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Comparison between Vitamn D Level of Asymptomatic Confirmed Covid-19 Patients with Symptomatic Confirmed Covid-19 Patients in Makassar

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Abstract

Vitamin D shows an important role in immune function. However,there is still little analysis regarding the role of vitamin D inpreventing infection and death fromCOVID-19.This researchaimed todetermine vitamin D levels inpatients with confirmed COVID-19. A cross-sectional study was conducted. Researcher collected blood fromthemedian cubital vein of COVID-19 patients and examined the vitamin D levels in patients using the ELISA method.This research showed a comparison of serum vitaminD based on clinical manifestations of COVID-19 patients in all subjects. In20 patients with symptomatic COVID-19 clinical manifestations there were 17 patients (85 %) with vitamin Ddeficiency, 3 patients (15 %) with vitamin D insufficiency; To the contrary, there were noasymptomatic COVID-19 patientswith vitamin D deficiency, 18 patients with vitamin D insufficiency, and 4 patients with normal vitamin D levels.There was a significant relationship between the clinicalmanifestations of patients with COVID-19 and the patient's serum vitamin D levels where asymptomatic patientshad higher levels than that of symptomatic patients (p-value with the Chi-square test was 0.000 with p <0.001).In addition, there was a significant difference between vitamin D levels in asymptomatic and symptomatic patients whereserum vitamin D levels were obtained to be higher in the asymptomatic patient group than in thesymptomaticgroup.

Keywords: Vitamin D, COVID-19, asymptomatic COVID-19, symptomatic COVID-19

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1. Introduction

Coronavirus is an RNA virus with a particle size of 120-160 nm. This virussubstantially infects animals, including bats and camels. Beforethe occurance of the Corona virus disease-19 (COVID-19) outbreak, there are 6 types of coronavirus have been identified that caninfect humans, namely Alpha-coronavirus 229E, Alpha-coronavirus NL63, Beta-coronavirusOC43, Beta-coronavirus HKU1, Severe Acute Respiratory Illness Coronavirus (SARS-CoV),and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The Coronavirusbeing the etiology of COVID-19 belongs to the Beta-coronavirus genus (Riedel et al., 2019; Zhuet al., 2020).

WHO declared that COVID-19 is caused by a Severe Acute Respiratory virusIllness Coronavirus 2 (SARS-CoV-2) as a worldwidepandemic.As of on January 23, 2021, Indonesia has confirmed as many as 977 thousand cases along with 27,664 deaths and791,000 recoveries recorded thus far. From the data collected by COVID-19 Response Acceleration Task Force in Indonesia, South Sulawesi is still in 4provinces with the highest number of COVID-19 cases in Indonesia in whichthe number of cases inMakassar is 44 thousand cases as of January 23, 2021. It is estimated that the number of cases will beincreasing even though there is still little analysis of covid treatment and handling thus far. Therefore, we must be able to describe theprotective factors of anti-infective agents which may protect against infection andfactors which increase the cure rate after infection occurs (SulselTanggapCOVID-19, 2020).

The most common symptoms of COVID-19 are fever,malaise and dry cough.Some patients may experience muscle or body aches,congestion nose, flu, headache,conjunctivitis, sore throat, diarrhoea, loss of taste or smell, or a rash on skin. In addition, serious symptoms such as acute respiratory distress syndrome (ARDS), sepsis and septic shock,multi-organ failure, including kidney failure or acute heart failure to death. Moreover,some studies show that SARS-CoV-2 can attack the peripheral nerves, spreadalong the nerve synapses, and enter into the central nervous system (CNS) such as one of the olfactory nervesin the nasal cavity resulting in symptoms of anosmia (WorldHealth Organization, 2020; Zubair et al., 2020).

The difference in the number and clinical manifestations of COVID-19 in the world is crucial to figure outthe causes of these differences. The enhancement of immunity through better nutritional intake probablyis the determining factor. Nutrient such as vitamin D showsthe prominent role in immune function. However,reearch about the role of vitamin D in preventing infection and death in COVID-19 is too little (Ali, 2020)

A recent review also stated thatthe Vitamin D has a role in decreasing risk COVID-19 infection and mortality, and people who are at risk of exposure influenza and COVID-19 are recommended to consume 10,000 IU / day of vitamin D3 for several weeks to increase the concentration of 25-hydroxy vitamin D (25 (OH) D) quickly, followed by 5,000 IU / day. The aim is to improve 25 (OH) D concentrations above 40–60 ng / mL (100–150 nmol / L). Furthermore, for the treatment of infected peoplewith COVID-19, higher dose of vitamin D3 may be useful (William et al., 2020). In a systematic review and meta-analysis of 25 randomized controlled studies, Martineau et al. described that vitamin D may protect against tract infectionrespiration (Martineau et al., 2017).

In a literature review, regarding the possible role of vitamin D in the prevention of Influenza virus infection, Gruber-Bzura (2018), noted that the data was still controversial.

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Calcitriol (1.25-dihydroxyvitamin D3) provided a significant effecton the Angiotensin converting enzyme 2 (ACE-2) axises by decreasing expression ACE-2. Itwas the host cell receptor which was responsible for infection by SARSCoV-2. This could lead to a higher risk of infection (Cui et al., 2019).

Evaluation of vitamin D supplementation as an adjuvant therapy is meaningful both clinically andeconomically in the management of COVID-19. Empirical intervention with vitamin D isa clinically justifiable decision to increase serum vitamin D levelsand reduce the risk of disorders of the immune system. Increased level 25 (OH) D inbloodmay retard disease progression or evenimprove patient survival (Ebadi& Montana, 2020).

In relation to above background, this research aims to assessvitamin D levels in sufferers of COVID-19 especially in Makassar and to compare vitamin D levelsofsymptomatic with asymptomatic Covid-19 paients.

2. Materials And Methods

2.1 Location and Time of Research

The research was conducted in the Covid-19 treatment room at Pelamonia Hospital and the Swiss Bellhotel Makassar from November 2020 to December 2020.

2.2 Research Design and Variable

This research was a cross sectional study. Research variable consisted of: independent variables(symptomatic and asymptomatic Covid-19 patients), dependent variables (Vitamin D), dan confounding variables (age, skin color, exposure, sunlight, and obesity).

2.3 Population and Sample

The population for this reaseach was patients with confirmed Covid-19 through nasaopharyngeal swab examination, both confirmed asymptomatic and symptomatic Covid-19 and treated in the Covid-19 treatment room at Pelamonia Hospital and Swiss Bellhotel Makassar. Samples were all Covid-19 patients who were confirmed positivethrough nasaopharyngeal swab examination, both asymptomatic and symptomatic, who were treated in the Codiv-19 treatment room at Pelamonia Hospital and Swiss Bellhotel Makassar who met the inclusion criteria and were willing to take part in the reseach.

2.4 Method of collecting data

Completing the questionnaire form that had been provided in the form of anamnesis, physical examination and additional examinations according to the attached list. Then the data were compiled, inputted and analyzed by using SPSS software.

2.5 Data Analysis Technique

The obtained data was recorded in a special form, processed, and the results were displayed in a narrative, table, or graphic form. Afterwards the statical test was carried out for

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descriptive analysis and hypothesis testing. This research was analyzed based on the objectives and measuring scale.

3. Results

A cross sectional study had been conducted to assess Vitamin D levels in Covid-19 sufferers especially in Makassar and to compare Vitamin D levels ofconfirmed asymptomatic Covid-19 patients with symptomatic Covid-19 patients. The research was conducted in the Covid-19 treatment room at Pelamonia Hospital and Swiss Bellhotel Makassar fromNovember 2020 to December 2020. The research sample consisted of 42 patients in which there were 20 Covid-19 symtomatic patients and 22 asymptomatic Covid-19 patients.

Covid-19 patients who experienced Vitamin D deficiency weregenerally found in the 21 – 30 year age group (97 patients). While patients with Vitamin D insufficiency commonly experienced in the age group of 41 – 50 years (7 patients), and patients with normal Vitamin D levels were mostly found in the age group of >50 years (2 patients); female patients (10 patients) were mostly obtained to be deficient in Vitamin D. Whereas male patients tended to experience Votamin D insufficiency (16 patients) and the group with normal Vitamin D levels (4 patients); Patients with deficiency (7 patients) and insufficiency 912 patients) of Vitamin D were mostly found in the undergraduates, while 4 patients with normal Vitamin D levels were found in the high school group; Patients with defeciency (14 patients) and insufficiency (14 patients) of Vitamin D were more common in the Indoor workers than in the Outdoor workers wih normal vitamin D levels (4 patients); Based on ethnicity, Buginese patients were more likely to have defecient, insuffucuent, or normal Vitamin D levels compared to other ethnic groups. Overall, there was a significant correlation between gender, type of work andserum Vitamin Dlevels of Covid-19 patients where the *p*-values were 0.024 and 0.007 ($p<0.05$). Meanwhile,among age, education, ethnicit and serum Vitamin Dlevels of Covid-19 patients, there was no significant correlation obtained where tha *p*-vallue is 0.000 ($p>0.05$). (Appendix, Table 1).

The comparison of serum Vitamin D levels by gender of COVID-19 patients in all subjects. The patients' serum Vitamin D levels experienced a significant difference in which male patients possessed higher serum vitamin D levels than female patients ($p<0.05$) (appendix, Table 2).

The comparison of serum Vitamin D levels by COVID-19 patients' occupation in all subjects. Patients' serum Vitamin D levels experienced a significant difference where patients who worked outdoors possessed higher serum Vitamin D levels than patients who worked indoors ($p<0.05$) (Appendix, Table 3).

The comparison of serum Vitamin D levels based on the clinical manifestations of Covid-19 patients in all subjects. The patients' serum Vitamin D levels experienced a significant difference where asymptomatic patients had higher levels than symptomatic patients ($p<0.001$) (Appendix, Table 4).

Regarding clinical manifestations of patients with of confirmedsymptomatic Covid-19, there were17 patients (%) with vitamin D deficiency, 3 patients (%) with vitamin D insufficiency, and there were no patients with normal serum vitamin D levels; Conversely regarding patients with asymptomatic Covid-19, there were not obtained patients with Vitamin D defeciency, but 18 patients with vitamin D insufficiency, and 4 patients with normal serum

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vitamin D levels. In summary, There was a significant correlation between the patient's clinical manifestations confirmed Covid-19 and the patient's serum Vitamin D levels where *p*-value and Chi-square test were 0.000 ($p<0.001$) (Appendix, Table 5).

4. Discussion

This research presents that there was a significant relationship between the clinical manifestations of patients with COVID-19 and the patient's serum vitamin D levels where asymptomatic patients had higher levels than symptomatic patients.

Regarding the results of this research, thedecrease in vitamin D levels did not have a significant relationship with age. However, based on the percentage of deficiency and insufficiency of vitamin D was more often found at an older age. This research is in line with research by Carpagno et al (2020), where vitamin D levels were lower at an older age (> 60 years) and a comorbid COVID-19 infection. The insignificant results were thought to be due to many confounding factors in measuring vitamin D levels of COVID-19 patients such as consumption of foods containing vitamin D, daily sun exposure, race and ethnicity as well as the severity of COVID-19 infection.

In this study, a significant relationship was found between gender and vitamin D levels where serum vitamin D was consistently lower in women than men. This research is in accord with research conducted by Horani et al (2016), where serum vitamin D in women were lower than men. The number of women experiencing vitamin D deficiency was 15.69%, while for men it was only 7.41%. In addition, they also examined the differences in vitamin D levels in women with Hijab and without Hijab. It was found that vitamin D levels in women who wore hijab were lower than those without Hijab.

According to Nimitipong (2013), the high prevalence of vitamin D deficiency in Southeast Asian women can be caused by several factors. Indonesia is on the equator, the amount of sunlight reaching the earth's surface is reduced due to air pollution in big cities that absorbs UVB rays. In addition, many women in Indonesia cover their entire bodies with Hijab so that their skin is only slightly exposed to the sun. Unusual sunbathing behaviors such as frequent shade and avoidance of sun exposure have an impact on decreasing vitamin D levels in the body.Based on the journal written by Mercola et al (2020), decreased vitamin D can worsen the clinical condition of COVID-19.

Based on the results of this research, there was a significant correlation between the work of COVID-19 patients and serum vitamin D levels, where indoor workers had lower serum vitamin D levels than outdoor workers. Moreover, The same research was also obtained by Sowah et al (2017), where the decreased levels of vitamin D (25- (OH) D <50 nmol / L) of indoor workers (78%) compared to outdoor workers (48%). The factor that leads vitamin D deficiency in indoor workers is lack of sun exposure (UVB). Insufficient sun exposure is caused by a lack of outdoor activity or working indoors for a long period of time, a lifestyle that tends to avoid sunlight, the use of clothing that is difficult to absorb sunlight or the habit of wearing long clothing, the use of body protection such as hats, umbrellas, sunscreen / sunblock which can cause a decrease in vitamin D levels. The use of this body armor causes less sun exposure. Furthermore, the use of sunscreens such as p-aminobenzoic acid inhibits the absorption of the spectrum of sunlight which is useful for the synthesis of vitamin D in the skin (UVB) and causes the mean serum value of 25 (OH) D in lesser chronic sunscreen users. In addition, the use of

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chronic sunscreens can cause vitamin D deficiency because using sunscreens with SPF 8 reduces vitamin D production in the skin by up to 93% and increases to 99% when using sunscreens with SPF (Whitney & Rofles, 2011).

In this research, it was found that there was a significant relationship between the clinical outcome of COVID19 patients and serum vitamin D levels. The results of the study are in accord with research conducted by Ye et al (2020) which revealed that COVID-19 patients with a serum concentration of 25 (OH) D below 41.19 nmol / L tended to be more severe Covid-19 infection and clinical outcome with more severe symptoms. Moreover, another research from Wuhan concluded that patients admitted to the ICU had significantly higher neutrophils and lower lymphocytes than patients who were not admitted to the ICU and the number of immune cells was closely related to case severity and mortality (Huang et al, 2020).

In this research, there was a significant difference in vitamin D levels of asymptomatic and symptomatic COVID-19 patients. This analysis managed to identify that 85% of the patients with symptoms had Vitamin D deficiency and no vitamin D deficiency was found in asymptomatic patients. This is in accordance with research conducted by Alipio (2020), where the majority of normal vitamin D levels were found in patients with mild symptoms, vitamin D insufficiency status was found in the majority of patients who had moderate symptoms and vitamin D deficiency was found in patients with severe symptoms. Other researches have also reported that normal vitamin D levels are mostly found in asymptomatic COVID-19 patients (without symptoms) and vitamin D deficiency is majority in patients with severe symptoms (Jain et al., 2020).

This research is also compatible with a retrospective cohort study in Indonesia, with a sample of 780 COVID-19 patients who examined the interconnection of vitamin D status and mortality of COVID-19 patients. After ruling out confounding factors such as age, sex, and comorbidities; To be concluded that Vitamin D status is closely related to mortality of Covid-19 oatients. The mortality rate was found to be higher in patients with vitamin D deficiency. When compared with COVID-19 patients with normal vitamin D status, the risk of death increased by 10.12 times in COVID-19 patients with vitamin D deficiency (Raharusun et al., 2020).

The significant difference of vitamin D levels in asymptomatic and symptomatic patients in this research is expectedt to be because vitamin D has acted in modulating the immune system by inhibiting the release of pro-inflammatory cytokines and increasing anti-inflammatory cytokines. Vitamin D holds on the expression of inflammatory cytokines such as IL-1α, IL-1β, tumor necrosis factor-α through decreased MAP kinase activation and decreased vitamin D levels are linked to overexpression of the T1 cytokine. The key pathogenic mechanism leading to severe symptoms of Sars Cov-2 infection and complications is the hyperinflammatory state (cytokine storm) that occurs during the first week of symptoms. These cytokine storms can lead to severe COVID-19 complications, such as ARDS, myocarditis, and acute heart and kidney failure leading to increased mortality, especially in the elderly or patients with previous cardiovascular comorbidities (Tay et al., 2020; Ahn et al., 2020). 1,25-dihydroxyvitamin D3 is capable of increasing the induction of regulatory T cells which function to inhibit the production of pro-inflammatory cytokines. Therefore, patients with severe symptoms will experience a decrease in vitamin D levels in the body as obtained in this analysis (Tramontana et al., 2020).

The limitation of this sresearch is that there is no analysis of confounding factors such as daily consumption of vitamin D from food or supplementation, daily behavior or activities of

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COVID-19 patients which can cause deficiency of serum vitamin D levels, sun exposure, and use of sunscreen or SPF. On the other hand, patient follow-up was not performed to observe the prognosis of each patient group to determine the relationship between vitamin D levels and disease prognosis. Thus further researcheswith more samples should be conducted with the aim of determining the correlation of vitamin D levels with the patient's clinical outcome which can be assessed objectively so that it can be analyzed through numerical regression analysis.

5. Conclusion and Suggestion

Researchers concluded that there was a significant difference between levels vitamin D of asymptomatic and symptomatic patients where serum vitamin D levels were obtained to be higher in the asymptomatic patients than that of in the symptomatic group. Researchers suggest that research be carried out further analysis regarding the confounding factors such as daily consumption of vitamin D from food and supplementation, behavior or daily activities of COVID-19 patients which can cause deficiency of serum vitamin D levels. Further researches with many samples to determine the cut-off point of Vitamin D level for each symptom level of Covid-19. To find out the clinical development of COVID-19 patients, it is expected that checking vitamin D levels is a routine check for every patient diagnosed with COVID-19.

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APPENDIX

Table 1. Characteristic Relationship with Serum Vitamin D Levels in COVID-19 Patients

Variabel			VitaminD			Total	p
			Deficiency	insufficiency	Normal		
Age	<20	n (%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	4 (100.0%)	*0.094
	21-30	n (%)	7 (77.8%)	1 (11.1%)	1 (11.1%)	9 (100.0%)	
	31-40	n (%)	2 (25.0%)	6 (75.0%)	0 (0.0%)	8 (100.0%)	
	41-50	n (%)	4 (36.4%)	7 (63.6%)	0 (0.0%)	11(100.0%)	
	>50	n (%)	2 (20.0%)	6 (60.0%)	2 (20.0%)	10(100.0%)	
Gender	Laki-laki	n (%)	7 (25.9%)	16 (59.3%)	4 (14.8%)	27(100.0%)	***0.024**
	perempuan	n (%)	10 (66.7%)	5 (33.3%)	0 (0.0%)	15(100.0%)	
Education	SD	n (%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (100.0%)	*0.205
	SMP	n (%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	
	SMA	n (%)	6 (35.3%)	7 (41.2%)	4 (23.5%)	17(100.0%)	
	Diploma	n (%)	3 (75.0%)	1 (25.0%)	0 (0.0%)	4 (100.0%)	
	Sarjana	n (%)	7 (36.8%)	12 (63.2%)	0 (0.0%)	19(100.0%)	
Occupation	Indoor	n (%)	14 (50.0%)	14 (50.0%)	0 (0.0%)	28(100.0%)	***0.007**
	Outdoor	n (%)	3 (21.4%)	7 (50.0%)	4 (28.6%)	14(100.0%)	
Ethics	Ambon	n (%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	2 (100.0%)	*0.938
	Bugis	n (%)	15 (40.5%)	18 (48.6%)	4 (10.8%)	37(100.0%)	
	Jawa	n (%)	1 (33.3%)	2 (66.7%)	0 (0.0%)	3 (100.0%)	

*Chi-square test

Table 2. The Comparison of Vitamin D Serum Levels Based on Gender of COVID-19 Patients

	Male (n=27)	Female (n=15)	P

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Serum Vitamin D levels	23.58 ± 6.08	16.75 ± 5.27	*0.001

*Independent-T test

Table 3. The Comparison of Vitamin D Serum Levels Based on the Occupation of COVID-19 Patients

	Indoor (n=28)	Outdoor (n=14)	p
Serum Vitamin D levels	18.92 ± 5.64	25.58 ± 6.35	*0.001

*Independent-T test

Table 4. The Comparison of Serum Vitamin D Levels Based on Clinical Manifestations of COVID-Patients

	Symptomatic (n=20)	Asymptomatic (n=22)	p
Serum Vitamin D levels	16.22 ± 4.81	25.61 ± 4.55	*0.000

*Mann-WhitneyTest

Table 5. The Relationship between Clinical Manifestations of COVID-19 Patients and Serum Vitamin D Levels

Clinical Manifestation of COVID-19 patients		Vitamin D serum			Total	* p
		Defisiensi	Insufusiensi	Normal		
Symptomatic	n	17	3	0	22	0.000
	%	85.0%	15.0%	0.0%	100.0%	
Asymptomatic	n	0	18	4	20	
	%	0.0%	81.8%	18.2%	100.0%	
Total	n	17	21	4	42	
	%	40.5%	50.0%	9.5%	100.0	

*Chi-square test