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Relationship between Vitamin D Levels and Platelet Function in the Elderly Patients with Coronary Heart Disease

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ABSTRACT

Introduction: Coronary Heart Disease (CHD) is a degenerative disease caused by fat accumulation and chronic inflammation. The elderly have a significant CHD risk due to the prevalence of vitamin D insufficiency and decreased platelet function, influencing the inflammatory response in this population. Therefore, this study aims to assess the relationship between vitamin D levels and platelet function measured by the mean platelet volume (MPV) and P-Selectin serum levels of CHD patients.

Method: The cross-sectional design was carried out on stable CHD patients aged 60-75. Meanwhile, vitamin D, MPV, and P-Selectin were taken through venous blood, and an ELISA examination was performed to determine their levels in serum. A correlation test using the Pearson product-moment was also conducted between vitamin D and platelet function.

Result: The subjects involved were 45 male and 16 female at 73.8% and 23.2%, respectively. The highest risk factor was dyslipidemia found in 45 (73.8%) and all subjects (100%) received statins. This study showed no correlation between vitamin D levels and MPV levels (p= 0.094) and P-Selectin (p=0.362).

Conclusion: Vitamin D levels are not associated with platelet function levels (MPV and P-Selectin) in elderly patients with CHD.

Keywords: inflammation, MPV, P-Selectin, vitamin D.
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INTRODUCTION

The elderly population is increasing in line with the rise in life expectancy. According to the World Health Organization (WHO), by 2050, it will increase to 33% of the world's population,[1] meaning an increase in degenerative coronary heart disease (CHD).[2] Atherosclerosis is the primary mechanism of CHD because fat accumulation with chronic and progressive inflammation will cause vascular endothelial dysfunction.[3,4] The risk factors include hypertension, diabetes mellitus, dyslipidemia, obesity, smoking, and vitamin D deficiency.[5,6] Pro-inflammatory cytokines will be activated with these risk factors, including TNF-α and IL-6.[7]

Platelets are one of the cells that play a role in hemostasis mechanisms to repair injuries to the endothelium of blood vessels. In intact and inactive endothelium, platelets will not be activated or attached.[8] Inflammation in the atherosclerotic process will cause the activation of various prothrombotic and pro-inflammatory mediators in the circulation or attached to the endothelium. This will cause P-selectin as an adhesion molecule to be expressed and increase platelet activity.[9]

The mean platelet volume (MPV) is one of the markers of platelet function and an indicator of its activity.[10] An increase in MPV levels indicates a larger and more reactive platelet size, leading to a poorer outcome in CHD patients. This is due to faster thrombus formation, increased platelet aggregation, and expression of adhesion molecules.[11]

Vitamin D is known for its influence on calcium metabolism, and bone also plays a role in atherothrombosis and causes CHD. Low vitamin D levels can affect the balance of pro-inflammatory cytokines and cause impaired platelet function.[12] The mechanism is by inhibiting the inflammatory response in various pathways, primarily by suppressing the NF-B pathway and reducing cytokine levels in CHD.[13]

Previous studies on vitamin D, platelet function, and CHD found that high platelet reactivity is associated with atherosclerosis severity.[14] Vitamin D is also related to the platelet aggregation level and can be reduced in the blood.[15,16] According to observations, no studies determined the relationship between vitamin D levels and platelet function in CHD patients. Therefore, this study aims to determine the relationship between vitamin D levels and platelet function measured by MPV and P-Selectin serum of the elderly CHD patients. It also provides additional knowledge and more comprehensive management of patients.

METHODS

Study design

This cross-sectional design was performed on 61 stable CHD patients aged 60-75, subjected to a percutaneous choreonergic

intervention procedure with stent placement (IKP-Stent) at the cardiac catheterization section of Dr. Kariadi Hospital, Semarang. The inclusion criteria used for the elderly were in accordance with the WHO, namely 60-75 years. Furthermore, the consecutive sampling method was conducted from February 2021 to 2022. The exclusion criteria included patients with grade IV-V chronic renal failure, acute heart failure, a history of heart bypass, a fever or active infection, acute stroke, valvular heart disease, autoimmune disease, acute bleeding or coagulation disorders, history of malignancy, chronic liver disease, long-term glucocorticoid therapy (>1 month), history of gastric bypass, and malnutrition.

The aims and procedures were explained directly and in writing to each subject. Furthermore, the subjects have agreed to participate by signing the informed consent. This study has received ethical clearance with code number 669/EC/KEPK-RSKD/2020 from the Health Research Ethics Committee, Dr. Kariadi Hospital, Semarang.

Vitamin D and Platelet Function

The samples were taken from the patient's venous blood, and the levels of vitamin D, TNF- α, and IL-6 were checked by the ELISA method. The results were read with a microplate reader ELX800 (Bio-Tech Instrument Inc) at a wavelength of 400 nm in the GAKI laboratory, Faculty of Medicine, Diponegoro University.

Statistical Analysis

The data was processed and analyzed using IBM SPSS version 26 software for windows. The results of vitamin D, MPV, and P-Selectin levels were presented in numerical data. Univariate analysis was conducted to describe the characteristics of the subjects in descriptive statistics and presented in tabular form. Using the Pearson Product-Moment test, a bivariate analysis was conducted to determine the relationship between the independent and dependent variables. Previously, the data normality test was carried out with the Kolmogorov Smirnov test, and the distribution of both independent and dependent variables is normal.

RESULTS

Based on the results of the univariate analysis, 61 subjects fulfilled the inclusion criteria, consisting of 45 (73.8%) males and 16 (23.2%) females with a median age of 64 years. The highest risk factors were dyslipidemia and hypertension among 45 (73.8%) and 43 (68.9%) people, respectively. Most of the drug use was statin found in all study subjects, namely 61 (100%), followed by aspirin, ACE-I, and ARB with 57 (93.4%), 31 (50.8), and 29 (47.5), respectively.

Vitamin D levels of the subjects were in insufficiency status with a median of 21.10 ng/mL. Platelet function levels were assessed, namely MPV and P-selectin

Table 1. Subject characteristics.

Variable name	Value
Gender, n (%)	
Male	45 (73,8)
Female	16 (23,2)
Age, median (RIK)	64 (60-75)
Obesity, n (%)	
Yes	28 (45,9)
No	33 (54,1)
Diabetes	
Yes	25 (41,0)
No	36 (59,0)
Hypertension	
Yes	42 (68,9)
No	19 (31,1)
Dyslipidemia	
Yes	45 (73,8)
No	16 (26,2)
Smoking	
Smoker	11 (18,0)
Former smoker	23 (37,7)
Do not smoke	27 (44,3)
LVEF, median (rik)	59 (33-84)
ACE-I, n (%)	
Yes	31 (50,8)
No	30 (49,2)
ARB	
Yes	29 (47,5)
No	32 (52,5)
CCB	
Yes	20 (32,8)
No	42 (67,2)
Statin	
Yes	61 (100,0)
Aspirin	
Yes	57 (93,4)
No	4 (6,6)
Clopidogrel	
Yes	26 (42,6)
No	35 (57,4)
Ticagregol	
Yes	14 (23,0)
No	47 (77,0)
Vitamin D (ng/ml), median (RIK)	21,10 (11,30-30,40)
MPV (fl), median (RIK)	9,90 (8,40-12,70)
P-Selectin (pg/ml), median (RIK)	32,00 (18,00-49,00)

Table 2. Correlation of Vitamin D with MPV.

Variable	Mean±SD	p
Vitamin D	20,81±5,25	<0,094
MPV	9,99±0,89	

Table 3. Correlation of Vitamin D with P-Selectin.

Variable	Mean±SD	p
Vitamin D	20,81±5,25	<0,362
P-Selectin	32,07±7,38	

with median of 9.90 fL and 32.00 pg/mL, respectively, (Table 1).

The results of bivariate analysis (Tables 2 and 3) with the Pearson Product-Moment test on a 95% confidence interval showed no correlation for both between vitamin D and MPV levels (p = 0.094) as well as vitamin D and P-Selectin levels (p=0,362).

DISCUSSION

CHD is one of the diseases with high morbidity and mortality, and the incidence is expected to increase annually. Therefore, further study and understanding of risk factors, diagnosis, prevention, and management are needed. According to epidemiological data, males suffer from CHD more often as the classic risk factor. In line with this study, 45 (73.8%) subjects were male, and age is also a risk factor for CHD. The mean patients' age was 64 years with a range between 60-75 years according to WHO criteria.[17]

Vitamin D deficiency occurs in all countries, specifically in the elderly. Deficiency occurs because, with aging, the concentration of 7-dehydrocholesterol in the skin decreases, adiposity, lack of nutrient intake, and less time exposed to the sun decrease vitamin D production.[18] In line with this study, all subjects involved experienced vitamin D insufficiency.

The hemostatic response to vascular damage is a complex process and involves many mechanisms, including platelet activation.[8] Platelet function can be assessed using MPV and P-Selectin levels. High MPV levels are associated with larger platelet sizes, having more granules, aggregating more quickly, and expressing more receptors.[19] P-Selectin in the thrombosis process activates neutrophils and monocytes' attachment to the vascular endothelial surface and the platelets' interaction with these cells. Furthermore, increased secretion of P-Selectin reflects platelet activation.[20]

Vitamin D has a close relationship with platelet activity and influences the pathological mechanism of CHD. For its role in inflammatory mechanisms, it has receptors found on platelets. This affects the platelets' activity in the formation and development of atherosclerosis and the atherothrombosis incidence in CHD proven *in vivo* and *in vitro*.[21] Moreover, vitamin D's anti-inflammatory effect can suppress pro-inflammatory cytokines such as TNF-α and IL-6 to inhibit megakaryopoiesis and oxidative stress. These processes affect the MPV because the megakaryopoiesis stimulation can lead to the release of young and larger-volume platelets.[22] An inverse relationship between MPV and vitamin D levels has been shown in various studies. Cure et al. reported that 438 subjects were found to have low vitamin D levels, therefore, MPV levels were higher.[23] The study conducted by Park et al. also showed a significant relationship between vitamin D levels and MPV.[24]

In the state of vitamin D deficiency, platelet activation and the P-Selectin expression increase.[20] In a study conducted by Lindqvist et al. in Sweden on 29,000 subjects, there is a "*D-lightful*" theory in which the thrombosis risk increases by 50% during winter compared to other seasons. The study concluded that sufficient exposure to sunlight (ultraviolet B radiation) could reduce the venous thrombosis risk by producing vitamin D, increasing levels of anti-inflammatory and antithrombotic cytokines, and increasing anticoagulant properties.[25]

There was no correlation between vitamin D levels with MPV (p = 0.094) and P-Selectin (p = 0.362) in line with Kucukay MB et al. The vitamin D replacement on platelet counts shows similar results, where the count and MPV were significantly lower in subjects after the administration. Even though there was no difference between MPV and age groups before treatment, a significant difference was found after vitamin D administration.[15] The study by Hejazi et al. also stated that there was no relationship between vitamin D deficiency and P-Selectin levels in patients even though they had been given vitamin D supplementation.[26] Although many studies showed a strong correlation between the anti-inflammatory effect of vitamin D and anticoagulants, there is a molecular mechanism. The relationship between vitamin D and thrombosis assessed by platelet function is also still inconclusive.

This study has limitations, such as a single-center with a cross-sectional design. Therefore, it does not describe the whole elderly with CHD. The variables used are only related to CHD, but there are probably many confounding variables, including genetics. The differences in genetics can also affect vitamin D receptors. Additionally, there was no further follow-up to determine the relationship between platelet function and future prognosis. This study does not explain the molecular mechanism as supporting data. Therefore, more clinical trials with a larger population are needed to understand the relationship with a complete mechanism and pathogenesis of the vitamin D effect on thrombosis.

CONCLUSION

Vitamin D levels are not linked with platelet function (MPV and P-Selectin) in elderly patients with CHD.

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We appreciate the participation of all the subjects who have agreed to be involved in this research.

ETHICAL CLEARANCE

This study has received ethical clearance with code number 669/EC/KEPK-RSKD/2020 from the Health Research Ethics Committee, Dr. Kariadi Hospital, Semarang.

CONFLICTS OF INTEREST

The author reports no conflicts of interest in this work.

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AUTHOR CONTRIBUTION

All authors were equally involved in this research.

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