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Vitamin D-Binding Protein and the Role of its Gene Polymorphisms in the Mortality of Sepsis Patients

Liliriawati Ananta Kahar[1], Yusrawati Yusrawati[2], Jamsari Jamsari[3], Tinni Maskoen[4], Kornelis Aribowo[5], Wiwi Monika Sari[6]

[1]Department of Anesthesiology and Intensive Care, Faculty of Medicine, Andalas University, M. Djamil Hospital, Padang, Indonesia, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University, Padang, Indonesia, [3]Department of Biotechnology, Postgraduate Program, Andalas University, Padang, Indonesia, [4]Faculty of Medicine, Padjadjaran University, Bandung, Indonesia, [5]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Andalas University, Padang, Indonesia, [6]Intensive Care Unit, M. Djamil Hospital, Padang, Indonesia

The study was conducted at Faculty of Medicine, Andalas University, Padang, Indonesia

Correspondence: *lili_anestintensivist@yahoo.com*; Tel.: + 62 813 63279385

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Abstract

Objective. This study aimed to determine the role of vitamin D-binding protein (VDBP) gene polymorphisms (especially at locus rs7041), vitamin D-binding protein levels, and vitamin D levels in mortality in sepsis patients. **Patients and Methods.** We performed the analytic observational study with a case-control approach. A total of 80 patients were included in this study, 40 patients were grouped as the case group and 40 patients were grouped as the control group. The patients were diagnosed with sepsis and treated in the Intensive Care Unit (ICU), M. Djamil Hospital, Indonesia. The VDBP rs7041 gene polymorphism was analyzed using the polymerase chain reaction procedure. VDBP and vitamin D levels were examined using the enzyme-linked immunosorbent assay (ELISA) method. **Results.** The case group showed lower mean vitamin D and VDBP levels than the control group ($P<0.05$). There were more variations in the rs7041 gene VDBP (mutant) locus in the case group than in the control group, and this difference was considered statistically significant, $P<0.05$. The results of this study indicate that the occurrence of polymorphism or variations at locus rs7401 (mutant) causes a decrease in VDBP and vitamin D levels. A decrease in vitamin D levels correlates with the incidence of mortality in sepsis patients. **Conclusion.** Polymorphism gene VDBP at locus rs7041 causes a decrease in the production of VDBP, a vitamin D carrier protein.

Key Words: Genetic Polymorphisms ▪ Intensive Care Units ▪ Sepsis ▪ Vitamin D ▪ Vitamin D-Binding Protein.

Introduction

Sepsis is a severe medical condition when the body responds to infection by releasing an excessive inflammatory reaction (1). This situation can cause organ damage, organ failure, and even mortality in patients (2). Sepsis is a significant global health problem, with high mortality rates worldwide (3, 4). Although many factors can influence the development of sepsis and patient outcomes, recent studies have shown that genetic polymorphisms in the immune system and vitamin D metabolism can contribute to the variability of patient response to sepsis (5, 6).

Vitamin D plays a role in regulating the immune system (7). Adequate levels of vitamin D help maintain a balance between inflammatory and anti-inflammatory responses. In septic patients, an exaggerated inflammatory response can lead to organ damage (8). Vitamin D is essential in protecting these organs and minimizing the damage caused by sepsis (9). Vitamin D deficiency or changes in vitamin D metabolism can affect the body's ability to fight infections associated with sepsis.

Vitamin D-binding protein (VDBP) is a protein produced by the liver and serves as the primary "vehicle" for transporting vitamin D in blood

circulation. Most of the vitamin D produced by the skin in response to sun exposure (vitamin D3) or taken from food (vitamin D2 or D3) must be bound to VDBP to circulate in the blood (7). This is a critical step in delivering vitamin D throughout the body. VDBP also has a role in protecting vitamin D from the degradation that can occur in the blood. This helps ensure that vitamin D remains active and ready for use by various cells in the body. Once bound to VDBP, vitamin D can easily be distributed to various cells and tissues in the body.

One gene that has caught the attention of researchers is the gene that encodes the vitamin D binding protein (VDBP). The genetic polymorphism at the rs7041 locus of the VDBP gene has been the subject of intense research because it is associated with variations in VDBP levels and blood levels of vitamin D (10, 11). VDBP, also known as Gc-globulin, plays a role in the transport of vitamin D in the blood, and can also have critical immunomodulatory effects (12). Vitamin D levels, which can also be affected by the rs7041 polymorphism, are essential in regulating the immune system and response to infection (11). Genetic polymorphisms are variations in the DNA sequence that can affect how specific genes function (13). Locus rs7041 is a VDBP gene locus with genetic variations that can affect VDBP expression and function (13). VDBP is a protein in the blood that binds to circulating vitamin D. Variations in the rs7041 locus can affect blood levels of VDBP. Some variants of rs7041 are associated with lower or higher blood levels of VDBP (14). VDBP levels strongly influence vitamin D levels in the blood (15). VDBP helps transport vitamin D from the skin and intestines throughout the body (12). Variations in VDBP levels can affect how efficiently vitamin D is distributed to the cells (16). Recent studies have shown that differences in the rs7041 polymorphism, VDBP levels, and vitamin D levels can affect an individual's susceptibility to sepsis, and the outcome (17, 18). These factors can affect the inflammatory response, the body's ability to overcome infection, and potential complications that may occur during sepsis.

This study aims to determine the role of the vitamin D binding protein polymorphism gene, vitamin D binding protein (VDBP) levels, and vitamin D levels in relation to mortality in sepsis patients.

Methods

Study Design and Participants

This study is an analytic observational study with a case-control approach. A total of 80 patients were included in this study, where the research subjects consisted of 40 patients grouped into the case group and 40 patients grouped into the control group. The patients were diagnosed as having sepsis and treated in the Intensive Care Unit (ICU), M. Djamil Hospital, Padang, Indonesia, from July to September 2022. The sampling process was carried out using consecutive sampling until the number of samples for the case and control groups was fulfilled. The inclusion criteria for the case group were sepsis patients aged 18-60 years who died in ICU care within 30 days of hospitalization, and who had complete medical record data, while the criteria for the control group were sepsis patients caused by bacterial infection, aged 18-60 years who did not die in ICU care and had complete medical record data. Sepsis diagnosis was based on Acute Physiology Age Chronic Health Evaluation (APACHE)-II and Sequential Organ Failure Assessment (SOFA) scores. Age and gender were matched in this study. Patients who received vitamin D supplementation and had viral, parasite, or fungal infections were excluded from this study. This study included observations on sociodemographic data as well as the clinical and laboratory test results of the patients, including routine blood laboratory tests and blood chemistry.

Gene Polymorphism Analysis

The VDBP rs7041 gene polymorphism was analyzed using the following procedure: genomic DNA was isolated from peripheral blood taken from

patients using a Vivantis Technologies, GF-1, and Blood DNA Extraction Kit, Malaysia, as instructed by the manufacturer. The amplification of 482 bp PCR rs7041 at the VDBP gene was accomplished using the following pairs of primers: F. Primer (5'AAATAATGAGCAAATGAAAGAAGAC3') R. Primer (5'CAATAACAGGAAAGAAATGAGTAG A3'). PCR reactions contained 10 µM of each primer, 12.5 µl of OnePCR™ Mix (2X) (GeneDirex Inc., Seoul, South Korea), 6.5 µl of nuclease-free water, and 3 µl of genomic DNA. A nuclease-free water test was run with each PCR experiment for contamination detection (negative control). PCR reactions were done using the Eppendorf Master Cycler. The amplified products were electrophoresed in 2% agarose gel containing ethidium bromide, visualized, and photographed using a UV transilluminator (Olympus, Tokyo, Japan). Each 482 bp PCR fragment was digested twice for all cases and controls using HaeIII restriction enzyme (Thermo Scientific, 2000 U) to determine genotypes of the c.1296 T > G variant (rs7041) according to the manufacturer's instructions. Fragments were analyzed by 2% agarose gel electrophoresis using a 100 bp DNA Ladder (Thermo Scientific, GeneRuler).

VDBP and Vitamin D Level Evaluation

VDBP and vitamin D levels were examined using the ELISA (Enzyme-Linked Immunosorbent Assay) method, using an ELISA kit (CloudClone®, Hangzhou, China) according to the manufacturer's instructions. According to the manufacturer's protocols (CloudClone®, Hangzhou, China), 50 µl of standard diluent or serum samples were added to the well and incubated at 37°C for 30 minutes. After the plates were washed, 100 µl of the biotinylated antibody solution was added and set for 30 minutes at 37°C. After washing three times, 50 ul avidin-peroxidase complex solution was added and incubated for 15 minutes at 37°C. After washing, 50 µl of tetramethylbenzidine color solution was added and set in the dark for 15 minutes at 37°C. Finally, a 50 ul stop solution was added to stop the reaction. The absorbance was measured at 450 nm using an ELISA reader (Epoch, Biotek, Winooski, VT, United States).

Ethical Approval

This study received approval from the medical and health research ethics committee of M. Djamil Hospital, Padang, Indonesia (LB.02.02/5/7/385/2022). In addition, participants, or their legal guardians, were informed about the study's objectives, and they provided informed consent to participate.

Statistical Analysis

Univariate and bivariate data analysis was performed using SPSS software version 25 (IBM, Jakarta, Indonesia). Univariate analysis was performed to present the distribution of data frequencies for each test variable. Meanwhile, bivariate analysis was carried out to determine the relationship between the test variables, where $P<0.05$.

Results

The basic clinical characteristics of research subject are summarized in Table 1. There was no statistically significant difference between the case and control groups in the variables age, gender, and body mass index (BMI), $P>0.05$. There was no difference between the case and control groups in the variables of past medical history (diabetes mellitus, chronic kidney injury, cardiovascular, and chronic obstructive pulmonary disease (COPD)), $P>0.05$. The study results showed no differences in the Acute Physiology Age Chronic Health Evaluation (APACHE)-II and Sequential Organ Failure Assessment (SOFA) scores between the case and control groups, $P>0.05$. Table 1 shows no difference between the case and control groups in the laboratory test results, $P>0.05$. The absence of statistically significant differences between the case and control groups in relation to demographic variables, medical history, and laboratory evaluation indicated that patients in the case and control groups were in equal and matching conditions.

Table 1. Basic Clinical Characteristics of Patients

Characteristics	Group		P-value
	Case, N (%)	Control, N (%)	
Age (years), Mean±SD	52.6±4.9	52.1±4.6	0.132*
Gender			
Male	15 (37.5)	15 (37.5)	1.000†
Female	25 (62.5)	25 (62.5)	
Body mass index			
Underweight	4 (10)	3 (7.5)	0.544†
Normoweight	14 (35)	15 (37.5)	
Overweight	22 (55)	22 (55)	
Diabetes mellitus	5 (12.5)	4 (10)	0.575†
Chronic kidney injury	4 (10)	5 (12.5)	0.537†
Cardiovascular disease	5 (12.5)	4 (10)	0.524†
COPD	3 (7.5)	3 (7.5)	1.000†
APACHE II Score, Mean±SD	21.3±1.4	21.8±1.6	0.622*
SOFA score, Mean±SD	7.6±0.7	7.1±0.8	0.562*
Hemoglobin, g/dL	10.24±1.4	10.64±1.6	0.122*
Leukocytes, $x10^3/mm^3$	14.11±1.9	13.96±1.8	0.132*
Platelets, $x10^3/mm^3$	208.9±16.2	208.2±17.7	0.089*
Procalcitonin, ng/mL	15.88±2.7	13.43±3.3	0.082*
Lactate, mmol/L	2.8±0.7	2.4±1.6	0.113*
Albumin, g/dL	2.69 ± 0.84	2.72±0.81	0.138*
Vitamin D, ng/mL	16.12±1.5	24.68±,97	0.002*
VDBP, ug/mL	161.29±14.12	224.36±17.98	0.003*
Variant locus rs7041			
Mutant (G/G)	28 (70)	14 (35)	0.011†
Wild type (T/T or T/G)	12 (30)	26 (65)	

*Independent t test; †Chi square test; APACHE II=Acute physiology age chronic health evaluation II; COPD=Chronic obstructive pulmonary disease; SD=Standard deviation; SOFA=Sequential organ failure assessment; VDBP=Vitamin D binding protein.

Table 1 also shows vitamin D levels, vitamin D binding protein (VDBP) levels, and variant locus rs7041. The case group showed lower mean vitamin D and VDBP levels than the control group, and they differed significantly statistically, P<0.05. The case group indicated a condition of vitamin D deficiency (16.12+1.5 ng/ml), whereas the control group exhibited a state of vitamin D insufficiency (24.68+1.97 ng/ml), when compared to the standard reference value for normal vitamin D levels in blood (30 ng/ml).There were more variations in the rs7041 gene VDBP (mutant) locus in the case group than in the control group, and this difference was also considered statistically significant, P<0.05. The study showed that in patients who died, there was polymorphism or variation at locus rs7401, and they had lower VDBP and vitamin D levels compared to patients who did not die.

A correlation test was performed to explore the correlation between the test variables, and this is presented in Table 2. Table 2 shows the very strong and statistically significant correlation between vitamin D and mortality. Strong correlations were also found between the mortality variables and VDBP levels. Moderate correlations were found between the mortality variables

Table 2. Correlation between Test Variables

Variables		Mortality	Vitamin D	VDBP	Variant rs7041
Mortality	r	-	-0.921	-0.911	0.490
	p value	-	0.000	0.000	0.001
Vitamin D	r	-0.921	-	0.971	-0.653
	p value	0.000	-	0.000	0.000
VDBP	r	-0.911	0.971	-	-0.891
	p value	0,000	0.000	-	0.000
Variant rs7041	r	0.490	-0.653	-0.891	-
	p value	0.001	0.000	0.000	-

*Pearson correlation-test; VDBP=Vitamin D binding protein.

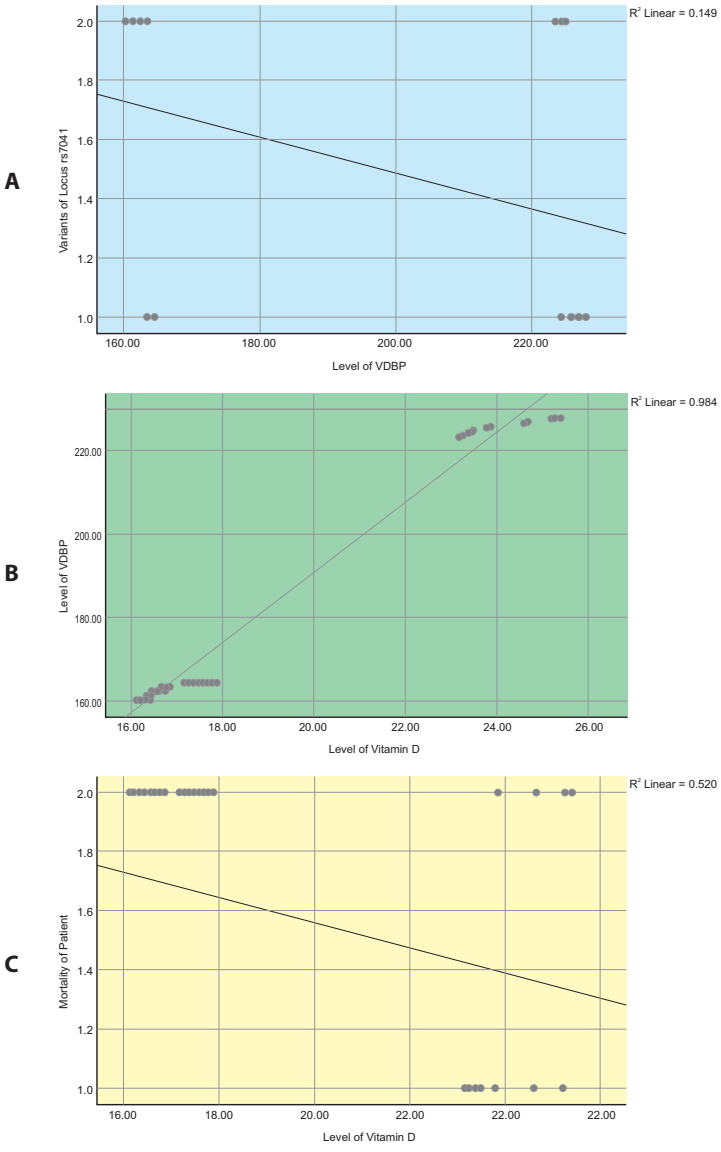

Figure 1. The graphs of correlation among the variable tests; (A) Graph of correlation between variant locus rs7041 and VDBP level; (B) Graph of correlation between VDBP level and vitamin D level; (C) Graph of correlation between vitamin D level and mortality.

and polymorphism or variations at locus rs7401. Polymorphism or variations at locus rs7401 had a strong and statistically significant correlation with VDBP levels. VDBP levels correlated strongly and statistically significantly with vitamin D levels. The correlation between polymorphism or variations at locus rs7401, VDBP levels, and vitamin D levels can be seen in Figure 1. The results of this study indicate that the occurrence of polymorphism or variations at locus rs7401 (mutant- G/G) causes a decrease in VDBP levels. In contrast, a decrease in VDBP protein also caused a decrease in vitamin D levels. A decrease in vitamin D levels correlated with the incidence of mortality in sepsis patients.

Discussion

This study shows that in sepsis patients who died, polymorphism or variation at the locus rs7401 of the VDBP gene was more dominant than in septic patients who did not die. VDBP protein levels and vitamin D levels also showed a decrease in septic patients who died compared to septic patients who did not die. Further studies were carried out to reconstruct the role of polymorphism or variations at the rs7401 locus of the VDBP gene, VDBP protein, and vitamin D in the incidence of mortality in sepsis patients. The correlation test showed that polymorphism or variations in the rs7401 gene VDBP locus caused a decrease in VDBP protein levels. Furthermore, decreased VDBP levels led to decreased vitamin D levels, which correlated with mortality in septic patients.

The rs7041 genetic polymorphism is a genetic variation in the human vitamin D binding protein (VDBP) gene (13). This gene encodes a protein essential for transporting vitamin D in the blood. There are two main variants of rs7041, namely T and G. Several studies have shown that individuals with the rs7041 G/G variant tend to have lower levels of vitamin D in the blood compared to individuals with the T/T or T/G variants (14, 19). The G/G variant is associated with the reduced capacity of VDBP to bind to vitamin D, so vitamin D may not be transported efficiently in the blood. Epidemiological studies have shown that individuals with the rs7041 G/G variant have a higher risk of developing vitamin D deficiency (20, 21). Vitamin D deficiency can contribute to several health problems, including an increased risk of bone diseases such as osteoporosis. Studies have also shown that individuals with different rs7041 polymorphisms may respond differently to vitamin D supplements (22, 23). G/G variants may require higher doses of vitamin D supplements to achieve the same levels as individuals with T/T or T/G variants (24).

A previous study stated that vitamin D can influence the activity of T cells, which are essential components of the adaptive immune system (8). In particular, vitamin D can help regulate the balance between regulatory T cells (Tregs) that inhibit inflammation, and cytotoxic T cells that trigger inflammatory responses (25, 26). It plays a role in controlling excessive inflammation, as occurs in sepsis. In particular, vitamin D has been associated with an increased number and activity of Tregs (27). This can result in a reduction in inflammation as Tregs inhibit the over activity of cytotoxic T cells (28).

One of the main mechanisms by which vitamin D reduces inflammation is by inhibiting the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (29, 30). It is a critical component in controlling the inflammatory response in the body. IL-6 is one of the main cytokines that trigger inflammation in the body. High levels of IL-6 may be associated with various inflammatory diseases, such as rheumatoid arthritis, heart disease, and autoimmune diseases (29). Vitamin D can inhibit the production of IL-6 by immune system cells. TNF-alpha is a cytokine that also plays a role in inflammation and can contribute to various chronic inflammatory diseases, such as Crohn's disease and psoriasis. Vitamin D has been shown to reduce TNF-alpha production in inflammatory responses. Macrophages are phagocytic cells that play an essential role in inflammation by producing large amounts of pro-inflammatory cytokines. Vitamin D can regulate the activity of macrophage cells and reduce the production of

pro-inflammatory cytokines by these cells (31). By inhibiting the production of pro-inflammatory cytokines, vitamin D helps maintain the proper balance between the inflammation that is needed to fight infection and excess inflammation that can damage body tissues (32). It is a crucial element in keeping the body healthy and preventing the development of chronic inflammatory diseases (32).

Prior studies supported the findings of our investigation. Shojaei et al. found a significant correlation between decreased blood vitamin D levels and mortality in individuals with sepsis (33). Multiple studies have also indicated that administering vitamin D supplements to individuals with sepsis might diminish the severity and enhance the prognosis of sepsis cases. A retrospective study by Guan et al., showed that vitamin D supplementation resulted in a lower risk of sepsis and a lower risk of mechanical ventilation requirement (34). Other studies by Bayat et al., and Rech et al., found an association between vitamin D and sepsis severity (35, 36). Furthermore, Rech et al., reported an increased risk of 30-day mortality for sepsis patients with vitamin D deficiency (36).

This study provides a pathway for connecting the rs7041 gene polymorphism, VDBP levels, vitamin D levels, and their correlation with mortality in sepsis patients. The limitation of this study is that it was conducted in a single center. In order to obtain results that may be applied to a wider population, it is necessary to conduct research involving multiple centers and greater sample sizes.

Conclusion

Polymorphism of the VDBP gene at locus rs7041 causes a decrease in the production of VDBP protein, a vitamin D carrier protein. This causes a decrease in vitamin D levels and plays a role in the incidence of mortality in sepsis patients. In future, more studies with larger samples and multiple centres in this field of expertise could yield useful data.

What Is Already Known on This Topic:

Vitamin D-binding protein is a protein produced by the liver essential for transporting vitamin D in the blood circulation. Vitamin D levels, which can also be affected by rs7041 polymorphism, are essential in regulating the immune system and response to infection.

What This Study Adds:

Our study reveals the vital role of vitamin D binding protein related gene polymorphisms (locus rs7041) in the mortality of sepsis patients. The correlation test emphasizes a pathway in the pathophysiology of patient death due to sepsis, namely a gene polymorphism at locus rs7041 that causes a reduction in VDBP; low VDBP will reduce vitamin D levels. Furthermore, a decrease in vitamin D will affect the immune system, which is necessary for the survival and recovery of sepsis patients.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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