COMPARING THE SEVERITY OF LONG COVID AND VITAMIN D LEVELS

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DOCTOR OF PHILOSOPHY
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THIS DISSERTATION IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANTI-AGING AND REGENERATIVE MEDICINE

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ABSTRACT

"Long COVID" is a condition where patients continue with persistent symptoms even after they have recovered from the initial COVID-19 infection. These ongoing symptoms might damage various systems in their body. Several risk factors contribute to long-term COVID-19 development, including vitamin and mineral deficiencies. Vitamin D is an important nutrient for maintaining overall health and may be associated with the onset of long COVID. As a result, we studied the prevalence and compared long COVID cases among individuals with varying vitamin D levels. We also examined the relationship between vitamin D levels and the prevalence of eight long-term COVID-19 symptom categories in 170 patients who had previously been infected with the COVID-19 virus and received treatment at Foresta Clinic. This study used a cross-sectional descriptive design, collecting data by using a case record form that included demographic data, eight long COVID symptom categories, and vitamin D levels.

The study results indicated a female-to-male ratio of 1.1:1 among the participants, with a mean age of 45.87±8.65 years. Additionally, 62.4% had received three doses of the COVID-19 vaccination. Long COVID was found in 64.7%, with the majority (50.0%) had mild long COVID, followed by 11.2% with moderate long COVID, and 3.5% with severe long COVID. The blood vitamin D level was 41.2% of participants had insufficient, 30.6% were deficiency, and 28.2% had sufficient status. All participants with severe long COVID had vitamin D deficiency, while 73.7% of moderate, 28.2% of mild, and 13.3% without long COVID were deficient with

statistical significance (P<0.001). Participants with long COVID symptoms had significantly lower blood vitamin D levels than those without symptoms (p<0.05) across all symptom categories. Multivariable analysis found that vitamin D deficiency was significantly associated with long COVID symptoms at the 0.05 level. Specifically, associations were found for general symptoms (Adj. OR 4.55 [95%CI 1.88, 10.87]), respiratory symptoms (Adj. OR 6.06 [95%CI 2.37, 15.54]), cardiovascular symptoms (Adj. OR 22.63, 95%CI [5.88, 87.14]), neurological symptoms (Adj. OR 16.22 [95%CI 4.81, 54.65]), musculoskeletal symptoms (Adj. OR 13.77 [95%CI 4.54, 41.82]), skin symptoms (Adj. OR 11.28 [95%CI 4.30, 29.57]), psychiatric symptoms (Adj. OR 3.97 [95%CI 1.56, 10.08]), and the overall occurrence of long COVID (Adj. OR 5.80 [95%CI 2.10, 16.13]). Therefore, assessing and maintaining vitamin D levels, vitamin D supplementation, and sunlight exposure in COVID-19 patients can reduce the risk and severity of long-term COVID symptoms.

Keywords: Long COVID, COVID-19, Post-COVID-19 Symptoms, Vitamin D

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance of the Problem

COVID-19, also known as Coronavirus Disease 2019, is a respiratory infection caused by a novel coronavirus strain, SARS-CoV-2, which mutated naturally into a new variant. The first reported cases were detected in December 2019 in Wuhan, the capital of Hubei province in central China. The ensuing outbreak quickly spread worldwide and had a significant impact on public health, the economy, and society. [1] The most common mild symptoms include fever (87.9%), dry cough (67.7%), fatigue (38.1%), and sputum (33.4%). [2] However, severe symptoms are more common in older people and those with preexisting conditions. COVID-19 constitutes a global pandemic, and measures were taken to curb its transmission, including screening tests, wearing face masks, frequent handwashing with soap or alcohol-based sanitizers, maintaining social distancing of 1-2 meters, quarantining of susceptible individuals, avoiding large gatherings, and COVID-19 vaccinations. [3] These efforts gradually led to a decline in cases and fatalities. The COVID-19 outbreak was declared an endemic disease in 2022 by the National Health Security Office, according to the Thai government gazette, resulting in people being able to start returning to normal in the "new normal" [4] while relaxing preventive measures against the virus.

The illness effects of COVID-19 can be both short-term and long-term. Some patients who recover from COVID-19 may not fully regain their pre-illness health and may develop new or persistent symptoms. This condition, known as Long COVID syndrome or Post-Acute Sequelae of SARS-CoV-2 infection (PASC), refers to symptoms that persist for more than 12 weeks following the initial infection. The World Health Organization (WHO) defines Long COVID as

"*A condition that occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, typically within three months of the initial infection, where*

symptoms persist for at least two months and cannot be attributed to another medical condition." [5]

Similarly, the National Institute for Health and Care Excellence (NICE) categorizes Long COVID into two phases: [6]

1. Post-acute COVID-19: Symptoms that persist or emerge between 4 to 12 weeks after infection

2. Chronic COVID-19: Symptoms that long-lasting beyond 12 weeks after infection.

Long COVID is estimated to affect approximately 10-30% of patients who have had COVID-19, often significantly impact their quality of life. [7] A survey-based study on the incidence of Long COVID among individuals with a history of COVID-19 in the United States reported rates of 18.9% (95% CI: 17.9-19.8) in 2022 and 11.0% (95%CI 10.4-11.6) in 2023, with a higher prevalence among those aged 60 years and older compared to younger age groups. Additionally, 26.4% (95%CI 24.0-28.9) of individuals with Long COVID experienced limitations in daily activities.[8] A systematic review and meta-analysis of 11,192 articles covering 120,970 participants found that the incidence of any Long COVID symptoms among individuals previously infected with SARS-CoV-2 was 56.9% (95%CI 52.2-61.6). [9] In China, studies reported a high 90.4% prevalence of Long COVID among COVID-19 patients was 90.4%, with 62.4% having moderate to severe symptoms and 31.0% suffering from severe symptoms. [10] In Thailand, 29.9% of recovered COVID-19 patients reported Long COVID symptoms. Among them, 81.7% reported respiratory symptoms, 27.9% reported general body symptoms, and 22.0% had neurological symptoms.[11] Long COVID has been associated with more than 200 symptoms affecting multiple organ systems [12], including general symptoms: (fatigue, fever, chills), respiratory symptoms (dyspnea, cough), cardiovascular symptoms (palpitations, tachycardia, chest pain), neurological symptoms (loss of smell and taste, headaches, dizziness, brain fog, difficulty concentration), gastrointestinal symptoms (diarrhea, nausea, vomiting, abdominal pain), dermatological symptoms (rashes, hair loss, peeling), Ears, eyes, nose, and throat symptoms (dysphagia, hearing loss, blurred vision, ear pain), musculoskeletal symptoms (muscle pain, joint pain, stiffness, muscle atrophy), immune system issues (increased allergic reactions, blistering skin),

reproductive system symptoms (testicular pain, erectile dysfunction), and psychiatric symptoms (anxiety, depression, insomnia). These findings highlight that Long COVID remains a significant global health concern, significantly affecting patients' quality of life and posing direct economic consequences due to healthcare costs and productivity loss. [13]

The statistically significant risk factors contributing to the development of long COVID include gender, with studies showing that women are 1.49 times more likely than men to develop long COVID (95%CI 1.13–1.95). [10] Age, as persons aged 50 and older are 2.44 times more likely to develop long COVID compared to those aged 15–30 (95%CI 1.29–4.66). [14] Additional risk factors include occupations in the transportation sector, smoking, the presence of underlying conditions, experiencing five or more symptoms during the acute phase of COVID-19, and the severity of the disease. [10-14] Additionally, abnormal blood test results, such as lymphopenia, neutrophil-to-lymphocyte ratio, thrombocytopenia, coagulation profile, liver enzymes, inflammatory markers, LDH, D-dimer, troponin, and creatine phosphokinase, are associated with poorer treatment outcomes for hospitalized patients and are linked to the occurrence of long COVID. [15] Furthermore, deficiencies or insufficiencies in vitamins and minerals, both water-soluble and fat-soluble, can adversely affect the body’s functions and contribute to the development of long COVID.

Vitamin D is an essential nutrient that plays a critical role in supporting overall health in various aspects. As a fat-soluble vitamin with a cholesterol-based chemical structure, it is primarily stored in adipose tissue. Most vitamin D is synthesized in the skin when exposed to sunlight (Ultraviolet B, UVB). Once in the bloodstream, it undergoes hydroxylation in the liver, converting it into calcidiol, an inactive form. In the kidneys, under the regulation of parathyroid hormone, calcidiol is further hydroxylated into $1,25(OH)_2D$, also known as calcitriol. Calcitriol enhances the absorption of nutrients, particularly calcium and protein, in the intestines, thereby increasing blood calcium levels. [16] Additionally, vitamin D is vital for immune system modulation, supporting both innate and adaptive immunity. It enhances the immune response to antimicrobial and antiviral agents and potentially influences the outcomes related to COVID-19. [17] Vitamin D may contribute to the production of antimicrobial peptides in the respiratory epithelium, maintain cellular junction integrity, regulate cell

proliferation, and support angiogenesis. Studies have demonstrated that blood vitamin D levels are associated with the severity of COVID-19. Individuals with sufficient vitamin D levels (>50 nmol/L) or insufficient levels (25–50 nmol/L) were found to have a 50% lower risk of developing severe COVID-19 symptoms (aOR 0.51 [95%CI 0.27–0.96] and 0.49 [95%CI 0.25–0.94], respectively) compared to those with vitamin D deficiency (<25 nmol/L). Male patients or individuals under 65 with sufficient vitamin D levels were found to have a lower risk of severe COVID-19 symptoms compared to females or those aged 65 and older. [18] Conversely, low blood vitamin D levels are associated with increased severity of COVID-19, leading to higher mortality rates, more frequent admission to critical care units, prolonged hospital stays, and greater reliance on ventilators. Evidence also suggests that severe COVID-19 is linked to the development of long COVID. Patients with severe or critical COVID-19 are 2.15- and 2.59 times more likely to develop long COVID compared to those with mild COVID-19. [10] This may be due to higher viral load during the acute phase, which is linked to the severity of long COVID symptoms. [13]

Although there are findings indicating a relationship between low blood vitamin D levels and COVID-19, But evidence regarding the role of vitamin D in persistent symptoms and signs after recovery from COVID-19 remains limited. Therefore, this research aimed to examine the prevalence and compare the occurrence of long COVID among those with vitamin D deficiency, insufficient vitamin D levels, and sufficient vitamin D levels. The study assessed the impact of blood vitamin D levels on the risk of developing long COVID in patients who had previously been infected with the COVID-19 virus. The evaluation covered symptoms across eight body systems associated with long COVID, evaluated three months post-recovery.

The findings of this study provide insights into the role of vitamin D deficiency or insufficiency in long COVID and contribute evidence of the potential relationship between vitamin D deficiency or insufficiency and the development of long COVID.

1.2 Research Questions

1. 2.1 Does the severity level of long COVID exhibit differences in vitamin D levels?

1.2.2 What is the prevalence of long COVID among those with vitamin D deficiency, insufficient vitamin D levels, and sufficient vitamin D levels?

1.2.3 Do differing blood levels of vitamin D affect the risk of long COVID?

1.3 Objective of the Research

1.3.1 To examine the association between the severity of long COVID in patients who previously contracted COVID-19 and vitamin D status.

1.3.2 To determine the prevalence of long COVID among those with vitamin D deficiency, insufficient vitamin D levels, and sufficient vitamin D levels in the blood.

1. 3.3 To investigate the relationship between blood vitamin D levels and symptoms of long COVID.

1.4 Hypothesis of the Research

1.4.1 Blood vitamin D is associated with the severity of long COVID.

1.4.2 Blood vitamin D is associated with general symptoms of long COVID.

1.4.3 Blood vitamin D is associated with respiratory symptoms of long COVID.

1.4.4 Blood vitamin D is associated with cardiovascular symptoms of long COVID.

1.4.5 Blood vitamin D is associated with neurological symptoms of long COVID.

1.4.6 Blood vitamin D is associated with gastrointestinal symptoms of long COVID.

1.4.7 Blood vitamin D is associated with musculoskeletal symptoms of long COVID.

1.4.8 Blood vitamin D is associated with dermatological symptoms of long COVID.

1.4.9 Blood vitamin D is associated with psychiatric symptoms of long COVID.

1.4.10 Blood vitamin D is associated with the occurrence of long COVID.

1.5 Scope of the Research

1.5.1 Population scope: The population for this study consists of patients who have previously contracted the COVID-19 virus.

1.5.2 Variable scope: The variables studied include blood vitamin D levels (25(OH)D) and symptoms of long COVID with eight body systems: general symptoms, respiratory symptoms, cardiovascular symptoms, neurological symptoms, gastrointestinal symptoms, musculoskeletal symptoms, dermatological symptoms, and psychiatric symptoms.

1.5.3 Study period scope: The data collection period is from September 2024 to November 2024.

1.6 Conceptual Frameworks in the Research

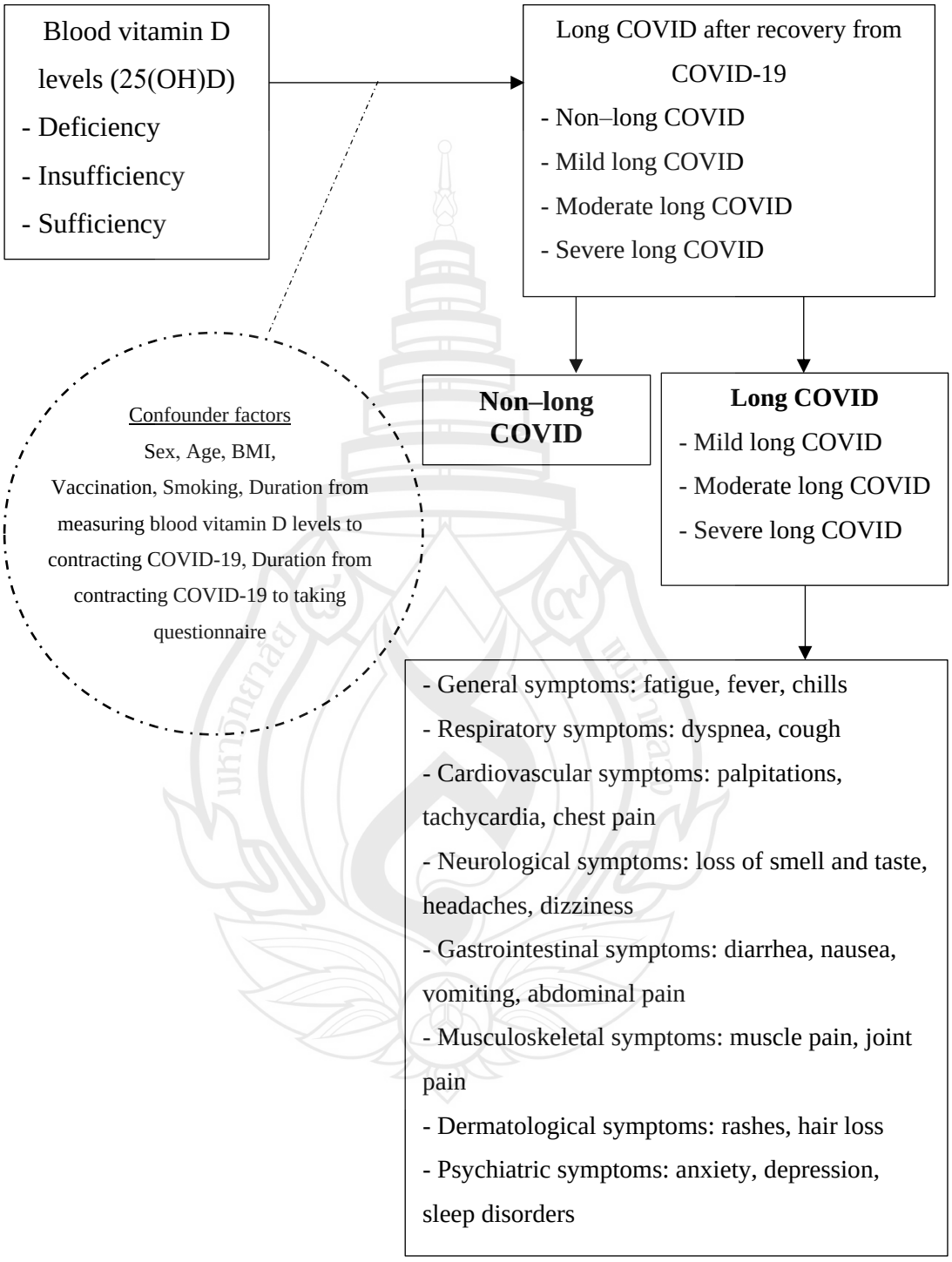


Figure 1.1 Conceptual frameworks in the research

1.7 Definition of Terms

1.7.1 Blood vitamin D levels refer to the concentration of 25-Hydroxyvitamin D (25(OH)D) in the blood, measured in ng/mL. This includes two types of vitamin D: Vitamin D2 (Ergocalciferol) and Vitamin D3 (Cholecalciferol). [16] According to the guidelines of the Endocrine Society of the United States, blood vitamin D levels are categorized as follows: [19]

Deficiency level	< 20 ng/mL (or <50 nmol/L)
Insufficiency level	20-30 ng/mL (or 50-75 nmol/L)
Sufficiency level	>30-100 ng/mL (or >75-250 nmol/L)
Toxic level	>100 ng/mL (or >250 nmol/L)

1.7.2 Long COVID is defined as a condition characterized by persistent symptoms or signs that last beyond recovery from COVID-19, or symptoms that continue for more than four weeks after acute infection. These symptoms can affect multiple organ systems and cannot be attributed to other diagnoses. [6]

In this study, the definition is based on the criteria established by NICE [6], which include both Post-acute COVID-19 (4-12 weeks) and chronic COVID-19 (> 12 weeks). The long COVID symptoms are grouped into eight symtemic symptoms, including general symptoms, respiratory symptoms, cardiovascular symptoms, neurological symptoms, gastrointestinal symptoms, musculoskeletal symptoms, dermatological symptoms, and psychiatric symptoms.

1.7.3 Long covid severity refers to the extent of persistent symptoms experienced by participants after recovering from acute COVID-19 infection. In this study, severity was categorized based on the number of reported symptoms from a long COVID symptom checklist, as follows:

Non–long COVID	no symptoms
Mild long COVID	1 to 7 symptoms
Moderate long COVID	8 to 14 symptoms
Severe long COVID	15 to 21 symptoms

1.7.4 Symptom severity refers to the intensity of symptoms compared to the pre-COVID-19 state. Severity is measured using a numerical rating scale (NRS) from

0 to 10, where 0 indicates the symptom's absence, and 10 indicates the most severe symptoms.

1.7.5 Prevalence of Long COVID refers to the proportion of patients with long COVID symptoms among the population of COVID-19 patients at a specific period. In this study, prevalence is assessed within groups categorized by vitamin D levels: deficiency, insufficiency, and sufficiency.

1.7.6 COVID-19 is a respiratory infectious disease caused by the SARS-CoV-2 virus. Symptoms may include fever, dry cough, and fatigue, and pneumonia in severe cases. However, some patients remain asymptomatic. [1]

1.7.7 Patients who had previously contracted COVID-19 refer to patients who were confirmed to have been infected with COVID-19 through either a Covid-Ag or PCR test. For this study, patients selected were those with mild symptoms and no significant risk factors for severe disease or major comorbidities.

1.7.8 Sex refers to the biological classification of the participants as either male or female, as documented in the case records form.

1.7.9 Age refer to the age of the participants at the time of data collection, recorded in completed years.

1.7.10 Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (kg/m²). In this study, the Asian BMI classification was applied and categorized into three group as follows:

Underweight	BMI < 18.5 kg/m²
Normal weight	BMI 18.5–22.9 kg/m²
Overweight	BMI 23.0–24.9 kg/m²

1.7.11 COVID-19 vaccination refers to the history of COVID-19 vaccination before or after infection, including the number of doses, and vaccine types, as reported by the participants.

1.7.12 Duration from measuring blood vitamin D levels to contracting COVID-19 refers to the time interval (in days) between the date of serum 25(OH)D level measurement and the confirmed date of COVID-19 infection diagnosis.

1.7.13 Duration from contracting COVID-19 to take questionnaire refers to the time interval (in days) from the confirmed date of COVID-19 infection to the date when the participant completed the long COVID symptom severity questionnaire.

1.8 Expected Outcome/Benefits of the Project

1.8.1 Provides medical evidence on blood vitamin D levels in patients who have previously contracted COVID-19, focusing on the prevalence and severity of long COVID in vitamin D deficiency and insufficient blood vitamin D levels.

1.8.2 Enhances understanding of the role of blood vitamin D levels in the symptoms and severity of long COVID, enabling healthcare professionals to plan treatments and provide better suggestions to patients.

CHAPTER 2

REVIEW LITERATURES

In this research, the researcher reviewed relevant literature and previous studies, with a focus on the following details:

2.1 Vitamin D

2.2 Long COVID

2.3 Vitamin D and COVID-19

2.4 Vitamin D and Long COVID

2.5 Related researches

2.1 Vitamin D

2.1.1 Vitamin D Metabolism

Vitamin D is an important nutrient that plays a crucial role in overall health. Vitamin D is not recognized as a true vitamin but rather as a prohormone belonging to the secosteroid group. It is fat-soluble and structurally formed from cholesterol. The main source of vitamin D is synthesis in the skin upon exposure to ultraviolet B (UVB) radiation from sunlight, though some can also be obtained from dietary sources. Once in the bloodstream, vitamin D undergoes hydroxylation in the liver, turning it into calcidiol (25(OH)D), its inactive form. A second hydroxylation occurs in the kidneys, regulated by parathyroid hormone, transforming calcidiol into $1,25(OH)_2D$ (calcitriol), the biologically active form. Calcitriol enhances nutrient absorption, especially calcium and phosphorus, in the intestines, promoting efficient uptake into the bloodstream and increasing blood calcium levels. [16] In the absence of vitamin D, the intestines can absorb only 10–15% of dietary calcium and 60% of dietary phosphorus, leading to potential deficiencies. [20]

Additionally, calcitriol synthesized in other tissues acts in autocrine and paracrine signaling, playing a key role in cellular regulation. Many cells express vitamin D receptors (VDR), and when activated by vitamin D, they affect genetic

transcription, regulating protein synthesis, cell proliferation, cell differentiation, and apoptosis. [20] Therefore, vitamin D deficiency impairs the body's ability to regulate physiological functions and respond to illness, highlighting its importance in maintaining overall health. [20]

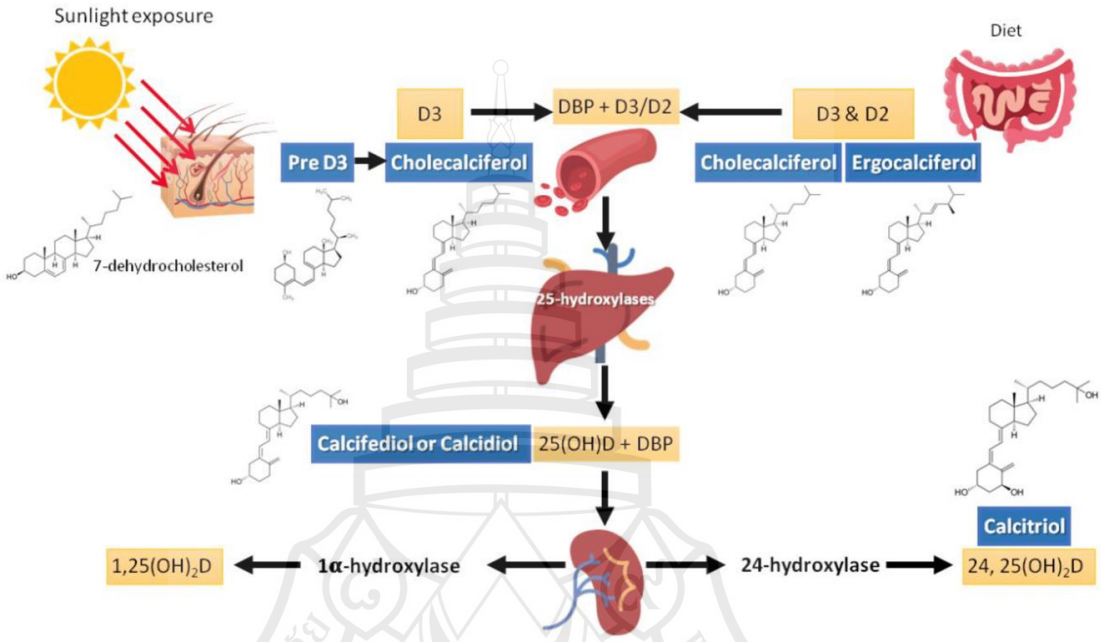


Figure 2.1 The synthesis and metabolism process of vitamin D in the body

2.1.2 Types of Vitamin D

Normally, the body acquires vitamin D in two forms: [20-21]

1. Vitamin D3 (Cholecalciferol): This form is obtained from two sources: animal-based nutrients, which are fat-soluble vitamin D3 with a steroid structure, and the skin's synthesis via UVB radiation from sunlight. Vitamin D synthesized in the skin can stay in the body 2-3 times longer than Vitamin D obtained from food and does not cause toxicity due to high levels of Vitamin D. This is because skin synthesis has a protective system that breaks down excess Vitamin D into an inactive form. Several factors impact the process of vitamin D production in the skin:

1) UVB wavelength: The UVB radiation should range between 290 and 315 nm.

2) Exposure Time to UVB: The skin should be exposed to 25-50% of the Minimal Erythema Dose (MED), which is the minimum amount of sunlight needed

to cause skin redness, to increase vitamin D synthesis. For example, if 1 MED is 30 minutes, the optimal exposure time would be 6-8 minutes.

3) Zenith Angle: This refers to the optimal angle of sunlight relative to the Earth's vertical axis. As the Zenith angle rises, the amount of UVB radiation reaching the Earth's surface decreases, reducing vitamin D synthesis. The angle rises at higher latitudes or during early morning and late afternoon. Therefore, in Thailand, the best time for vitamin D production is between 10:00 AM and 3:00 PM.

4) Environmental Factors: Dust, air pollution, glass, plastic barriers, and sunscreen can lower UVB exposure, which negatively affects the efficiency of vitamin D synthesis.

5) Reflective Surfaces: including snow, sand, water, glass, and plastic reflect UVB, which can increase the amount of UVB exposure.

6) Melanin Content: Higher levels of melanin in the skin lower the effectiveness of vitamin D synthesis. Skin types can be classified into six categories according to the amount of melanin (Fitzpatrick's classification). In Thailand, skin types are mostly types 4 and 5. Melanin functions as a natural sunscreen, absorbing UVB rays to prevent sunburn. As a result, those with darker skin require more sun exposure than those with lighter skin to synthesize the same quantity of vitamin D.

7) Age: As people age, the amount of cholesterol in the skin (7-dehydrocholesterol) decreases, as it is the precursor for Vitamin D3. This reduction increases the risk of Vitamin D deficiency in the elderly.

2. Vitamin D2 (Ergocalciferol): This type is obtained from plant-based foods, vitamin D supplements, or fortified foods. The body cannot synthesize vitamin D2 on its own.

For those concerned about extended sun exposure because of its potential harms, such as skin cancer, vitamin D3 can be obtained from sources like cod liver oil, egg yolks, and skin synthesis. Vitamin D2 is found in yeast, and both types of vitamin D can also be present in milk. [22]

2.1.3 Appropriate Vitamin D Intake for the Body

The appropriate amount of vitamin D for each individual may vary. The recommended daily intake of vitamin D for the general population, as well as for those with vitamin D deficiency, depends on age. The maximum daily intake should not

exceed 1,000-4,000 IU (25-100 mcg) for those aged 0-18 years, and 4,000 IU (100 mcg) for aged 19 years and older. The details are given in Tables 2.1 and 2.2 [23-24]

Table 2.1 Recommended daily intake of vitamin D for the general population

Age group	Recommended daily intake
0–12 months	400 IU (10 mcg)
1–13 years	600 IU (15 mcg)
14-18 years	600 IU (15 mcg)
19-50 years	600 IU (15 mcg)
51-70 years	600 IU (15 mcg)
>70 years	800 IU (20 mcg)

Note *40 IU = 1 mcg

Table 2.2 Recommended daily intake of vitamin D for vitamin D deficiency individuals

Age group	Recommended daily intake
0–6 months	1,000 IU (25 mcg)
7–12 years	1,500 IU (38 mcg)
1-3 years	2,500 IU (63 mcg)
4-8 years	3,000 IU (75 mcg)
9-18 years	4,000 IU (100 mcg)
>18 years	4,000 IU (100 mcg)

Note *40 IU = 1 mcg

2.1.4 Assessment of Vitamin D Levels in the Body

To assess vitamin D levels in the body, it is recommended to use the level of 25(OH)D or total vitamin D (25(OH)D2 and 25(OH)D3). Several methods are used to examine 25(OH)D, including: [21]

1. Immunoassay: This method is based on the concept of antigen binding to antibodies. Various techniques include:

1) Radioimmunoassay (RIA): This method measures both 25(OH)D2 and 25(OH)D3 together, involving protein precipitation with acetonitrile before analysis. A limitation is that it is unsuitable for laboratories without space for working

with radioactive substances. RIA gives results that are close to those from high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, at low vitamin D concentrations, RIA tends to give lower results compared to LC-MS/MS, and at high concentrations, RIA gives higher results. On average, RIA yields about 13% higher vitamin D levels than LC-MS/MS.

2) Enzyme-Linked Immunosorbent Assay (ELISA): This method is less commonly used and has a low recovery rate, with 56.0% recovery for 25(OH)D2 and 79.0% for 25(OH)D3. It also provides lower Vitamin D concentrations compared to RIA by approximately 5.2-6 ng/mL and lower than LC-MS/MS by about 21.0%.

3) Chemiluminescent Immunoassay: This modern technology allows for automated analysis, which saves time and makes the process more convenient and efficient, suitable for general laboratories. However, a disadvantage is that the antibody may also bind to other Vitamin D metabolites, potentially resulting in higher-than-normal readings.

2. Chemistry-Based Assay: This method uses the chemical properties of substances for separation, including HPLC and LC-MS/MS techniques. Both methods can accurately separate and evaluate 25(OH)D2 and 25(OH)D3 levels. LC-MS/MS is more sensitive than HPLC for detecting low levels of 25(OH)D2 or 25(OH)D3. Currently, LC-MS/MS is widely accepted as the reference method for assessing Vitamin D levels. However, its limitation lies in the cost of the equipment and the need for skilled technicians to operate the instruments.

2.1.5 Optimal Vitamin D Levels in the Body

The normal levels of 25(OH)D are calculated differently in each country, as vitamin D levels in populations vary depending on various factors. [21] Generally, a vitamin D level greater than 20 ng/mL is considered adequate for the general population, while levels above 30 ng/mL are considered appropriate for individuals over 65 years old, patients receiving antiresorptive or anabolic bone-forming agents to reduce the risk of fractures due to fragility, or those under treatment that increases the risk of fragility fractures, such as glucocorticoids or cancer treatments with hormone-blocking drugs. However, national and international agencies define different 25(OH)D

status levels, including varying definitions of Vitamin D deficiency and insufficiency, as shown in Table 2.3. [25]

Table 2.3 Criteria for assessing blood vitamin D levels (25(OH)D) by international organizations

25(OH)D ng/mL	NAM/NIH	ES	NOS	SACN	AGS*	ESE
<10	Deficiency	Deficiency	Deficiency	Deficiency	Deficiency	Deficiency
10-20	Inadequacy risk	Deficiency	Inadequacy risk	Sufficiency	Deficiency	Deficiency
20-30	Sufficiency	Insufficiency	Sufficiency	Sufficiency	Deficiency risk	Insufficiency
30-50	Sufficiency	Desirable concentration	Sufficiency	Sufficiency	Minimally acceptable concentration	Sufficiency
50-100	Possible excess adverse events	Desirable concentration			Possible onset of toxicity	
100-150	Possible excess adverse events				Possible onset of toxicity	
>150					Toxicity	

Note NAM: National Academy of Medicine (former Institute of Medicine, IOM), USA; NIH: National Institute of Health, USA; ES: Endocrine Society, USA; NOS: National Osteoporosis Society, UK; SACN: Scientific Advisory Committee on Nutrition, UK; American Geriatrics Society, USA; ESE: European Society of Endocrinology

*for elder

At the international meeting on Controversies in Vitamin D held in Pisa, Italy, in 2017, the status of Vitamin D levels in adults was agreed upon as follows:

Sufficient Vitamin D is defined as 25(OH)D > 20 ng/mL,

Vitamin D Insufficiency is defined as 25(OH)D 12-20 ng/mL,

Vitamin D Deficiency is defined as 25(OH)D < 12 ng/mL,

Risk of Toxicity is defined as 25(OH)D > 100 ng/mL for adults with high calcium intake. [26]

2.1.6 The Role of Vitamin D in the Body

It is well-known that vitamin D plays a crucial role in the absorption of calcium and phosphate from the digestive system, which is then used to strengthen bones. This is the classical effect of Vitamin D, important in treating bone diseases and chronic kidney disease patients. At the same time, Vitamin D also has a non-classical effect, which plays a role in cardiovascular diseases, the immunological system, cancer, the neurological system [27], and the muscular system. The details are as follows: [20]

1. Role in Cardiovascular Diseases

Vitamin D helps regulate the Renin-angiotensin hormone system, which affects the development of high blood pressure. The release of Renin-angiotensin is controlled by the cyclic AMP signaling system. Vitamin D prevents cyclic AMP response element-binding protein (CREB) from binding to the renin gene promoter. Vitamin D reduces the formation of free radicals in the kidney vasculature in hypertension patients by inhibiting the enzyme NADPH-oxidases (NOXs), which increase the production of free radicals. These free radicals then stimulate calcium signaling to cause vasoconstriction, leading to high blood pressure. Additionally, Vitamin D enhances the activity of the Superoxide dismutase 1 enzyme to eliminate free radicals more efficiently. Vitamin D helps reduce calcium influx into the endothelial walls of blood vessels, thus reducing the release of vasoconstrictor substances and helping the blood vessels to relax, which lowers blood pressure. Furthermore, Vitamin D reduces the levels of angiotensin II and endothelin-1, which decreases the risk of heart muscle hypertrophy and heart failure. Vitamin D also reduces elevated endothelin-1 levels that could stimulate InsP3Rs (atrial heart rhythm regulators), reducing the incidence of atrial arrhythmias.

2. Role in the Immune System

Vitamin D regulates both adaptive immunity and innate immunity. Vitamin D deficiency is associated with immune system diseases, such as rheumatoid arthritis, multiple sclerosis, and chronic inflammatory bowel disease. Autoimmune conditions tend to increase when there is a deficiency of Th1 cells, which attack proteins produced by our body. Vitamin D can control the immune system by inhibiting changes in

antigen-presenting dendritic cells and reducing the activation of T cells and macrophages, which can lead to inflammation. Additionally, Vitamin D also helps to improve the general immune system by increasing the production of antimicrobial peptides that the body uses to fight off microorganisms.

3. Role in Cancer

Vitamin D is related to several types of cancer. Mortality rates from certain cancers increasing in areas with low UV light, such as colorectal, breast, and prostate cancers. Vitamin D has been found to have anti-cancer properties in breast, ovarian, lung, and prostate cancers. These effects are mediated through mechanisms where vitamin D works with Klotho and Nrf2 to reduce cancer cell division, formation of new blood vessels that supply tumors, and prevent cancer metastasis. Vitamin D limits the proliferation of endothelial cells (cells that form blood vessels) by inhibiting Vascular Endothelial Growth Factor (VEGF), a substance that promotes the growth of blood vessels to support cancer cells. Vitamin D plays an important role in increasing the strength of cell adhesion through mechanisms that produce substances such as E-cadherin, Zonula occludens-1 (ZO-1), ZO-2, and Vinculin. It also reduces the ability of cancer cells to migrate, invade, or develop into new cancers by inhibiting the secretion of Metalloproteinase 2 and 9. Additionally, vitamin D helps regulate calcium balance, and since calcium (Ca^{2+}) signaling is involved in cancer cell migration, thus making vitamin D a control mechanism that helps control cancer metastasis.

4. Role in the Nervous System

Nerve cells have vitamin D receptors on their surface. It has been found that genetic diversity of vitamin D receptors (VDR polymorphisms) is associated with Parkinson's, autism, and cognitive loss in the elderly. Neurodegenerative diseases are often related to increased calcium signaling movement and the production of free radicals, which suggests a decline in vitamin D activity. This decline results in inhibiting these signals and stimulations, thus leading to neurological diseases.

5. Role in the Muscular System

Patients with nonspecific muscle weakness or muscle pain often have low vitamin D levels. Vitamin D receptors are also found in muscle cells, which play a role in ensuring efficient muscle function. These receptors decrease with age, leading to a decline in muscle strength as well.

2.2 Long COVID

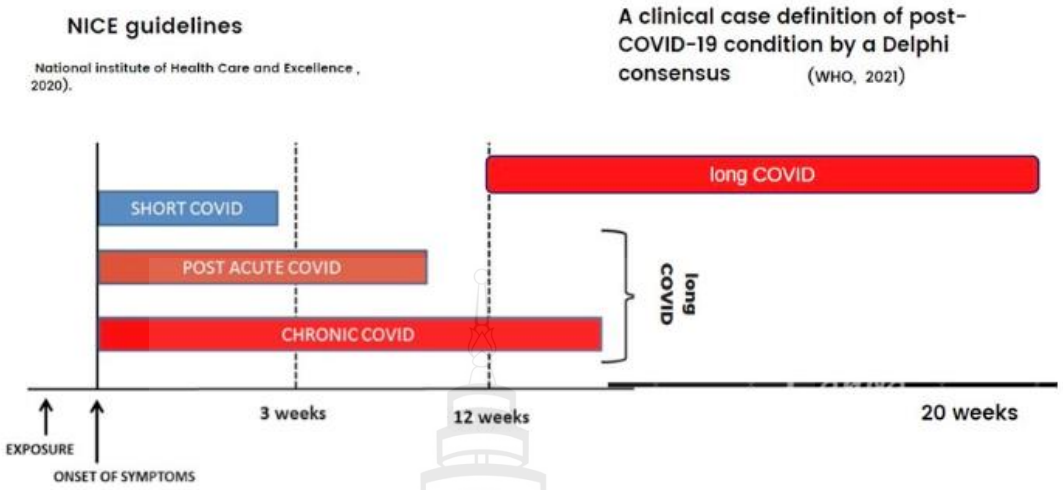
2.2.1 Definition

Patients who have previously been infected with the coronavirus 2019 (COVID-19) and have been properly treated until recovery, may still have symptoms that were present during the infection. This condition is known as “Long COVID-19” or by other names such as Post-COVID condition, Long-haul COVID, post-acute COVID-19, post-COVID-19 syndrome, or chronic COVID. [28] The World Health Organization (WHO) defines Long COVID as “a condition that occurs new or continues after the COVID-19 disease in individuals who have a possible or confirmed history of SARS-CoV-2 infection, typically occurring within 3 months from the onset of infection, with symptoms lasting at least 2 months and cannot be explained by other diagnostic cause” [5] The National Institute for Health and Care Excellence (NICE) has stated that it is “symptoms or signs that persist or develop after COVID-19 for more than 4 weeks, divided into 2 phases:

Post-acute COVID-19: symptoms or signs that persist or develop after COVID-19 for 4-12 weeks after the initial infection.

Chronic COVID-19: symptoms or signs that persist or develop after COVID-19 more than 12 weeks after the initial infection. [6]

The U.S. Department of Health and Human Services (DHHS) has defined Long COVID as “symptoms, signs, and conditions that continue or emerge after a SARS-CoV-2 infection in its initial phase. These symptoms appear from 4 weeks onward after the initial infection and may affect multiple body systems. They may flare up, worsen, or become more severe over time. Long COVID can potentially lead to serious or even life-threatening conditions, even months or years after the initial infection. It is a complex condition with multiple overlapping symptoms, which may include biological causes, risk factors, and different outcomes”. [29]



Source [7]

Figure 2.2 Timeline of long COVID onset according to definitions by NICE and the World Health Organization (WHO)

2.2.2 Prevalence and Risk Factors of Long COVID

A meta-analysis and systematic literature review of 41 research articles on the prevalence of long COVID from 2020 to 2022 [30] found an overall prevalence of long COVID was found to be 43.0% (95%CI 39.0-46.0). It was found in 54.0% (95%CI 44.0-63.0) of hospitalized patients and 34.0% (95%CI 25.0-46.0) of non-hospitalized patients. It was more prevalent in females than males (females 49.0% (95%CI 35.0-63.0) and males 37.0% (95%CI 24.0-51.0)). When categorized by region, it was found most frequently in Asia at 51.0% (95%CI 37.0-65.0), followed by Europe at 44.0% (95%CI 32.0-56.0), and America at 31.0% (95%CI 21.0-43.0), respectively. The prevalence also varied by follow-up duration, 37.0% (95%CI 26.0–49.0) at 30 days post-infection, 25.0% (95%CI 15.0–38.0) at 60 days, 32.0% (95% CI 14.0–57.0) at 90 days, and 49.0% (95% CI: 40.0–59.0) at 120 days.

The relatively high prevalence at the 120-day follow-up period was because most studies focused on the population group admitted to hospitals. The prevalence of long COVID symptoms, categorized by body systems, was further analyzed in the meta-analysis and systematic review. The findings are summarized as follows:

Table 2.4 Prevalence of symptoms and signs of long COVID

Systems	Gennaro FD, et al [9]	Chen C, et al [30]
General	31.0 (27.1-35.1)	
Fatigue	31.4 (95%CI 27.1-35.8)	23.0 (95%CI 17.0-30.0)
Pain	19.9 (95%CI 14.7-25.6)	
Flulike symptoms	16.5 (95%CI 9.7-25.4)	
Poor QoL	16.0 (95%CI 9.0-24.7)	
Myalgia	15.5 (95%CI 13.0-18.3)	6.0 (95%CI 4.0-9.0)
Arthralgia	15.0 (95%CI 11.6-18.9)	10.0 (95%CI 4.0-22.0)
Sore throat	7.6 (95%CI 6.2-9.2)	3.0 (95%CI 2.0-5.0)
Fever	7.9 (95%CI 5.2-11.0)	2.0 (95%CI 1.0-4.0)
Neurological	19.7 (95%CI 17.4-22.1)	
Difficulty concentrating	14.6 (95%CI 11.7-17.9)	9.00 (95%CI 5.00-15.00)
Memory deficits	13.5 (95%CI 10.5-16.9)	14.0 (95%CI 10.0-19.0)
Cognitive impairment	13.5 (95%CI 10.5-16.8)	
Smell disorder	13.1 (95%CI 11.1-15.3)	7.0 (95%CI 5.0-11.0)
Taste disorder	12.8 (95%CI 10.7-15.0)	8.0 (95%CI 4.0-13.0)
Headache	12.4 (95%CI 10.5-14.4)	5.0 (95%CI 3.0-7.0)
Respiratory	24.5 (95%CI 21.3-27.9)	
Dyspnea	24.1 (95%CI 20.5-27.9)	13.0 (95%CI 11.0-15.0)
Cough	13.1 (95%CI 11.0-15.5)	7.0 (95%CI 5.0-9.0)
Nasal congestion	6.3 (95%CI 5.0-7.7)	
Heart	11.0 (95%CI 8.9-13.3)	
Palpitations	11.2 (95%CI 8.7-14.1)	6.0 (95%CI 3.0-11.0)
Chest pain	10.6 (95%CI 8.2-13.3)	5.0 (95%CI 4.0-7.0)
Hypertension (new onset)	6.4 (95%CI 1.5-14.3)	
Psychiatric	20.3 (95%CI 17.4-23.3)	
Anxiety	18.9 (95%CI 15.2-22.2)	8.0 (95%CI 4.0-16.0)
Sleep disorder	17.8 (95%CI 14.8-21.0)	11.0 (95%CI 5.0-23.0)
Depression	16.1 (95%CI 12.8-19.8)	7.0 (95%CI 3.0-15.0)

Table 2.4 (continued)

Systems	Gennaro FD, et al [9]	Chen C, et al [30]
PTSD	13.6 (95%CI 8.9-19.3)	
Digestive	7.7 (95%CI 6.4-9.1)	
Loss of appetite	7.1 (95%CI 5.2-9.4)	
Diarrhea	5.9 (95%CI 4.9-7.1)	3.0 (95%CI 1.0-5.0)
Abdominal pain	5.2 (95%CI 4.0-6.5)	4.0 (95%CI 1.0-9.0)
Skin	8.5 (95%CI 6.8-10.3)	
Hair loss	8.8 (95%CI 6.8-11.1)	7.0 (95%CI 2.0-24.0)
Rash	4.1 (95%CI 2.9-5.5)	

Understanding the risk factors associated with long COVID, which may include pre-existing conditions, age, treatment, genetics, and lifestyle, helps guide clinical diagnosis and treatment of long COVID. These risk factors can be categorized into four factors as follows: [13]

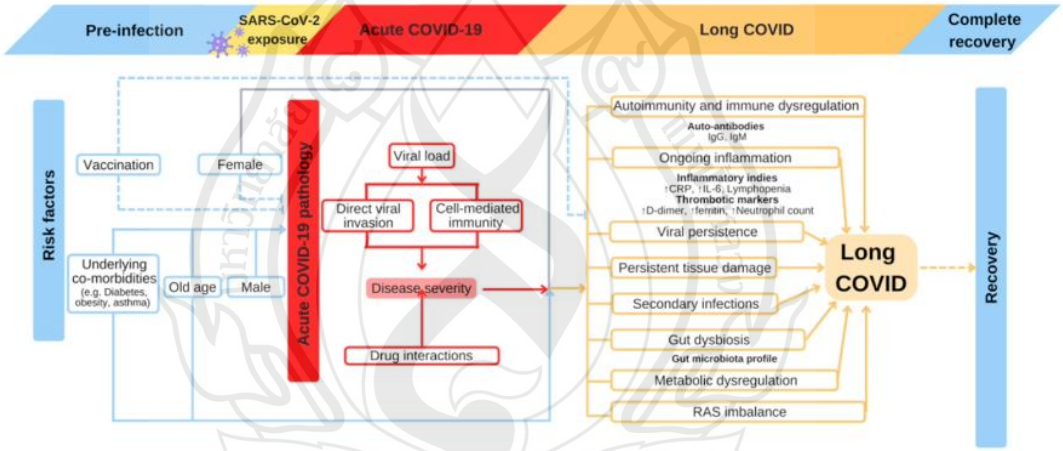
1. Severity of coronavirus 2019 infection: Studies indicate that patients who required ventilators during COVID-19 show an association between symptoms during this phase and the prevalence and severity of long COVID. Similarly, viral load during COVID-19 is related to long COVID severity, where lower viral loads or early viral clearance lead to reduced inflammatory responses, minimizing tissue damage. Additionally, the number of COVID-19 symptoms within the first week can also predict the duration of Long COVID.

2. Pre-existing conditions: Underlying health conditions are strongly linked to severe COVID-19 cases and are risk factors for long COVID. In ten longitudinal studies from the United Kingdom examining risk factors for long COVID, several pre-existing conditions were identified as risks, including female, poor mental health before the pandemic, overall poor health, asthma, and overweight or obese. [31] The complex interactions between asthma severity, underlying diseases, and corticosteroid use should be considered when treating long COVID. Additionally, type 2 diabetes increases the risk of long COVID due to chronic inflammation associated with insulin resistance, leading to increased immune response during SARS-CoV-2 infection.

Moreover, those with a history of poor mental health have a 50% increased risk of developing long COVID and more severe symptoms.

3. Age: Advancing age is a major factor in the severity of COVID-19 and the risk of long COVID. However, older adults tend to have pre-existing conditions and more severe acute responses to infections. Therefore, increasing age may be a secondary factor in the risk of long COVID.

4. Gender and sex hormones: Epidemiological has shown that female COVID-19 patients under 50 years old are up to five times more likely than males to have long COVID symptoms after hospital discharge. Studies suggest that the integrity of ACE2 in ovarian granulosa cells and hormone disruption from SARS-CoV-2 may both contribute to ovarian dysfunction. Women in the premenopausal and menopausal stages are particularly susceptible to long COVID because the virus disrupts ovarian hormone function, disrupting body system balance and leading to inflammation.



Source [13]

Figure 2.3 Risk factors for developing long COVID

2.2.3 Symptoms and Manifestations of Long COVID

Long COVID is a condition that affects multiple organ systems, often with severe symptoms following a serious SARS-CoV-2 infection. Biomedical research has found that many patients experience a wide range of symptoms affecting different organs. These symptoms may persist for several years. The symptoms and their pathological effects on various organ systems can be summarized as follows: [12]

Table 2.5 Symptoms of long COVID and impact on various organs

Organs	Symptoms	Pathology
Heart	- Chest pain	- Cardiac impairment
	- Palpitations	- Myocardial inflammation
		- POTS
Lunge	- Cough	- Abnormal gas exchange
	- Dyspnea	
Immune system		- Autoimmunity
		- MCAS
Pancreas		- Diabetes
		- Pancreas injury
Gastrointestinal tract	- Abdominal pain	- Gut dysbiosis
	- Nausea	- Viral persistence and viral reservoir
Neurological system	- Cognitive impairment	- Dysautonomia
	- Fatigue	- ME/CFS
	- Disordered sleep	- Neuroinflammation
	- Memory loss	- Reduced cerebral blood flow
	- Tinnitus	- Small fiber neuropathy
Kidney, Spleen, Liver		- Organ injury
Blood vessels	- Fatigue	- Coagulopathy
		- Deep vein thrombosis
		- Endothelial dysfunction
		- Microangiopathy
		- Microclots
		- Pulmonary embolism
		- Stroke

Table 2.5 (continued)

Organs	Symptoms	Pathology
Reproductive system	- Erectile dysfunction - Increased severity and number of premenstrual symptoms - Irregular menstruation	- Reduced sperm count

2.3 Vitamin D and COVID-19

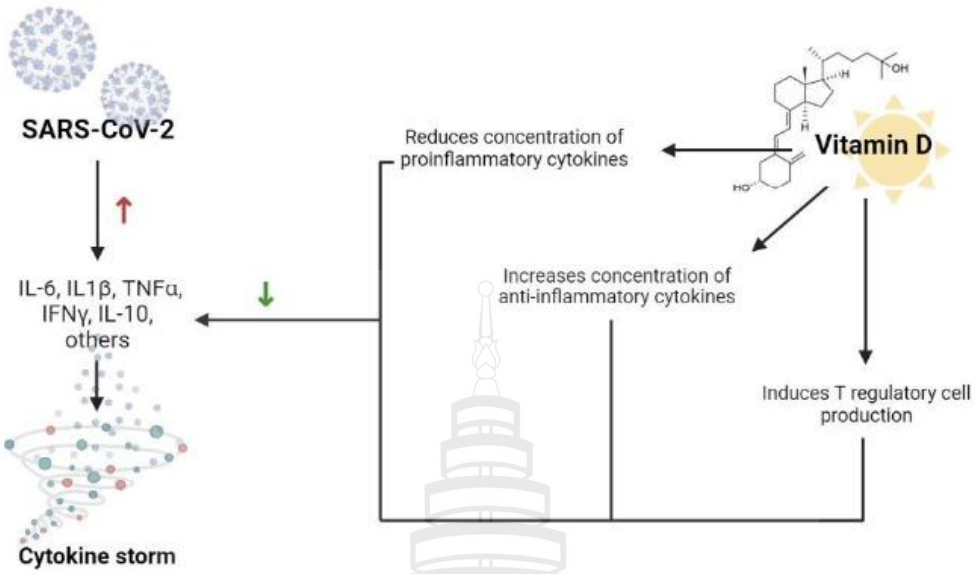
At the beginning of the COVID-19 pandemic, people were required to quarantine and adjust their daily routines, such as eating habits, exercise, and working from home, which became the new normal. These lifestyle changes resulted in less time spent outdoors, leading to reduced sun exposure and decreased vitamin D synthesis. Vitamin D deficiency is associated with various diseases, including infectious diseases, preeclampsia, cancer, dental caries and periodontitis, autoimmune diseases, cardiovascular diseases, chronic inflammation, type 1 and type 2 diabetes, neurological disorders, and an increased risk of death from respiratory infections in healthy individuals. [32] Although these conditions increase the risk of COVID-19, Vitamin D deficiency itself is also a significant factor in disease susceptibility. A clinical study across 20 countries found a significant negative correlation between average vitamin D levels and the number of COVID-19 cases ($r = -0.477$, $p=0.033$). This suggests that lower Vitamin D levels are associated with an increased incidence of COVID-19. However, there was no significant relationship between average Vitamin D levels and COVID-19 mortality rates. [33]

It is well known that Vitamin D helps regulate both the adaptive immunity and innate immunity systems. [20] The potential benefits of Vitamin D in COVID-19 outcomes may be due to its role in preventing an overactive immune response, such as cytokine storms in severe COVID-19 cases. Vitamin D may play a role in producing antimicrobial peptides in respiratory epithelial cells, maintaining the integrity of cellular junctions, and regulating cellular proliferation and angiogenesis. Additionally,

genetic variations in the Vitamin D-binding protein gene, particularly SNP in the rs7041 locus, are associated with the prevalence and mortality rate of COVID-19, indicating that genetic factors may also play a role. [18]

Vitamin D has multiple mechanisms that can reduce the risk of microbial infections and mortality. These mechanisms can be categorized into
physical barrier, natural cellular immunity, and adaptive immunity. Vitamin D protects the respiratory system by maintaining the integrity of cellular junctions and eliminating enveloped viruses through Cathelicidin and Defensins. Additionally, Vitamin D helps reduce the production of pro-inflammatory cytokines by the innate immune system and reduces the concentration of proinflammatory cytokines, which can lead to pneumonia. The immune-boosting role of Vitamin D enhances innate immunity through the secretion of antiviral peptides, which increase mucosal resistance and affect both Tumor Necrosis Factor (TNF)-α and Interferon-γ. [32]

In severe acute respiratory syndrome (SARS) caused by SARS-CoV-2, the immune response plays a crucial role during infection. This is accompanied by the excessive release of inflammatory cytokines such as TNF-α, Interleukin (IL)-6, and IL-1β, which contribute to increased vascular permeability, lung injury, multi-organ failure, and severe COVID-19 symptoms, followed by hyper-reaction and cytokine storms in some patients, which can lead to acute respiratory distress syndrome (ARDS). Cytokine storm can lead to abnormal stimulation of the adaptive immune system, causing an excessive release of pro-inflammatory cytokines and chemokines. Therefore, improving immunity through proper nutrition is an essential factor to consider, and vitamin D plays a crucial role in immune system function, [33] as illustrated in Figure 2.4.



Source [32]

Figure 2.4 Mechanisms of vitamin D in reducing cytokine storms

2.4 Vitamin D and Long COVID

A review of risk factors for Long COVID studied with various biomarkers including D-dimer, interleukin-6 (IL-6), C-reactive protein (CRP), procalcitonin, and neutrophil count. A study in Mexico on outpatients with COVID-19 compared 22 patients who received Vitamin D supplementation with 20 patients who did not. It was found that ferritin levels were significantly lower in the Vitamin D supplementation group, while D-dimer levels showed no significant difference. [34] Similarly, an India study compared inflammatory markers in COVID-19 patients who received vitamin D supplements at doses of 1,000 IU/day versus 5,000 IU/day. The results showed that D-dimer levels did not significantly differ between the two groups, but IL-6 levels decreased significantly in both groups. [35] A Turkish study of 95 hospitalized COVID-19 patients who received Vitamin D supplementation for 14 days found that Vitamin D had no effect on ferritin or D-dimer levels, but CRP and Fibrinogen levels decreased significantly. [36] One reason why vitamin D supplementation may not be effective in treating Long COVID is that SARS-CoV-2 can control vitamin D receptors, a

phenomenon also observed in infections such as Cytomegalovirus, Hepatitis B, and Hepatitis C. [32]

However, a study examining the relationship between low vitamin D levels and long COVID symptoms in 100 COVID-19 patients found that 25(OH)D levels at a 6-month follow-up were significantly lower in those with Long COVID compared to those without Long COVID (20.1 [95% CI 13.6-21.8] ng/mL vs. 23.2 [95% CI 16.7-26.6] ng/mL). However, hospitalization period 25(OH)D levels did not differ between the two groups. [17] This suggests that Vitamin D may function as an immunomodulator, but it also plays roles in cell growth regulation, neuromuscular function, immune system function, and inflammation reduction. Importantly, 25(OH)D levels may decrease during acute inflammation.

Fatigue is a common symptom of Long COVID. Before the COVID-19 pandemic, it was well-known that low 25(OH)D levels were associated with fatigue and muscle weakness in the general population. An experimental study on the effects of Ergocalciferol supplementation for 5 weeks on fatigue symptoms in individuals with low Vitamin D levels, was assessed using the Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF). The results showed that MFSI-SF scores significantly improved after 5 weeks of Ergocalciferol in general, physical, emotional, mental, and vigor domains. This improvement is because vitamin D helps regulate calcium balance by enhancing calcium absorption in the intestines and kidneys while also releasing calcium from bones. When Vitamin D levels are low, total and ionized calcium levels decrease, leading to increased parathyroid hormone (PTH), decreased bone density, decreased urinary calcium excretion, increased phosphate excretion, and decreased blood phosphate levels. This leads to reduced bone mineralization and muscle weakness, with fatigue being a direct consequence of muscle fatigue. Additionally, low vitamin D levels are associated with dysregulation of the renin-angiotensin system, impairing its ability to inhibit abnormal cell proliferation. [37]

However, a study on the relationship between 25(OH)D levels and fatigue/physical performance conducted with an average follow-up of 79 days post-COVID-19 used the Chalder Fatigue Scale, Six-Minute Walk Test, and Modified Borg Scale. The study found blood vitamin D status was not associated with fatigue as

assessed by the Chalder Fatigue Score and Modified Borg scale, nor with physical performance as assessed by the Six-minute walk test. [38]

2.5 Related Researches

2.5.1 Prevalence of Long COVID in Thailand

Supanan Wongsermsin et al. [11] conducted a study on long COVID symptoms and related risk factors in 364 who had previously received treatment for COVID-19 in Thailand. Data were collected through telephone interviews. The research design was a prospective cohort study with purposive sampling using stratified random sampling according to the proportion of COVID-19 severity to obtain a sample group with a realistic distribution of disease severity. Patients were categorized into four groups: those with asymptomatic, those with mild symptoms without lung infection, those with lung infection without requiring oxygen therapy, and those with lung infection requiring oxygen therapy. The study utilized a long COVID symptom record form divided into 4 parts: Part 1 – Demographic data including gender, age, underlying diseases, and COVID-19 vaccination history; Part 2 - Clinical data including illness history and length of hospitalized; Part 3 - post-COVID symptom assessment with 16 symptom-related questionnaires; Part 4 - Additional patient history including CT ratio from RT-PCR testing, hospitalization details, oxygen therapy, medication use, and chest X-ray results.

Long COVID symptoms were categorized into six systems: (1) respiratory symptoms including shortness of breath, fatigue, and chronic cough; (2) general symptoms including muscle aches, joint pain, and abnormal taste/smell; (3) neurological symptoms including memory loss, sudden weakness, headache, dizziness, muscle atrophy; (4) mental health symptoms including anxiety, insomnia, depression; (5) skin and hair symptoms including hair loss; and (6) cardiovascular symptoms including palpitations, chest pain, chronic fatigue.

The results showed that of the 364 recovered COVID-19 patients, 54.9% were female, and 45.1% were male, most were under 60 years old (92.6%). 22.3% had high-risk underlying diseases, and 34.3% had a CT ratio of ≤ 20. Regarding COVID-19

vaccination status, 31.1% had received two doses, 18.1% had received one dose, and 50.8% had not been vaccinated. The overall prevalence of Long COVID in the study was 29.9%. When divided by severity of symptoms during COVID-19 infection, it was found that 14 asymptomatic patients did not develop long COVID, 20.9% of the 234 patients with mild symptoms had Long COVID; 39.4% of the 71 patients with lung infection not requiring oxygen had Long COVID; and 71.1% of the 45 patients with lung infection requiring oxygen had Long COVID. The most common Long COVID symptoms were respiratory system (81.7%), followed by general symptoms (27.9%), neurological symptoms (22.0%), mental health symptoms (21.3%), skin and hair symptoms (10.6%), and cardiovascular symptoms (6.7%), respectively. Logistic regression analysis identified significant risk and protective factors for Long COVID. Being male (RR 0.48 [95%CI 0.31-0.78]) and receiving two doses of the COVID-19 vaccine (RR 0.49 [95%CI 0.29-0.83]) were identified as protective factors. In contrast, receiving only one vaccine dose (RR 2.22 [95%CI 1.10-4.48]) and being unvaccinated (RR 1.85 [95% CI 1.04-3.32]) were significant risk factors for developing Long COVID. Factors such as age, obesity, high-risk underlying diseases, CT ratio $\leq$20, and smoking, were not statistically significant in affecting the occurrence of Long COVID symptoms.

The Institute of Medical Research and Technology Assessment, Department of Medical Services, [39] conducted a study in 2023 on long COVID prevalence in Thailand among 24,370 who had been infected with COVID-19. The findings revealed that the overall prevalence of Long COVID was 40.49%. Among those with persistent long COVID symptoms for more than five months post-infection, the most commonly reported symptom was fatigue/exhaustion (55.3%), followed by shortness of breath (42.3%), cough (39.2%), sleep disorder (30.2%), memory/concentration problems (27.2%), hair loss (23.2%), muscle pain (21.9%), anxiety/stress (21.2%), headache (21.2%), and dizziness (19.4%), respectively. Additionally, 21.4% of participants reported a decline in quality of life after COVID-19. Regarding severe Long COVID symptoms (rated 5 or higher in severity), the most reported were sleep disorder (65.1%), anxiety/stress (63.9%), fatigue (57.3%), muscle pain (54.4%), and memory/concentration problems (51.7%), respectively. In the subgroup of 2,608 patients aged 60 years and older, the prevalence of long COVID was 30.0%. Among

those experiencing symptoms five months post-infection, the most reported were fatigue/exhaustion (57.2%), followed by shortness of breath (34.9%), sleep disturbances (27.6%), cough (26.3%), muscle pain (19.1%), dizziness (18.4%), short-term memory/concentration problems (17.8%), hair loss (14.5%), anxiety/stress (13.8%), and joint and bone pain (12.5%), respectively. Among severe long COVID symptoms (rated 5 or higher in severity) in the elderly group, the most common were joint and bone pain (63.2%), followed by sleep disorder (62.5%), fatigue/exhaustion (62.1%), muscle pain (55.2%), and anxiety (47.6%), respectively.

Methavee Wangchalabor et al. [40] studied the prevalence of long COVID in patients with a history of COVID-19 infection at a 3-month follow-up period in a sample of 220 people in Saraburi. The study utilized a long COVID interview form consisting of the mMRC assessment, Fatigue Severity Score, Post-Exertional Malaise (PEM) assessment, and an individual symptom questionnaire conducted via telephone. The findings revealed that the weighted prevalence of long COVID was 64.6%. The most common reported symptoms were hair loss (32.5%), post-exertional malaise (PEM) (32.0%), shortness of breath (21.6%), fatigue (16.5%), and insomnia (13.8%). The female gender was significantly associated with at least one symptom of Long COVID.

2.3.2 Relationship between Vitamin D and Long COVID and COVID-19

Filippo LD and et al. [17] studied the relationship between vitamin D 25(OH)D levels and long COVID in 100 COVID-19 patients who had been hospitalized and 6-month follow-up in Italy. The study used a matched-pair design, 50 patients with Long COVID and 50 patients without Long COVID, assessing their vitamin D levels during hospitalization and at 6-month follow-up. The results showed that the most common long COVID symptoms were weakness (38.0%), followed by loss of taste (34.0%), and difficulty breathing (34.0%), respectively. The median vitamin D levels during hospitalization and at the 6-month follow-up were 14.7 (IQR 9.3-21.7) ng/mL and 20.6 (IQR 15.2-25.2) ng/mL, respectively. Patients with long COVID had significantly lower vitamin D levels than those without long COVID (20.1 vs. 23.2 ng/mL, P=0.030). Vitamin D levels in patients with neurological symptoms were significantly lower than in patients without neurological symptoms (14.6 vs. 20.6 ng/mL, P=0.042). Among 42 patients with vitamin D deficiency (<20 ng/mL) both during hospitalization and at 6-

month follow-up, those with long COVID had lower vitamin D levels than those without long COVID (12.7 vs. 15.2 ng/mL, P=0.041). Vitamin D levels during hospitalization and at 6-month follow-up were negatively correlated with blood glucose levels (r=-0.29, r=-0.220). Multivariable logistic regression analysis showed that vitamin D levels at 6-month follow-up were associated with long COVID (OR 1.09 [95%CI 1.01-1.16], P=0.008).

Nielsen NM et al. [18] did a study on the relationship between COVID-19 severity and vitamin D levels in 447 patients. The study categorized 25(OH)D levels into 3 groups: >50 nmol/L (sufficient vitamin D), 30-49.9 nmol/L (insufficient vitamin D), and <30 nmol/L (vitamin D deficiency). The findings showed that low 25(OH)D levels were associated with severe COVID-19, particularly in males under 65 years of age. Additionally, higher 25(OH)D levels were observed in non-hospitalized patients compared to those who were hospitalized, admitted to intensive care, or died. Patients with sufficient or insufficient vitamin D levels had a 50% reduction in risk of developing severe COVID-19 compared to those with vitamin D deficiency. However, there was no significant difference in protection against severe COVID-19 between vitamin D levels of 50-75 nmol/L and >75 nmol/L.

Gönen MS et al. [36] investigated the effects of 25(OH)D levels in COVID-19 patients and the effectiveness of vitamin D3 supplementation on clinical outcomes in COVID-19. The study involved a retrospective review of 867 COVID-19 patients with no underlying diseases, followed by a randomized controlled trial (RCT) in 210 COVID-19 patients who received vitamin D3 supplementation (2,000-100,000 IU) and were monitored for 14 days. A healthy control group of 23 individuals was also included. Blood samples were collected from the study group before receiving vitamin D3 (1-3 days) and at 7 and 14 days post-supplementation. In the retrospective study, patients were categorized into four groups based on their 25(OH)D levels: <12 ng/mL, 12-20 ng/mL, 20-30 ng/mL, and >30 ng/mL. The results showed no statistically significant difference in ICU admission rates or length of hospital stay among the groups. However, the overall mortality rate was 11.2% (97/867), and vitamin D supplementation significantly reduced mortality risk by 2.14 times (95%CI 1.06-4.33).

In a study of 162 COVID-19 patients without underlying diseases, significant differences were found in Ca^{2+} and nitrate-nitrite levels between groups. Comparing

two vitamin D level groups (<12 ng/mL vs. >12 ng/mL), patients with higher 25(OH)D levels (>12 ng/mL) had significantly higher levels of vitamin D-binding protein and NOS1, and lower levels of parathyroid hormone.

In a separate comparison of COVID-19 patients (with no underlying diseases and 25(OH)D levels <30 ng/mL), those who received vitamin D supplementation had a significantly lower proportion of hospital stays longer than 8 days. Patients who did not receive vitamin D were 1.91 times more likely (95% CI 1.19-3.06) to have hospital stays longer than 8 days. The RCT results showed that 25(OH)D levels increased from 16.62 ng/mL to 35.46 ng/mL after 14 days of vitamin D supplementation. However, there were no statistically significant changes in Ca^{2+}, PTH, IL-6, ferritin, or D-dimer levels, while CRP and fibrinogen levels significantly decreased. The relationship between 25(OH)D levels and inflammatory markers revealed a positive correlation between 25(OH)D levels and Ca^{2+}, DBP, IL-1β, and ICAM-1, while a negative correlation was observed between 25(OH)D levels and PTH, nitrate-nitrite. In the pre-supplementation phase of the study group, 25(OH)D levels showed a negative correlation with PTH in the control group. No significant correlations were found between 25(OH)D levels and IL-6, IL-17, S100B, or VCAM-1 in any group.

Townsend L et al. [38] did a study on the relationship between vitamin D and persistent symptoms following SARS-CoV-2 infection. The study included 149 outpatients with confirmed SARS-CoV-2 infection by PCR who were treated in Ireland. They measured fatigue and physical performance using the Chalder Fatigue Score (CFQ-11), Six-minute walk test and Modified Borg scale (MBS). Vitamin D levels were evaluated based on 25-hydroxy-vitamin D (25(OH)D) (D2 and D3), with vitamin D status defined as: >50 nmol/L (sufficient vitamin D), 30-49.9 nmol/L (insufficient vitamin D), and <30 nmol/L (vitamin D deficiency). The median follow-up period after COVID-19 was 79 days. The median 25(OH)D level was 62 nmol/L, with 24.0% in the 30-49 nmol/L and 9.0% in the <30 nmol/. Fatigue was noted in 58.0% of patients. A multivariable regression analysis study revealed that 25(OH)D levels were not associated with fatigue and physical performance.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

Cross sectional descriptive study

3.2 Population and Sample

Study population: COVID-19 patients confirmed by RT-PCR.

Sample: 170 COVID-19 patients confirmed by RT-PCR, who received treatment at Foresta Clinic.

3.2.1 Inclusion Criteria

1. Aged between 18-59 years.
2. Confirmed COVID-19 diagnosis by Covid-Ag or RT-PCR method.
3. Patients with mild symptoms who received only symptomatic treatment only.
4. Having a 25(OH)D level result within 60 days before COVID-19 diagnosis.
5. Patients who can be followed up for at least 4 weeks after COVID-19 infection.
6. Communicate in Thai language.

3.2.2 Exclusion Criteria

1. Having underlying diseases or conditions that increase the risk of severe COVID-19.
2. Previously taking vitamin D supplements.
3. Incomplete questionnaire responses.
4. Unwilling to participate in the study.

3.2.3 Withdrawal Criteria

Participants who decide to withdraw during the data collection process.

3.3 Sample Size Calculation

Previous studies of Fillppo LD. and et al. 17 in the title Low Vitamin D Levels Are Associated with Long COVID Syndrome in COVID-19 Survivors. The finding revealed that the blood vitamin D levels in patients with long COVID were 20.1±6.22 ng/mL, while in patients without long COVID, they were 23.2±7.33 ng/mL. This difference was statistically significant at the 0.05 level. The sample size can be calculated using the comparison of two independent means formula, as follows:

Formula [41] $n_1 = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$

$r = \frac{n_2}{n_1}$, $\Delta = \mu_1 - \mu_2$

Notation: n_1 = sample size per group; $n_2 = n_1$

$z_{1-\frac{\alpha}{2}}$ = probability of type I error ($\alpha = 0.05$); Z(0.975) = 1.96

$z_{1-\beta}$ = probability of type II error ($\beta = 0.20$); Z(0.80) = 0.84

Δ = difference between $\mu_1 - \mu_2 = |23.2 - 20.1| = 3.1$

σ_1 = standard deviation of patients with long COVID at 6.22 ng/mL

σ_2 = standard deviation of patients without long COVID at 7.33 ng/mL

r = ratio of sample size group assuming equal allocation would be 1:1 or $n_1 = n_2$

Hence: $n_1 = \frac{(1.96+0.84)^2[6.22^2+\frac{7.33^2}{1}]}{3.1^2} = 75.40 \sim 76$

$n_2 = (1)\, n_1 = 76$

To reduce the problem of incomplete data or withdrawal, the sample size is adjusted 10% (R)

$n_{adj} = n / (1\text{-}R)$

Hence: 76 / (1-0.10) = 84.4 ~ 85

Therefore, the total sample size is 85x2 = **170 samples**.

3.4 Sampling

This study uses non-probability sampling, specifically the purposive sampling method, selecting participants who met the inclusion criteria. The participants were selected by the treating physician from the patient database at Foresta Clinic.

3.5 Research Tools and Equipment

The research tools consist of a data collection divided into 3 parts, as follows:

1. Demographic data of COVID-19 patients, consisting of 4 items: gender, age, body mass index, and COVID-19 vaccination status.

2. Long COVID Symptoms, categorized into 8 body systems with 21 items: general symptoms (3 items), respiratory symptoms (2 items), cardiovascular symptoms (3 items), neurological symptoms (3 items), gastrointestinal symptoms (3 items), musculoskeletal symptoms (2 items), skin symptoms (2 items), and psychiatric symptoms (3 items). A Numerical Rating Scale was used to measure symptom levels from 0-10 points, where 0 points means no symptoms and 10 points means the most severe symptoms. The questionnaire was adapted from the Department of Medical Services' Long-term Health Impact Questionnaire for COVID-19 Survivors with Kuder-Richarson coefficient of reliability (KR-20) was 0.864. 42

3. Blood vitamin D level (25(OH)D) 1 item, which was collected from the patient database at Foresta Clinic, and COVID-19 diagnosed period

3.6 Research Process and Data Collection

This study followed these research processes, as detailed below:

1. The researcher studied theories and reviewed literature related to this study and developed the research's conceptual framework and proposal.

2. Ethical approval for human research was acquired from the Research Ethics Committee of Mae Fah Luang University.

3. A formal request for permission to collect research data was submitted to the Director of Foresta Clinic.

4. The researcher explained the details about objectives, conceptual framework, procedures, and research tools to the research assistants to ensure they followed the research guidelines correctly.

5. The researcher selected the target population according to the inclusion criteria, which were patients receiving follow-up treatment at the hospital where the researcher worked. Purposive sampling was used to recruit 170 participants.

6. Data collection consisted of two parts: demographic data and long COVID symptoms, both obtained directly from participants through primary data collection. In contrast, blood vitamin D levels (25(OH)D) were secondary data collection from the patient's database records at Foresta Clinic.

7. Both the researcher and research assistants explained the details about objectives, procedures, and benefits to the participants.

8. Participants were asked to read, understand, and sign the consent. They were freedom to withdraw from the study at any time, even after signing the consent form.

9. Participants began answering the questionnaire independently, with the option to ask the researcher for clarification if needed. The process took approximately 15 minutes.

10. The researcher recorded the blood vitamin D levels in the participants' questionnaires from the hospital's Electronic Health Record database.

11. To ensure data confidentiality, each participant was assigned an anonymous code, and questionnaires were securely stored in a restricted and protected area that others could not access.

12. The researcher collected data and entered the data into Microsoft Excel electronic files, then prepared the data before analyzing it according to the research hypotheses.

13. The collected data was used to summarize and discussion of the research findings.

3.7 Ethical Consideration

This study adheres to the three fundamental principles outlined in the Belmont Report:

3.7.1 Respect for Persons

In this research, all demographic data and research data of the participants will be kept confidential throughout the processes of data collection, recording, analysis, and reporting. The participants' names and addresses will not be included in the record forms. Only the necessary information will be specified, such as coded participant numbers, will be used. Data analysis and research findings will be presented as an overview for academic purposes only and will not impact the participants in any way. Participation in this study is entirely voluntary, with participants will be required to sign an informed consent form, which explains their right to accept or decline participation without any coercion. No authority from any party will decide to participate or withdraw. Withdrawal from the study will not damage their career, personal standing, or result in any negative consequences.

3.7.2 Beneficence/Non-Maleficence

In this study, participants will not gain immediate advantages from participating in this study. However, the research findings will contribute to new knowledge valuable to the overall population who have been infected with COVID-19. The study does not pose any harm or suffering to participants, as it only involves completing a questionnaire and does not include any experimental interventions. Additionally,

vitamin D level data will be collected retrospectively from medical records during the participants' COVID-19 treatment period, without additional blood tests.

3.7.3 Justice

The study ensures fair selection of participants, including all eligible persons who meet the inclusion criteria established by the research protocol. Every qualified individual has an equal opportunity to participate in the study. There is no conflict of interest or personal advantage involved in conducting the research.

3.8 Statistical Analysis

This study analyzed data using STATA (StataCorp. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC; 2023.). All statistical tests were conducted as two-tailed tests, with a statistical significance level set at 0.05 ($\alpha = 0.05$). The data analysis procedures were as follows:

1. Demographic data and long COVID symptoms were analyzed using descriptive statistics. Continuous variables were provided as means, standard deviations, medians, and interquartile ranges. Categorical variables were presented as frequencies and percentages.

2. Comparison of long COVID and Vitamin D Levels was performed using an independent t-test for normally distributed continuous data, while the Mann-Whitney U test was used for non-normally distributed continuous data. The Chi-square test or Fisher's exact test was applied for categorical data.

3. Analysis of vitamin D levels as a factor influencing long COVID symptoms was conducted using binary logistic regression in both univariable and multivariable analysis, with results provided as crude odds ratios and adjusted odds ratios with 95% confidence Intervals (95% CI).

4. Data distribution was normality tested by Kolmogorov-Smirnov. A P-value less than 0.05 indicated a normal distribution of the data.

CHAPTER 4

RESEARCH FINDINGS

This study aims to determine the prevalence of long COVID among those with vitamin D deficiency, insufficient vitamin D levels, and sufficient vitamin D levels in the blood. It also examines the influence of blood vitamin D levels on long COVID. Data were collected through questionnaires from 170 participants infected with COVID-19 and confirmed by RT-PCR or Rapid Antigen testing. The researcher conducted statistical analysis and reported the research findings in 7 sections as follows:

4.1 Demographic data and history of COVID vaccination

4.2 Symptoms and Severity of Long COVID

4.3 Blood vitamin D levels and comparison of blood vitamin D levels between 2 subject groups (Present and Absent long COVID symptoms)

4.4 Comparison of long COVID severity and blood vitamin D levels

4.5 Comparison of duration from measuring blood vitamin D levels to contracting COVID-19

4.6 Comparison of duration from contracting COVID-19 to taking the questionnaire

4.7 The blood vitamin D levels related to the symptoms of long COVID

4.1 Demographic Data and COVID Vaccination

Table 4.1 Demographic data (n=170)

	Total (n=170)	**Deficiency (n=52)**	**Insufficiency (n=70)**	**Sufficiency (n=48)**	**P-value**
Sex, n(%)					
Male	83 (48.8)	26 (50.0)	34 (48.6)	23 (47.9)	0.977
Female	87 (51.2)	26 (50.0)	36 (51.4)	25 (52.1)	

Table 4.1 (continued)

	Total (n=170)	**Deficiency (n=52)**	**Insufficiency (n=70)**	**Sufficiency (n=48)**	**P-value**
Age (years), mean±SD	45.87±8.65	45.85±8.87	45.17±8.85	46.92±8.18	0.563
Height (cm), mean±SD	162.±6.07	163.12±6.58	162.06±5.94	162.21±5.73	0.612
Weight (kg), mean±SD	65.42±11.48	65.94±11.37	64.03±11.30	66.88±11.85	0.387
Body mass index (kg/m^2), mean±SD	24.90±4.72	24.87±4.55	24.48±4.67	25.54±5.01	0.489
< 18.5	17 (10.1)	4 (7.8)	8 (11.4)	5 (10.4)	
18.5 – 22.9	47 (27.8)	16 (31.4)	20 (28.6)	11 (22.9)	0.866
$\geq$ 23.0	105 (62.1)	31 (60.8)	42 (60.0)	32 (66.7)	
Number of COVID-19 vaccine doses, n(%)					
1 dose	11 (6.5)	4 (7.7)	4 (5.7)	3 (6.3)	0.911
2 doses	30 (17.7)	9 (17.3)	12 (17.1)	9 (18.8)	
3 doses	106 (62.4)	30 (57.7)	44 (62.9)	32 (66.7)	
4 doses	23 (13.5)	9 (17.3)	10 (14.3)	4 (8.3)	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	38.5	37	41	38	0.585

Table 4.1 (continued)

	Total (n=170)	**Deficiency (n=52)**	**Insufficiency (n=70)**	**Sufficiency (n=48)**	**P-value**
Duration from contracting COVID-19 to take questionnaire (day), median	58.5	54.5	59.5	57.5	0.622

Note n(%): number(percentage); SD: standard deviation
Data were analyzed with the Chi-square test and One-way ANOVA

Table 4.1 presents the demographic data of 170 COVID-19-infected patients. The female-to-male ratio was 1.1:1. The mean age was 45.87±8.65 years, the mean height was 162.0 ± 6.07 cm, and the mean weight was 65.42±11.48 kg. The mean body mass index (BMI) was 24.90 ± 4.72 kg/m². Most of the participants have an overweight BMI ($\geq$ 23.0 kg/ m^2), at 62.1%, followed by those within the normal BMI (18.5-22.9 kg/m^2) at 27.8%, and underweight (<18.5 kg/m^2) at 10.1%. Regarding the number of COVID-19 vaccinations received during the data collection period, most of the participants received 3 doses (62.4%), followed by 2 doses (17.7%), 4 doses (13.5%), and only 1 dose (6.5%). When comparing the demographic data between participants with vitamin D deficiency, insufficiency, and sufficiency, there were no statistically significant differences in sex, age, height, weight, BMI, and the number of COVID-19 vaccinations (P>0.05).

Table 4.2 Type of COVID-19 vaccination (n=170)

Type of vaccines, n(%)	**Dose 1**	**Dose 2**	**Dose 3**	**Dose 4**
AstraZeneca	79 (46.5)	64 (40.3)	3 (2.3)	-
Sinovac	60 (35.3)	28 (17.6)	-	-
Sinopharm	31 (18.2)	7 (4.4)	-	-
Pfizer	-	37 (23.3)	68 (52.7)	11 (47.8)
Moderna	-	23 (14.5)	58 (45.0)	12 (52.2)

Note n(%): number(percentage)

Table 4.2 shows the history of COVID-19 vaccination received during the data collection period. It was found that most participants received the AstraZeneca in the 1st, 2nd, and 3rd doses at 46.5%, 40.3%, and 2.3%. Pfizer and Moderna were first received in the 2nd dose at 23.3% and 14.5%, respectively, and in the 3rd dose at 52.7% and 45.0%, respectively. Among the 23 participants who received 4 vaccinations, 52.2% received Pfizer, and 47.8% received Moderna. Meanwhile, participants who received Sinovac and Sinopharm vaccines in the 1st dose were 35.3% and 18.2%, respectively, and in the 2nd dose were 40.3% and 17.6%, respectively.

4.2 Long COVID Severity and Symptoms

Table 4.3 Long COVID severity (n=170)

Long COVID severity	n (%)
Non–long COVID (no symptoms)	60 (35.3)
Mild long COVID (1 - 7 symptoms)	85 (50.0)
Moderate long COVID (8 to 14 symptoms)	19 (11.2)
Severe long COVID (15 to 21 symptoms)	6 (3.5)
Number of long COVID symptoms, median (range)	2 (0, 17)

Note n(%): number(percentage)

Table 4.3 presents the severity levels of long COVID. It was found that 64.7% of patients experienced long COVID symptoms. The majority, 50.0%, had mild long COVID, followed by 11.2% with moderate long COVID, and 3.5% with severe long COVID, respectively. Meanwhile, 35.3% of patients reported no long COVID symptoms. The median number of long COVID symptoms was 2 (range 0–17).

Table 4.4 Long COVID symptoms and severity level (n=170)

Long COVID symptoms	**n (% [95%CI])**	**Severity level, mean±SD**
General symptoms	67 (39.4 [95%CI 32.0, 47.2])	
Fatigue	67 (39.4)	3.84±1.62
Fever	22 (12.9)	
Chills	0 (0.0)	
Respiratory symptoms	94 (55.3 [95%CI 47.5, 62.9])	
Dyspnea	67 (39.4)	3.85±1.84
Cough	88 (51.8)	3.94±1.92
Cardiovascular symptoms	20 (11.8 [95%CI 7.3, 17.5])	
Palpitation	12 (7.1)	2.17±1.03
Tachycardia	12 (7.1)	2.33±0.98
Chest pain	9 (5.3)	1.33±0.5
Neurological symptoms	45 (26.5 [95%CI 20.0, 33.8])	
Loss of smell and taste	28 (16.5)	
Headaches	17 (10.0)	3.47±1.42
Dizziness	14 (8.2)	3.50±1.56
Gastrointestinal symptoms	2 (1.2 [95%CI 0.1, 4.2])	
Diarrhea	2 (1.2)	
Nausea/Vomiting	0 (0.0)	
Abdominal pain	1 (0.6)	
Musculoskeletal symptoms	23 (13.5 [95%CI 8.8, 19.6])	
Muscle pain	18 (10.6)	2.89±1.18
Joint pain	20 (11.8)	2.60±1.19
Skin symptoms	86 (50.6 [95%CI 42.8, 58.3])	
Rashes	34 (20.0)	
Hair loss	81 (47.7)	

Table 4.4 (continued)

Long COVID symptoms	**n (% [95%CI])**	**Severity level, mean±SD**
Psychiatric symptoms	51 (30.0 [95%CI 23.2, 37.4])	
Anxiety	26 (15.3)	3.12±1.07
Depression	12 (7.1)	2.67±1.78
Sleep disorders	36 (21.2)	4.44±2.08
Long COVID	**110 (64.7 [95%CI 57.0, 71.9])**	

Note n(%): number(percentage); 95%CI: 95% Confidence interval ;SD: standard deviation

Table 4.4 shows the symptoms and severity of Long COVID. It was found that 64.7% (95%CI 57.0, 71.9) of COVID-19 patients experienced Long COVID symptoms. General symptoms were found in 39.4% (95%CI 32.0, 47.2), with fatigue or exhaustion was 39.4%. The mean severity score was 3.84±1.62. Fever was reported by 12.9%, Chills was absent. Respiratory symptoms were found in 55.3% (95%CI 47.5, 62.9), with dyspnea reported by 39.4%, and the mean severity score was 3.85 ± 1.84. Coughing was reported by 51.8%, with a mean severity score of 3.94 ± 1.92. Cardiovascular symptoms were observed in 11.8% (95%CI 7.3, 17.5), with palpitations reported by 7.1%, and a mean severity score of 2.17±1.03. Tachycardia was reported by 7.1%, with a mean severity score of 2.33 ± 0.98, and chest pain was reported by 5.3%, with a mean severity score of 1.33±0.5. Neurological symptoms were found in 26.5% (95%CI 20.0, 33.8), with loss of smell/taste reported by 16.5%, headache reported by 10.0%, with a mean severity score of 3.47 ± 1.42, and dizziness reported by 8.2%, with a mean severity score of 3.50 ± 1.56. Gastrointestinal symptoms were found in 1.2% (95%CI 0.1, 4.2), with frequent diarrhea reported by 1.2% and abdominal pain by 0.6%. No symptoms of nausea/vomiting were observed. Musculoskeletal symptoms were found in 13.5% (95%CI 8.8, 19.6), with muscle pain reported by 10.6%, with a mean severity score of 2.89±1.18, and joint and bone pain with a mean severity score of 2.60 ± 1.19. Dermatological symptoms were found in 50.6% (95%CI 42.8, 58.3), with rashes reported by 20.0%, and hair loss reported by 47.7%. Psychiatric

symptoms were found in 30.0% (95%CI 23.2, 37.4), with anxiety reported by 15.3%, with a mean severity score of 3.12± 1.07. Depression was reported by 7.1%, with a mean severity score of 2.67± 1.78, and sleep disorder was reported by 21.2%, with a mean severity score of 4.44 ± 2.08.

4.3 Blood Vitamin D Levels (25(OH)D)

Table 4.5 Blood vitamin D levels (n=170)

25(OH)D levels	n (%)	Median
Deficiency (< 20 ng/mL)	52 (30.6)	16.98
Insufficiency (20-30 ng/mL)	70 (41.2)	23.34
Sufficiency (>30-100 ng/mL)	48 (28.2)	34.15
Overall	170 (100.0)	22.96

Note n(%): number(percentage)

Table 4.5 demonstrates the Vitamin D Level in COVID-19-infected patients. The median vitamin D level was 22.96 ng/mL. Most patients (41.2%) had insufficient vitamin D levels (20-30 ng/mL), with a median vitamin D level of 23.34 ng/mL. The next group was those with vitamin D deficiency (<20 ng/mL) was 30.6%, with a median vitamin D level of 16.98 ng/mL. The sufficient vitamin D levels (>30-100 ng/mL) had a median vitamin D level of 34.15

4.4 Comparison of Long COVID Severity and Blood Vitamin D Levels

Table 4.6 Comparison of long COVID severity and blood vitamin D levels

25(OH)D levels	Long COVID severity				Median[a]	P-value
	None	Mild	Moderate	Severe		
Deficiency	8 (13.3)	24 (28.2)	14 (73.7)	6 (100.0)	$6^{(1,2)}$	<0.001[#]
Insufficiency	30 (50.0)	35 (41.2)	5 (26.3)	0 (0.0)	$1^{(1)}$	

Table 4.6 (continued)

25(OH)D levels	Long COVID severity				Median[a]	P-value
	None	Mild	Moderate	Severe		
Sufficiency	22 (36.7)	26 (30.6)	0 (0.0)	0 (0.0)	$1^{(2)}$	
Median[b]	$25.46^{(1,2,3)}$	$26.20^{(1,4,5)}$	$16.42^{(2,4)}$	$15.58^{(3,5)}$		$<0.001^{‡}$

Note Median value represented number of long COVID symptoms[a], and vitamin D levels[b]

Data were analyzed with Chi-square test[#], and Kruskal–Wallis H test[‡]

Identical numbers indicate statistically significant differences between groups at the 0.05 level.

Table 4.6 presents the comparison between long COVID severity and vitamin D levels. All participants classified with severe long COVID were found to have vitamin D deficiency. Among those with moderate long COVID, 73.7% were vitamin D deficient. In the mild long COVID group, 28.2% had vitamin D deficiency, whereas only 13.3% of participants without long COVID were deficient. These differences were statistically significant at the 0.05 level ($P < 0.001$).

Similarly, the vitamin D levels between long COVID severity groups showed a statistically significant difference at the 0.05 level ($P < 0.001$). The median vitamin D levels were highest among participants without long COVID (25.46 ng/mL) and those with mild long COVID (26.20 ng/mL), while lower levels were observed in participants with moderate (16.42 ng/mL) and severe long COVID (15.58 ng/mL).

Furthermore, the number of long COVID symptoms between vitamin D levels showed a statistically significant difference at the 0.05 level ($P < 0.001$). Participants with vitamin D deficiency had a higher median number of symptoms (6 symptoms) compared to those with insufficient or sufficient vitamin D levels (1 symptom).

Table 4.7 Prevalence of long COVID symptoms among vitamin D deficiency, insufficient vitamin D levels, and sufficient vitamin D levels participants (n=170)

Long COVID symptoms	Deficiency (n=52)	Insufficiency (n=70)	Sufficiency (n=48)
General symptoms, n(%)	31 (59.6)	24 (34.3)	12 (25.0)
fatigue	31 (59.6)	24 (34.3)	12 (25.0)
fever	12 (23.1)	9 (12.9)	1 (2.1)
Chills	0 (0.0)	0 (0.0)	0 (0.0)
Respiratory symptoms, n(%)	42 (80.8)	31 (44.3)	21 (43.8)
Dyspenea	42 (80.8)	19 (27.1)	6 (12.5)
Cough	36 (69.2)	31 (44.3)	21 (43.8)
Cardiovascular symptoms, n(%)	17 (32.7)	3 (4.3)	0 (0.0)
Palpitation	9 (17.3)	3 (4.3)	0 (0.0)
Tachycardia	9 (17.3)	3 (4.3)	0 (0.0)
Chest pain	9 (17.3)	0 (0.0)	0 (0.0)
Neurological symptoms, n(%)	29 (55.8)	12 (17.1)	4 (8.3)
Loss of smell and taste	12 (23.1)	12 (17.1)	4 (8.3)
Headaches	17 (32.7)	0 (0.0)	0 (0.0)
Dizziness	14 (26.9)	0 (0.0)	0 (0.0)
Gastrointestinal symptoms, n(%)	2 (3.9)	0 (0.0)	0 (0.0)
Diarrhea	2 (3.9)	0 (0.0)	0 (0.0)
Nausea/Vomiting	0 (0.0)	0 (0.0)	0 (0.0)
Abdominal pain	1 (1.9)	0 (0.0)	0 (0.0)
Musculoskeletal symptoms, n(%)	18 (34.6)	5 (7.1)	0 (0.0)
Muscle pain	16 (30.8)	2 (2.9)	0 (0.0)
Joint pain	15 (28.9)	5 (7.1)	0 (0.0)
Skin symptoms, n(%)	41 (78.9)	32 (45.7)	13 (27.1)
Rashes	24 (46.2)	10 (14.3)	0 (0.0)
Hair loss	39 (75.0)	29 (41.4)	13 (27.1)

Table 4.7 (continued)

Long COVID symptoms	Deficiency (n=52)	Insufficiency (n=70)	Sufficiency (n=48)
Psychiatric symptoms, n(%)	24 (46.2)	18 (25.7)	9 (18.8)
Anxiety	14 (26.9)	8 (11.4)	4 (8.3)
Depression	10 (19.2)	2 (2.9)	0 (0.0)
Sleep disorders	21 (40.4)	10 (14.3)	5 (10.4)
Overall, n(%)	**44 (84.6)**	**40 (57.1)**	**26 (54.2)**

Note n(%): number(percentage)

Table 4.7 presents the prevalence of Long COVID symptoms among participants with deficient, insufficient, and sufficient levels of vitamin D. The findings indicate that participants with vitamin D deficiency exhibited a higher prevalence of Long COVID symptoms, as detailed in each system below. General symptoms 59.6%, respiratory symptoms 80.8%, cardiovascular symptoms 32.7%, neurological symptoms 55.8%, gastrointestinal symptoms 3.9%, musculoskeletal symptoms 34.6%, dermatological symptoms 78.9%, psychiatric symptoms 46.2%, and overall Long COVID symptoms 84.6%. For participants with insufficient vitamin D levels, the prevalence of symptoms was general symptoms 34.3%, respiratory symptoms 44.3%, cardiovascular symptoms 4.3%, neurological symptoms 17.1%, musculoskeletal symptoms 7.1%, dermatological symptoms 45.7%, psychological symptoms 25.7%, and overall Long COVID symptoms 57.1%. For participants with sufficient vitamin D levels, the prevalence of symptoms was general symptoms 25.0%, respiratory symptoms 43.8%, neurological symptoms 8.3%, dermatological symptoms 27.1%, psychiatric symptoms 18.8%, and overall Long COVID symptoms 54.2%. It indicates that the prevalence of Long COVID symptoms is higher in participants with vitamin D deficiency compared to those with insufficient and sufficient vitamin D levels across all eight symptom groups.

Table 4.8 Comparison of blood vitamin D levels between with and without long COVID symptoms participants (n=170)

Long COVID symptoms	Yes		No		P-value
	n	Median, (ng/mL)	n	Median, (ng/mL)	
General symptoms	67	21.21	103	25.46	<0.001
Respiratory symptoms	94	21.32	76	26.65	<0.001
Cardiovascular symptoms	20	15.33	150	25.28	<0.001
Neurological symptoms	45	17.03	125	26.67	<0.001
Gastrointestinal symptoms	2	12.27	168	23.12	0.002
Musculoskeletal symptoms	23	16.36	147	25.46	<0.001
Skin symptoms	86	21.0	84	27.82	<0.001
Psychiatric symptoms	51	20.91	119	25.46	<0.001
Overall	110	21.52	60	25.46	<0.001

Note Data were analyzed with Mann Whitney U test

n : amount of participants, Median : Median Vitamin D level

Table 4.8 shows the comparison of vitamin D levels in the blood between patients with and without symptoms of Long COVID. It was found that participants with general symptoms, respiratory symptoms, cardiovascular symptoms, neurological symptoms, gastrointestinal symptoms, musculoskeletal symptoms, skin symptoms, and psychiatric symptoms had significantly lower median vitamin D levels compared to those without symptoms in all eight groups of Long COVID symptoms ($P < 0.05$).

4.5 Comparison of Duration from Measuring Blood Vitamin D Levels to Contracting COVID-19

Table 4.9 Comparison of duration from measuring blood vitamin D levels to contracting COVID-19 between with and without long COVID symptoms participants (n=170)

Long COVID symptoms	Yes		No		P-value
	n	Median	n	Median	
General symptoms	67	36	103	40	0.236
Respiratory symptoms	94	39	76	38.5	0.706
Cardiovascular symptoms	20	41	150	38	0.757
Neurological symptoms	45	37	125	40	0.245
Gastrointestinal symptoms	2	37.5	168	38.5	0.834
Musculoskeletal symptoms	23	41	147	38	0.462
Skin symptoms	86	38	84	39.5	0.863
Psychiatric symptoms	51	40	119	38	0.829
Overall	110	39.5	60	38	0.728

Note Unit: day

Data were analyzed with Mann Whitney U test

Table 4.9 shows a comparison of the duration between measuring blood vitamin D levels and contracting COVID-19 in participants with and without long COVID symptoms. The findings indicate that the median duration from vitamin D level measurement to COVID-19 infection is not statistically different ($P > 0.05$) between those who had general symptoms, respiratory symptoms, cardiovascular symptoms, neurological symptoms, gastrointestinal symptoms, musculoskeletal symptoms, dermatological symptoms, psychiatric symptoms, and long COVID compared to those without these symptoms. In other words, the duration between measuring blood vitamin

D levels and contracting COVID-19 does not influence the occurrence of long COVID symptoms.

Table 4.10 Comparison of duration from measuring blood vitamin D levels to contracting COVID-19 by blood vitamin D levels (n=170)

25(OH)D levels	n	Median	P-value
Deficiency (< 20 ng/mL)	52	37	
Insufficiency (20-30 ng/mL)	70	41	0.586
Sufficiency (>30-100 ng/mL)	48	38	

Note Unit: day

Data were analyzed with Kruskal–Walli test

* Statistically significant at the 0.05 (α=0.05)

Table 4.10 compares the duration between measuring blood vitamin D levels and contracting COVID-19 for participants with different vitamin D levels. The findings reveal that there is no statistically significant difference (P>0.05) in the median duration from the measurement of vitamin D levels to the onset of COVID-19 among those with deficiency, insufficiency, and sufficient levels of vitamin D. In other words, the time interval between measuring blood vitamin D levels and contracting COVID-19 does not influence the vitamin D levels in the blood.

4.6 Comparison of Duration from Contracting COVID-19 to Taking the Questionnaire

Table 4.11 Comparison of duration from contracting COVID-19 to taking questionnaires between participants with and without long COVID symptoms. (n=170)

Long COVID symptoms	Yes		No		P-value
	n	Median	n	Median	
General symptoms	67	59	103	58	0.984
Respiratory symptoms	94	57	76	59	0.473
Cardiovascular symptoms	20	49.5	150	59	0.236

Table 4.11 (continued)

Long COVID symptoms	Yes		No		P-value
	n	Median	n	Median	
Neurological symptoms	45	54	125	59	0.685
Gastrointestinal symptoms	2	34	168	59	0.099
Musculoskeletal symptoms	23	44	147	59	0.083
Skin symptoms	86	58.5	84	58	0.875
Psychiatric symptoms	51	53	119	59	0.230
Overall	110	58	60	59	0.508

Note Duration from contracting COVID-19 to take questionnaire, median= 58.5 days
Unit: day
Data were analyzed with Mann Whitney U test

Table 4.11 offers a compelling analysis of the duration from contracting COVID-19 to completing the questionnaire, comparing participants with long COVID symptoms to those without. The results reveal that the median duration between contracting COVID-19 and questionnaire completion shows no statistically significant difference ($P > 0.05$) among individuals experiencing a range of symptoms—whether general, respiratory, cardiovascular, neurological, gastrointestinal, musculoskeletal, dermatological, or psychiatric—when compared to those without these symptoms. Thus, it is evident that the duration from infection to survey completion does not affect the likelihood of developing long COVID symptoms.

4.7 The Blood Vitamin D Levels Related to the Symptoms of Long COVID

4.6.1 General Symptoms

Table 4.12 Multivariable analysis of blood vitamin D levels affecting general symptoms of long COVID (n=170)

Factors	General symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	31 (46.3)	21 (20.4)	4.55 (1.88, 10.87)	0.001
Insufficiency	24 (35.8)	46 (44.7)	1.65 (0.72, 3.79)	0.239
Sufficiency	12 (17.9)	36 (34.9)	Ref.	
Sex				
Male	30 (44.8)	53 (51.5)	0.74 (0.39, 1.44)	0.387
Female	37 (55.2)	50 (48.5)	Ref.	
Age (years)				
< 45	28 (41.8)	43 (41.8)	Ref.	
≥ 45	39 (58.2)	60 (58.3)	0.96 (0.49, 1.93)	0.904
Body mass index (kg/m^2)				
< 18.5	5 (7.6)	12 (11.7)	0.87 (0.24, 3.10)	0.826
18.5 – 22.9	17 (25.8)	30 (29.1)	Ref.	
≥ 23.0	44 (66.7)	61 (59.2)	1.56 (0.72, 3.36)	0.256
Number of COVID-19 vaccine doses				
1 dose	5 (7.5)	6 (5.8)	1.12 (0.23, 5.32)	0.890
2 doses	10 (14.9)	20 (19.4)	0.72 (0.22, 2.39)	0.591
3 doses	42 (62.7)	64 (62.1)	0.97 (0.36, 2.58)	0.951
4 doses	10 (14.9)	13 (12.6)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	36	40	0.98 (0.95, 1.01)	0.228

Table 4.12 (continued)

Factors	General symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Duration from contracting COVID-19 to take questionnaire (day), median	59	58	1.00 (0.99, 1.01)	0.899

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.12 presents an analysis of blood vitamin D levels as a factor influencing general symptoms of long COVID. The multivariable analysis found that vitamin D deficiency significantly increased the likelihood of general long COVID symptoms at the 0.05 level (Adjusted OR 4.55 [95% CI 1.88, 10.87], P=0.001). In other words, patients with vitamin D deficiency were 4.55 times more likely to develop general long COVID symptoms compared to those with sufficient vitamin D levels.

4.6.2 Respiratory Symptoms

Table 4.13 Multivariable analysis of blood vitamin D levels affecting respiratory symptoms of long COVID (n=170)

Factors	Respiratory symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	42 (44.7)	10 (13.2)	6.06 (2.37, 15.54)	<0.001
Insufficiency	31 (32.0)	39 (51.3)	1.10 (0.51, 2.37)	0.807
Sufficiency	21 (22.3)	27 (35.5)	Ref.	
Sex				
Male	49 (52.1)	34 (44.7)	1.37 (0.70, 2.67)	0.358
Female	45 (47.9)	42 (55.3)	Ref.	

Table 4.13 (continued)

Factors	Respiratory symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Age (years)				
< 45	33 (35.1)	38 (50.0)	Ref.	
≥ 45	61 (64.9)	38 (50.0)	1.98 (0.99, 3.95)	0.052
Body mass index (kg/m^2)				
< 18.5	7 (7.5)	10 (13.2)	0.98 (0.28, 3.50)	0.978
18.5 – 22.9	23 (24.7)	24 (31.6)	Ref.	
≥ 23.0	63 (67.8)	42 (55.3)	1.97 (0.90, 4.28)	0.089
Number of COVID-19 vaccine doses				
1 dose	7 (7.5)	4 (5.3)	2.09 (0.38, 11.39)	0.394
2 doses	17 (18.1)	13 (17.1)	1.32 (0.40, 4.41)	0.652
3 doses	57 (60.6)	49 (64.5)	1.22 (0.45, 3.30)	0.694
4 doses	13 (13.8)	10 (13.2)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	39	38.5	0.99 (0.96, 1.02)	0.657
Duration from contracting COVID-19 to take questionnaire (day), median	57	59	1.00 (0.99, 1.02)	0.873

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.13 presents an analysis of blood vitamin D levels as a factor influencing respiratory symptoms of long COVID. The multivariable analysis found that vitamin D deficiency remained a significant increase in the likelihood of respiratory symptoms at the 0.05 level (Adjusted OR 6.06 [95% CI 2.37, 15.54], P<0.001). In other words, patients with vitamin D deficiency were 6.06 times more likely to develop respiratory symptoms of long COVID compared to those with sufficient vitamin D levels.

4.6.3 Cardiovascular Symptoms

Table 4.14 Multivariable analysis of blood vitamin D levels affecting cardiovascular symptoms of long COVID (n=170)

Factors	Cardiovascular symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	17 (85.0)	35 (23.3)	22.63 (5.88, 87.14)	<0.001
Insufficiency	3 (15.0)	67 (44.7)	Ref.	
Sufficiency	0 (0.0)	48 (32.0)		
Sex				
Male	9 (45.0)	74 (49.3)	0.62 (0.20, 1.94)	0.413
Female	11 (55.0)	76 (50.7)	Ref.	
Age (years)				
< 45	6 (30.0)	65 (43.3)	Ref.	
≥ 45	14 (70.0)	85 (56.7)	2.13 (0.64, 7.09)	0.218
Body mass index (kg/m^2)				
< 18.5	1 (5.0)	16 (10.7)	1.05 (0.08, 14.47)	0.970
18.5 – 22.9	4 (20.0)	43 (28.9)	Ref.	
≥ 23.0	15 (75.0)	90 (60.4)	2.21 (0.58, 8.47)	0.246
Number of COVID-19 vaccine doses				
1 dose	2 (10.0)	9 (6.0)	0.84 (0.08, 8.56)	0.879

Table 4.14 (continued)

Factors	Cardiovascular symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
2 doses	2 (10.0)	28 (18.7)	0.22 (0.03, 1.78)	0.157
3 doses	11 (55.0)	95 (63.3)	0.53 (0.13, 2.28)	0.398
4 doses	5 (25.0)	18 (12.0)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	41	38	1.00 (0.96, 1.05)	0.907
Duration from contracting COVID-19 to take questionnaire (day), median	49.5	59	0.98 (0.96, 1.01)	0.154

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.14 presents an analysis of blood vitamin D levels as a factor influencing cardiovascular symptoms of long COVID. The multivariable analysis found that vitamin D deficiency significantly increase in the likelihood of cardiovascular symptoms at the 0.05 level (Adjusted OR 22.63 [95% CI 5.88, 87.14], P<0.001). In other words, patients with vitamin D deficiency were 22.63 times more likely to develop cardiovascular symptoms of long COVID compared to those with sufficient vitamin D levels.

4.6.4 Neurological Symptoms

Table 4.15 Multivariable analysis of blood vitamin D levels affecting neurological symptoms of long COVID (n=170)

Factors	Neurological symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	29 (64.4)	23 (18.4)	16.22 (4.81, 54.65)	<0.001
Insufficiency	12 (26.7)	58 (46.4)	2.49 (0.74, 8.43)	0.142
Sufficiency	4 (8.9)	44 (35.2)	Ref.	
Sex				
Male	18 (40.0)	65 (52.0)	0.50 (0.22, 1.13)	0.097
Female	27 (60.0)	60 (48.0)	Ref.	
Age (years)				
< 45	17 (37.8)	54 (43.2)	Ref.	
≥ 45	28 (62.2)	71 (56.8)	1.36 (0.60, 3.07)	0.465
Body mass index (kg/m^2)				
< 18.5	4 (9.1)	13 (10.4)	1.30 (0.28, 6.03)	0.736
18.5 – 22.9	12 (27.3)	35 (28.0)	Ref.	
≥ 23.0	28 (63.6)	77 (61.6)	1.32 (0.53, 3.27)	0.542
Number of COVID-19 vaccine doses				
1 dose	2 (4.4)	9 (7.2)	0.40 (0.05, 3.00)	0.373
2 doses	7 (15.6)	23 (18.4)	0.77 (0.19, 3.23)	0.725
3 doses	29 (64.4)	77 (61.6)	1.13 (0.36, 3.55)	0.836
4 doses	7 (15.6)	16 (12.8)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	37	40	0.98 (0.95, 1.01)	0.251

Table 4.15 (continued)

Factors	Neurological symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Duration from contracting COVID-19 to take questionnaire (day), median	54	59	0.99 (0.98, 1.01)	0.825

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.15 presents an analysis of blood vitamin D levels as a factor influencing neurological symptoms of long COVID. The multivariable analysis confirmed that vitamin D deficiency remained a significantly increased the likelihood of neurological symptoms at the 0.05 level (Adjusted OR 16.22 [95%CI 4.81, 54.65], P<0.001). In other words, patients with vitamin D deficiency were 16.22 times more likely to develop neurological symptoms of long COVID compared to those with sufficient vitamin D levels.

4.6.5 Gastrointestinal Symptoms

Table 4.16 Multivariable analysis of blood vitamin D levels affecting gastrointestinal symptoms of long COVID (n=170)

Factors	Gastrointestinal symptoms		Univariable	
	Yes, n(%)	No, n(%)	Crude OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	2 (100.0)	50 (29.8)	N/A	0.171#
Insufficiency	0 (0.0)	70 (41.7)		
Sufficiency	0 (0.0)	48 (28.6)		

Table 4.16 (continued)

Factors	Gastrointestinal symptoms		Univariable	
	Yes, n(%)	**No, n(%)**	**Crude OR (95%CI)**	**P-value**
Sex				
Male	2 (100.0)	81 (48.2)	N/A	0.237 #
Female	0 (0.0)	87 (51.8)		
Age (years)				
< 45	0 (0.0)	71 (42.3)	N/A	0.511#
≥ 45	2 (100.0)	97 (57.7)		
Body mass index (kg/m^2)				
< 18.5	0 (0.0)	17 (10.2)	N/A	1.000#
18.5 – 22.9	0 (0.0)	47 (28.1)		
≥ 23.0	2 (100.0)	103 (61.7)		
Number of COVID-19 vaccine doses				
1 dose	0 (0.0)	11 (6.6)	N/A	0.391#
2 doses	0 (0.0)	30 (17.9)		
3 doses	1 (50.0)	105 (62.5)		
4 doses	1 (50.0)	22 (13.1)		
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	37.5	38.5	0.99 (0.88, 1.12)	0.889

Table 4.16 (continued)

Factors	Gastrointestinal symptoms		Univariable	
	Yes, n(%)	No, n(%)	Crude OR (95%CI)	P-value
Duration from contracting COVID-19 to take questionnaire (day), median	34	59	0.92 (0.81, 1.04)	0.178

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Chi-square test (exact sig.)[#] and Binary logistic regression (Enter method)

Table 4.16 presents an analysis of blood vitamin D levels as a factor influencing gastrointestinal symptoms of long COVID. The univariable analysis found that vitamin D levels did not have a statistically significant effect on the gastrointestinal symptoms of long COVID (P = 0.171).

4.6.6 Musculoskeletal Symptoms

Table 4.17 Multivariable analysis of blood vitamin D levels affecting musculoskeletal symptoms of long COVID (n=170)

Factors	Musculoskeletal symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	18 (78.3)	34 (23.1)	13.77 (4.54, 41.82)	<0.001
Insufficiency	5 (21.7)	65 (44.2)	Ref.	
Sufficiency	0 (0.0)	48 (32.7)		

Table 4.17 (continued)

Factors	Musculoskeletal symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Sex				
Male	13 (56.5)	70 (47.6)	1.30 (0.47, 3.66)	0.613
Female	10 (43.5)	77 (52.4)	Ref.	
Age (years)				
< 45	10 (43.5)	61 (41.5)	Ref.	
≥ 45	13 (56.5)	86 (58.5)	0.83 (0.29, 2.33)	0.719
Body mass index (kg/m^2)				
< 18.5	1 (4.4)	16 (11.0)	0.49 (0.04, 5.73)	0.569
18.5 – 22.9	6 (26.1)	41 (28.0)	Ref.	
≥ 23.0	16 (69.6)	89 (61.0)	1.29 (0.40, 4.16)	0.672
Number of COVID-19 vaccine doses				
1 dose	2 (8.7)	9 (6.1)	1.54 (0.17, 13.93)	0.699
2 doses	2 (8.7)	28 (19.1)	0.37 (0.56, 2.77)	0.331
3 doses	15 (65.2)	91 (61.9)	1.16 (0.28, 4.71)	0.839
4 doses	4 (17.4)	19 (12.9)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	41	38	1.01 (0.97, 1.06)	0.534

Table 4.17 (continued)

Factors	Musculoskeletal symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Duration from contracting COVID-19 to take questionnaire (day), median	44	59	0.98 (0.96, 1.00)	0.071

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.17 presents an analysis of blood vitamin D levels as a factor influencing musculoskeletal symptoms of long COVID. The multivariable analysis found that vitamin D deficiency remained a significantly increased the likelihood of musculoskeletal symptoms at the 0.05 level (Adjusted OR 13.77 [95%CI 4.54, 41.82], P<0.001). In other words, patients with vitamin D deficiency were 13.77 times more likely to develop musculoskeletal symptoms of long COVID compared to those with sufficient vitamin D levels.

4.6.7 Skin Symptoms

Table 4.18 Multivariable analysis of blood vitamin D levels affecting skin symptoms of long COVID (n=170)

Factors	Skin symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	41 (47.7)	11 (13.1)	11.28 (4.30, 29.57)	<0.001
Insufficiency	32 (37.2)	38 (45.2)	2.44 (1.08, 5.50)	0.032
Sufficiency	13 (15.1)	35 (41.7)	Ref.	

Table 4.18 (continued)

Factors	Skin symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Sex				
Male	43 (50.0)	44 (52.4)	1.07 (0.54, 2.10)	0.850
Female	43 (50.0)	40 (47.6)	Ref.	
Age (years)				
< 45	32 (37.2)	39 (46.4)	Ref.	
≥ 45	54 (62.8)	45 (53.6)	1.64 (0.81, 3.32)	0.166
Body mass index (kg/m^2)				
< 18.5	8 (9.4)	9 (10.7)	1.81 (0.51, 6.43)	0.358
18.5 – 22.9	20 (23.5)	27 (32.1)	Ref.	
≥ 23.0	57 (67.1)	48 (57.1)	2.20 (0.99, 4.89)	0.053
Number of COVID-19 vaccine doses				
1 dose	7 (8.1)	4 (4.8)	2.01 (0.37, 10.98)	0.422
2 doses	14 (16.3)	16 (19.0)	0.83 (0.24, 2.83)	0.768
3 doses	52 (60.5)	54 (64.3)	1.00 (0.36, 2.76)	0.999
4 doses	13 (15.1)	10 (11.9)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	38	39.5	0.99 (0.97, 1.03)	0.846
Duration from contracting COVID-19 to take questionnaire (day), median	58.5	58	1.00 (0.99, 1.02)	0.758

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.18 presents an analysis of blood vitamin D levels as a factor influencing skin symptoms of long COVID. The multivariable analysis found that vitamin D deficiency remained a significantly increased the likelihood of skin symptoms at the 0.05 level (Adjusted OR 11.28 [95%CI 4.30, 29.57], P<0.001). In other words, patients with vitamin D deficiency were 11.28 times more likely to develop skin symptoms of long COVID compared to those with sufficient vitamin D levels. Similarly, insufficient vitamin D levels also remained a significant factor (Adjusted OR 2.44 [95% CI 1.08, 5.50], P=0.032), indicating that patients with insufficient vitamin D levels were 2.44 times more likely to develop skin symptoms of long COVID compared to those with sufficient vitamin D levels.

4.6.8 Psychiatric Symptoms

Table 4.19 Multivariable analysis of blood vitamin D levels affecting psychiatric symptoms of long COVID (n=170)

Factors	Psychiatric symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	24 (47.1)	28 (23.5)	3.97 (1.56, 10.08)	0.004
Insufficiency	18 (35.3)	52 (43.7)	1.51 (0.61, 3.76)	0.335
Sufficiency	9 (17.7)	39 (32.8)	Ref.	
Sex				
Male	25 (49.0)	58 (48.7)	0.89 (0.44, 1.78)	0.741
Female	26 (51.0)	61 (51.3)	Ref.	
Age (years)				
< 45	19 (37.3)	52 (43.7)	Ref.	
≥ 45	32 (62.7)	67 (56.3)	1.25 (0.61, 2.56)	0.536
Body mass index (kg/m^2)				
< 18.5	4 (7.8)	13 (11.0)	0.93 (0.24, 3.67)	0.919
18.5 – 22.9	13 (25.5)	34 (28.8)	Ref.	
≥ 23.0	34 (66.7)	71 (60.2)	1.30 (0.58, 2.91)	0.522

Table 4.19 (continued)

Factors	Psychiatric symptoms		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Number of COVID-19 vaccine doses				
1 dose	4 (7.8)	7 (5.9)	1.00 (0.20, 4.92)	0.999
2 doses	8 (15.7)	22 (18.5)	0.61 (0.18, 2.11)	0.439
3 doses	30 (58.8)	76 (63.9)	0.73 (0.27, 1.97)	0.538
4 doses	9 (17.7)	14 (11.8)	Ref.	
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	40	38	1.00 (0.97, 1.03)	0.998
Duration from contracting COVID-19 to take questionnaire (day), median	53	59	0.99 (0.97, 1.00)	0.146

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.19 presents an analysis of blood vitamin D levels as a factor influencing psychiatric symptoms of long COVID. The multivariable analysis confirmed that vitamin D deficiency remained a significantly increased the likelihood of psychiatric symptoms at the 0.05 level (Adjusted OR 3.97 [95%CI 1.56, 10.08], P=0.004). In other words, patients with vitamin D deficiency were 3.97 times more likely to develop psychiatric symptoms of long COVID compared to those with sufficient vitamin D levels.

4.6.9 Long COVID (Any Symptoms)

Table 4.20 Multivariable analysis of blood vitamin D levels affecting the occurrence of long COVID (n=170)

Factors	Long COVID		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Blood vitamin D levels				
Deficiency	44 (40.0)	8 (13.3)	5.80 (2.10, 16.01)	0.001
Insufficiency	40 (36.4)	30 (50.0)	1.25 (0.57, 2.74)	0.576
Sufficiency	26 (23.6)	22 (36.7)	Ref.	
Sex				
Male	59 (53.6)	24 (40.0)	1.83 (0.91, 3.69)	0.089
Female	51 (46.4)	36 (60.0)	Ref.	
Age (years)				
< 45	42 (38.2)	29 (48.3)	Ref.	
≥ 45	68 (61.8)	31 (51.7)	1.68 (0.82, 3.42)	0.157
Body mass index (kg/m^2)				
< 18.5	9 (8.3)	8 (13.3)	1.26 (0.36, 4.41)	0.722
18.5 – 22.9	25 (22.9)	22 (36.7)	Ref.	
≥ 23.0	75 (68.8)	30 (50.0)	2.74 (1.24, 6.09)	0.013
Number of COVID-19 vaccine doses				
1 dose	9 (8.2)	2 (3.3)	5.89 (0.86, 40.39)	0.071
2 doses	19 (17.3)	11 (18.3)	1.58 (0.46, 5.45)	0.468
3 doses	68 (61.8)	38 (63.4)	1.73 (0.62, 4.81)	0.293
4 doses	14 (12.7)	9 (15.0)	Ref.	

Table 4.20 (continued)

Factors	Long COVID		Multivariable	
	Yes, n(%)	No, n(%)	Adjusted OR (95%CI)	P-value
Duration from measuring blood vitamin D levels to contracting COVID-19 (day), median	39.5	38	1.00 (0.97, 1.04)	0.838
Duration from contracting COVID-19 to take questionnaire (day), median	58	59	1.00 (0.99, 1.02)	0.872

Note n(%): number(percentage); 95%CI: 95% confidence interval; OR: odds ratio; Ref: reference

Data were analyzed with Binary logistic regression (Enter method)

Table 4.20 presents an analysis of blood vitamin D levels as a factor influencing long COVID. The multivariable analysis found that vitamin D deficiency remained a significantly increased the likelihood of long COVID at the 0.05 level (Adjusted OR 5.80 [95%CI 2.10, 16.01], P=0.001). In other words, patients with vitamin D deficiency were 5.80 times more likely to develop long COVID compared to those with sufficient vitamin D levels.

CHAPTER 5

CONCLUSION

This research is a cross-sectional descriptive study aimed at examining the prevalence of long COVID among those with vitamin D deficiency, insufficient vitamin D levels, and sufficient vitamin D levels. It also explores the investigating the relationship between blood vitamin D levels and symptoms of long COVID in patients who tested positive for COVID-19 using the RT-PCR method and received treatment at the Foresta Clinic. A total of 170 participants were included in the study. The sampling method used was non-probability sampling, specifically purposive sampling. Data collection involved three sections: demographic data, long COVID symptoms and severity (8 body systems), and blood vitamin D levels (25(OH)D).

Data analysis was conducted using the STATA statistical software (StataCorp. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC; 2023). Descriptive statistics were used for data analysis. For categorical data presented by frequency and percentage. For Continuous data measures, such as mean, standard deviation, and median, were presented. Inferential statistics were applied using the Mann-Whitney U test to compare blood vitamin D levels between patients with and without long COVID symptoms across the eight symptom groups. Binary logistic regression was used to analyze the factors related to blood vitamin D levels that affect long COVID symptoms. A significance level set at 0.05 ($\alpha = 0.05$). The results of the study are summarized as follows:

5.1 Summary of the Findings

5.1.1 Demographic Data and COVID Vaccination

A total of 170 COVID-19-infected patients in the study, with a female-to-male ratio was 1.1:1. The mean age was 45.87±8.65 years, and the mean body mass index (BMI) was 24.90±4.72 kg/m². Most participants had a BMI of overweight (62.1%). The number of COVID-19 vaccine doses vaccinated during the data collection period was

3 doses (62.4%) with the AstraZeneca vaccine used for the first, second, and third doses in 46.5%, 40.3%, and 2.3%, respectively. Pfizer and Moderna vaccines were used starting from the second dose, with 23.3% and 14.5% of participants vaccinated with these vaccines, respectively. For the third dose, 52.7% and 45.0% of participants received Pfizer and Moderna vaccines, respectively.

5.1.2 Long COVID Severity and Symptoms

Among the 170 COVID-19 patients, 64.7% (95%CI 57.0, 71.9) experienced long COVID symptoms. Of the participants, 50.0% had mild long COVID, 11.2% had moderate, 3.5% had severe, and 35.3% had no symptoms. The median number of symptoms was 2 symptoms (range 0–17). These symptoms were classified into 8 body systems as follows:

General symptoms were found in 39.4% (95%CI 32.0, 47.2), with fatigue/tiredness reported by 39.4% (severity: 3.84±1.62), fever was reported by 12.9%, and no symptoms of chills.

Respiratory symptoms were found in 55.3% (95%CI 47.5, 62.9), with difficulty breathing/shortness of breath reported by 39.4%, with a mean severity score was 3.85±1.84. Cough was reported by 51.8%, with a mean severity score of 3.94±1.92.

Cardiovascular symptoms were found in 11.8% (95%CI 7.3, 17.5), with palpitations in 7.1% (severity: 2.17±1.03), rapid heartbeat in 7.1% (severity: 2.33 ± 0.98), and chest pain in 5.3% (severity: 1.33 ± 0.5).

Neurological symptoms were found in 26.5% (95%CI 20.0, 33.8), with loss of smell/taste in 16.5%, headache in 10.0% (severity: 3.47 ± 1.42), and dizziness in 8.2% (severity: 3.50±1.56).

Gastrointestinal symptoms were found in 1.2% (95%CI 0.1, 4.2), with frequent diarrhea in 1.2% and abdominal pain in 0.6%. No reports of nausea/vomiting.

Musculoskeletal Symptoms were found in 13.5% (95%CI 8.8, 19.6), with muscle pain in 10.6% (severity: 2.89 ± 1.18) and joint/bone pain with an average severity score of 2.60±1.19.

Skin symptoms were found in 50.6% (95%CI 42.8, 58.3), with rashes reported by 20.0% and hair loss by 47.7%.

Psychiatric symptoms were found in 30.0% (95%CI 23.2, 37.4), with anxiety in 15.3% (severity: 3.12±1.07), depression in 7.1% (severity: 2.67± 1.78), and sleep problems in 21.2% (severity: 4.44±2.08).

5.1.3 Blood Vitamin D Levels (25(OH)D)

The median blood vitamin D level in COVID-19 patients was 22.96 ng/mL. The majority had insufficient vitamin D levels was 41.2% (median 23.34 ng/mL). The next group was vitamin D deficiency was 30.6% (median 16.98 ng/mL), followed by Vitamin D sufficiency was 28.2% (median of 34.15 ng/mL).

5.1.4 Comparison of Long COVID Severity and Blood Vitamin D Levels

The study found a significant association between long COVID severity and vitamin D levels ($P < 0.001$). All participants with severe long COVID had vitamin D deficiency, while 73.7% of moderate, 28.2% of mild, and only 13.3% without long COVID were deficient. Median vitamin D levels were higher in those without long COVID (25.46 ng/mL) and with mild symptoms (26.20 ng/mL) compared to moderate (16.42 ng/mL) and severe cases (15.58 ng/mL). Additionally, participants with vitamin D deficiency exhibited a higher median number of symptoms (6 symptoms) compared to those with insufficient or sufficient levels (1 symptom).

Participants had general symptoms, respiratory symptoms, cardiovascular symptoms, neurological symptoms, gastrointestinal symptoms, musculoskeletal symptoms, skin symptoms, and psychiatric symptoms, and long COVID had significantly lower median blood vitamin D levels compared to those without symptoms across all 8 body systems, with statistical significance at the 0.05 level ($P < 0.05$).

Those with vitamin D deficiency had a higher prevalence of general symptoms (59.6%), respiratory symptoms (80.8%), cardiovascular symptoms (32.7%), neurological symptoms (55.8%), gastrointestinal symptoms (3.9%), musculoskeletal symptoms (34.6%), skin symptoms (78.9%), psychiatric symptoms (46.2%), and long COVID symptoms (84.6%), which were higher than those observed in with insufficient or sufficient vitamin D levels across all 8 body systems.

5.1.5 The Blood Vitamin D Levels Related to the Symptoms of Long COVID

Vitamin D deficiency was found to significantly affect long COVID symptoms at the 0.05 level of statistical significance. The factors include general symptoms (Adjusted OR 4.55 [95%CI 1.88, 10.87] P=0.001), respiratory symptoms (Adjusted OR 6.06 [95%CI 2.37, 15.54] P<0.001), cardiovascular symptoms (Adjusted OR 22.63 [95% CI 5.88, 87.14] P< 0.001), neurological symptoms (Adjusted OR 16.22 [95% CI 4.81, 54.65] P< 0.001), musculoskeletal symptoms (Adjusted OR 13.77 [95% CI 4.54, 41.82] P<0.001), skin symptoms (Adjusted OR 11.28 [95% CI 4.30, 29.57] P< 0.001), psychiatric symptoms (Adjusted OR 3.97 [95% CI 1.56, 10.08] P = 0.004), and long COVID (Adjusted OR 5.80 [95% CI 2.10, 16.13] P=0.001).

Additionally, insufficient vitamin D levels were found to significantly affect skin symptoms in long COVID patients (Adjusted OR 2.44 [95% CI 1.08, 5.50] P = 0.032).

5.2 Discussion

5.2.1 COVID-19 Vaccination

The study found that among COVID-19 patients, 53.5% received the inactivated vaccine type (Sinovac and Sinopharm) as their first dose, while 46.5% received the recombinant viral vector vaccine (AstraZeneca). For the second dose, most participants (40.3%) received the recombinant viral vector vaccine, while 37.8% received the mRNA vaccine. This is consistent with the report on the progress of the COVID-19 vaccination service by the Department of Disease Control, which found that 53.5% of the Thai population had been vaccinated with an inactivated vaccine for their first dose, and 24.5% vaccinated with a viral vector vaccine. For the second dose, 52.9% were vaccinated with a viral vector vaccine, and 26.4% were vaccinated with an mRNA vaccine. 43 The Thai government began providing COVID-19 vaccines to the public in early 2021, facilitating emergency access to the vaccine. The Ministry of Public Health considered both safety and the benefits to the public when approving vaccines for emergency use, issuing Conditional Approval for Emergency Use Authorization by the

Food and Drug Administration (FDA) during the COVID-19 pandemic. Six vaccine companies received approval: AstraZeneca (January 20, 2021), Sinovac (February 22, 2021), Johnson & Johnson (March 25, 2021), Moderna (May 13, 2021), Sinopharm (May 28, 2021), and Pfizer (June 24, 2021). 44

5.2.2 Long COVID Severity and Symptoms

The study found that among COVID-19 patients, the prevalence of long COVID was 64.74% (95%CI 57.0, 71.9), with 50.0% had mild long COVID, 11.2% had moderate, 3.5% had severe, and 35.3% had no symptoms. The median number of symptoms was 2 symptoms (range 0–17). Patients with mild long COVID most experienced general and respiratory symptoms, such as cough (74.1%), fatigue (49.4%), and dyspnea (49.4%).

The highest prevalence of respiratory symptoms was at 55.3% (95%CI 47.5, 62.9), followed by skin symptoms at 50.6% (95%CI 42.8, 58.3), and general symptoms at 39.4% (95%CI 32.0, 47.2). Considering individual symptoms, cough was the most common at 51.8%, followed by hair loss at 47.7%, and fatigue/tiredness at 39.4%. The mean severity of long COVID symptoms was no higher than 5 points, indicating that the symptoms were generally mild to moderate level. Long COVID refers to the condition that occurs after a patient recovers from COVID-19, and may still have symptoms that were present during the infection. These symptoms can affect multiple systems of the body and cannot be explained by other diagnoses. They may be caused by fragments of the viral genome or viral antigens, which no longer affect the infection but can impact the immune system, leading to inflammation in various parts of the body. 45 The prevalence of long COVID in this study is like that reported in the study by Methavi Wangchalabvor 40, which showed a prevalence of 64.9%. The most common symptoms in that study were hair loss (32.5%), fatigue after activities (32.0%), and dyspnea (21.6%). This is higher than the study by Supanan Wongsermsin 11, which reported a prevalence of 29.9% but also found the most common to be respiratory symptoms, followed by general symptoms and neurological symptoms. Meanwhile, the study on long COVID prevalence by the Medical Technology Assessment and Research Institute found a prevalence of 40.5%. 39

International studies have shown varying prevalence of long COVID symptoms. In the UK, self-reported long COVID symptoms were found in 40.7% of COVID-19

patients 31, while in Brazil, the prevalence was 29.6%.14 A systematic review and meta-analysis by Chen C et al., which examined 41 research articles, found a long COVID prevalence of 43.0% (95%CI 39.0, 46.0). 30 The prevalence was highest in Asia at 51.0% (95% CI 37.0, 65.0), followed by Europe at 44.0% (95% CI 32.0, 56.0), and the Americas at 31.0% (95% CI 21.0, 43.0). Additionally, a study by Gennaro FD et al. involving a sample of 120,970 participants, found a prevalence of 56.9% (95%CI 52.2, 61.6) of long COVID. The most common symptoms reported were general symptoms at 31.0% (95%CI 27.1, 35.1), followed by respiratory symptoms at 24.5% (95% CI 21.3, 27.9), and psychiatric symptoms at 20.3% (95%CI 17.4, 23.3). 9 In China, long COVID was found in up to 90.4% of COVID-19 patients, with 62.4% had moderate to severe symptoms and 31.0% with severe symptoms. The most common symptoms were fatigue (33.7%), cough (31.9%), and sore throat (31.0%). 10 A study in Malaysia reported that 54.0% of participants had fatigue, 16.1% had sleep disorders, and 12.9% had memory problems. 46

The analysis of long COVID prevalence based on the demographic data of COVID-19 patients reveals that all eight body systems of long COVID are more commonly found in females, aged 45 years or older, and those with a body mass index (BMI) of overweight (> 23.0 kg/m²). COVID-19 patients with all three of these factors have a 1.18 times higher likelihood of developing long COVID. This is due to sex-related biological and hormonal factors specific to females, particularly estrogen and Progesterone, which may enhance immune system responses. Additionally, the functioning of the ACE-2 Receptor (which plays a crucial role in allowing the COVID-19 virus to enter cells) is regulated by estrogen, affecting both the severity and persistence of symptoms. 47 Furthermore, the immune system in females, with an increasing in IL-1 signaling and XIST expression, is related to autoimmune conditions, which could be another contributing factor to the development of long COVID. 48 These findings are consistent with research by Thompson EJ 31, Caze AB 14, and Wong MCS 10, which demonstrated that females have significantly higher risk of developing long COVID compared to males. The study revealed a 1.49 to 1.55 times increased likelihood of long COVID among females. Therefore, confirming the importance of considering sex-related factors when assessing and managing long COVID.

Aging leads to a weakened immune system through a process called Immunosenescence, which may increase the risk of severe initial-stage COVID-19 infection. This results in an inability to effectively eliminate the virus and a longer recovery period. 49 Elderly individuals are also more likely to have pre-existing chronic conditions such as cardiovascular disease, diabetes mellitus, or obesity, leading to low-grade chronic inflammation. This condition may contribute to the development of long COVID symptoms such as fatigue, brain fog, muscle pain, and psychiatric symptoms. 50 This finding aligns with Caze AB 14, which found that individuals aged 41-50 and over 50 had a 2.04- and 2.44-times higher likelihood of experiencing long COVID symptoms compared to those aged 15-30. This study highlights the relationship between aging and the increased risk of long COVID, which may result from physiological changes and the presence of comorbidities in older adults.

Individuals with an overweight BMI often experience chronic low-grade inflammation due to excess fat tissue. This fat tissue secretes inflammatory substances, such as cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). 51, which can affect various systems in the body, including immune and metabolic system dysfunctions, such as insulin resistance, metabolic syndrome, and type 2 diabetes. 52 Additionally, excess fat tissue increases the risk of hypertension, atherosclerosis, and other cardiovascular diseases, as well as respiratory complications like sleep apnea. Moreover, fat tissue can influence the endocrine system, leading to hormonal changes that may reduce physical activity levels. When combined these factors may contribute to an increased likelihood of developing long COVID in individuals with an overweight BMI. However, the findings of this study are inconsistent with those of Caze AB, 14 Supanan Wongsermsin, 11 and Methavi Wangchalabvor, 40 suggesting that further research is needed to clarify this relationship and reach a more definitive conclusion.

5.2.3 Blood Vitamin D Levels

The study found that the median blood vitamin D level in participants was 22.96 ng/mL, which falls within the insufficient vitamin D range (20-30 ng/mL) according to the American Endocrine Society's guidelines. 19 The majority of participants had insufficient vitamin D levels (41.2%), followed by those with vitamin D deficiency (30.6%) and sufficient vitamin D levels (28.2%). As the participants in this study reside

in Bangkok, a metropolitan area with environmental factors and lifestyle patterns typical of city dwellers, their exposure to direct sunlight is limited due to indoor living and working in buildings. Sunlight serves as a primary source of vitamin D, but is further hindered by high air pollution in the city, which obstructs its penetration. Furthermore, the widespread use of sunscreen to protect the skin from sunlight and heat—while effective in preventing skin cancer—may also reduce vitamin D production. The diet of city dwellers in Bangkok is often low in vitamin D, due to the fast-paced lifestyle, with many opting for fast food that tends to be nutritionally poorer. The high population density also makes it more challenging to access sunny or outdoor spaces compared to other regions. These combined factors contribute to the insufficient vitamin D levels observed in people living in Bangkok.

These findings are consistent with a study by Chailurkit L et al., 53 who examined vitamin D levels in Thai people between 2019-2020. They found that people living in Bangkok or central Thailand had lower blood vitamin D levels than those in other regions. Overall, 31.0% of the Thai population had insufficient vitamin D levels (< 30 ng/mL), which is an improvement compared to the 45.2% reported in 2009. The mean vitamin D level was 26.0 (SE=0.3) ng/mL, also lower than in other regions. 54 This agrees with the study by Siwamogsatham O et al., 55 who found that 45.2% of Thai people had insufficient vitamin D levels, with 5.7% being deficient. Factors contributing to lower vitamin D levels included being female, adolescent, and living in urban areas.

The blood vitamin D levels by sex revealed that the median vitamin D level was 22.2 ng/mL for males and 24.5 ng/mL for females. There was no statistically significant difference between sexes (P=0.516). When classified by vitamin D status, both males and females showed similar proportions. Among males, 31.3% had vitamin D deficiency, 41.0% had insufficient levels, and 27.7% had sufficient levels. Among females, 29.9% had a deficiency, 41.4% had insufficient levels, and 28.7% had sufficient levels. Again, no statistical difference was found (P = 0.977). These results differ from previous studies. Research conducted in 2009 54 and 2019 56 found that vitamin D levels in males in Bangkok were significantly higher than in females (males 27.6 ng/mL vs. females 24.5 ng/mL in 2009; males 29.4 ng/mL vs. females 25.2 ng/mL in 2019). Additionally, a study by Jeenduang N and Sangkaew B 57 in Southern

Thailand also showed that vitamin D levels in males were significantly higher than in females (male 37.46 ± 10.61 ng/mL vs. female 28.84 ± 6.65 ng/mL). The difference between the findings in this study and previous research may be attributed to changes in urban lifestyles. Nowadays, the patterns of life, sun exposure, and dietary behaviors between males and females in urban societies are becoming more similar, which may have led to a reduction in the gap in vitamin D levels between the sexes.

When comparing vitamin D levels across various regions was found that 5.0% of the population in North America (United States) had vitamin D levels below 12 ng/mL (indicating vitamin D deficiency), while prevalence in Canada was 8.8%, more commonly found in non-Caucasian populations than in Caucasians. In South America, Argentina's adult population had vitamin D levels between 10-20 ng/mL, with a prevalence ranging from 15.0% to 55.0%, which increased to 15.0%-73.0% among the elderly. In Brazil, vitamin D deficiency in patients and the elderly was reported at 2.0%-16.0%.

In Europe, 16.5% of the population in the UK and 15.0% in Germany had vitamin D levels below 10-12 ng/mL, while 12.0% of the population in Ireland, 21.5% in Portugal, and only 1.0% in Finland had low vitamin D levels. In Oceania, 4.5% of the population in Australia had vitamin D levels below 12 ng/mL, and 20.1% had levels below 20 ng/mL. In New Zealand, 4.9% of those aged 15 and above had vitamin D levels below 10 ng/mL, and 27.1% had levels between 10-19.9 ng/mL.

In Africa, an analysis of 119 studies found that 18.5% of the population had vitamin D levels below 12 ng/mL, and 34.2% had levels below 20 ng/mL. In Western and Central Asia, Kazakhstan had 27.5% of the population with vitamin D levels below 10 ng/mL, and 42.4% had levels between 10-20 ng/mL. In South Korea, 9.4% had levels below 10 ng/mL, and 57.5% had levels between 10-20 ng/mL. In Mongolia, 61.0% of infants, 75.4% of pregnant women, and 40.4% of men aged 14-49 years had vitamin D levels below 20 ng/mL. In China, 30.0% of the population had vitamin D levels below 10 ng/mL, while in Japan, 40.8% had levels below 20 ng/mL.

In South Asia, Pakistan had 61.0%-65.0% of the population with vitamin D levels below 10 ng/mL, while in India, the prevalence ranged from 31% to 65%. In Iran, 63.0% of the population had vitamin D levels below 10 ng/mL in winter and 26.6% in summer. In Southeast Asia, Vietnam showed a vitamin D deficiency

prevalence of 11.2%-20.6% in children and 17.3% in women. In Malaysia and Indonesia, vitamin D levels below 10 ng/mL were found in 0.0% and 4.1% of the population, respectively, while levels between 10-20 ng/mL were found in 44.0% and 39.6%, respectively. 58 These findings highlight that vitamin D deficiency is a widespread issue in many regions globally, with South Asia and Southeast Asia showing a higher prevalence compared to other regions.

The median duration from the blood vitamin D level test to COVID-19 infection was 38.5 days. This duration did not statistically affect the onset of long COVID symptoms across all 8 body systems. This is because vitamin D levels in the body are an indicator of the overall immune system. Low vitamin D levels are a consistent and ongoing risk factor that is independent of the time elapsed. The impact of low vitamin D levels on immune response and long-term inflammation, as well as the increased risk of long COVID, is not reliant on a specific timeframe between testing and COVID-19 infection. Furthermore, blood vitamin D levels usually do not change significantly over a short timeframe unless supplemented. Consequently, testing vitamin D levels at a particular point in time can indicate the general trend of an individual's vitamin D status.

Furthermore, the median time from COVID-19 infection to responding to the long COVID symptom questionnaire was 58.5 days. Similarly, this time gap did not statistically affect the onset of long COVID symptoms across all 8 body systems. This indicates that long COVID symptoms can occur and persist independently of the time elapsed after infection. The complexity of long COVID, which may involve various mechanisms of disease, suggests that it is not solely dependent on time. Instead, factors such as the severity of the initial infection, genetic, or immune system responses may play a more significant role in determining the onset and severity of symptoms.

5.2.4 Long COVID Severity and Blood Vitamin D Levels

The study found a significant association between long COVID severity and vitamin D levels ($P < 0.001$). All participants with severe long COVID had vitamin D deficiency, while 73.7% of moderate, 28.2% of mild, and only 13.3% without long COVID were deficient. Median vitamin D levels were higher in those without long COVID (25.46 ng/mL) and with mild symptoms (26.20 ng/mL) compared to moderate (16.42 ng/mL) and severe cases (15.58 ng/mL). Additionally, participants with vitamin

D deficiency had more symptoms (6 symptoms) than those with insufficient or sufficient levels (1 symptom). In addition, participants with long COVID symptoms (having symptoms from any one system) had a median blood vitamin D level of 21.52 ng/mL, significantly lower than those without long COVID symptoms, who had a median of 25.46 ng/mL. The findings also revealed that participants with vitamin D deficiency were 5.80 times more likely to develop long COVID compared to those with sufficient vitamin D levels.

These results suggest a clear association between lower vitamin D levels and the increased severity and likelihood of long COVID, emphasizing the potential role of vitamin D in mitigating or exacerbating post-viral symptoms. Sufficient vitamin D levels help reduce the risk of long COVID, as vitamin D plays a crucial role in supporting the immune system and the body's response to COVID-19 infection and its post-infection complications. It helps decrease the inflammation caused by the virus. It may lessen the severity of the initial infection by enhancing immune response and reducing cytokine storms (the release of pro-inflammatory cytokines), 32,59, which could reduce the likelihood of long-term complications. These findings were studied by Nielsen NM et al., which showed that participants with sufficient and insufficient vitamin D levels had a 50% lower risk of severe COVID-19 compared to those with vitamin D deficiency, with a reduction in their risk by 63.5% in males, significantly more than the 14.0% in females. 18 Similarly, Filippo LD et al. found that long COVID patients had significantly lower vitamin D levels than those without long COVID (20.1 vs. 23.2 ng/mL, P=0.030), with lower vitamin D levels increasing the risk of long COVID by 1.09 times. 17 Additionally, Cardoso F et al. found that patients with severe COVID-19 pneumonia had a mean vitamin D level of 26.8±7.6 ng/mL, which was lower than the control group (28.6±7.4 ng/mL). Participants with vitamin D deficiency were three times (95%CI 1.79, 5.10) more likely to develop severe COVID-19 pneumonia than the control group, with statistical significance. 60

Vitamin D benefits include enhancing the function of monocytes and macrophages, essential components of the immune system. It also helps prevent autoimmune reactions that could damage the body's tissues, which are associated with long COVID. 61 Moreover, vitamin D reduces the production of pro-inflammatory cytokines, which are responsible for inflammation, and helps modulate the body's

inflammatory response to appropriate levels. 62 This reduction in inflammation can help reduce the risk of long COVID. Additionally, vitamin D helps maintain the integrity of epithelial cells in the respiratory system, 63 playing a role in preventing viral intrusion into cells and potentially reducing the severity of infections. Reducing viral load and inflammatory response can further decrease the risk of long COVID. Vitamin D also supports overall health, including bone health, muscle function, and mental health, aiding the body's recovery from infection and reducing chronic symptoms associated with long COVID. 64

5.2.5 The Blood Vitamin D Levels Related to the Symptoms of Long COVID in 7 Body Systems

The classification of long COVID symptoms according to body systems allows for a discussion of blood vitamin D levels that affect these symptoms as follows:

1. General symptoms

The study found that participants with general symptoms had a median blood vitamin D level of 21.21 ng/mL, which was significantly lower than those without general symptoms, who had a median of 25.46 ng/mL. This result suggests that vitamin D levels affect the occurrence of general symptoms of long COVID, including fatigue and fever. Participants with vitamin D deficiency were 4.55 times more likely to experience general symptoms compared to those with sufficient vitamin D levels. Vitamin D plays a crucial role in maintaining the balance of inflammatory responses in the body by both stimulating and inhibiting inflammatory processes. Vitamin D deficiency can lead to chronic inflammation, which is commonly found in long COVID patients who continue to experience inflammation even after recovering from the infection and resulting in persistent fatigue. Additionally, vitamin D is important for energy production and mitochondrial function. When low vitamin D levels affect to decrease mitochondrial efficiency, leading to reduced energy production in the body, which can manifest as fatigue. Another symptom commonly seen in long COVID is fever, which is a general response to infection and inflammation, triggered by the release of cytokines. Low vitamin D levels may lead to the continuous release of cytokines, causing prolonged or recurrent fever. On the other hand, maintaining sufficient vitamin D levels can help reduce the production of pro-inflammatory cytokines and control fever. In summary, sufficient vitamin D levels are important for

regulating inflammation, mitochondrial function, and cytokine production. These processes are associated with chronic fatigue and fever in long COVID.

The results of this study differ from the findings of Townsend L et al., 38 who studied the relationship between vitamin D levels and post-SARS-CoV-2 infection symptoms. Their research found that one-third of patients with fatigue 79 days after infection had insufficient vitamin D and vitamin D deficiency. However, multivariable regression analysis did not show a statistical relationship between vitamin D levels and Chalder fatigue score, 6MWT, 6MWT modified Borg scale, or the prevalence of fatigue. In contrast, the experimental study by Chaoenporn V et al. 65 investigated the effects of vitamin D supplementation on fatigue in post-COVID patients. The study showed a statistically significant decrease of 3.5 points on the Chalder Fatigue Scale among participants who received 60,000 IU of vitamin D supplementation for 8 weeks. This indicates that the relationship between vitamin D levels and fatigue in long COVID remains inconsistent and varies between observational and experimental studies. This discrepancy may be influenced by other factors and may require further investigation.

2. Respiratory symptoms

The study found that participants with respiratory symptoms had a median blood vitamin D level of 21.32 ng/mL, which was significantly lower than those without respiratory symptoms, whose median was 26.65 ng/mL. This result suggests that blood vitamin D levels influence the occurrence of respiratory symptoms in long COVID, such as dyspnea/shortness of breath and coughing. Participants with vitamin D deficiency were 6.06 times more likely to experience respiratory symptoms compared to those with sufficient vitamin D levels. Long COVID patients often present with respiratory symptoms, such as dyspnea and coughing, which are reflected in lung function tests and/or chest X-rays showing abnormalities. These patients tend to have irregular and fluctuating breathing patterns, which lead to reduced control over their breathing. This could be due to damage to the central nervous system caused by COVID-19 infection. 66 Additionally, vitamin D is essential for optimal lung function as it aids in the creation and repair of lung tissue, as well as maintaining the integrity of the lung epithelial barrier, which helps prevent pathogens from entering. Those with low vitamin D levels may struggle to repair the lung damage caused by the virus effectively, weakening the lungs' defense and increasing the risk of infection and

inflammation. 67 Furthermore, long COVID is associated with inflammatory responses (such as cytokine storms), which may damage lung tissue. Vitamin D helps modulate the immune system and reduce the chance of cytokine storms, which are responsible for excessive inflammation, leading to increased dyspnea and coughing. Vitamin D deficiency may also contribute to chronic inflammation, which could cause fibrosis in the lungs. This fibrosis can reduce lung capacity and elasticity, further impairing breathing. 68

Therefore, vitamin D plays a critical role in supporting lung health and function, which may be related to the respiratory symptoms observed in long COVID patients. In the study by Hikmet RG et al. 69 was found that participants with blood vitamin D levels <20 ng/mL had a 1.06- and 1.14-times higher risk of experiencing dyspnea and coughing, respectively, compared to those with levels >20 ng/mL. However, these results were not statistically significant.

3. Cardiovascular symptoms

The study found that participants with cardiovascular symptoms had a median blood vitamin D level of 15.33 ng/mL, which was significantly lower than those without cardiovascular symptoms, who had a median of 25.28 ng/mL. This result suggests that blood vitamin D levels affect the occurrence of cardiovascular symptoms in long COVID, such as palpitations, tachycardia, and chest pain. Participants with vitamin D deficiency were 22.63 times more likely to experience cardiovascular symptoms compared to those with insufficient/sufficient vitamin D levels. Vitamin D plays a critical role in cardiovascular health through several mechanisms, which may contribute to symptoms in long COVID patients. First, vitamin D helps regulate the renin-angiotensin-aldosterone system (RAAS), which affects blood pressure and fluid balance in the body. Vitamin D deficiency can lead to overactivation of the RAAS system, resulting in elevated blood pressure. Second, vitamin D is essential for the contraction and function of the myocardium. A deficiency in vitamin D can impair heart function, potentially leading to cardiovascular complications. 70 Vitamin D also has anti-inflammatory properties and helps to reduce cytokine levels that trigger inflammation. A deficiency may lead to chronic inflammation and increase the risk of cardiovascular complications. 71 Furthermore, vitamin D influences the autonomic nervous system, which controls heart rate and blood pressure. Vitamin D deficiency

may cause dysfunction in this system, particularly by increasing sympathetic nervous system activity. 72 Vitamin D also supports the blood vessel walls function and any dysfunction here can increase the risk of atherosclerosis and coronary artery disease. 73

Lastly, vitamin D is necessary for calcium absorption and regulation, which is the role for the electrical conduction system of the heart. A deficiency in vitamin D may disrupt calcium balance, leading to arrhythmias. 61 Given these mechanisms, vitamin D deficiency can lead to cardiovascular symptoms in long COVID patients, such as palpitations, tachycardia, and chest pain. These symptoms are attributed to various pathways, including excessive RAAS activity, inflammation, autonomic nervous system dysfunction, impaired vascular wall function, and abnormal calcium regulation.

4. Neurological symptoms

The study found that participants with neurological symptoms had a median blood vitamin D level of 17.03 ng/mL, which was significantly lower than those without neurological symptoms, who had a median of 26.67 ng/mL. This result suggests a link between vitamin D levels and the occurrence of neurological symptoms associated with long COVID, such as loss of smell or taste, headaches, and dizziness. Participants with vitamin D deficiency were found to be 16.22 times more likely to develop neurological symptoms than those with sufficient vitamin D levels. Vitamin D plays a vital role in the central nervous system—it supports the function of nerve cells, protects neurons, and affects neurotransmitters that are essential for sensory perception, cognitive function, and emotional control. Neurological symptoms like headaches, dizziness, and sensory loss are common in long COVID patients and are thought to be caused by the virus's impact on the nervous system. A deficiency of vitamin D can hinder neurological recovery in these patients. 74 Vitamin D is also crucial for reducing inflammation and modulating the immune response. It helps reduce the production of pro-inflammatory cytokines. 62 When low vitamin D levels, it can increase inflammation in the nervous system, potentially damaging the cranial and olfactory nerves. This damage may worsen symptoms such as dizziness, headaches, and the loss of smell or taste. 32

These findings are consistent with a systematic review by Nowaczewska M et al. 75 where 18 out of 22 studies reported that low vitamin D levels were associated

with headaches and migraines. In those studies, 67.2–73.0% of patients with headaches had vitamin D levels between 13.5 and 16.9 ng/mL. Another study by Sencan Z et al. 76 revealed that participants with vitamin D3 deficiency had significantly smaller olfactory bulbs (which transmit scent signals to the brain) and shallower olfactory sulci (brain grooves associated with the sense of smell) compared to those without deficiency.

Furthermore, vitamin D influences the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, which is crucial in the body's stress response. A deficiency in vitamin D can disrupt the HPA axis, leading to increased stress, emotional imbalances, and possibly intensifying symptoms like dizziness and headaches. Since vitamin D also supports nerve repair, its deficiency may delay the recovery process, prolonging or worsening neurological symptoms in long COVID patients. 77

5. Musculoskeletal symptoms

The study found that participants with musculoskeletal symptoms had significantly lower vitamin D levels of 16.36 ng/mL, compared to those without musculoskeletal symptoms, who had a median of 25.46 ng/mL. This result suggests that blood vitamin D levels play a role in the occurrence of musculoskeletal symptoms in long COVID patients, such as muscle pain and joint or bone pain. Participants with vitamin D deficiency were 13.77 times more likely to experience musculoskeletal symptoms than those with no deficiency vitamin D level. Vitamin D has several key functions related to the musculoskeletal system. One of its primary roles is regulating calcium and phosphate levels in the body. It helps absorb calcium from the intestines and maintains phosphate balance. Both minerals are crucial for bone strength and muscle function. A vitamin D deficiency can reduce calcium absorption and phosphate levels, leading to hypocalcemia, 21 which effects to bone mineral loss and muscle weakness and pain. 32 In addition, vitamin D is involved in bone mineralization and the process of bone remodeling. A deficiency can led to conditions such as osteomalacia in adults, rickets in children, and osteoporosis, which are often characterized by bone and joint pain. This is particularly relevant for long COVID patients who may also experience prolonged weakness or fatigue. 78 Vitamin D also directly affects muscle strength and function by interacting with vitamin D receptors (VDRs) in muscle tissues.

A deficiency can cause muscle weakness, pain, and hinder the muscle recovery process. 79

The study also revealed that 78.3% of participants with musculoskeletal symptoms experienced fatigue. Chronic fatigue and musculoskeletal discomfort are prevalent symptoms among long COVID patients. 32 Vitamin D deficiency can more severe these symptoms by intensifying muscle pain, fatigue, and weakness. In severe cases, it may lead to a condition known as vitamin D deficiency myopathy, which further worsens muscle pain and weakness. 80 Moreover, vitamin D contributes to joint and cartilage by protecting the cartilage that cushions the joints. A deficiency can accelerate cartilage degeneration, leading to worsened joint pain, especially in long COVID patients who may already be dealing with musculoskeletal symptoms. A study by Razzaque MA et al. 81 found a high prevalence of low vitamin D levels in patients with unexplained bone pain and cramps, with 84.9% and 79.8% of patients, respectively, showing deficiencies. Similarly, a study by Chaudhari SS et al. 82 found that 82.4% of patients with unexplained musculoskeletal symptoms had low vitamin D levels. Notably, 49.1% of these patients were deficient, while 33.3% had insufficient levels.

Finally, vitamin D plays a role in regulating the nervous system and pain perception. A deficiency can contribute to mood disturbances and increase the risk of depression, 83 which can further impair pain perception. This is particularly relevant for long COVID patients often had psychiatric symptoms. If these patients also have vitamin D deficiency, it may intensify the muscle and joint pain they experience.

6. Skin symptoms

The study found that participants with skin symptoms had significantly lower vitamin D levels of 21.0 ng/mL, compared to those without skin symptoms, who had median of 27.82 ng/mL. This result suggests that blood vitamin D levels influence the occurrence of skin symptoms in long COVID, such as rashes and hair loss. Those with vitamin D deficiency and insufficient vitamin D levels more likely to occur skin symptoms were 11.28 and 2.44 times compared to those with sufficient vitamin D levels. Vitamin D plays a crucial role in skin and hair health, particularly in the context of long COVID, where symptoms like rashes and hair loss are common. Vitamin D deficiency affects the skin's function, including the activity of keratinocytes, the

strength of the skin's protective barrier, and the regulation of inflammation. Long COVID patients with low vitamin D levels often prevalent skin rashes, dryness, and irritation, due to impaired skin repair and regeneration. 84 Additionally, vitamin D affects immune system function by regulating cytokines that stimulate and inhibit inflammation in a balanced manner, thereby reducing the risk of autoimmune diseases, which may contribute to skin rashes in long COVID patients. 85 Regarding hair growth, vitamin D is essential for the functioning of hair follicles and vitamin D receptors (VDRs) in the follicles, which are necessary for initiating new hair growth. A deficiency can led to telogen effluvium, a common type of hair loss observed in long COVID patients.

This aligns with a study by EI-Tatawy et al. 86 which found that vitamin D levels in patients with telogen effluvium were significantly lower than in healthy participants (13.31±5.8 ng/ml vs. 33.61 ± 8.16 ng/ml). Vitamin D deficiency may also increase the risk of developing alopecia areata, an autoimmune condition where the immune system attacks hair follicles. A study by Cerman AA et al. 87 revealed a significantly higher prevalence of vitamin D deficiency among patients with alopecia areata (91.0%) compared to healthy participants (33.3%). Furthermore, the stress of illness combined with vitamin D deficiency can cause hair follicles to enter the resting phase (telogen phase), leading to more severe hair loss and delaying the transition to the growth phase (anagen phase). This can result in thinning hair and continuous hair loss. 88 Inflammation of the skin and scalp is another factor contributing to skin and hair loss issues in long COVID patients. Therefore, vitamin D deficiency may lead to skin and scalp inflammation, resulting in rashes and hair loss. 85,89

7. Psychiatric symptoms

The study found that participants' psychiatric symptoms had significantly lower blood vitamin D levels of 20.91 ng/mL, compared to those without psychiatric symptoms, who had a median of 26.67 ng/mL. This result suggests that blood vitamin D levels influence the occurrence of psychiatric symptoms in long COVID patients, including anxiety, depression, and sleep disorders. Those with vitamin D deficiency were 3.86 times more likely to experience psychiatric symptoms compared to those with sufficient vitamin D levels. This finding is consistent with a study by Algin S et al. 90 found that vitamin D levels in patients with psychiatric disorders averaged

19.9±11.8 ng/mL, with 87.8% of these patients having low vitamin D levels, including 62.0% with levels below 20 ng/mL and 25.8% with levels between 21-30 ng/mL. Vitamin D plays a crucial role in mental health and the nervous system particularly in the context of long COVID. A deficiency in vitamin D can affect the synthesis of neurotransmitters, particularly serotonin, which plays a crucial role in regulating mood, risks of depression and anxiety. Moreover, vitamin D has protective properties for the nervous system, helping to shield brain cells from damage and supporting brain development and function. A deficiency of vitamin D may thus contribute to neurological disorders that affect cognition and mood. 91 In long COVID, chronic inflammation is a key factor linking psychiatric symptoms. Vitamin D helps modulate the immune response by reducing the levels of pro-inflammatory cytokines. When there is a deficiency in vitamin D can make inflammation worsen, negatively affecting brain function and leading to emotional disturbances. 92 Vitamin D is also involved in regulating the Hypothalamic-Pituitary-Adrenal (HPA) axis, which plays a critical role in the stress response. A vitamin D deficiency can disrupt the HPA axis and increase the risk of mental health issues. 77

In a randomized controlled trial, supplementation with 60,000 IU of vitamin D per week significantly reduced anxiety levels but did not significantly affect depression. 65 However, other studies have shown that vitamin D levels are not always correlated with anxiety, depression, or sleep disorders. 69 Sleep disorders are another concern related to vitamin D because vitamin D receptors are in areas of the brain that regulate sleep. It is believed that vitamin D influences the production of melatonin, a hormone that controls the sleep-wake cycle. A vitamin D deficiency can disrupt melatonin production, leading to sleep disorders. 93 Even in long COVID patients, a vitamin D deficiency may worsen symptoms, increasing stress from managing various symptoms, which in turn deteriorates mental health, increases anxiety, and worsens sleep disorders. 94 A study by Hikmet RG et al. 69 found that 89.0% of participants with low vitamin D levels experienced sleep disorders.

5.3 Recommendation

Recommendations of the study and further research are followed as:

5.3.1 Implication of the Study

1. This study found that vitamin D deficiency is significantly associated with various symptoms of long COVID. It is recommended to test vitamin D levels in COVID-19 patients and in high-risk groups potentially deficient in Vitamin D, especially among the elderly, those with chronic conditions, and individuals with obesity, who have a high risk of Vitamin D deficiency. Providing guidance on sun exposure and Vitamin D supplementation is crucial in reducing Long COVID risks.

2. Measurement of Vitamin D levels should be considered in patients with symptoms persisting more than 4 weeks (long COVID) to help identify those at higher risk of developing more severe conditions.

3. The findings suggest that vitamin D deficiency may increase the risk of developing long COVID. Vitamin D supplementation before and after infection could be a method to reduce long COVID risk. Developing prevention strategies that focus on increasing vitamin D can enhance immunity and reduce Long COVID risks.

4. The results highlight the importance of maintaining sufficient vitamin D levels to prevent and reduce the severity of long COVID symptoms. Therefore, public health agencies should promote campaigns to increase awareness about vitamin D benefits, including testing for vitamin D levels in high-risk populations, especially those experiencing long COVID or those at high risk of musculoskeletal, skin, and fatigue-related symptoms. Vitamin D supplementation in these groups may help reduce symptom severity and increase the chances of faster recovery.

5. This finding can be applied to planning treatment and rehabilitation for COVID-19 patients. It is recommended that healthcare providers consider vitamin D supplementation as part of post-infection care, particularly for patients at risk of developing long COVID.

6. The findings from this study should be shared with the public and public health organizations to raise awareness about importance of vitamin D for health,

especially during the COVID-19 pandemic that continues to have widespread health impacts.

5.3.2 Suggestion for Further Research

1. The sample size should be increased to obtain more comprehensive and diverse data. The current study's sample may lead to conclusions that do not fully capture demographic or environmental changes.

2. A longitudinal study design should be considered to evaluate changes in vitamin D levels and long COVID symptoms over time periods. Long-term studies will help understand the effects of vitamin D levels changes at various time points.

3. Additional in-depth research should be conducted on the biological mechanisms associated with vitamin D levels to the symptoms of long COVID, particularly in the nervous, musculoskeletal, and cardiovascular systems. This will help understand the role of Vitamin D in reducing symptom severity and accelerating recovery.

4. It is recommended to use medical tools, examination by a physician specialist, or internationally recognized questionnaires to assess long COVID symptoms. This will ensure the data is more detailed and reliable.

5. Additional factors should be included in the analysis of potential confounding variables that may impact vitamin D levels, such as physical activity levels, nutrition, and sunlight exposure. Collecting comprehensive data will help control for confounding factors and provide a more accurately assess of the effect of vitamin D on long COVID symptoms.

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APPENDIX

FIGURES

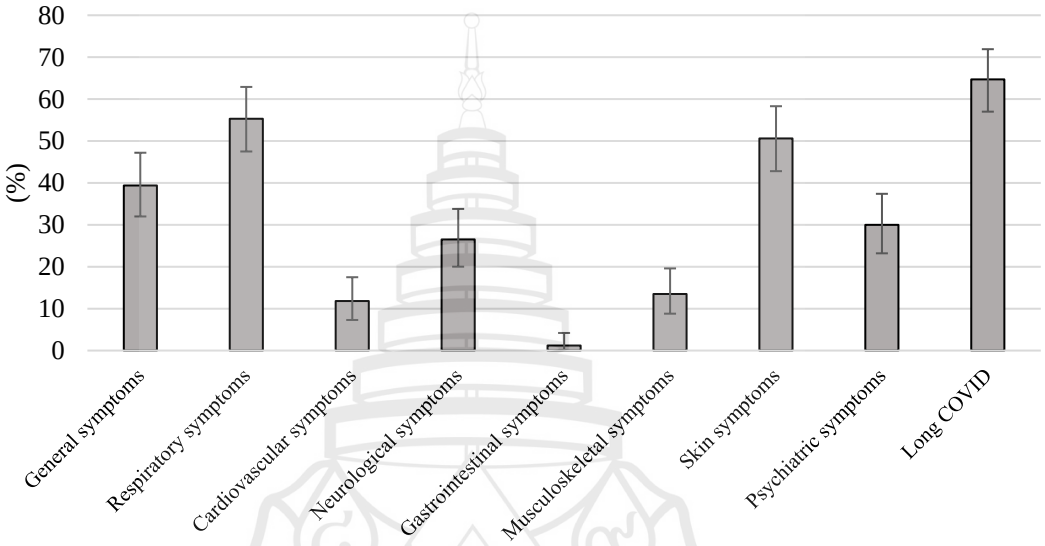

Figure 1 Prevalence of eight systemic symptoms of long COVID

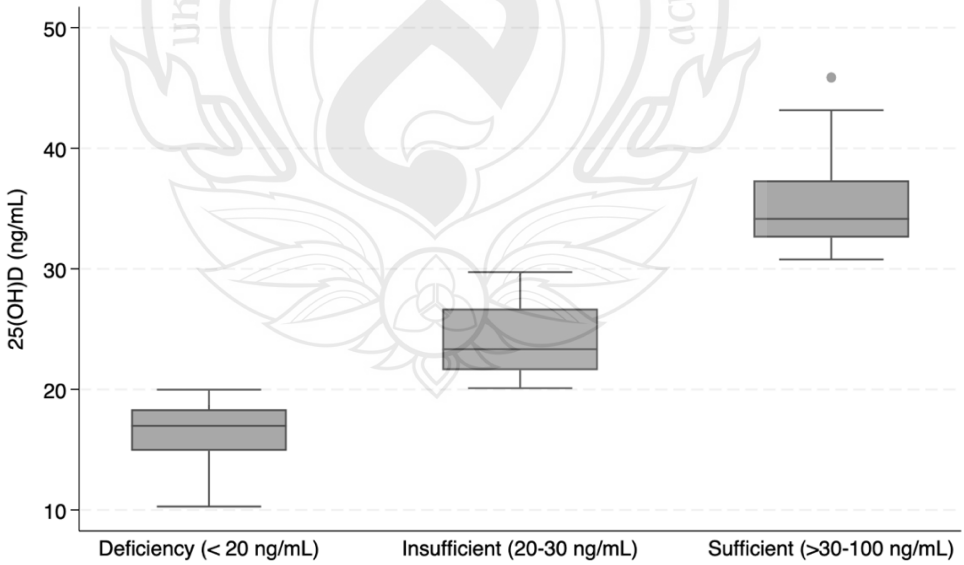

Figure 2 Blood vitamin D levels categorized by vitamin D status

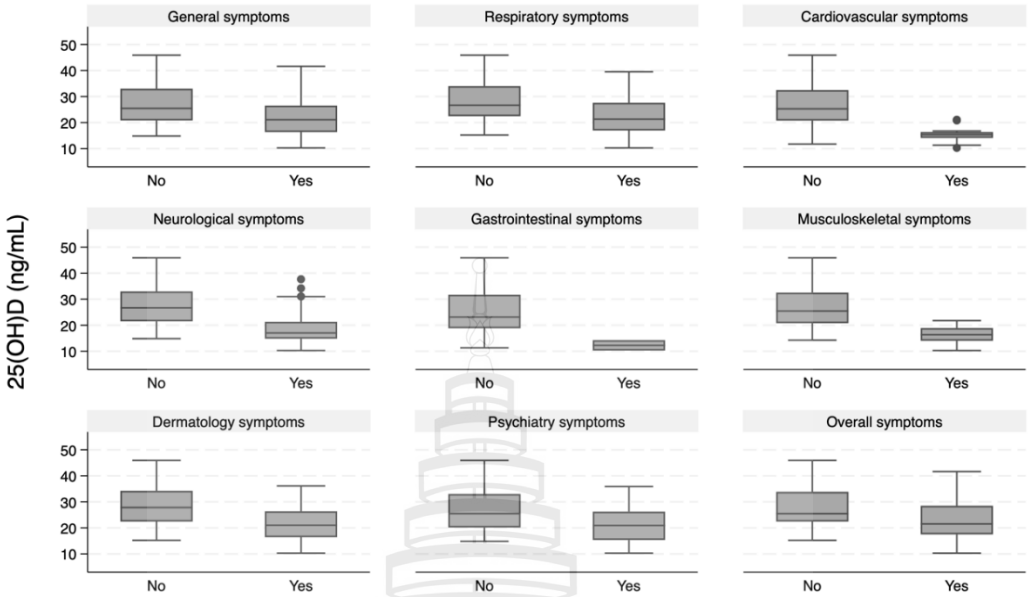

Figure 3 Comparison of blood vitamin D levels between with and without long COVID symptoms participants, categorized by eight systemic symptoms