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Vitamin D and Phagocytic Function in Patients with Sepsis: A Cross-Sectional Study

Vitamina D y función fagocítica en pacientes con sepsis: un estudio transversal

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SUMMARY

Background: *Sepsis is a leading cause of mortality in intensive care units. Several recent studies have shown that Vitamin-D deficiency is associated with the malfunctioning of macrophages and neutrophils. We determined whether Vitamin D levels affect phagocyte function in patients with sepsis. We evaluated serum vitamin-D levels and phagocyte function in patients with sepsis in Dr. Sardjito Hospital.* **Method:** *The sample was 38 adult patients treated with sepsis from June to November 2018. Patients on long-term steroid therapy or other immunosuppressants, suffering from chronic granulomatous disease, and tuberculosis, were excluded. A blood sample was drawn for vitamin D levels and Nitro Blue Tetrazolium (NBT) tests.* **Results:** *Of the 38 sepsis patients who were involved in this study, 26 (68.4 %) had Vitamin-D deficiency, 5 (13.2 %) had Vitamin-D insufficiency, and 7 (18.4 %) were normal, with a median phagocyte function of 0.21 (0.11-0.59). Spearman correlation analysis showed no relationship between vitamin D levels and phagocyte function in sepsis patients (r = 0.082; p> 0.05).* **Conclusion:** *Although there was no direct correlation between vitamin D levels and phagocytic function in sepsis patients, it was found that septic patients with low vitamin D levels were ten times more likely to have a low phagocytic function.*

Keywords: *Sepsis, vitamin D, phagocyte function, critical illness, immune function.*

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RESUMEN

Antecedentes: *La sepsis es una de las principales causas de mortalidad en las unidades de cuidados intensivos. Varios estudios recientes han demostrado que la deficiencia de vitamina D está asociada con el mal funcionamiento de los macrófagos y los neutrófilos. Determinamos si los niveles de vitamina D afectan la función de los fagocitos en pacientes con sepsis. Evaluamos los niveles séricos de vitamina D y la función fagocitaria en pacientes con sepsis en el Dr. Sardjito Hospital.* **Método:** *La muestra fueron 38 pacientes adultos tratados con sepsis de junio a noviembre de 2018. Se excluyeron los pacientes*

con terapia prolongada con esteroides u otros inmunosupresores, con enfermedad granulomatosa crónica y tuberculosis. Se extrajo una muestra de sangre para los niveles de vitamina D y las pruebas Nitro Blue Tetrazolium (NBT). **Resultados:** *De los 38 pacientes con sepsis que participaron en este estudio, 26 (68,4 %) tenían deficiencia de vitamina D, 5 (13,2 %) insuficiencia de vitamina D y 7 (18,4 %) eran normales, con una función fagocitaria media de 0,21 (0,11-0,59). El análisis de correlación de Spearman no mostró relación entre los niveles de vitamina D y la función de los fagocitos en pacientes con sepsis (r = 0,082; p > 0,05).*

Conclusión: *Aunque no hubo una correlación directa entre los niveles de Vitamina D y la función fagocítica en pacientes con sepsis, se encontró que los pacientes sépticos con niveles bajos de Vitamina D tenían diez veces más probabilidades de tener una función fagocítica baja.*

Palabras clave: *Sepsis, vitamina D, función fagocitaria, enfermedad crítica, función inmune.*

INTRODUCTION

Sepsis remains the leading cause of mortality in critical care units. Sepsis cases that occur worldwide each year are estimated to reach 189 cases per 100 000 person-year, with the overall mortality rate due to sepsis being relatively high, about 26.7 %, and this rate is even higher in the elderly, immunocompromised, and critically ill (1). Sepsis develops from the overactivation of host body defense mechanisms in response to systemic infection and does not directly affect these microorganisms. As a result of this infection, there are acute changes in organs, even those located far from the source of infection.

Over the last decade, the perspective on how vitamin D affects human health has changed dramatically based on the finding that vitamin-D receptors and vitamin-D 1-α-hydroxylase activating enzymes are expressed in many cell types (2). This reveals that vitamin D has a considerably larger influence on human health than was previously understood. Particularly in human immunology, extra-renal production of the active metabolite calcitriol - 1,25 (OH) 2D - by immune cells and peripheral tissues is thought to exhibit immunomodulatory qualities comparable to those of locally active cytokines (3,4). The reduced levels of 25-hydroxyvitamin D3 (25 (OH) D) due to the pleiotropic effect cause decreased signals to stimulate the innate immune system (5). Therefore, we aimed to determine the relationship between vitamin D levels and phagocytic function in patients with sepsis.

Vitamin D is found effective for the regulation of hormone secretion, immune functions, and cell proliferation along with differentiation. Its role as an immune modulator is based on the presence of receptors on many immune cells and the synthesis of its active metabolite from these cells. Vitamin D is an immune system modulator (6).

Vitamin D is an important immune modulator that is linked to infection susceptibility. It has been suggested that vitamin D deficiency plays a role in sepsis and septic shock because vitamin D-related pathways are associated with various immunological, endocrine, and endothelial functions (7).

MATERIALS AND METHODS

Patient

This study was an observational study with a cross-sectional design. All adult patients with sepsis at Dr. Sardjito Hospital who met the criteria from June to November 2018 were included. The inclusion criteria were adult patients (>18 years) diagnosed with sepsis based on the criteria for sepsis-3 and agreed to join the study. Patients on long-term steroid therapy or other immunosuppressants, suffering from chronic granulomatous disease and tuberculosis, and patients with incomplete data were excluded. There were 48 sepsis patients during the study period. However, ten were excluded due to incomplete data.

The Institutional Review Board of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital gave their approval to this study (KE/FK/0910/EC/2018).

Vitamin-D Levels and Phagocyte Function

All patients who meet the criteria will be examined for phagocyte function and serum Vitamin D (25(OH) D) levels. Serum vitamin D was assessed using ELISA, while phagocyte function was assessed calorimetrically with Nitroblue Tetrazolium (NBT) reduction test. The nitroblue tetrazolium test (NBT), based on the formation of a purple formazan precipitate following neutrophil-mediated reduction of the NBT

dye, is commonly used method to test for neutrophil respiratory burst and phagocytic function measuring intracellular O_2 production in various phagocytic cells. The sample is the patient's peripheral blood which is activated to produce superoxide which is detected by the NBT, which is converted from a yellow water-soluble compound to a dark-blue insoluble formazan that can be quantified. The dissolved NBT solution was transferred to a 96-well plate and absorbance was read on a microplate reader at 620 nm.

Serum vitamin D is considered normal if >30 ng/dL, deficiency <20 ng/dL, and insufficiency 20-30 ng/dL (8,9). Because there is no standard limit for phagocyte function, the value of phagocyte function was categorized based on ROC analysis with the determination of the optimal cut-off based on the Youden Index (sensitivity-(1-specificity)) obtained a cut-off of 0.215 (Figure 1).

Table 1. Clinical Characteristics

CharacteristicsMean ± SD or Median (min-max) or N(%)	
Age	60 (20 – 85)
Gender	
Male	20 (52.6)
Female	18 (47.4)
BMI (kg/m^2)*	20.5 ± 4.0
Hb (g/dL)*	9.9 ± 2.8
Leukocyte count (10^3/µL)*	14.7 (2.4 – 178.7)
Thrombocyte count (10^3/µL)*	164 (6.5 – 833)
Neutrophils (%)	87.7 (2.5 – 99.7)
SOFA score	7.21 ±3.55
Vitamin D (25 (OH) D)	
Deficiency	26 (68.4)
Insufficiency	5 (13.2)
Normal	7 (18.4)
Phagocyte Function	0.21 (0.11 – 0.59)

*BMI: Body Mass Index; Hb: Hemoglobin.

Statistical Analysis

The data were presented in the form of numbers and percentages in tables and diagrams. The relationship between vitamin D levels and phagocyte function was determined using the Spearman rank correlation test. The cut-off point for phagocyte function is determined by looking at the intersection of the ROC curve. The Fisher Exact Test was used to assess the difference in vitamin D levels based on the cut-off value of phagocyte function.

RESULT

We identified 48 patients with sepsis during the six months study period. Ten patients were excluded due to incomplete data. Seven (18.4 %) patients had normal vitamin D levels. The remaining 5 (13.2 %) and 26 (68.4 %) had insufficiency and deficiency, respectively (Table 1). The median phagocyte function is 0.21 (0.11-0.59). Phagocyte function is presented in median form because the data are abnormally distributed. The value of phagocyte function was categorized based on ROC analysis by determining the optimal cut-off based on the Youden Index (sensitivity-(1-specificity)), and the cut-off value was 0.215 (Figure 1). Actual Spectroscopic Values Data Obtained in the Vit D and Phagocytosis Function Determinations (Table 4).

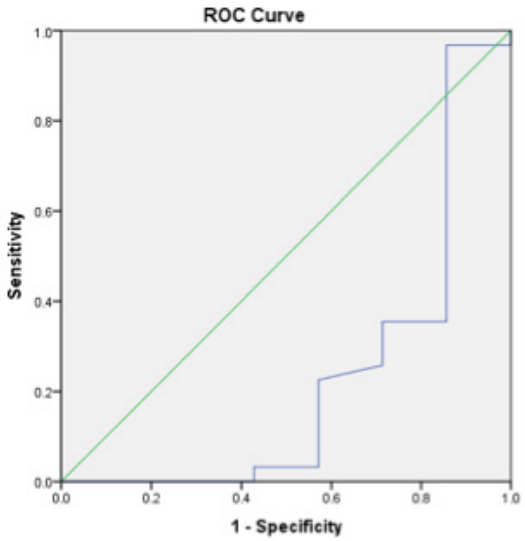

Figure 1. ROC Curve of Phagocyte Function Based on Vitamin D Levels.

The rank Spearman correlation test results showed no relationship between vitamin D levels and phagocyte function in patients with sepsis (r=0.082, p=0.311) (Table 2).

Table 2. Relationship between Vitamin D and Phagocyte Function in Sepsis Patients

Variable	r *	p **
Vitamin D (25 (OH)D) and Phagocyte Function (NBT test)	0.082	0.311

* Correlation coefficient
** Spearman correlation test is significant if p<0.05

Vitamin D levels were then grouped according to phagocytic function. Bivariate analysis was performed using the Fisher Exact test, and the results showed that sepsis patients with low vitamin-D levels would have a low phagocytic function (≤0.215), and this was statistically significant (p<0.05). In addition, sepsis patients with low vitamin D levels are ten times more likely to have a low phagocytic function than sepsis patients with normal vitamin D levels (RR:10.91) (Table 3).

Table 3. Comparison of vitamin-D levels based on phagocyte function

		Phagocyte function (NBT test)				p*	RR**	CI 95 %
		>0.215		≤0.215				
		N	%	N	%			
Vitamin D (25 (OH) D)	Normal	6	35.50	1	4.80	0.022	10.91	1.16-102.59
	Deficiency/ insufficiency	11	64.70	20	95.20			

* Fisher Exact Test sig if p<0.05
**Relative Risk

DISCUSSION

Our results showed that most of the septic patients (81.6 %) had low vitamin D levels (deficiency and insufficiency). This is consistent with previous studies, which also found that vitamin D levels in septic patients tend to be lower and can be used as a predictor of outcomes in septic patients (10-12).

Several studies have shown that age is one factor that affects vitamin D levels, where the older a person tends to have low vitamin D levels (13). This is also evident in the results of our study, where the median age in our study was 60 years.

This cross-sectional study shows the relationship between vitamin D levels and phagocyte function in sepsis patients. There is a trend toward lower vitamin D levels in septic patients and decreased immune status (chemotaxis, phagocytosis, and pro-inflammatory cytokines), leading to multiple organ failures, need for mechanical ventilation and catecholamines, increased length of stay, and increased mortality, the differences were not statistically significant (14-16). However, our results showed no direct relationship between vitamin D levels and phagocyte function in patients with sepsis ($r = 0.082$; $p > 0.05$). This is because the level of vitamin D and the function of phagocytes can be influenced by many factors (17,18).

Normal vitamin D levels can improve spontaneous phagocyte and lymphoproliferative function compared to low vitamin D levels (19). Serum vitamin D has significant effects on maintaining the body's immunity, including inducing antimicrobial peptides (AMPs) and suppressing T-cell proliferation, as well as in the phagocyte system (20).

Although it does not have a direct relationship, our results show that lower vitamin D levels will lower phagocyte function (Figure 1). Subgroup analysis showed that the sepsis patients with low (deficiency/insufficiency) vitamin-D levels had a 10.91 times higher risk of having low phagocyte function than samples with normal vitamin-D levels

Table 4. The Original Spectroscopic Values Data Obtained in the Vit D and Phagocytosis Function Determinations

Organism Test	Vit D	NBT
Achrombacter denitrificans	13	0.195
Acinetobacter baumani	23.6	0.133
Staphylococcus hominis ssp hominis	22.2	0.146
Pseudomonas aeruginosa	30.7	0.115
-	21.3	0.13
Staphylococcus cohnii ssp urealyticum	14.8	0.132
-	18	0.135
-	11.5	0.113
-	<8.1	0.223
-	11.2	0.154
Klebsiella pneumonia	13.8	0.217
-	34.5	0.242
Burkholderia cepacia group	8.6	0.168
-	<8.1	0.17
Staphylococcus haemoliticus; Pseudomonas aeruginosa	11.9	0.3
-	<8.1	0.716
No germ growth	<8.1	0.227
-	17.9	0.267
-	17.9	0.279
-	35.1	0.22
Staphylococcus hominis ssp hominis	18.6	0.169
-	14.2	0.345
-	<8.1	0.214
-	<8.1	0.129
Pseudomonas aeruginosa	<8.1	0.154
-	33.1	0.558
-	9.7	0.589
-	12.6	0.220
-	29.2	0.213
Klebsiella pneumonia	<8.1	0.197
-	<8.1	0.223
-	<8.1	0.237
Eschericia coli	<8.1	0.197
-	21	0.211
-	17.2	0.218
-	13.1	0.219
-	53.6	0.216
-	<8.1	0.198
Sphingomonas paucimobilis	<8.1	0.194
-	18	0.237
-	34.5	0.596
-	<8.1	0.394
-	18.2	0.572
-	<8.1	0.243
-	23.2	0.318
-	<8.1	0.275
-	10.9	0.224
Streptococcus pneumonia	26.5	0.361

or vice versa. Vitamin D deficiency causes decreased secretion of the antimicrobial peptide cathelicidins, leading to impaired auto/xenophagy. This situation results in disordered phagocytosis, cytotoxicity, antigen processing, and antigen presentation, besides vitamin-D deficiency affects T- and B-lymphocyte activation, as well as cell quantity, maturation, and regulatory function (7,20,21).

Our results are in line with Chae et al. (22) who showed that there is scant and conflicting evidence connecting vitamin D deficiency to sepsis mortality. However, Vitamin D is an important immune modulator that is linked to infection susceptibility (23). It was found that septic patients with low vitamin D levels were ten times more likely to have a low phagocytic function. Patients with sepsis who are vitamin D deficient are more likely to develop secondary infections (24). The prevalence of vitamin D deficiency ranges from 30 % to 70 % in the majority of pediatric intensive care units (PICUs) worldwide (25).

Multicenter studies with larger sample sizes and improvement in research methods are needed to provide stronger evidence regarding the relationship between serum vitamin D levels and phagocyte function in septic patients.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethical approval

This study was approved by the Institutional Review Board of the Faculty of

Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital (KE/FK/0910/EC/2018).

Authors' contributions

JL, DPM, and RH conceived the study. JL drafted the manuscript, and DPM and RH critically revised the manuscript for important intellectual content. JL collected samples and analyzed data. DPM and RH facilitated all project-related tasks. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

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