mortality rate, social distancing, face masks, and personal hygiene measures were introduced. Those measures, combined with herd immunity produced by vaccination were seen as the most effective preventive measures (4). One major concern for infected COVID-19 patients with comorbidities including chronic obstructive pulmonary disease (COPD), diabetes mellitus, hypertension, and especially cancer, was the risk of developing severe symptoms (5). Cancer patients could potentially become immunocompromised by the effects of antineoplastic therapy, supportive medications such as steroids, and the immunosuppressive properties of the cancer itself, factors which increase the risk for COVID-19-related morbidity and mortality (6). Saini et al. (7) performed a systematic review of 52 studies involving a total of 18,650 cancer patients infected with COVID-19 and found the probability of death to be as high as 25.6%. Patients with cancer can benefit from having good knowledge, attitudes, and practices (KAP) related to the disease and are a key target population for COVID-19 vaccination. A study in India surveyed these issues in 521 gynecologic cancer patients and found overall good KAP related to the COVID-19 pandemic (8). Regarding COVID-19 vaccine acceptance among cancer patients, Chun et al. (9) recently reported the acceptance of COVID-19 vaccination among cancer patients in Korea was only 61.8%. A study by Prabani et al. (10) included a systematic review of 29 studies and found the pooled prevalence of COVID-19 vaccination acceptance was 59%. However, the data on KAP and COVID-19 vaccination acceptance in Thai gynecologic cancer patients was limited. Understanding the level of KAP in a population and knowing the COVID-19 vaccination rate is helpful for designing preventive strategies and health promotion guidelines.

METHODS

Patients Selection

This study multicenter cross-sectional study included five gynecologic cancer institutes in four provinces representing the 4 regions of Thailand: Chiang Mai University Hospital (Chiang Mai, northern region), Khon Kaen University Hospital (Khon Kaen, northeast region), Khon Kaen Hospital (Khon Kaen, northeast region), and Prince of Songkhla University Hospital (Songkhla, southern region), plus the National Cancer Institute (NCI) (Bangkok, central region). After receiving approval from each local Research Ethics Committee, this cross-sectional study was conducted between February and December 2022. The inclusion criteria consisted of gynecologic cancer patients treated at one of the institutes who were able to verbally communicate well in the Thai language.

Patients who received the COVID-19 vaccine before being diagnosed with gynecologic cancer were excluded.

Eligible patients were invited to participate in the study and all provided informed consent. The patients were interviewed for 15-20 minutes by well-trained interviewers, completing the World Health Organization (WHO) Regional Office for Europe survey tool (11) while they were waiting to see physicians at the outpatient unit. The WHO survey instrument served as a guide for conducting COVID-19 behavioral insights research. The questionnaire covered of 16 issues: 1) demographic data, 2) history of COVID-19 infection, 3) data on COVID-19 vaccination status, 4) health literacy regarding COVID-19 from most difficult to easiest rating scale 1 to 7), 5) probability and severity of COVID-19 infection (rating scale 1 to 7 from never to the most, 6) behavior related to prevention of COVID-19 infection in the previous seven days (rating scale 1 to 7 from never to always), 7) impact of COVID-19 (rating scales 1 to 7 from the most to least), 8) level of trust in the source of information (rating scale 1 to 7 from the least to most), 9) level of trust in the Institute to handle COVID-19 infection (rating scale 1 to 7 from the least to the most), 10) the lifting of regular rules for the COVID-19 pandemic (rating scale 1 to 7 from strongly disagree to strongly agree), 11) unwanted behavior in the previous two weeks during the COVID-19 pandemic (3 options: yes, no, not regularly), 12) general level of well-being in the previous two weeks during the COVID-19 pandemic (rating scale 1 to 7 from always to never), 13) opinion regarding not receiving COVID-19 vaccination (rating scale 1 to 7 from strongly disagree to strongly agree), 14) factors related to affecting the decision to vaccinate (rating scale 1 to 7 from least influential to most influential), 15) impact of side effects of COVID-19 vaccine (rating scale 1 to 7 from the lowest to highest) 16) the encouraging factors to receive the COVID-19 vaccine (2 choice answer; yes or no).

Following the validation of the Thai translation of the original English version by forward and backward translations and a pilot study with 63 participants and further study with 300 participants at Chiang Mai University Hospital, all interviews were conducted in Thai and were compiled for further analysis. Details of COVID-19 vaccination status and basic clinical data such as age, type of cancer, education level, underlying diseases, and the status of cancer treatment were reviewed as well.

Sample size calculation

The primary outcome of this study was the proportion of Thai gynecologic oncology patients who were willing to receive the COVID-19 vaccine. A previous study in Korea found 62% of cancer patients were amenable to obtaining the COVID-19 vaccine (9). The sample size for this study was calculated using the formula for estimating an infinite population proportion (12). This study needed 1,200 participants divided into 4 regions.

Statistical analysis

Statistical analysis was carried out using IBM SPSS statistics V22.0 for Windows program (IBM, Armonk, New York, USA). Means and standard deviations were calculated for descriptive analysis. The Chi-square or Fisher's exact test was used for comparative analysis of aspects of the factors, and the ANOVA test was used to compare differences in attitude among the provinces from four regions and differences in attitude between the COVID-19 vaccinated and non-vaccinated groups. A $p < 0.05$ was considered statistically significant.

Ethical approval

The study was approved by Research Ethics Committees as follows:

Chiang Mai University: Research ID: 08662 Study Code: OBG-2564-08708

Prince of Songkla University: Research ID: REC.65-119-12-1

Khon Kaen University: Research ID: HE651078

Khon Kaen Hospital: Research ID:KE-MOU65014

The National Cancer Institute Research ID:EC COA 013/2022

RESULTS

Demographic data of the participants

A total of 1,263 patients participated in this study. The number of patients in each center

was as follows: Chiang Mai University Hospital (363), Khon Kaen University Hospital (150), Khon Kaen Hospital (150), Prince of Songkhla University Hospital (300), and the National Cancer Institute (NCI) (300). Demographic data of the participants are presented in Table 1. The mean age was 54.56 years old and the most common type of gynecologic cancer was cancer of the cervix. Approximately 40% of the participants had completed elementary school. Most of the participants did not work in the medical field. Half the subjects had been diagnosed with an underlying condition, and roughly 60% were presently under cancer surveillance.

Table 1. Demographic data of all participants

	N (%)
Mean age ± SD (years)	54.46±12.58
Type of cancer	
Cervix	507 (40.1)
Uterus	316 (25.0)
Ovary	355 (28.1)
Fallopian tube	23 (1.9)
Other*	62 (4.9)
Education	
No formal education	42 (3.3)
Elementary	497 (39.4)
High school	342 (27.1)
Bachelor	297 (23.5)
Beyond bachelor	85 (6.7)
Health-professional	
Yes	71 (5.6)
No	1186 (93.9)
Missing	6 (0.5)
Underlying disease	
Yes	641 (50.8)
No	587 (46.5)
Unknown	35 (2.7)
Household membership	
Alone	107 (8.5)
Children under 18-year-old	256 (20.3)
People over 65 years and/or with chronic disease	216 (17.1)
Other	684 (54.1)
Personal financial situation over the past 3 months	
Improved	63 (5.0)
Unchanged	718 (56.8)
Worse	441 (34.9)
Unknown	42 (3.3)
Cancer treatment status	
Currently undergoing treatment	468 (37.1)
Surveillance	795 (62.9)

*Other: primary peritoneal carcinoma (9), vulvar cancer (14), vaginal cancer (11), gestational trophoblastic neoplasia (28)

COVID-19 infection experience

Regarding COVID-19 experience, only 357 (28.3%) had been previously infected with COVID-19 and most of those cases (330) were a mild form. Only 27 cases (2.1%) presented with a severe form; of those, only two had not received the COVID-19 vaccine. Eight of the 27 severe cases of COVID-19 infection were currently receiving cancer treatment. The incidence of COVID-19 infection was not significantly different between participants who were vaccinated for COVID-19 and those who were unvaccinated (28.7% versus 23.6%, p = 0.577 (95% confidence interval 0.544-0.598) by chi-square test).

COVID-19 vaccination

Most of the patients (73.8%) had received two to three doses of COVID-19 vaccine as of the time of the survey (Table 2). The highest vaccination rate (at least one dose) was in Bangkok (93.3%) followed by Khon Kaen (87.7%), Chiang Mai (82.9%), and Songkhla (80.8%). This difference was statistically significant (p < 0.001, 95% confidence interval 0.000-0.002) by Chi-square test. The majority of the vaccinations were Sinovac and AstraZeneca (Table 3). A significantly higher proportion of the participants who were currently under surveillance status for their cancer had been vaccinated than those in the ongoing cancer treatment phase (94.5% vs.82.9%, p < 0.001 (95% confidence interval <0.000-0.000), Chi-square test).

Table 2. COVID-19 vaccination status

Doses	N (%)
Never	179 (14.2)
1st dose	50 (4.0)
2nd dose	492 (39.0)
3rd dose	440 (34.8)
4th dose	99 (7.8)
More than 4 doses	3 (0.2)
Total	1,263

Vaccination received during cancer surveillance = 751 cases (94.5% of participants in this status)
Vaccination received during treatment = 388 cases (82.9% of participants in this status)

Table 3. Details of COVID-19 vaccine by type and dosage

	Dose 1 (%)	Dose 2 (%)	Dose 3 (%)	Dose 4 (%)	Dose 5 (%)
Chiang Mai					
Sinovac	110 (30.3)	31 (8.5)	-	-	-
AstraZeneca	117 (32.2)	144 (39.7)	31 (8.5)	-	-
Pfizer	26 (7.2)	66 (18.2)	101 (27.8)	11 (3.0)	-
Moderna	14 (3.9)	16 (4.4)	23 (6.3)	9 (2.5)	-
Sinopharm	34 (9.4)	33 (9.1)	1 (0.3)	-	-
Not received	62 (17.0)	73 (20.1)	207 (57.1)	343 (94.5)	363 (100.0)
Khon Kaen					
Sinovac	93 (31.0)	24 (8.0)	-	1 (0.3)	-
AstraZeneca	90 (30.0)	119 (39.7)	14 (4.7)	3 (1.0)	1 (0.3)
Pfizer	38 (12.8)	57 (19.0)	82 (27.3)	13 (4.3)	1 (0.3)
Moderna	13 (4.3)	10 (3.3)	38 (12.7)	9 (3.0)	2 (0.7)
Sinopharm	28 (9.3)	27 (9.0)	1 (0.3)	-	-
Missing	1 (0.3)	-	-	-	-
Not received	37 (12.3)	63 (21.0)	165 (55.0)	274 (91.4)	296 (98.7)
Songkhla					
Sinovac	101 (33.7)	30 (10.0)	-	-	
AstraZeneca	77 (25.7)	134 (44.7)	20 (6.7)	2 (0.7)	-
Pfizer	42 (14.0)	43 (14.3)	60 (20.0)	5 (1.7)	-
Moderna	1 (0.3)	4 (1.3)	7 (2.3)	2 (0.6)	-
Sinopharm	13 (4.3)	16 (5.3)	1 (0.3)	-	-
Missing data	6 (2.0)	4 (1.3)	1 (0.3)	-	-
Not received	60 (20.0)	69 (23.1)	211 (70.4)	291 (97.0)	300 (100.0)
Bangkok					
Sinovac	75 (25.0)	38 (12.7)	2 (0.7)	-	-
AstraZeneca	151 (50.3)	164 (54.7)	45 (15.0)	7 (2.3)	-
Pfizer	17 (5.7)	31 (10.3)	87 (29.0)	19 (6.3)	1 (0.3)
Moderna	4 (1.3)	6 (2.0)	19 (6.3)	6 (2.0)	2 (0.7)
Sinopharm	29 (9.7)	30 (10.0)	5 (1.7)	-	-
Missing data	4 (1.3)	-	-	-	-
Not received	20 (6.7)	31 (10.3)	142 (47.3)	268 (89.4)	297 (99.0)

Knowledge, Attitude, and Practices (KAP) data

KAP data (Table 4) shows the mean level of these factors for all the participants combined and stratified by provinces. Overall, most of the participants reported they felt great ease in achieving health literacy, a moderate probability of getting a severe COVID-19 infection, indicated they exhibited proper behavior for the prevention of infection, reported they felt little stress of regarding the risk of COVID infection, had quite a lot of trust in healthcare workers, quite agreed were in agreement with suspending some regular rules as a means of controlling the COVID-19 pandemic, and often with had a feeling of general well-being. However, for some of the issues there were significant differences among the regions, e.g., participants from Songkhla Province felt finding information about COVID-19 to be more difficult than did participants in other regions. Participants from the northern region revealed the in general reported exhibiting more proper behavior for the prevention of COVID-19 infection in the last seven days than participants in the other areas. Participants from Khon Kaen Province felt the lowest impact from COVID-19. Most participants indicated they had not exhibited unwanted inappropriate behaviors related to the COVID-19 pandemic within the previous two weeks (Table 5).

The opinions on these issues of the 179 participants who were unvaccinated for COVID-19 are summarized in Table 6 (level 0 = strongly disagree; level 6 = strongly agree). For the statement, "If other people are vaccinated, there is no need for me to be vaccinated," the

Table 4. Mean level of knowledge, attitudes, and practices (KAP) of COVID-19 stratified by province

Item, Mean (95% confidence interval)	Chiang Mai	Khon Kaen	Songkhla	Bangkok	Total	*p*-value*
Health literacy#						
Ease of finding information about COVID-19	4.03 (3.90-4.17)	3.98 (3.82-4.14)	1.88 (1.76-2.00)	4.00 (3.86-4.13)	3.50 (3.41-3.58)	< 0.001
Knowledge of what to do if infected with COVID-19	4.04 (3.92-4.16)	4.13 (4.00-4.27)	4.17 (4.03-4.32)	4.06 (3.93-4.19)	4.10 (4.03-4.16)	0.445
Evaluation of the reliability of COVID-19 vaccine	3.56 (3.43-3.69)	3.89 (3.73-4.04)	4.36 (4.07-4.65)	3.58 3.45-3.70	3.83 (3.74-3.92)	< 0.001
Practice recommendations for COVID-19 prevention	4.14 (4.02-4.27)	4.20 (4.06-4.34)	3.58 (3.44-3.72)	4.18 (4.05-4.31)	4.03 (3.96-4.10)	< 0.001
Understanding and following the "work from home" policy	4.26 (4.15-4.38)	4.28 (4.15-4.41)	4.47 (4.34-4.60)	4.23 (4.10-4.36)	4.31 (4.25-4.37)	0.50
Understanding recommendations for COVID-19 prevention	4.32 (4.20-4.44)	4.29 (4.15)	4.28 (4.14-4.41)	4.39 (4.26-4.53)	4.32 (4.25-4.39)	0.646
Understanding and following the social distancing policy	4.33 (4.21-4.45)	4.26 (4.12-4.40)	4.34 (4.21-4.47)	4.22 (4.08-4.36)	4.29 (4.22-4.36)	0.51
Data on COVID-19 vaccine	4.15 (4.02-4.28)	4.16 (4.01-4.30)	4.23 (4.09-4.37)	4.18 (4.04-4.32)	4.18 (4.11-4.25)	0.86
Understanding recommendations for COVID-19 vaccination	4.27 (4.15-4.39)	4.27 (4.13-4.41)	4.34 (4.21-4.47)	4.18 (4.05-4.31)	4.26 (4.20-4.33)	0.41
Following COVID -19 vaccination guidelines	4.32 (4.20-4.44)	4.25 (4.11-4.39)	4.41 (4.28-4.53)	4.33 (4.19-4.47)	4.33 (4.26-4.39)	0.47
Probability and Severity of COVID-19 infection#						
Probably of COVID-19 infection	2.75 (2.59-2.91)	2.40 (2.22-2.58)	2.41 (2.23-2.58)	3.11 (2.95-3.28)	2.67 (2.58-2.76)	<0.001
Risk of having COVID-19 infection	2.84 (2.68-3.00)	2.47 (1.81-3.13)	2.51 (2.33-2.69)	2.83 (2.66-3.00)	2.67 (2.50-2.84)	0.28
Probability of severe COVID-19 infection	2.98 (2.82-3.14)	2.09 (1.93-2.26)	2.54 (2.37-2.71)	2.90 (2.75-3.06)	2.65 (2.56-2.73)	<0.001
Behavior to prevent COVID-19 infection in the last 7 days#						
Wash hands with soap for at least 20 seconds	5.03 (4.90-5.16)	4.32 (4.14-4.51)	4.04 (3.87-4.21)	3.92 (3.75-4.08)	4.36 (4.28-4.45)	< 0.001
Avoid contact with eyes, nose, and mouth with dirty hands	4.79 (4.63-4.95)	4.61 (4.44-4.77)	4.23 (4.05-4.40)	4.20 (4.03-4.36)	4.47 (4.39-4.56)	< 0.001
Use disinfectants to clean hands when soap and water are not available	4.97 (4.83-5.12)	4.78 (4.62-4.94)	4.50 (4.34-4.66)	4.22 (4.05-4.38)	4.63 (4.55-4.71)	< 0.001
Avoid social events	5.07 (4.71-5.44)	4.52 (4.35-4.69)	4.45 (4.27-4.630	4.06 (3.87-4.24)	4.55 (4.42-4.68)	< 0.001
Work/study from home	4.71 (4.52-4.89)	4.37 (4.18-4.56)	4.52 (4.32-4.71)	3.75 (3.52-3.99)	4.36 (4.25-4.46)	< 0.001
Used antibiotics to prevent or treat COVID-19	4.72 (4.53-4.90)	4.48 (4.31-4.66)	4.31 (4.11-4.51)	3.79 (3.55-4.02)	4.35 (4.24-4.45)	< 0.001
Wear a mask in public	5.79 (5.72-5.85)	5.36 (5.23-5.49)	5.78 (5.70-5.87)	5.44 (5.31-5.57)	5.60 (5.55-5.65)	< 0.001
Ensure physical distancing in public	5.60 (5.52-5.69)	5.22 (5.10-5.33)	5.56 (5.46-5.65)	5.23 (5.10-5.37)	5.41 (5.36-5.47)	< 0.001
Disinfect surfaces	5.35 (5.25-5.45)	4.87 (4.70-5.04)	5.19 (5.07-5.31)	4.67 (4.50-4.84)	5.04 (4.97-5.11)	< 0.001

*Comparison between groups, #scale 0 = lowest, 6 = highest

Table 4. Mean level of knowledge, attitudes, and practices (KAP) of COVID-19 stratified by province (continued)

Item, Mean (95% confidence interval)	Chiang Mai	Khon Kaen	Songkhla	Bangkok	Total	*p*-value*
Impact of COVID-19#						
Impact is close to me	1.69 (1.54-1.84)	2.19 (1.98-2.40)	2.30 (2.11-2.49)	2.07 (1.89-2.25)	2.05 (1.95-2.14)	< 0.001
Spread rate	1.55 (1.42-1.68)	1.97 (1.78-2.16)	2.10 (1.93-2.27)	1.87 (1.71-2.03)	1.86 (1.77-1.94)	< 0.001
Thinking of COVID-19	1.85 (1.70-2.00)	3.10 (2.91-3.28)	2.17 (1.99-2.36)	1.89 (1.74-2.04)	2.23 (2.14-2.32)	< 0.001
Fear-inducing	2.39 (2.23-2.54)	3.50 (3.32-3.69)	2.42 (2.23-2.60)	2.25 (2.09-2.41)	2.63 (2.65-2.72)	< 0.001
COVID-19 media is addictive	2.93 (2.78-3.08)	4.01 (3.82-4.19)	3.12 (2.95-3.29)	2.84 (2.68-3.00)	3.21 (3.12-3.29)	< 0.001
Feeling hopeless	4.01 (3.85-4.18)	4.72 (4.57-4.87)	4.33 (4.18-4.49)	3.93 (3.76-4.10)	4.24 (4.16-4.32)	< 0.001
Feeling stress	3.75 (3.58-3.93)	4.60 (4.43-4.76)	4.08 (3.92-4.25)	3.69 (3.52-3.87)	4.02 (3.93-4.11)	< 0.001
Level of trust in sources of information#						
Media (television, radio, newspapers)	3.31 (3.16-3.45)	3.99 (3.83-4.15)	3.70 (3.55-3.84)	3.65 (3.51-3.80)	3.64 (3.57-3.72)	< 0.001
Healthcare workers	4.13 (3.99-4.28)	4.38 (4.23-4.53)	4.23 (4.08-4.38)	3.89 (3.74-4.04)	4.16 (4.08-4.23)	< 0.001
Social media	3.11 (2.95-3.27)	4.00 (3.83-4.17)	3.17 (3.00-3.34)	3.29 (3.13-3.45)	3.38 (3.30-3.46)	< 0.001
Actors, internet idols	2.15 (2.00-2.30)	3.42 (3.24-3.60)	2.48 (2.33-2.63)	2.66 (2.51-2.82)	2.65 (2.57-2.73)	< 0.001
Ministry of Health	3.86 (3.70-4.02)	4.20 (4.05-4.35)	4.33 (4.19-4.47)	3.75 (3,59-3.92)	4.03 (3.95-4.10)	< 0.001
Department of Disease Control	3.77 (3.61-3.93)	4.15 (3.99-4.30)	4.31 (4.16-4.46)	3.81 (3.64-3.97)	4.00 (3.92-4.08)	< 0.001
World Health Organization (WHO)	3.59 (3.42-3.76)	4.10 (3.93-4.26)	4.12 (3.96-4.28)	3.77 (3.59-3.94)	3.88 (3.79-3.96)	< 0.001
National COVID-19 information application	3.54 (3.38-3.70)	4.13 (3.97-4.29)	3.98 (3.82-4.14)	3.27 (3.07-3.47)	3.72 (3.63-3.81)	< 0.001
Frequency of seeking COVID-19 information	3.38 (3.23-3.54)	3.61 (3.42-3.79)	3.55 (3.40-3.70)	3.51 (3.37-3.66)	3.51 (3.43-3.59)	0.194
Level of trust in agencies treating COVID-19 infection#						
Family doctors	4.55 (4.43-4.66)	4.54 (4.40-4.68)	4.46 (4.31-4.60)	4.29 (4.14-4.43)	4.46 (4.39-4.53)	0.030
Employers	2.81 (2.63-3.00)	3.47 (3.28-3.67)	3.47 (3.29-3.65)	3.28 (3.11-3.46)	3.24 (3.14-3.33)	< 0.001
Hospitals	4.25 (4.12-4.37)	4.35 (4.21-4.50)	4.45 (4.32-4.58)	4.27 (4.14-4.41)	4.33 (4.26-4.39)	0.192
Ministry of Health	4.03 (3.89-4.17)	4.31 (4.17-4.44)	4.35 (4.21-4.48)	4.05 (3.90-4.21)	4.18 (4.11-4.25)	0.003
Department of disease control	3.89 (3.74-4.04)	4.22 (4.08-4.36)	4.27 (4.13-4.42)	4.00 (3.85-4.16)	4.09 (4.01-4.16)	0.001
Schools	2.57 (2.39-2.76)	3.65 (3.49-3.81)	3.42 (3.25-3.59)	3.04 (2.87-3.21)	3.14 (3.05-3.23)	< 0.001
Places of worship (temple/church etc.)	2.45 (2.26-2.62)	3.52 (3.35-3.69)	3.28 (3.11-3.45)	2.78 (2.61-2.94)	2.98 (2.89-3.06)	< 0.001
Opinion of COVID-19 infection control efforts#						
Proper strategy	3.57 (3.43-3.71)	4.29 (4.15-4.43)	4.00 (3.84-4.16)	3.95 (3.82-4.09)	3.93 (3.86-4.01)	< 0.001
Can convince other people	3.58 (3.45-3.72)	4.32 (4.19-4.46)	4.10 (3.96-4.25)	4.11 (3.99-4.23)	4.01 (3.94-4.08)	< 0.001

*Comparison between groups, #scale 0 = lowest, 6 = highest

Table 4. Mean level of knowledge, attitudes, and practices (KAP) of COVID-19 stratified by province (continued)

Item, Mean (95% confidence interval)	Chiang Mai	Khon Kaen	Songkhla	Bangkok	Total	*P*-value*
Agreement with suspending regular rules to help control the COVID-19 pandemic#						
Vaccination along with the recommendation	4.94 (4.78-5.09)	4.93 (4.75-5.10)	4.28 (4.08-4.49)	4.96 (4.80-5.12)	4.79 (4.70-4.87)	< 0.001
More strictly recommendation	3.08 (2.93-3.22)	2.03 (1.81-2.24)	3.06 (2.88-3.23)	3.61 (3.45-3.76)	2.95 (2.86-3.04)	< 0.001
Increased screening, testing	4.23 (4.10-4.37)	4.27 (4.13-4.41)	4.10 (3.94-4.26)	4.39 (4.26-4.52)	4.25 (4.18-4.32)	0.046
Financial impact	4.08 (3.93-4.23)	4.10 (3.94-4.27)	4.19 (4.03-4.36)	4.36 (4.21-4.51)	4.18 (4.10-4.26)	0,050
Requirement to wear a mask in a closed environment	4.69 (4.56-4.82)	5.09 (4.96-5.23)	5.04 (4.91-5.17)	5.07 (4.94-5.20)	4.96 (4.89-5.03)	< 0.001
Reopening of schools	3.36 (3.22-3.51)	4.15 (4.01-4.29)	4.37 (4.22-4.52)	4.47 (4.33-4.61)	4.05 (3.97-4.13)	< 0.001
Opening borders with more countries	2.41 (2.25-2.57)	3.78 (3.61-3.94)	3.44 (3.25-3.63)	3.59 (3.40-3.77)	3.26 (3.17-3.35)	< 0.001
Feeling of general well-being within 2 weeks (during COVID-19 pandemic)#						
Joyful	2.85 (2.70-3.00)	2.81 (2.66-2.96)	2.19 (2.03-2.35)	2.99 (2.34-3.64)	2.72 (2.55-2.89)	0.007
Peaceful	2.64 (2.48-2.80)	2.75 (2.61-2.90)	2.05 (1.90-2.20)	2.91 (2.25-3.56)	2.59 (2.42-2.76)	0.004
Enthusiastic	2.85 (2.70-3.00)	2.84 (2.70-2.99)	2.09 (1.94-2.24)	2.93 (2.27-3.58)	2.69 (2.52-2.85)	0.002
Fresh	2.54 (2.38-2.70)	2.72 (2.58-2.87)	1.96 (1.81-2.10)	2.89 (2.24-3.54)	2.53 (2.36-2.70)	0.001
Interesting	2.72 (2.56-2.87)	2.82 (2.67-2.96)	2.03 (1.88-2.18)	2.68 (2.52-2.84)	2.57 (2.49-2.65)	< 0.001

Table 5. Inappropriate behavior within the past two weeks (during the COVID-19 pandemic)

Item	Yes (%)	No (%)	Not regularly (%)	Missing data (%)
Reducing exercise	437 (34.6)	416 (32.9)	402 (31.8)	8 (0.7)
Drinking alcohol	18 (1.4)	226 (17.9)	1015 (80.4)	4 (0.3)
Eating unhealthy food	133 (10.5)	385 (30.5)	741 (58.6)	4 (0.4)
Smoking more	7 (0.6)	205 (16.2)	1046 (82.8)	5 (0.4)
Postponing other vaccinations (including for family members)	73 (5.8)	626 (49.6)	560 (44.3)	4 (0.4)
Avoiding going to the doctor for a non-COVID-19-related problem	105 (8.3)	703 (55.7)	449 (35.6)	6 (0.4)
Buying drugs that I heard are good for treating COVID-19	137 (10.8)	481 (38.1)	635 (50.3)	10 (0.8)

average score ranged from 2.25 to 3.57.

Comparison of the level of agreement with statements related to the decision to vaccinate or not between unvaccinated and vaccinated participants found significant differences (0 = strongly disagree; 6 = strongly agree) as follows: agree with health ministry recommendations (3.92 vs. 4.16), the vaccine is easy to get (3.6 vs. 3.9), no need to vaccinate for a rare disease (2.6 vs. 2.2), stress made me not want to vaccinate (2.6 vs. 2.1), if everyone is vaccinated, there no need for me to vaccinate (2.5 vs. 1.9), and COVID-19 vaccines are important (3.7 vs. 4.2). Details are shown in Table 7.

Of the factors that encouraged the 1,083 participants to get the COVID-19 vaccine, the

Table 6. Opinion of participants Who were unvaccinated for COVID-19 (N=179)

Item Mean (95% confidence interval)	Chiang Mai (N=62)	Khon Kaen (N=37)	Songkhla (N=60)	Bangkok (N=20)	Total (N=179)	*p*-value
Level of agreement#						
Vaccine can control the spread of COVID-19	3.29 (2.92-3.66)	2.95 (2.49-3.40)	3.50 (3.14-3.86)	3.30 (2.64-3.96)	3.29 (3.08-3.50)	0.317
Once infected with COVID, there is no need to vaccinate	2.63 (2.26-3.00)	3.16 (2.65-3.67)	2.85 (2.42-3.28)	2.75 (2.09-3.41)	2.83 (2.60-3.05)	0.414
If other people are vaccinated, there is no need for me to vaccinate	2.15 (1.85-2.44)	2.38 (1.95-2.80)	2.38 (1.98-2.79)	1.95 (1.39-2.51)	2.25 (2.05-2.45)	0.516
If a COVID-19 vaccine is availble and recommended for me, I think most of my family and friends would want me to get it	3.56 (3.22-3.91)	3.19 (2.68-3.70)	3.53 (3.13-3.93)	3.10 (2.46-3.74)	3.42 (3.21-3.64)	0.477
If a COVID-19 vaccine is available and recommended, I think that other people whose opinions I value would want me to get it	3.58 (3.24-3.92)	4.11 (2.40-5.81)	3.62 (3.25-3.99)	3.35 (2.63-4.07)	3.68 (3.29-4.06)	0.669
Trust in vaccine manufacturers##						
Pfizer	3.84 (3.52-4.15)	3.32 (2.90-3.75)	3.48 (3.13-3.84)	3.45 (2.80-4.10)	3.57 (3.38-3.76)	0.234
Moderna	3.77 (3.46-4.09)	3.35 (2.91-3.79)	3.32 (2.96-3.67)	3.40 (2.75-4.05)	3.49 (3.30-3.69)	0.222
Astra Zeneca	3.45 (3.17-3.73)	3.16 (2.73-3.60)	3.15 (2.81-3.49)	3.40 (2.75-4.05)	3.28 (3.10-3.47)	0.505
Sinovac	3.37 (3.08-3.67)	3.11 (2.67-3.55)	3.03 (2.68-3.39)	3.20 (2.46-3.94)	3.18 (2.99-3.38)	0.541
Sinopharm	3.27 (2.98-3.57)	3.16 (2.71-3.62)	3.15 (2.79-3.51)	3.40 (2.75-4.05)	3.22 (3.03-3.42)	0.869

scale 0 = strongly disagree, 6 = strongly agree, ##scale 0 = lowest, 6 = highest

Table 7. Factors affecting decision regarding getting the COVID-19 vaccination among unvaccinated (N=179) and vaccinated (N=1,083) participants (scale 0 = least affect, 6 = greatest affect)

Factor	Unvaccinated (%)	Vaccinated (%)	*p*-value
The country that produced the vaccine	3.16	3.45	0.429
Type of vaccine	3.30	3.78	0.733
Producer of the vaccine	3.30	3.53	0.377
Effectiveness	3.71	4.14	0.121
Recommend from your doctor	4.03	4.29	0.234
Recommendation of the Ministry of Health	3.92	4.16	0.030
A long-lasting vaccine	3.42	3.62	0.970
Possibility of serious side effects	3.96	3.95	0.652
Number of people vaccinated	3.76	3.82	0.588
Risk of COVID-19 infection	3.24	3.61	0.807
Ease of getting the vaccine	3.59	3.92	0.004
Allowed to see family and friends	3.39	3.78	0.538
Allowed to travel	3.50	3.69	0.198
Allowed gathering in groups	3.55	3.76	0.075
Safety of the vaccine	3.05	3.68	0.084
No need to vaccinate as the infection is rare	2.66	2.21	< 0.001
Stress made me not want to vaccinate	2.58	2.14	< 0.01
Comparison of the benefits and risks of vaccinating	3.69	3.63	0.115
If everyone is vaccinated, no need for me to vaccinate	2.47	1.93	< 0.001
Due to the pandemic, everyone should be vaccinated	3.77	4.52	0.096
COVID-19 vaccines are important for your health	3.70	4.20	0.020
Vaccination prevents COVID-19 infection in the community	3.53	4.07	0.091
Worry about serious vaccine side effects	3.85	3.32	0.610
Trust in the health worker who vaccinated you	3.75	4.34	0.789

factor rated most effective was "easy access to the vaccination site" (79.1%) followed by "receiving a phone call from a nurse when you are eligible to schedule a vaccine appointment" (54.35), "being able to schedule a vaccine appointment online/via mobile application" (43.6%), "receiving a text notification when you are eligible to schedule a vaccine appointment" (32.6%), "being given a day off of work after getting the vaccine" (27.1%), and "free public transportation to a vaccination site" (21.2%).

DISCUSSION

The COVID-19 vaccination rate

The present study was a cross-section survey of gynecologic cancer patients from the 4 regions of Thailand during the Omicron wave. In this survey, we found acceptance rate of the COVID-19 vaccine (at least one dose) among the participants was 85.8%, with the highest rate in Bangkok (the nation's capital). However, among all the participants 23.3% of refused the COVID-19 vaccine during their cancer treatment. The factor regarding accepting vaccination with the greatest difference between the unvaccinated and vaccinated groups was the belief in the unvaccinated group that if everyone is vaccinated, there was no need for them to vaccinate. Concerns regarding the possibility of serious side effects was not significantly different between the groups. Prabani et al. (10) recently published a systematic review and meta-analysis about COVID-19 acceptance and hesitancy among cancer patients. That study searched papers from PubMed, Science Direct, and Cochrane about this issue published between 25 April 2021 and 21 May 2022, selecting 29 studies from different geographical areas for review, but not from Thailand. The study reported the pooled prevalence of COVID-19 vaccine acceptance was 59% (95% confidence interval 52-67%), I2:99%. The pooled etiology of vaccine hesitancy was fear of vaccine-related side effects (53%) and uncertainty about the vaccine's effectiveness (36%). For patients actively undergoing cancer treatment, seven studies were pooled for the final analysis which found that these patients were more likely to be hesitant about COVID-19 vaccination than patients not currently undergoing treatment. The difference in the vaccine acceptant rate of our report and the Prabani et al. (10) study might be due to the different periods when the surveys were done and to differences in culture of the surveyed populations. In Thailand, from 3 January 2020 to 1 June 2023, a total of 139,247,715 vaccine doses were administered (1).

The COVID-19 experience

Regarding the COVID-19 infection experience, our study found 28.3% of the participants had previously been infected with COVID-19 and that most of those cases (330 cases) were the mild form. Only 27 cases (2.1%) presented with a severe form of COVID-19, but there was no difference in the rate of infection between the vaccinated and unvaccinated in this group. Pinato et al. (13) recently reported on the prevalence and impact of COVID-19 sequelae in 1,557 cancer patients at a median time after infection of 44 days (28-329 days). Of those patients, 234 (15%) were reported to have COVID-19 sequelae, with the most common symptom being respiratory tract problems and fatigue. Unfortunately, our study did not include COVID-19 sequelae data.

Knowledge, Attitude, and Practices (KAP) issues

The KAP survey of gynecologic cancer patients in the present study found positive responses in terms of health literacy issues and appropriate behavior for the prevention of COVID-19, while there was only moderate concerned about the severity of COVID-19 infection that might have resulted from less morbidity the perceived lower risk of death or hospitalization of the Omicron wave. The results of the present study are quite similar to a study from India. Poddar et al. (8) surveyed KAP in relation to COVID-19 among 521 Indian gynecologic cancer patients from February to May 2021. That study reported good overall knowledge, attitudes, and practices with a high score of 80%. However, that positive response was quite different from a Romanian study (14) in which a cross-sectional multicentric study of 1,585 Romanian cancer patients during April-May 2020 found only 10.8% had good knowledge about COVID-19 infection. The difference in the Romanian study and the present study might be related to the different

periods in which the surveys were conducted: the present study surveyed the epidemic outbreak in February to December 2022, a year later than the Romanian study. At the time of the present study, most populations had faced COVID-19 for more than two years and would be expected to understand more about the COVID-19 infection.

The strengths and the limitations

A strength of the present study is that the survey was conducted by well-trained interviewers in each of the four regions of Thailand and included a total of 1,263 participants, helping to assure that the data are reliable and to provide important information about KAP among Thai gynecologic cancer patients during the COVID-19 pandemic. However, the survey reflects only short-term responses; some knowledge, attitudes and practice behavior might change with alterations in the current situation.

CONCLUSIONS

During the Omicron epidemic wave, most Thai gynecologic oncology patients from all four regions reported good knowledge, attitudes, and practices related to the COVID-19 outbreak and most received the COVID-19 vaccine, especially those who were in a cancer surveillance situation. The highest acceptance rate of COVID-19 vaccination was in Bangkok, the capital of Thailand.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ADDITIONAL INFORMATION

Authors Contribution Statement

P.S., V.C., and R.P: conceived the presentation idea. V.C., A.R., A.A., K.V. and W.K.: collected the data. P.S.: verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

Data availability statement

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

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Connections Between Vitamin D Deficiency and Brain Structural Changes: Implications for Neurocognitive Function and Neurodegenerative Disorders

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ABSTRACT

In the general world population, vitamin D insufficiency frequently occurs. Severe vitamin D deficiencies are more common in individuals who also have critical illnesses. Ultraviolet B (UVB) radiation applied to the skin has an impact on the production of vitamin D. Through its anti-inflammatory and anti-autoimmune effects, vitamin D plays an immunomodulatory role. The regulation of calcium-mediated neuronal excitotoxicity, the decrease of oxidative stress, the induction of synaptic structure proteins as well as inadequate neurotransmitters and neurotrophic factors are all aspects of how vitamin D functions in the nervous system. A lack of dietary consumption and inadequate sun exposure can cause vitamin D deficiency. Vitamin D is essential for preserving brain health and function. Vitamin D deficiency can worsen the neurocognitive effects of disorders like Parkinson's disease, Alzheimer's disease, and other dementias. Mild cognitive impairment (MCI) refers to the earliest stage of memory impairment or other cognitive function loss. It has been found that the volume of the hippocampus and white matter integrity are both on the decline which is related to this cognitive impairment. There has been only limited exploration of the brain-specific areas that undergo structural change in response to vitamin D status. The objective of the present article was to review the connections between vitamin D deficiency and structural changes in the brain including implications for neurocognitive and neurodegenerative disorders in order to provide additional understanding, especially of brain areas that are involved with neurocognitive functioning or neurodegenerative disorders.

KEYWORDS vitamin D deficiency, 25-hydroxyvitamin D, 25 (OH)D), vitamin D deficiency and brain image, vitamin D deficiency and neurodegenerative disorders

INTRODUCTION

UVB radiation from sunlight induces 7-dehydrocholesterol (7-DHC) in the epidermis to be converted into pre-vitamin D, the method by which vitamin D is generated endogenously, after which it is then thermally isomerized to generate vitamin D2 and vitamin D3, resulting, through two stages, in an active form of vitamin D. In the liver, vitamin D2 and D3 are first converted into calcidiol (25-hydroxyvitamin D; 25(OH)D), which is the major form of vitamin D in circulating blood, thus serum 25(OH)D concentration is a measure of vitamin D status. The scientific community considers that serum 25(OH)D concentrations <25 or 30 nmol/L (<10 or 12 ng/L) should be prevented and treated. Several guidelines have set targets of serum 25(OH)D concentrations of ≥50 nmol/L (≥20

ng/mL) (1, 2).

Geographic location and demographic factors affect the incidence of severe vitamin D deficiency. Numerous variables, such as the time of day, season, latitude, altitude, extent of cloud cover, level of air pollution, type of clothing, and the use of sunscreen can all affect the level of skin exposure to UVB radiation (1, 3). The extensive benefits of vitamin D as a hormone precursor are becoming more widely recognized (4, 5). According to several studies (6, 7), active vitamin D might influence the brain through a variety of mechanisms, including regulating the activity of neuronal growth factors, reducing inflammation, and preventing thrombosis. In the search for ways to reduce the risk of dementia and stroke, a variety of brain morphometry characteristics have been identified which may indicate a decline in cognition and the onset of neurocognitive disorders.

The author conducted a comprehensive search for references using reputable research databases in the fields of healthcare, medicine, and biomedical research, including PubMed, EMVASE, PMC, Cohhrane Library, Science Direct, and Google Scholar. The search focused on articles published within the previous year and included those from the past 5 years or more as deemed necessary. In compiling information on each topic, the author selected research articles that presented a variety of study designs, including systematic reviews and meta-analyses as well as retrospective, prospective, and cross-sectional studies. The selected articles were then used to consolidate information and provide a robust foundation of content related to various topics. In addition to the research articles, the author also gathered data and statistical information from relevant organizational websites to enhance the depth of information included in this review.

VITAMIN D SOURCES AND BIOSYNTHESIS

Vitamin D is produced in the epidermis layer of the skin and can also be found in foods and dietary supplements such as vitamin D2 (ergocalciferol) and D3 (cholecalciferol). The only form of vitamin D produced through the skin is vitamin D3, which is also found in plants, including potatoes, tomatoes, and peppers. Vitamin D2 is also produced in yeast and fungi through UVB exposure to the steroid ergosterol, a component of the fungi's cell membrane. While farmed mushrooms, which are grown in the dark, do not contain substantial amounts of vitamin D2, UVB-treated vitamin D2-boosted mushrooms are now commercially available. Wild mushrooms are a rich natural source of vitamin D2, containing approximately 13-30 µg (520-1200 IU) per 100 g of fresh weight. Most of the vitamin D in the body is created in the skin through the photochemical reaction of UV light impact on its precursor, 7-dehydrocholesterol (7-DHC), when the skin is exposed to UV radiation from the sun (wave length 290-315 nm) or from artificial UVB light. Previtamin D is created from 7-DHC in the epidermis and reaches its peak level in the skin within a few hours. Even after continuous sun exposure, however, previtamin D production is limited to 12 to 15 percent of the beginning 7-DHC. For those with darker skin pigmentation, cutaneous vitamin D production is less effective (1, 8). Both vitamin D2 and D3 must be transformed twice before becoming physiologically active. The main form of vitamin D that circulates in the blood is calcidiol (25(OH)D), which is first transformed in the liver by an enzyme known as 25-hydroxylase (CYP27A1) and has a half-life of approximately 13-15 days. Several tissues, such as fat tissue, muscle tissue, and the liver, absorb 25(OH)D from the circulation and store it there as well. 25(OH)D is commonly used as a biomarker to assess a person's vitamin D level because it is a particularly stable and frequent vitamin D component in serum. The majority of 25(OH)D is converted to calcitriol (1,25(OH)2D), which is created in the second stage by the kidney enzyme 1-hydroxylase (CYP27B1). While performing its roles, calcitriol interacts with the vitamin D receptor (VDR) in several types of tissues across the body (1, 8-13). The tissues with the highest levels of VDR are the kidney's distal tubules, the islets of Langerhans in the pancreas, intestinal absorptive cells, and osteoblast cells (14). Additionally, a variety of immune cells, including lymphocytes (B and T cells), monocytes, macrophages, and dendritic cells, also contain VDR (15). The nuclear steroid

receptor VDR serves as a functional component in the brain. It has been demonstrated that various parts of the brain are where vitamin D production and destruction take place (16). In the human brain, the location of VDR and an enzyme needed to produce the hormone 1-hydroxylase's active form (CYP27B1) have been identified. Research has also demonstrated that VDR is present in a variety of cells in the brain, including astrocytes, oligodendrocytes, microglia, and neurons (9, 17-19). Active vitamin D may influence the brain through a variety of mechanisms, including the regulation of neurotrophic growth factors, effects on cognitive function, and white matter hyperintensity in patients with mild Alzheimer's disease (20). Brain morphometry can reveal cognitive decline and neurocognitive disorders through numerous methods. Reduced volumes of gray matter in the olfactory and hippocampus are as have been linked to low serum 25 (OH)D in older adults with cognitive impairment (21). The high density of vitamin D receptors (VDR) in the hippocampus, hypothalamus, thalamus, cortex, and substantia nigra suggests the potential of vitamin D to influence neurological conditions (22).

VITAMIN D SUFFICIENCY

Different societies have different minimum standards for adequate serum 25(OH)D. For example, the European Food Safety Authority and the American Institute of Medicine (the National Academy of Medicine) both define sufficiency as >50 nmol/L 25(OH)D, in contrast to the US Endocrine Society's definition of >75 nmol/L (23). The optimum vitamin D level has been determined to be 50 nmol/L (20 ng/mL) by the World Health Organization (WHO) and the Institute of Medicine (IOM) USA in their "Dietary Reference Intakes." A public health plan should likely steer away from vitamin D concentrations <30 nmol/L (or 12 ng/mL) (24-26). Based on 25(OH)D serum levels, vitamin D conditions have been classified into three groups: individuals with sufficient levels of vitamin D (>50 nmol/L), insufficient levels (30-50 nmol/L), and deficient levels (<30 nmol/L). People with adequate vitamin D levels don't require as much vitamin D supplementation as those who are vitamin D deficient.

Vitamin D toxicity is evidenced by hypercalcemia, a serum level of 25(OH)D >250 nmol/L, and hypercalciuria, so it is important to beware of the negative impact of an overdose of vitamin D (27). The <50 nmol/L definition of 25(OH)D inadequacy has a considerable body of evidence to support it in the UK. A "white" and "nonwhite" categorization was used in that analysis as different ethnic categories were used in the population samples. The nonwhite group was comprised of people who were listed as being black, Asian, or other, including mixed race. According to the findings, people with dark skin, including Asians, and other minorities, and people who work in buildings or who get little direct sunlight, are at a much higher risk of insufficiency based on their 25(OH)D serum levels. Insufficiency (<50 nmol/L) has been reported to be present in 45.7% of cases in the summer and 69.3% in the winter, whereas deficiency (<30 nmol/L) was present in 31.8% of cases in the winter and 15.3% in the summer (28). Ninety-two percent of the 6,433 South Asians who live in the UK, based on a recent report from the UK Biobank cohort, have 25(OH)D levels <50 nmol/L (29). Serum 25(OH)D levels <50 nmol/L (<20 ng/mL) have been linked to unfavorable skeletal outcomes, such as fractures and bone loss. The main therapeutic objective is a 25(OH)D level >50 nmol/L (>20 ng/mL), while some studies indicate a benefit from a higher threshold (3).

DEFINITION OF VITAMIN D DEFICIENCY

Low levels of serum 25(OH)D concentrations in the bloodstream indicate a vitamin D deficiency. Estimates of the prevalence of vitamin D insufficiency and, consequently, the magnitude of the low vitamin D status public health problem, are significantly impacted by diverse definitions which are based on different serum 25(OH)D thresholds. It is widely accepted that vitamin D deficiency exists in both high- and low-income nations and that it is necessary to address this deficiency with the strictest serum 25(OH)D criteria of 25 or 30 nmol/L (23). The 2016 report of the Scientific Advisory Committee on Nutrition (SACN) (p. 46, section 6.34) on vitamin D recommendations states that, "In

the UK, the serum 25(OH)D level of 25 nmol/L was defined as a minimum level selected to identify an increased risk of rickets." (1, 30). The vitamin D nutritional guidelines and Nature Reviews Endocrinology agree that blood 25(OH)D concentrations below 25 nmol/L (less than 10 ng/mL) need to be avoided at all ages (31). Severe vitamin D deficiency can be verified using a cutoff of <25 or 30 nmol/L (<10 or 12 ng/ml), a level which considerably increases the risk of nutritional rickets and osteomalacia (13, 32). The Global Consensus Recommendations on Prevention and Management of Nutritional Rickets Research advises categorizing vitamin D status according to 25(OH)D levels as follows: more than 50 nmol/L is sufficient, 30-50 nmol/L is insufficient, and less than 30 nmol/L is deficient (27). Whenever possible, severe vitamin D deficiency that has 25(OH) D concentrations less than 30 nmol/L, should be prevented, as that greatly raises the possibility of mortality, infection, and several other illnesses (3).

VITAMIN D DEFICIENCY ESTIMATES WORLDWIDE

Geographical and demographic factors affect how common vitamin D deficiency is, according to Amrein et al.'s study Vitamin D deficiency 2.0. That study reviewed the current situation globally and found the incidence of severe vitamin D deficiency, defined as 25(OH)D lower than 30 nmol/L, was 5.9% in the US, 7.4% in Canada, and 13% in Europe. An estimated 24% of Americans, 37% of Canadians, and 40% of Europeans had levels of 25(OH)D less than 50 nmol/L. Age, with lower levels in childhood and the elderly, as well as ethnicity, may also affect this. A very high prevalence of low vitamin D status has been reported in several countries, e.g., in India, Tunisia, Pakistan, and Afghanistan it is estimated that >20% of the population has 25(OH)D levels <30 nmol/L. In addition, there is a high prevalence of vitamin D deficiency in some patient subgroups that frequently exhibit organ dysfunction or deficiency affecting the metabolism of vitamin D. The prevalence of vitamin D deficiency in patients with chronic renal failure, who are on hemodialysis, who are renal transplant recipients affected by liver disease, and patients following liver transplantation can range from 85 to 99% (3).

THE FUNCTION OF VITAMIN D ON THE NERVOUS SYSTEM

A research evaluation by Bivona et al. was conducted to provide a summary of the roles that vitamin D plays in the brain as well as to clarify the possibility that it may contribute to neurological illnesses. Vitamin D increases brain function in both embryonic and adult brains, enhancing the connections of neural circuits involved in movement, emotion, and reward-driven behavior. This role appears in the form of interaction between vitamin D and the nervous system. Low vitamin D serum levels have been observed in people with schizophrenia, multiple sclerosis, Parkinson's disease, autism spectrum disorders, and Alzheimer's disease (33). In the neurological system, vitamin D may have an impact on neuronal development, differentiation, neural protection, neurotransmission, and neuroplasticity. Vitamin D also has an impact on how the brain develops and functions. Vitamin D can enhance the expression of synaptic structures of proteins, neurotrophic factors, and can help alleviate the lack of neurotransmitters in some neurodegenerative illnesses (34-37). In a randomized clinical study contrasting vitamin D with a placebo, it was discovered that vitamin D had a significant impact on neuroplasticity as determined by improved corticospinal transmission (38). It has also been reported that vitamin D protects neurons from apoptosis and axon destruction (37, 39). Additionally, vitamin D may also influence intracellular calcium homeostasis by regulating calcium neuronal excitotoxicity (37).

LOW VITAMIN D AND NEUROLOGICAL DISORDERS

The hypothesis that vitamin D is associated with cognitive decline, behavioral abnormalities, and attention deficits has been substantiated by numerous analyses of preclinical research. According to cross-sectional studies, patients with cognitive impairment and Alzheimer's disease have much lower vitamin D levels than healthy individuals. Low vitamin D has also been

linked in longitudinal studies and meta-analyses to cognitive impairment and related neurological illnesses, including Alzheimer's disease (33, 40-43). Cross-sectional studies have suggested a strong link between low vitamin D and mild cognition problems according to systematic reviews and meta-analyses, but interventional studies are still required to demonstrate the specific impacts of vitamin D supplementation on cognition (44). After adjusting for age, educational level, and brain volume, Shivakumar et al. discovered a positive correlation between 25(HO)D level and hippocampus volume in antipsychotic-naive schizophrenic patients, 84% of whom were 25(HO)D deficient (p = 0.018) (45). That research also found a relationship between vitamin D levels and alterations in brain volume in individuals with schizophrenia and bipolar disorder (46, 47). Vitamin D has been shown to promote the growth of neurons by regulating the creation of neurotransmitters including acetylcholine, dopamine, gamma-aminobutyric acid (GABA), and nerve growth factor (NGF) (22, 48). In one study, participants with low levels of vitamin D performed poorly on cognitive tests and processed information more slowly than those with normal vitamin D levels (49). A higher risk of cognitive impairment has been shown to be linked to low vitamin D levels (50). Another study found an independent relationship between olfactory dysfunction and low serum vitamin D concentrations in individuals with Parkinson's disease (51). In subjects with Alzheimer's disease, which is frequently characterized by medial temporal atrophy, it has been discovered that the left parahippocampal gyrus, the fusiform, and the hippocampus are as are positively correlated with 25(OH)D level. The medial and lower parts of the left temporal lobes have been linked to the memory domain of cognitive function. In other words, as the level of atrophy of the medial parts of the temporal lobe, which includes hippocampus, increases, the vitamin D deficiencies in patients with Alzheimer's disease also increases. The concept that vitamin D can help prevent or reduce neurodegenerative processes is supported by these findings. Additionally, as the affected areas are essential to normal cognitive abilities (which include executive functions, memory consolidation, and behavioral regulations), the level of brain atrophy of parts of the brain responsible for neurocognitive functions might be useful in describing cognitive impairment in people with low 25(OH)D levels. The pathogenesis of Alzheimer's disease, which results in neuronal degeneration, may be influenced by vitamin D levels (21). However, despite some encouraging findings, there is still inadequate proof that vitamin D treatment can help treat Alzheimer's disease or reduce its symptoms (52).

LOW VITAMIN D AND BRAIN STRUCTURAL CHANGES

Neurodegenerative processes and decreased neurocognitive performance have both been linked to vitamin D deficiency. According to current research, it is debatable whether vitamin D deficiency has structural correlations in the brain. A study by Terock et al. on the association of vitamin D levels with aging brain imaging patterns was published in 2022. That study investigated correlations between vitamin D status and brain aging imaging patterns obtained from 1,865 individuals from the general population with an average age of 51.6 years and mean vitamin D level of 60.75 nmol/L. Various combinations of covariates were used. Findings from an extensive general population sample composed of adults of various ages supports the concept that vitamin D is crucial for preserving the health of brain neurons. The association of vitamin D deficiency with hippocampus volume was discovered, and the volume of the entire brain and of the gray matter were found to be significantly correlated with linear vitamin D levels. However, as these results are cross-sectional, it would be difficult to establish whether vitamin D levels can be used as an indicator of brain health or as a predictor of the progression of brain aging (53) Table 1. The association between 25(OH)D, neuroimaging features and the likelihood of dementia and stroke was investigated in a study by Navale et al. Prospective data from the UK Biobank were used to examine the relationship between 25(OH)D concentrations and neuroimaging findings, as well as the risk of dementia and stroke. In this observational

research, factors including age, sex, ethnicity, socioeconomic status, lifestyle, sun exposure, and illnesses were included. Out of a total of 427,690 subjects with illness, there were 3,414 occurrences of dementia and 5,339 incidents of stroke. A total of 33,523 people were enrolled in the brain neuroimaging subsample. Low vitamin D status was found to be associated with neuroimaging findings, dementia, and stroke risks, with the strongest associations for those with 25(OH)D less than 25 nmol/L and adjusted HR: 1.79; 95% CI: 1.57, 2.04 (40) Table 1. A study by Lee et al. explored the connection between blood levels of 25(OH)D and structural changes in the brain. That study included 201 participants (10 community-based normal healthy subjects, 33 subjects with subjective cognitive decline, 97 with mild cognitive impairment, and 61 with Alzheimer's disease) and elderly patients with MRI scans. The mean age was 74.91± 9.21 years and the mean 25(OH) D level was 18.05 nmol/L. That study found no correlation between 25 (OH)D levels and white matter area. However, it was found that decreased 25(OH)D levels were linked to smaller right olfactory and rectus gray matter regions. In SCD patients, there was no association between 25(OH)D and total brain volume. The right olfactory, rectus, supplementary motor region, left medial cingulate, superior temporal, and Rolandic operculum areas all showed a positive correlation with 25(OH)D levels in participants with MCI. It has also been demonstrated that the left parahippocampal, fusiform, cerebellum, and hippocampus regions are positively associated with 25(OH)D serum levels in Alzheimer's disease patients (21) Table 1.

Higher levels of vitamin D were found to be associated with increased gray matter volume in a cross-sectional study of 217 Dutch community-dwelling individuals 65 years old and older, but that study did not measure the entire brain volume or white matter volume (54) Table 1. In research on a large sample of healthy Dutch individuals without dementia, vitamin D insufficiency was found to be associated with a reduction in the volume of the hippocampus, white matter, and brain tissue (55) Table 1. The 376 publications chosen were reviewed statistically and systematically for data on the extent and types of morphometric alterations in the brain related to vitamin D deficiency or supplementation. In the studies there were from 20 to 333 clinical patients, all adults. Two studies included young to middle-aged individuals, five studies included elderly people, and 40-79% of the participants in the studies were female. The findings demonstrated that vitamin D deficiency was linked to larger lateral ventricles and reduced brain capacity. The ventricular volume was 1.01 SD greater with vitamin D depletion and the pooled effect size was 1.01 (95% CI: 0.62; 1.41), which is a "large" effect size. In summary, systematic reviews and quantitative syntheses of information on the areas affected and the morphometric alterations in the brain associated with vitamin D depletion or repletion showed that low levels of vitamin D are related to brain atrophy in both parts of the lateral ventricles and throughout the brain (5). There is evidence that vitamin D and its active form can directly affect the health of neurons, the shape of the brain, and memory function in both humans and animals. For example, using structural and diffusion MRI datasets of older patients, Al-Amin et al. conducted a study on people who visited the Memory Clinic at the Konkuk University Hospital (KUH) in South Korea who complained about their memory. A total of 56 MCI patients were divided into serum 25(OH)D deficient (less than 30 nmol/L, n = 27) and not-deficient (>30 nmol/L, n = 29) groups. Voxel-based morphometric analysis was used to examine the relationship between vitamin D levels and focal brain atrophy. The findings revealed that low vitamin D (25(OH) D <30 nmol/L) was associated with decreased hippocampus subfield volume and connection deficits in older MCI patients and may worsen neurocognitive outcomes (56) Table 1. According to Miller et al., accelerated cognitive decline was linked to low vitamin D levels (57). After adjusting for age, education level, and brain volume (p = 0.018), a study by Shivakumar et al. found a positive connection between 25(HO)D level and hippocampus volume in antipsychotic naive schizophrenia patients, 84% of whom were 25(HO)D deficient (45). A blood test and a brain MRI were performed on 215 elderly Caucasian community residents

Table 1. Studies of serum 25(OH)D concentration deficiency and brain volume by magnetic resonance imaging (MRI) scanning

Reference	Design	Population/characteristics	Brain volume areas	Findings
Terock et al. 2022 (53)	Cohort study	1,865 subjects from the general population, age 51.6±13.7 years, 52.3% female	Gray matter and hippocampus	- The average serum 25 (HO)D concentration was 24.3 ng/mL (60.75 nmol/L), range 15.5 to 159.25 nmol/L (6.2 to 63.7 ng/ml) - Increased brain age was strongly linked to vitamin D deficiency and was significantly correlated with the total volume of the brain as well as the gray matter, and the hippocampus volume
Navale et al. 2022 (40)	Cohort study	427,690 subjects; risk of dementia = 3,414 cases, stroke = 5339 cases, age 37-73 years, (99.5% aged 40–69 years), 53.4% female	White matter, gray matter, and hippocampus	The strongest relationships were found in individuals with serum 25(OH)D concentrations < 25 nmol/L compared with 50-75.9 nmol/L and adjusted HR: 1.79; 95% CI: 1.57, 2.04 and HR: 1.40; 95% CI: 1.26, 1.56, respectively, for vitamin D deficiency and a higher risk of dementia and stroke.
Croll et al. 2021 (55)	Cross-sectional study	2,716 individuals without dementia from the Rotterdam population, age 56.6 ± 6.4 years, 55.4% female	White matter, gray matter, and hippocampus	- Serum 25(OH)D average 60.9 nmol/L - Reduced brain tissue volume, white matter, and hippocampus volume were associated with serum 25(OH)D concentrations <30 nmol/L compared to a sufficient vitamin D status (≥50 nmol/L)
Lee et al. 2021 (21)	Cross-sectional study	201 subjects (10 community-based normal healthy subjects, 33 with subjective cognitive decline, 97 with mild cognitive impairment, and 61 with Alzheimer's disease) from the dementia registry database age 74.9 ± 9.2 years, 68.1% female	Gray matter, olfactory areas, cingulate gyrus, temporal lobe, parahippocampus, hippocampus, cerebellum	- The mean serum 25(OH)D was 18.05 nmol/L; reduced brain volume was linked to lower 25(OH)D levels in the right olfactory and rectus gray matter areas, (family-wise error; FWE-corrected, $p < .05$) for all the subjects. - There were no white matter regions associated with 25(OH)D levels. - Left parahippocampus, fusiform, and hippocampal regions had a significant association with 25(OH)D in Alzheimer's disease patients (FWE-corrected, p. 05).
Ali et al. 2020 (59)	Cross-sectional study	53 older adults did not meet the consensus DSM IV criteria for clinical dementia and did not suffer from any neurological, depressive, or orthopedic conditions, age 72±5 years, 38% female	White matter, gray matter	- Serum 25(OH)D levels averaged 67.3 nmol/L - In older individuals, lowered serum 25(OH)D concentrations were linked with atrophy of the calcarine sulcus and a positive correlation was found between serum 25(OH)D and gray matter volume in the left calcarine sulcus ($p < 0.05$), threshold- free cluster enhancement (TFCE), FWE-corrected.

Table 1. Studies of serum 25(OH)D concentration deficiency and brain volume by magnetic resonance imaging (MRI) scanning (continuous)

Reference	Design	Population/Characteristics	Brain volume areas	Findings
Al-Amin et al. 2019 (56)	Cross-sectional study	56 patients who have mild cognitive impairment, age N/A, 67.8% female	White matter, gray matter, hippocampus, dentate gyrus, fimbria	- The average serum 25-OHD concentration was 15.41 ng/mL (37.85 nmol/L) - A significant decrease in the volume of the entire hippocampus, particularly in the dentate gyrus and fimbria, in the serum 25(OH)D deficient group. An absence of connections in 13 areas, with the right hippocampus as the center of the network disruption
Foucault et al. 2019 (58)	Cross-sectional analysis	215 Older Caucasian community-dwellers, age 72.1±5.5 years, 40% female	White matter, gray matter, perigenual anterior cingulate cortex, midcingulate cortex, posterior cingulate cortex	- The total cingulate thickness in subjects with vitamin D insufficiency (<50nmol/L) was lower than that of the other cingulate part (24.6±1.9mm versus 25.3±1.4mm, P=0.001). A significant difference was found for the perigenual anterior (p = 0.011), midcingulate (P=0.013) and posterior cingulate cortex (p = 0.021)
Brouwer-Brolsma et al. 2015 (54)	Cross-sectional study	217 community-dwelling elderly people, age 72± 6 years, 57% male	White matter, gray matter	- The average level of serum 25(OH)D was 61± 23 nmol/L. after adjusting for intracranial volume. Serum 25(OH)D was significantly linked with gray matter volume (β±SE: 0.25 0.09 mL; p = 0.005) and total brain volume (β±SE: 0.26 0.11 mL; p = 0.02) but not with white matter volume.

as part of an investigation by Foucault et al. Using FreeSurfer and T1-weighted MR images, the thickness of the cingulate cortex (perigenual anterior, middle, and posterior) was evaluated. It was found that a smaller cingulate cortex was associated with vitamin D insufficiency (<50 nmol/L, n = 80) in the sample of elderly people studied. A smaller average cingulate thickness was cross-sectionally linked to vitamin D deficiency (β = -0.49, p = 0.028). The thickness of the perigenual anterior, midcingulate, and posterior cingulate cortex was strongly linked with serum 25(OH)D concentration (p = 0.011, p = 0.013, and p = 0.021, respectively) (58) Table 1. In addition, Ali et al. found that in older individuals, a smaller left calcarine sulcus volume was linked to lower serum 25(OH)D levels (59) Table 1.

The upshot is that vitamin D is crucial for the function of various systems such as the skeletal system, nervous system, and immune system, particularly in patients with Alzheimer's disease, Parkinson's disease, and neurocognitive disorders, and results in a decline in the brain's effectiveness. The brain volume also decreases in various areas, resulting in cognitive process being impaired. Cognitive impairment affects daily life and social interaction. Patients with MCI are at a higher risk of vitamin D deficiency, which is associated with focal brain atrophy and reduced brain gray matter volume in the relevant regions, including the hippocampus. Cognitive impairment patients have deficits in perceptual-motor, language, executive function, complex attention, memory and learning, and social cognition.

CONCLUSIONS

Vitamin D deficiency is characterized as a serum 25 (HO)D concentration <30 nmol/L, insufficiency to be between 30 and 50 nmol/L, and sufficiency to be >50 nmol/L. A severe vitamin D deficiency is indicated by serum 25(OH)D levels <25 or <30 nmol/L, which significantly increases the risk of osteomalacia and nutritional rickets. Vitamin D status of 25 (HO)D <30 nmol/L is linked with greater levels of decreased brain tissue volume compared to a sufficient vitamin D status (>50 nmol/L) and is associated with reduced volume of hippocam-

pal subfields and connection deficits in older people with MCI. 25(OH)D level is positively correlated with regions of low gray matter volume. A reduced area of the brain where neurocognitive functions are involved in response, including gray matter, white matter, the hippocampus, the cingulate cortex, the calcarine sulcus, the parahippocampus, cerebral cortex, and the olfactory regions, are associated with a low serum level of 25(OH)D. Additionally, as declining brain regions are responsible for reductions in typical cognitive states (such as executive processes, memory consolidation, and behavioral regulation), it is likely that those individuals have a low serum 25(OH)D concentration resulting in cognitive impairment.

Determination of the adequate intake of vitamin D is an important area for future investigation related to preventing decline in or improvement of neurological function. The results of such a study should be valuable for the general population, the elderly, patients with neurodegenerative disease, and neurocognitive disorders. Dementia is a major neurocognitive disorder. Each of the three categories of dementia (delirium, MCI, dementia), can occur in elderly patients and can cause increased disabilities and dependence which also have a significant impact on families, relatives, caregivers, and a country's health service system. Further investigation is needed to determine the most effective ways to use vitamin D for the prevention, treatment and/or alleviation of neurodegenerative diseases and neurocognitive disorders even though the condition is currently incurable. There are limitations to cross-sectional studies, so longitudinal imaging studies are required as well.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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