Relationship Between Stress Level, Salivary Cortisol Levels, and Platelet-Lymphocyte Ratio on Post-Covid-19 Syndrome Occurrence

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Abstract
Introduction: COVID-19 is a disease that affects multiple organs and presents with various symptoms, including Post-COVID-19 Syndrome, which is characterized by inflammation, immunological dysregulation, neuroendocrine dysfunction, and hypometabolism. Aim and Objective: This study aimed to investigate the relationship between stress, salivary cortisol, platelet-lymphocyte ratio, and Post-COVID-19 Syndrome Materials and Methods: A cross-sectional, analytical observational study was conducted on COVID-19 patients treated and followed up between May and July 2021 at Dr. Soetomo General Hospital Surabaya. EZR (Easy R) was used to analyze demographic data, stress levels, salivary cortisol, platelet-lymphocyte ratio, and post-COVID-19 Syndrome. The study included 46 participants, with 29 (63%) in the post-COVID-19 Syndrome group and 17 (37%) in the control group. Results and Conclusion: There were no statistically significant differences in age, gender, and platelet levels between the two groups ($p>0.05$). Stress levels and salivary cortisol levels were not significantly correlated with Post-COVID-19 Syndrome on days 0, 3, and 6 ($p>0.05$). However, the platelet-lymphocyte ratio on days 0, 3, and 6 was significantly correlated with Post-COVID-19 Syndrome ($p<0.05$). The study found a significant correlation between the platelet-lymphocyte ratio and Post-COVID-19 Syndrome.
Keywords: Cortisol Level, Platelet-Lymphocyte Ratio, Post-COVID-19 Syndrome, Stress Level

1. Introduction

COVID-19 is a persistent global issue with over 112 million cases and 2.5 million deaths as of mid-January 2021 (1). Isolation is required for all confirmed cases to prevent virus transmission, with the decision for hospital or home isolation based on the patient's clinical condition, need for supportive care, risk factors related to disease severity, and living conditions (2). Isolation in hospitals or homes can be a stressor for patients and affect their psychological condition, which in turn can impact their metabolism and immune system and contribute to disease severity.

Stress is a response to any form of trauma, surgery, or infection that can cause disruption to the body's homeostasis mechanism, which aims to maintain and initiate the healing process (3). Stressors can cause different behavioral and physiological responses that vary from person to person (4), and can manifest as anxiety, depression, fear, anger, or dissociation (5). COVID-19 patients in isolation rooms commonly experience anxiety and depression symptoms related to psychological stress (Mazza et al., 2020). This stress condition triggers a neuroendocrine response in the body through the Hypothalamic-Pituitary-Adrenal (HPA) axis, which releases the hormone cortisol (6).

Stress and increased cortisol levels can affect lymphocytes and platelets. The rise in cortisol hormone reduces telomerase activity in lymphocytes, which plays a critical role in cell proliferation and preventing aging. As T lymphocytes age, they become less responsive to antigen stimulation, which reduces their ability to fight infections (7). Additionally, stress is linked to platelet function, as epinephrine released during stress leads to platelet activation and increased serotonin binding to its receptors, resulting in the activation of other platelets and thrombus formation (8). Both platelets and lymphocytes can be described using the platelet-lymphocyte ratio (PLR), which can help determine the inflammatory status of COVID-19 patients. Previous research has shown that this ratio correlates with various diseases, including inflammation-related diseases, cardiovascular diseases, cancer, and type 2 diabetes (9).

The next phenomenon that appears in patients who

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have recovered from COVID-19 is Post-COVID Syndrome. This condition is characterized by COVID-19 symptoms that persist for more than 12 weeks. The pathophysiology and factors influencing Post-COVID Syndrome are still not fully understood. However, it has been reported that patients with Post-COVID Syndrome experience a decrease in their quality of life. In a study conducted by Moreno-Pérez et al. (10), Post-COVID Syndrome is associated with a decrease in patients' quality of life.

The SARS-CoV-2 virus is a positively charged single-stranded RNA (ssRNA) with spike glycoproteins that attach to host cell receptors, allowing the virus to enter and attack target cells that express ACE-2 (11). These target cells include the lungs, heart, kidneys, and gastrointestinal tract, among others. The virus initially enters the body through the mucous membrane, especially the nasal and laryngeal mucosa, and then enters the lungs. Clinical symptoms typically appear 7-14 days after infection (12).

Clinical symptoms of SARS-CoV-2 infection include fever, non-productive cough, shortness of breath, myalgia, and fatigue, which are accompanied by lymphopenia, increased pro-inflammatory mediators (Interleukin-6/ IL-6), and infiltrate images in both lung fields on chest X-ray. This disease can be divided into three phases based on its clinical course: viremia phase, pneumonia phase (acute), and recovery phase. If the patient's immune system is robust enough, their body can fight the virus and enter the recovery phase; otherwise, they will fall into a critical condition characterized by Acute Respiratory Distress Syndrome/ARDS, requiring intensive care (13,14). This critical condition is triggered by the cytokine storm mechanism that results from the immune response to the viral infection (12).

According to De Gaudio & Romagnoli (3), stress is defined as a response to trauma, surgery, and infection that triggers significant neural and hormonal responses, leading to disturbances in homeostasis mechanisms that aim to restore the body to its healing process. Furthermore, Wibowo et al. (15) stated that stress can contribute to physical diseases by inducing negative effects such as anxiety and depression, which may impact biological processes or behavioral patterns and exacerbate the severity of a disease. Stressors encountered in daily life can range from time constraints, work, and household responsibilities to financial problems, loneliness, poor health, and interpersonal conflicts. In some cases, rare stressors such as natural disasters, violence, and accidents can trigger a "fight or flight" response and cause traumatic memories that may lead to Post-Traumatic Stress Disorder (PTSD). The brain is responsible for determining whether a condition is stressful or not, and it can undergo architectural, molecular, and neurochemical changes during acute and chronic stress. These changes can have short- and long-term consequences for various systems in the body, including the immune, cardiovascular, and metabolic systems (5).

There are three types of stress, which are good stress, tolerable stress, and toxic stress. Good stress is also known as "*eustress*", and it refers to stress that is manageable, challenging, and can lead to a sense of accomplishment. It can help individuals develop resilience and improve their decision-making abilities, leading to personal growth. This type of stress has a positive impact on an individual's well-being (5).

The adrenal glands are small, triangular-shaped organs located above each kidney. They are composed of two distinct regions, the outer adrenal cortex and the inner adrenal medulla. The cortex accounts for about 90% of the adrenal gland and synthesizes several classes of steroid hormones, including glucocorticoids (such as cortisol), mineralocorticoids (such as aldosterone), and androgens (such as testosterone and estrogen precursors). On the other hand, the medulla is responsible for the production and release of catecholamines, including adrenaline (epinephrine) and noradrenaline (norepinephrine). All steroid hormones are synthesized from cholesterol, which is converted into pregnenolone in the mitochondria by the enzyme cytochrome P450. This conversion is the initial step in the synthesis of all steroid hormones, and from pregnenolone, specific enzymes in the adrenal cortex determine which type of steroid hormone will be produced (16).

Cortisol has various effects on the body, including the breakdown of proteins and the production of glucose through gluconeogenesis. It also mobilizes fatty acids and inhibits muscle protein synthesis, leading to the breakdown of muscle and bone mass at high concentrations (16). In addition to its metabolic effects, cortisol also plays a role in regulating the immune system. Cortisol has anti-inflammatory effects on almost all types of immune cells.

According to Prakoeswa (17), lymphocytes play a crucial role in adaptive immunity. They are the only cells in the body that can express clonal antigen receptors specific for different antigens. In a healthy adult, the total number of lymphocytes is about 5 x 10. Of these, approximately 2% are in the blood, 4% in the skin, 10% in the bone marrow, 15% in the gastrointestinal and respiratory mucosal lymphoid tissue, and 65% in lymphoid organs such as the spleen and lymph nodes. Lymphocytes are divided into two main classes based on their functions. B lymphocytes produce antibodies, while T lymphocytes mediate cellular immunity. T lymphocytes are produced from precursor cells in the bone marrow, migrate to the thymus, and mature there (18).

The Platelet-Lymphocyte Ratio (PLR) is a measurement that compares the absolute counts of platelets and lymphocytes in the blood, and it has been associated with various diseases related to inflammation, cardiovascular disease, cancer, and type 2 diabetes. PLR can be obtained through a

routine complete blood count, and the normal values of PLR can vary between different racial groups. According to a study by Wu et al. (19) conducted on 2500 men and 2500 women in the Chaoshan area of southern China, the normal range of PLR for men was between 36.63 and 149.13, and for women, it was between 43.36 and 172.68. The study also found that women aged 30-60 had a higher PLR than men, but in women over 50 years of age, PLR decreased.

The definition of Post-COVID-19 Syndrome is well-supported by the National Institute for Health and Care Excellence (NICE) and Venkatesan (2021), which states that this condition is characterized by persistent signs and symptoms that occur during or after a COVID-19 infection, last for more than 12 weeks, and cannot be explained by any other diagnosis. These symptoms can affect various systems of the body, and they can overlap, fluctuate, or change over time. Post-COVID-19 Syndrome has been observed to affect patients with mild to severe COVID-19, including those who were not hospitalized (20,21).

A study by Moreno-Pérez et al. (10) examined the incidence and risk factors of acute post-COVID-19 syndrome in patients who had been diagnosed with COVID-19 or discharged from the hospital. The study found that out of 277 patients, 141 (50.9%) experienced post-COVID-19 syndrome, which was associated with a 9-point reduction in quality of life (EuroQol VAS), indicating a decline in their health perception after COVID-19 infection. The severe pneumonia group had two independent factors associated with post-COVID-19 syndrome: opacity on chest X-ray > 50% (OR 2.87 (1.13-7.32), p = 0.027) and increased heart rate at admission (OR 1.03 (1.01–1.06), p = 0.04). Meanwhile, in the group with neurological symptoms, 70-90% of patients experienced symptoms of depression and anxiety (10).

The Post-COVID-19 Syndrome is a complex condition that remains poorly understood and requires further research. In Indonesia, there is currently no data available on the incidence of Post-COVID Syndrome. The pathophysiology and risk factors associated with this condition are still unclear. Therefore, researchers aim to investigate the relationship between stress levels, salivary cortisol levels, and the platelet-lymphocyte ratio with the incidence of Post-COVID Syndrome.

There is evidence to suggest that stress levels, cortisol levels, and the platelet-lymphocyte ratio are associated with various diseases, including those related to inflammation and immune dysfunction, which may be relevant to Post-COVID Syndrome. Previous studies have shown that stress can lead to dysregulation of the hypothalamic-pituitary-adrenal axis, resulting in increased cortisol levels. Additionally, the platelet-lymphocyte ratio has been associated with inflammation and immune dysfunction in various diseases .

Therefore, investigating the relationship between stress levels, salivary cortisol levels, and the platelet-lymphocyte ratio with the incidence of Post-COVID Syndrome may provide valuable insights into the pathophysiology of this condition and its risk factors in the Indonesian population.

2. Research Method

This study utilized an analytical observational approach with a cross-sectional design to examine the relationship between stress levels, saliva cortisol levels, platelet-to-lymphocyte ratio, and the occurrence of post-COVID-19 syndrome. The study was conducted in Isolation Wards 1, 2, 3, 4, and 5 at Dr. Soetomo Hospital Surabaya during the period from May to July 2021. The data were obtained from medical records of patients who were treated and post-treated in the isolation wards.

The intended sample for this study included all eligible patients who met the sample acceptance criteria from the reachable population. We used a total sampling technique to include all samples that met the inclusion criteria until the minimum sample size was reached. EZR (Easy R) was used to analyze demographic data, stress levels, salivary cortisol, platelet-lymphocyte ratio, and Post-COVID-19 Syndrome (22).

The variables in this study were stress level, saliva cortisol levels, platelet-to-lymphocyte ratio, and post-COVID-19 syndrome. The medical records data included gender, age, DASS-21 scores (measured on days 0, 3, and 6 of treatment), saliva cortisol levels, lymphocytes, platelets, platelet-to-lymphocyte ratio (measured on days 0, 3, and 6 of treatment), and data on patients who experienced post-COVID-19 syndrome.

3. Results and Discussion

Demographic Characteristics of Research Subjects

This study is a retrospective study that assesses the relationship between stress level, saliva cortisol levels, and platelet-to-lymphocyte ratio with the occurrence of post-COVID-19 syndrome. Normality tests were performed using the Shapiro-Wilk test, and the results showed that lymphocyte, saliva cortisol, and platelet-to-lymphocyte ratio variables were not normally distributed, and hence presented using median and interquartile range (IQR). The platelet and age variables were found to be normally distributed, and hence presented using mean and standard deviation (SD).

Table 1 and 2 show the characteristics of COVID-19 patients who were treated and post-treated in Isolation Wards 1, 2, 3, 4, and 5 at Dr. Soetomo Hospital Surabaya during the period from May to July 2021. Table 1 shows that there was no significant difference in patient characteristics between those with post-COVID-19 syndrome and those without. Table 2 presents the laboratory results of the patients. There was a significant difference in lymphocyte levels on days 0, 3, and 6 between patients with post-COVID-19 syndrome and those without ($p<0.05$). The lymphocyte levels in patients with post-COVID-19 syndrome were lower compared to the control group.

Table 1: Characteristics of COVID-19 Patients				
Variable	Total N (%)	Post-COVID-19 syndrome		p
		No 17 (37,0%)	Yes 29 (63,0%)	
Age (years; mean [SD])	**41,59 (11.23)**	**39,59 (11.97)**	**42,76 (10,82)**	**0,361[a]**
Gender				
Man	26 (56.5%)	8 (47.1%)	18 (62,1%)	0,322[a]
Woman	20 (43.5%)	9 (52.9%)	11 (37,9%)	
Comorbid	13 (28.3%)	3 (17.6%)	10 (34,5%)	0,221[a]
Diabetes mellitus	8 (17.4%)	2 (11.8%)	6 (20,7%)	0,441[a]
Hypertension	9 (19.6%)	2 (11.8%)	7 (24,1%)	0,307[a]
Clinical Manifestations				
Respiration		-	21 (72,4%)	-
General		-	14 (48,3%)	-
Nerve		-	7 (24,1%)	-
Gastrointestinal		-	2 (6,9%)	-
Musculoskeletal		-	3 (10,3%)	-
Ear, nose, throat		-	2 (6,9%)	-
Skin		-	1 (3,4%)	-
Chi-square test				

Table 2: Laboratory Results for COVID-19 Patients				
Variable	Total	Post-COVID-19 syndrome		p value
		No	Yes	
PLT D0 (mg/dL; mean [SD])	291260,87 (108975,52)	276117,65 (93626,17)	300137,93 (117711,43)	0,477a
PLT D3 (mg/dL; mean [SD])	360043,48 (128570,08)	331058,82 (84322,65)	377034,48 (147290,16)	0,246a
PLT D6 (mg/dL; mean [SD])	384739,13 (122352,03)	352882,35 (107106,77)	403413,79 (128554,47)	0,179a
D0 lymphocytes (mg/dL; median [IQR])	1085,00 (850,00-1380,00)	1250,00 (940,00-1490,00)	980,00 (840,00-1150,00)	0,030b
D3 lymphocytes (mg/dL; median [IQR])	1275,00 (1060,00-1970,00)	1820,00 (1260,00-2270,00)	1180,00 (850,00-1510,00)	0,007b
D6 lymphocytes (mg/dL; median [IQR])	1255,00 (1080,00-1950,00)	2110,00 (1400,00-2830,00)	1180,00 (990,00-1360,00)	0,001b
Independent sample t test Mann-Whitney U Test PLT = Platelets; D0 = day 0; D3 = 3rd day; D6 = 6th day				

Relationship of Stress Levels to Post-COVID-19 Syndrome Events

Table 3 shows the relationship between stress levels and the occurrence of post-COVID-19 syndrome. In this study, there was no significant relationship between stress levels and the occurrence of post-COVID-19 syndrome on days 0, 3, and 6 of treatment (p>0.05). Patients were predominantly in the normal stress category, with percentages of 73.9% on day 0, 78.3% on day 3, and 84.8% on day 6.

Table 3: Correlation of Stress Levels to Post-COVID-19 Syndrome Events		
Variable	Correlation coefficient	p[a] Value
Stress category d0	-0,185	0,219
Stress category d3	-0,019	0,901
Stress category d6	0,029	0,848
Spearman Correlation Stress category (DASS-21) D0 = 0th day, D3 = 3rd day, D6 = 6th day		

Relationship of Salivary Cortisol to Post-COVID-19 Syndrome

In table 4, the relationship between salivary cortisol and the incidence of post-COVID-19 syndrome is shown. In this study, there was no significant relationship between salivary cortisol and the incidence of post-COVID-19 syndrome on day 0, day 3, and day 6 of treatment (p>0.05).

Table 4: Relationship of Salivary Cortisol to Post-COVID-19 Syndrome				
Variable	Total	Post-COVID-19 Syndrome		p[a] Value
		No	Yes	
Salivary cortisol D0, ng/dL (median [IQR])	0,23 (0,16-0,45)	0,23 (0,17-0,42)	0,23 (0,15-0,47)	0,973
Salivary cortisol D3, ng/dL (median [IQR])	0,18 (0,08-0,37)	0,17 (0,11-0,33)	0,20 (0,08-0,37)	0,625
Salivary cortisol D6, ng/dL (median [IQR])	0,25 (0,15-0,46)	0,27 (0,15-0,37)	0,25 (0,16-0,46)	0,585
Mann Whitney U test D0 = day 0; D3 = 3rd day; D6 = 6th day				

Relationship of Platelet-Lymphocyte Ratio to Post-COVID-19 Syndrome

In table 5, the relationship between platelet-to-lymphocyte ratio and the occurrence of post-COVID-19 syndrome is shown. In this study, there is a significant relationship between platelet-to-lymphocyte ratio and the occurrence of post-COVID-19 syndrome on days 0, 3, and 6 of treatment ($p<0.05$).

Table 5: Relationship of Platelet-Lymphocyte Ratio to Post-COVID-19 Syndrome

Variable	Total	Post-COVID-19 Syndrome		pa Value
		No	Yes	
PLR D0 (median [IQR])	247.53 (208.78-208.78)	208.78 (170.27-222.61)	285.22 (234.38-331.78)	0.005a
PLR D3 (median [IQR])	233.00 (184.30-342.00)	192.50 (151.33-220.48)	278.61 (219.47-396.83)	0.003a
PLR D6 (median [IQR])	179.63 (144.44-311.39)	157.60 (144.44-262.20)	212.44 (157.33-361.22)	0.001a

Mann Whitney U test
PLR = platelet-lymphocyte ratio; D0 = day 0; D3 = 3rd day; D6 = 6th day

4. Discussion

Relationship between Stress Levels and Post-COVID-19 Syndrome Events

According to this study, there was no significant association found between stress levels, assessed using the DASS-21 questionnaire, and post-COVID-19 syndrome. One possible reason for this outcome is that most of the patients included in the study were classified as having normal levels of stress based on the DASS-21. This finding contrasts with previous studies (23–25) which reported that "COVID-19, post-COVID-19 syndrome, and stress are interconnected". It is worth noting that patients with post-COVID-19 syndrome may experience neuropsychological symptoms, including stress, which are not necessarily linked to the severity of COVID-19 (26).

The study found that on day 0 of treatment, only 2 (4.35%) patients had severe stress levels. The first patient with severe stress levels until day 6 of treatment did not develop Post-COVID-19 Syndrome, while the second patient with severe stress levels returned to normal by day 6 of treatment and did develop Post-COVID-19 Syndrome. The underlying mechanism for these two different outcomes remains unclear, which may be partly attributed to the limitations of the self-reported stress level questionnaire (DASS-21). Clinical diagnosis by a doctor could potentially provide more accurate stress levels. A study conducted in Italy reported that COVID-19 survivors experienced psychiatric disorders such as Post-Traumatic Stress Disorder (PTSD), anxiety, and insomnia (27). These diagnoses were made by psychiatrists rather than through questionnaires. It's worth noting that various factors could influence the questionnaire results, such as patient factors like fear or stigma, and provider or doctor factors such as support from healthcare workers or family members, and good doctor management during treatment.

It's important to note that this study did not investigate stressful conditions that may have existed prior to COVID-19 infection. This is noteworthy because earlier research has indicated that patients with pre-existing mental health disorders are more likely to develop post-COVID-19 syndrome (28–33). Additionally, psychological stress has been linked to longer hospital stays (34,35). In the present study, most of the patients (86.9%) were discharged after receiving treatment for six days.

The presence of a gap between the multifactorial pathophysiology of Post-COVID-19 Syndrome and the unknown causes of post-COVID-19 stress makes it important to invest in both areas of disease complexity. The pathophysiology of Post-COVID-19 Syndrome includes ischemia and microvascular injury, immobility, and metabolic changes during critical illness. In contrast, the pathogenesis of psychiatric complications involves immune dysregulation, inflammation, microvascular thrombosis, iatrogenic drug effects, and psychosocial impact of infection (36). Previous studies have shown a relationship between inflammation, immune dysregulation, and stress-related to Post-COVID-19 Syndrome. Evidence indicates that stressed patients have sustained production of proinflammatory cytokines and reactive oxygen species, which can cause Post-COVID-19 Syndrome, and vice versa, although this theory remains controversial (37–39). Other possible explanations include the Hypothalamic-Pituitary-Adrenal axis, autoantibodies, and hypometabolism in the frontal lobes and cerebellum, which may contribute to Post-COVID-19 Syndrome and stress (40–44). In this study, most of the patients did not experience stress, and the sample consisted of COVID-19 patients with mild to moderate symptoms.

Relationship of Salivary Cortisol to Post-COVID-19 Syndrome

The study found no significant relationship between salivary cortisol levels in the Post-COVID-19 Syndrome group and the control group. The salivary cortisol levels in this study on days 0, 3, and 6 were within the normal range and showed no difference between the two groups. These findings are in line

with previous studies that also found no significant difference in cortisol levels between patients with mild-to-moderate COVID-19 who later developed Post-COVID-19 Syndrome and a control group (45). However, stress has been shown to affect cortisol levels in previous research. In this study, the patients had normal stress levels, which may have contributed to the lack of difference in cortisol levels between the two groups. Another study also found that cortisol levels in patients with Post-COVID-19 Syndrome were normal (46). However, it is worth noting that the results of this study may have been influenced by factors related to sample collection, such as the effects of oxygen supplementation and dehydration on salivary cortisol levels, as observed in previous studies that also used salivary cortisol samples (47).

Immune dysfunction has been associated with damage to the hypothalamic-pituitary-adrenal (HPA) gland axis and adrenal gland insufficiency resulting in hypocortisolemia (48). Cortisol, known as the stress hormone, is also involved in inflammation, glycemia, and sleep regulation. Therefore, hypocortisolemia can be associated with difficulty in reducing inflammatory responses. In addition, reactivation of Epstein and herpes viruses has been associated with low levels of cortisol (49). The underlying causes of hypocortisolemia can be multifactorial, such as direct infection of the adrenal gland, resistance of alpha glucocorticoid receptor tissue, secondary adrenal gland insufficiency due to glucocorticoid therapy, and the emergence of anti-adrenocorticotropic hormone (ACTH) hormone antibodies (50). Hypocortisolism is also found in conditions such as chronic fatigue syndrome, encephalomyelitis, or fibromyalgia. Thus, hypocortisolemia may occur due to HPA axis insufficiency after a prolonged period of stress, and this change may help identify patients at risk of post-COVID-19 syndrome (48). However, this theory is still debated as other studies have also found normal cortisol levels in patients with post-COVID-19 syndrome, regardless of the severity of their acute infection.

The measurement of HPA axis activity, which measures stress, can be done non-invasively through the collection of saliva cortisol using the ELISA method (51,52). In this study, stress in research subjects was measured using saliva cortisol. Assessment of stress can also be measured using psychological assessment parameters such as DASS-21, which was used in this study. The HPA axis is a set of systems that have reciprocal influence and interaction between the hypothalamus (in the brain), the pituitary gland (in the brain), and the adrenal gland (above the kidneys). This section is important for the neuroendocrine system to control an individual's response to stress (53). Therefore, saliva cortisol and DASS-21 can represent the activity of the HPA axis.

Cortisol release is pulsatile, influenced by the sleep-wake cycle, and the perception of stress itself (54,55). Under normal conditions, cortisol levels show a clear circadian rhythm, with an increase of around 50-75% during the first 30 minutes after waking up, followed by a sharp decline, which then gradually decreases throughout the rest of the day, reaching a minimum point at night (56). The increase upon waking is genetic, while the variability of the decrease throughout the day is related to environmental influences. In stable physiological conditions, this rhythm varies greatly, as it is influenced by age, sex, developmental stage, or body composition (57). The morning response of saliva cortisol secretion has been suggested as an indicator of chronic stress or depression (58,59). Other cortisol measurements can be taken with variations of cortisol throughout the day that represent negative mood or acute perceived stress at that time (60). In this study, the morning cortisol secretion indicator was taken between 7-9 a.m. with the hope of representing long-term stress and not mood or stress perceived at that time.

There is a lot of evidence showing that psychosocial stress can affect various immune functions through neuroendocrine processes, such as the activation of the HPA axis or the adrenergic system (61). The neuroendocrine system is also influenced by the immune system, including the secretion of antibodies, the function of T helper cells, or the reactivity of macrophages or eosinophils (62). When cortisol is excessively secreted or hypersecreted due to chronic stress, negative effects such as decreased immune system function have been observed (61). In chronic stress, negative feedback can occur, resulting in a decrease in cortisol levels or the return of cortisol to normal levels, but still accompanied by a decrease in immune system function (63). This can then serve as a secondary indicator of chronic stress (64).

In this study, the cortisol levels of the research subjects were still considered normal. Therefore, the research subjects have two possibilities, which are experiencing chronic stress or normal. This is supported by the analysis results using DASS-21, which found that the research subjects were normal or not experiencing stress. This can support the notion that normal cortisol levels occur due to the absence of stress in the research subjects. However, the statement regarding the presence of Post-COVID-19 Syndrome in research subjects with normal cortisol levels and stress supports the statement that the pathophysiology of Post-COVID-19 Syndrome is more related to inflammation than the neuroendocrine system. Prolonged inflammation can cause Post-COVID-19 Syndrome.

This is different from previous findings that post-COVID-19 syndrome can indeed cause psychological problems, such as stress, after three months of observation (65,66). This is attributed to a persistent inflammatory mechanism. This inflammation is evidenced by increased ferritin levels associated with stress levels (67). However, stress during hospitalization was not associated with the occurrence of post-COVID-19 syndrome as found in

this study. This indicates the presence of a complex pathophysiology of post-COVID-19 syndrome involving inflammation. The inflammation also affects the central nervous system (neuroinflammation) which has an impact on psychological conditions, such as stress, and not the other way around.

Relationship of Platelet-Lymphocyte Ratio to Post-COVID-19 Syndrome

This study found a relationship between Platelet-Lymphocyte Ratio (PLR) on day 0, 3, and 6 between the Post-COVID Syndrome group and the control group. The study found that PLR values were higher in patients with Post-COVID Syndrome compared to the control group. In this study, PLR on day 0, 3, and 6 were 285.22 (234.38-331.78), 278.61 (219.47-396.83), and 212.44 (157.33-361.22), respectively. These values are consistent with previous studies (68). Clinically, these values are higher than normal values (36.63-172.68) (69). This is caused by a decrease in lymphocytes or lymphopenia. However, as the healing process occurs, the production of proinflammatory cytokines and lymphocytes will slowly return to normal levels (Liu et al., 2020). This explains the decrease in PLR values or a return to normal levels. Retrospective studies have shown no significant differences in lymphocyte, platelet, and platelet-lymphocyte ratio levels in patients with Post-COVID Syndrome at 6 and 12 months of observation (70).

A decrease in lymphocytes can be explained by the pathophysiology of Sars-CoV-2 viral infection. When Sars-CoV-2 enters the respiratory system, the host's immune system responds. First, the spike protein-S binds to the ACE2 receptor. Then the innate immune system responds to the invasion as a form of protection against the virus. This response involves cytolytic activity through type I interferon (IFN) and natural killer cells (NK-cells). This is important for destroying virus-infected cells and producing antibody-producing cells that specifically target Sars-CoV-2. In addition, during COVID-19 infection, there is systemic inflammation or Systemic Inflammatory Response Syndrome (SIRS). This inflammation then causes a cytokine storm and leads to lymphopenia. The decrease in lymphocytes can be caused by increased lymphocyte apoptosis and inflammatory markers such as TNF alpha and IL-6 (71). In this study, the median lymphocyte level in patients with mild-to-moderate COVID-19 was 1085.00 (850.00-1380.00), 1275.00 (1060.00-1970.00), and 1255.00 (1080.00-1950.00) on days 0, 3, and 6, respectively. These values are consistent with previous studies that showed lower lymphocyte levels in severe COVID-19 patients, with an average (SD) of 510 (65). Based on this, low lymphocyte levels are associated with the occurrence of Post-COVID-19 Syndrome. The findings of this study also suggest that the decreased lymphocyte levels at admission in patients with mild-to-moderate COVID-19 are associated with Post-COVID-19 Syndrome 12 days later.

In addition to the decreased lymphocyte count, the platelet count also affects the PLR value. Research has shown that platelet count decreases significantly in COVID-19 patients, especially those who are critically ill (72). This is consistent with the findings of this study, which showed that platelet count was within normal limits in both the post-COVID-19 syndrome group and the control group. The mechanism of thrombocytopenia in COVID-19 patients is multifactorial and refers to a combination of viral infection and the use of mechanical ventilators, which can cause endothelial damage and trigger activation, aggregation, and microvascular thrombosis in the lungs, leading to high consumption of platelets (73). Thrombocytopenia can also be caused by platelet consumption due to COVID-19, given the similarity of the mechanism of thrombocytopenia in COVID-19 and SARS. In addition, coronavirus can infect the bone marrow and suppress hematopoiesis or trigger an autoimmune response against blood cells (73,74). Increased consumption and decreased production of platelets due to cytokine storm have also been observed in COVID-19 patients.

Research related to the relationship between PLR and post-COVID-19 syndrome is still limited. PLR has been known as a marker for predicting mortality and severity in COVID-19 patients (Paul Simon et al., 2022). The sample in this study consisted of mild to moderate COVID-19 patients, so the mortality rate was low. Compared to the control group, the post-COVID-19 syndrome group in this study had higher PLR values.

PLR is known as an inflammation marker and is calculated by dividing the platelet count by the lymphocyte count. Previously, PLR has been used in various diseases, such as cardiovascular disease and autoimmune disease, as a predictor of inflammation and thrombocyte mortality with lymphocyte count (75,76). In previous studies, PLR was associated with the inflammation process, C-reactive protein (CRP), and Erythrocyte Sedimentation Rate (ESR) in COVID-19 patients (77). Additionally, the PLR value in this study was higher compared to the cut-off value of PLR as an inflammation marker, which is 180 with a specificity of 44% and sensitivity of 77% (77). This may indicate the presence of inflammation processes in patients with post-COVID-19 syndrome, but further testing is needed. Furthermore, further research is needed to determine the clinically and statistically significant cut-off value.

This study also found an increase in PLR in patients with mild-moderate degrees of COVID-19. This supports the findings of previous research that there is no relationship between PLR and the degree of COVID-19 (78). This is the reason why the subjects of this study still have a high PLR, even though the degree of COVID-19 is mild-moderate. This also opens up opportunities for the prognostic value of PLR in the incidence of post-COVID-19 syndromes. In COVID-19 patients, mild-moderate degrees with a

high PLR value have a relationship with the incidence of post-COVID-19 syndrome 12 days later. Research related to the risk of post-COVID-19 syndrome and high PLR values in mild-moderate COVID-19 patients can be carried out in the future.

The benefits of PLR can be applied in clinical practice because it is known to be associated with post-COVID-19 syndromes. First, PLR is an inflammatory marker, that is, a high PLR value indicates a high inflammatory process. This supports the role of inflammation in the pathophysiology of the post-COVID-19 syndrome. Second, the role of the immune system represented by PLR also plays a role in the pathophysiology of the post-COVID-19 syndrome, namely a decrease in lymphocytes. PLR has been known to be sensitive to represent the innate and acquired immune systems (79). Third, PLR is an indicator that is cheap, affordable, and available. This is useful if implemented in Indonesia, where facilities are still limited. Fourth, this study examined PLR at three times, and showed similar results. It can be concluded that evaluating PLR at one time, for example PLR when admitted to the hospital or ICU is sufficient to describe the incidence of post-COVID-19 syndrome in the future.

5. Conclusion

In conclusion, our study aimed to investigate the relationship between stress levels, salivary cortisol levels, and Platelet-Lymphocyte Ratio (PLR) with the incidence of post-COVID-19 syndrome. Based on our statistical analysis and discussion, we found the following: Firstly, we found no significant relationship between stress levels and post-COVID-19 syndrome. This suggests that stress may not be a significant factor in the development of post-COVID-19 syndrome. Secondly, we found no significant relationship between salivary cortisol levels and the incidence of post-COVID-19 syndrome. This suggests that cortisol levels may not be a significant predictor of post-COVID-19 syndrome. Finally, we found a significant relationship between PLR and the incidence of post-COVID-19 syndrome. This finding supports the hypothesis that PLR may be a useful biomarker for predicting the development of post-COVID-19 syndrome.

Conflict of Interest:

The authors hereby declare that there is no conflict of interest in this study.

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Author Contribution

A) Mahisa Pribadi Brahmana -contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B) Anna Surgean Veterini -contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

C) Edward Kusuma -contributed in study design, guiding the research work, proofreading and manuscript correction.

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