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Evaluation of the Relationship Between Serum Vitamin D Level and Severity of Sepsis

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Abstract

Background: On the basis of the literature, vitamin D is known as an important medium in bodi[illegible]mune function, and it therefore may play a role in the pathogenesis of sepsis.

Objectives: In this study, we aimed to evaluate the relationship between vitamin D se[illegible]and sepsis severity.

Patients and Methods: This study was a case-control study that evaluated adult patient[illegible]dmi[illegible]he emergency department of Imam Reza hospital with suspected sepsis. These patients were enrolled in the study as the case[illegible]p. In addition, healthy individuals without the sepsis diagnostic criteria were included in the control group. For all of the study par[illegible]pants, vitamin D levels were evaluated. The acute physiology age chronic health evaluation (APACHE) was used to e[illegible]disease seve[illegible]ty in the case group. A difference of $P < 0.05$ was regarded as statistically significant.

Results: A total of 112 patients were assessed: 56 in the control grou[illegible]d 56 in t[illegible]case group. In the case group, 18 patients had sepsis, 25 patients had severe sepsis, and 13 patients were in septic sho[illegible]The m[illegible]n age[illegible]the patients in the case and control groups were 57.7 ± 15.15 and 58.6 ± 15.05 years, respectively ($P = 0.741$). Vitami[illegible]levels in th[illegible]group were lower than in the control group (16.3 ± 10.7 versus 27.9 ± 11.46 ng/mL), and the difference between the g[illegible]ps w[illegible]significant ($P < 0.001$). Mean vitamin D levels in the severe sepsis and septic shock groups were lower than in the sepsis gro[illegible]and[illegible]mean l[illegible]el in the septic shock group was lower than in the severe sepsis group ($P = 0.001$). In the case group, there was a sig[illegible]cant reve[illegible]rrelation between APACHE II criteria and vitamin D levels ($P < 0.001$, $r = -0.586$).

Conclusions: The results of this study indicated that p[illegible]ients with sepsis had lower serum vitamin D levels than healthy controls. Also, patients with more severe disease h[illegible]er serum vitamin D levels, but to evaluate causation and determine whether vitamin D supplementation could be effective in r[illegible]ucing the risk or severity of sepsis, randomized controlled trials should be conducted.

Keywords: Vitamin D, Sepsis, Septic Sho[illegible]APAC[illegible]II Criteria

1. Background

Vitamin D has be[illegible]t des[illegible]bed and investigated for its role in bone[illegible]d ca[illegible]um-p[illegible]sphate homeostasis. After researchers f[illegible]d[illegible]min D receptors and 25-hydroxyvitam[illegible]n D-1α-[illegible]droxylase (1α-OHase) are distributed th[illegible]out bod[illegible]issues, not only in the skeleton, assessing the[illegible]tionship between this vitamin and several diseases bec[illegible]e an attractive field of research. Scientists h[illegible]e also demonstrated that the vitamin D response e[illegible]ent ex[illegible]s in more than 900 genes (1).

Cu[illegible]knowledge about the other important roles of this vitamin in other body systems and mechanisms is p[illegible], but several studies have shown that vitamin D has an influence on several chronic diseases, such as diabetes, cardiovascular disease, lung disease, autoimmune disorders, and common cancers (2-6).

Recent studies and clinical trials provide evidence that vitamin D affects the innate and adaptive immune system activity, including activation and differentiation of dendritic cells, macrophages, and B and T lymphocytes (7).

The prevalence rates in an Iranian population of mild, moderate, and severe vitamin D deficiency were 14.2%, 57.6%, and 9.5%, respectively (8). Chapuy et al. reported similar results of a high prevalence of vitamin D insufficiency in normal adults (9).

Recently, researchers have found an association between low levels of serum 25 (OH) D and increased risk of infection (10). The severity of an infection is negatively correlated to serum vitamin D concentrations. These findings proposed another important role for this vitamin in the progression of sepsis pathogenesis (11-13). The

incident of influenza A infections were also reduced with vitamin D supplementation in a clinical trial (14).

In 2011, Ginde et al. conducted a study to assess the severity of infection in patients with serum 25 (OH) D levels of < 75 nmol/L, and found that patients with a basic 25 (OH) D level of < 75 nmol/L, compared to those with >75 nmol/L, were more likely to develop severe sepsis (P = 0.006) and to have a sepsis-related organ failure assessment (SOFA) score of ≥ 2 (P = 0.049) and an Acute Physiology Age Chronic Health Evaluation (APACHE II) score of ≥ 25 (P = 0.06) (15).

There is evidence of the significant relationship of vitamin D serum levels and the risk of infection and its consequences; however, some studies had different results and did not reach the significance level (16-19). Due to this controversy, there should be more studies to investigate this relationship, given the importance of its application in critically ill patients at risk for infection or sepsis.

2. Objectives

The aim of this study was to provide additional evidence about serum vitamin D levels and their correlation with the severity of sepsis in patients referred to the emergency department.

3. Patients and Methods

3.1. Study Design

This was a case-control study. All patients over 18 years of age who were referred to the emergency department of Imam-Reza hospital, and hospitalized due to possible sepsis, were assessed for enrollment in the study as the case group (n = 56). We divided these 56 patients into three subgroups: sepsis, severe sepsis, and septic shock. Patients that met two or more criteria for systemic inflammatory response syndrome, and who were diagnosed with sepsis due to an infectious cause, were designated as the first subgroup. Sepsis patients were assigned to the second subgroup (severe sepsis) if there were one or more signs of organ dysfunction or hypoperfusion, such as metabolic acidosis, acutely altered mental status, oliguria, or acute respiratory distress syndrome. Patients with severe sepsis that did not respond to fluid resuscitation were considered to have septic shock and were placed in the third subgroup. Patients who did not sign the consent form, or who refused to continue in the study, were excluded. For each patient in the case group, we enrolled a healthy, age- and sex-matched individual without any of the diagnostic sepsis criteria, to form the control group.

3.2. Investigated Variables

We measured APACHE II scores, which are approved for determining the severity of infection or disease, and the serum vitamin D levels for each patient. Before any treatments, 5 ml of blood was drawn from each patient, then centrifuged (4,000 RPM for 5 minutes) to separate blood cells from serum. The serum samples were then stored at -80°C until analysis. The samples were sent to the central laboratory of Imam-Reza hospital for serum vitamin D assays. Vitamin D was determined with the chemiluminescence immunoassay (CLIA) technique using LIAISON® 25 OH Vitamin D Total assay (DiaSorin) kits on the LIAISON analyzer. Other demographic variables, such as age and sex, were collected by a questionnaire.

3.3. Ethical Issues

The study was conducted in accordance with the principles of the 1996 declaration of Helsinki and good clinical practice standards, under the supervision of the Mashhad University of Medical Sciences ethics committee. All patients signed informed consent.

3.4. Statistical Analysis

To compare quantitative variables between the two groups, if the data were normally distributed, an independent-samples t test was used. To compare quantitative variables between more than two groups, one-way ANOVA was used to analyze the data. If the data were not normally distributed, equivalent non-parametric tests were used. All data were analyzed with SPSS 16.0 statistical software (SPSS Inc., Champaign, IL, USA).

We used the Pearson chi-squared test to analyze qualitative variables, and if the data were not eligible for the test, the appropriate tests were used, such as Fisher's exact or the likelihood ratio. To assess correlations between quantitative data, the Pearson correlation test was used. In all cases, a significance level of P < 0.05 was set.

4. Results

A total of 112 eligible individuals were enrolled in this study, with 56 patients in the case group and 56 subjects in the control group. The case group included 18 patients with sepsis, 25 with severe sepsis, and 13 with septic shock. The demographic and baseline characteristics of the two groups are shown in Table 1.

The mean ages of the case and control groups were not significantly different (P = 0.741). Also, in the case group, the mean age of patients with septic shock was greater than in the other two groups, but the difference was not significant (P = 0.131). Males accounted for 48.2% and 53.6% of the patients in the case and control groups, respectively. No significant difference in sex distribution was observed between the case and control groups (P = 0.571) or among the case subgroups (P = 0.240) (Table 1).

The number of patients with underlying disease in the septic shock group was higher than in the other two groups. This number was greater in patients with severe sepsis compared to patients with sepsis. Statistical comparisons showed a significant difference between these groups (P = 0.024) (Table 2).

Serum vitamin D levels in the control group were higher than in the case group (P < 0.001). Patients with sepsis

had higher levels of vitamin D compared with the other two groups. Similarly, the patients with severe sepsis had higher levels of vitamin D compared to the patients suffering from septic shock (P = 0.001) (Figure 1, Table 2).

Vitamin D deficiency was considered to be present when serum levels were < 30 ng/mL. The number of patients in the case group who were deficient for vitamin D was higher than in the control group (P = 0.002). However, no significant differences were observed between the case subgroups (P = 0.144).

APACHE II score comparisons between the case subgroups showed a significant difference (P < 0.001), with a higher score in the septic shock patients compared to the two other subgroups. In patients with severe sepsis, APACHE II scores were greater than in the sepsis group (Table 2).

There was a significant negative correlation (correlation coefficient = -0.586) between vitamin D levels and APACHE II scores (As seen in Table 3, the APACHE II classification (≥ 25 or < 25) and vitamin D levels were measured in all patients (n = 56), and the u-djusted odds ratio was calculated.(Figure 2) (OR = 5.47).

There was a negative correlation between serum vitamin D levels and age. The qualitative analysis also showed that vitamin D deficiency in patients aged 65 years or older was greater than in patients younger than 65. Serum vitamin D levels were higher in men than in women (P < 0.001).

Table 1. Characteristics of the Subjects in the Case and Control Groups (N = 56)[a]

Variable	Case Group	Control Group	P Value
Age, y[b]	57.7 ± 15.15	58.6 ± 15.05	0.741
Gender, %			0.571
Male	48.2		
Female	51.8	46.2	
Temperature (rectal)[b]	38.37 ± 0.898	-	-
Mean arterial pressure[b]	80.6 ± 16.49	-	-
AaDO$_2$ or PaO$_2$[b]	75.1 ± 12.56	-	-
Heart rate, median [IQR]	109 (104 - 111)	-	-
Respiratory rate, median [IQR]	29 (26 - 39)	-	-
Hematocrit[b]	33.2 ± 5.82	-	-
Sodium (serum)[b]	135.2 ± 1	-	-
Potassium (serum)[b]	4.1 .26	-	-
White blood cell count[b]	153	-	-
Glasgow Coma Scale, median (IQR)	12 (11 -	-	-
Ph (arterial)[b]	7.33 ± 0.11	-	-
Serum vitamin D levels, ng/mL[b]	1 ± 10.70	27.91 ± 11.46	< 0.001
Serum vitamin D levels, No. (%)			0.002
Deficiency	48 (85.7)	33 (58.9)	-
Normal	8 (14.3)	23 (41.1)	-

[a] < 30 ng/mL was considered vita deficiency, 0 ng/mL was considered normal.
[b] Values are expressed as mean ± S

Table 2. Variables fo e Gro Patients[a,b]

Variable	Sepsis (n = 18)	Severe Sepsis (n = 25)	Septic Shock (n = 13)	P Value
Age, y	55.2 ± 12.69	55.6 ± 17.32	65.1 ± 12.10	0.131
Gender				0.240
Male	11 (61.1)	9 (36)	7 (53.8)	
ale	7 (38.9)	16 (64)	6 (46.2)	
hron ealth problem				0.024
	27.8	11 (44)	10 (76.9)	
N	72.2	14 (56)	3 (23.1)	
Serum itamin D levels, ng/mL	23.6 ± 11.31	13.5 ± 9.16	11.5 ± 7.36	0.001, P1 = 0.003, P2 = 0.003, P3 = 0.809
S m vitamin D levels				0.144
Deficiency	13 (72.2)	23 (92)	12 (92.3)	
Normal	5 (27.8)	2 (8)	1 (7.7)	
APACHE II, median (IQR)	10 (8 - 12.25)	28 (25 - 32)	32 (28 - 35.5)	< 0.001, P1 < 0.001, P2 < 0.001, P3 = 0.029

[a] Values are expressed as No. (%) unless otherwise indicated as mean ± SD.
[b] Below 30 ng/mL was considered vitamin D deficiency and 30 ng/mL or higher was considered normal. Post hoc analysis was done with the Tukey test.
P1 = sepsis vs. severe sepsis, P2 = sepsis vs. septic shock, P3 = severe sepsis vs. septic shock.

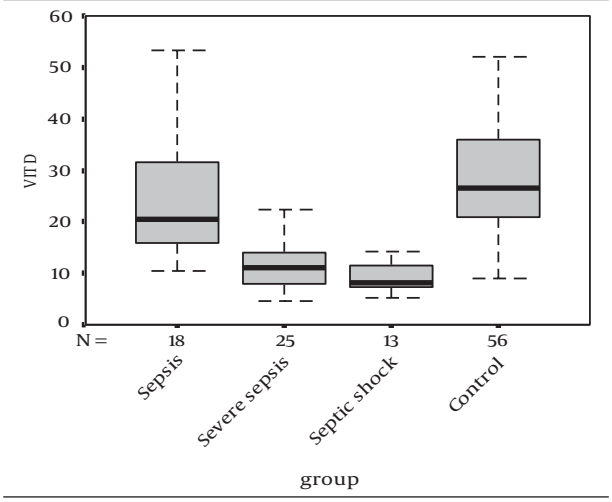

Figure 1. Serum Vitamin D levels in the Control Group and the Case Subgroups

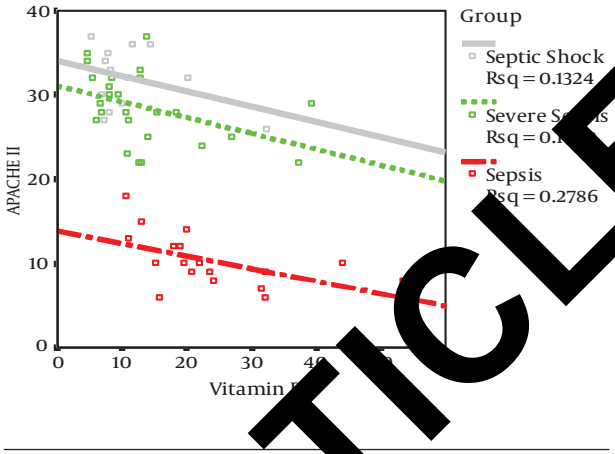

Figure 2. Scatter-Dot Di[illegible] of Serum [illegible]min D Levels and APACHE II Scores

Table 3. Cross-Tabulated Serum Vitamin D Levels and APACHE II Scores (N = 56)[a]

APACHE II	Vi[illegible] D	
	Deficient	Normal
≥ 25	[illegible]	2
< 25	17	6

[a]Odds ratio (CI95%): 5.47(0.99 - 30.13).

5. Discussion

We conducted a case-control study t[illegible]luate th[illegible] lationship between serum vitamin D and [illegible]sis severity. The results showed that seru[illegible]els of vit[illegible]in D in patients with sepsis compared [illegible]th the control group of healthy individuals were signifi[illegible]ntly lo[illegible]r. Also, serum vitamin D levels were lower in pa[illegible]ts [illegible]n severe sepsis or septic shock. On the [illegible]nd, by considering 30 ng/mL of vitamin D as the l[illegible]al concentration, we demonstrated t[illegible] the p[illegible]ntage of patients deficient for vitamin D [illegible]as s[illegible]ifica[illegible]ly higher in the case group than in the [illegible]ntr[illegible]but no significant difference was ob[illegible]ved b[illegible]ween the three case subgroups. The differe[illegible] these f[illegible]dings (vitamin D levels in the form of qua[illegible]tati[illegible]r quantitative variables) can be justified by [illegible]e fact tha[illegible]ng quantitative data is more accurate in a sta[illegible]tical analysis and leads to more precise results. [illegible]lso, u[illegible]g a cut-off point of 30 ng/mL as the lowest normal [illegible]ncentration for vitamin D was based on previous st[illegible]es, and may not be the lowest amount of vitamin D [illegible]cessary for optimal immune system function.

We also investigated the relationship between vitamin D levels and APACHE II scores, and found that a there is a significant inverse correlation between these two variables. In patients with more severe sepsis (higher APACHE II scores), we saw lower serum vitamin D levels.

Jeng et al.'s study found that critically ill patients hospitalized in the ICU (with or without sepsis) had significantly lower levels of 25 (OH) D plasma concentrations compared to healthy individuals, and that plasma concentrations of proteins that bind to vitamin D were significantly lower in severely ill patients with sepsis than in severely ill patients without sepsis (13).

Chinese researchers conducted a study to assess the association between vitamin D levels and risk of infection, disease severity, and mortality rates. Su et al. enrolled 50 healthy individuals, 51 patients with sepsis in the ICU, and 105 patients with sepsis in the ICU in this study. In general, ICU patients had lower vitamin D levels ($P < 0.01$), but no significant difference was observed between those with sepsis and those without it. A weak negative relationship was observed between APACHE II scores, the simplified acute physiology score (SAPS) II, SOFA scores, and vitamin D concentrations. Also, 25 (OH) D levels were not significantly different with regard to the 28-day ($P = 0.776$) and 90-day ($P = 0.389$) survival rates. However, APACHE II and SAPS II scores were identified as risk factors for mortality due to sepsis (16).

All of these findings are consistent with our study results. Higgins et al. assessed the prevalence and severity of vitamin D deficiency in ICU patients, and the deficiency's relationship to disease morbidity and mortality. Out of 196 ICU patients, 26% were vitamin D defi-

cient (< 30 nmol/L) and 56% had insufficient levels (30 - 60 nmol/L). In addition, 25 (OH) D levels decreased in all patients after admission to the ICU, and were significantly lower 10 days later (P < 0.001). 25 (OH) D status had no association with any cause of death after 28 days. However, higher levels of 25 (OH) D were associated with early discharge from the ICU. Also, patients with 25 (OH) D deficiency showed no significant differences with patients who had adequate levels with regard to acquiring infections (P = 0.11) (20).

Many studies have shown that a lack of vitamin D is common in patients admitted to the ICU (21-24). Several additio-l studies have shown that the prevalence of vitamin D deficiency is high in patients admitted to the hospital (25-27).

Ginde et al. investigated the hypothesis that in patients with suspected infection and a serum 25 (OH) D level of < 75 nmol/L, a more severe infection might be present. Eighty-one patients with a median age of 62 years were included in the study and scored with APACHE II and SOFA. The results of that study suggested that patients with a basic 25 (OH) D level of < 75 nmol/L, compared to patients with > 75 nmol/L, were more likely to develop severe sepsis (69% vs. 29%; P = 0.006), and more likely to have a SOFA score of ≥ 2 (44% vs. 18%; P = 0.049) and an APACHE II score of ≥ 25 (19% vs. 0%; P = 0.06). In addition, after 24 hours, those patients with 25 (OH) D levels of < 75 nmol/L were more likely to experience failure of two or more organs (50% vs. 18%; P = 0.02). four of the study's patients who died during hospit-za-tion had 25 (OH) D levels of < 75 nmol/L (15). That id- showed the most similarity to the design of o- study; 75 nmol/L (approximately 30 ng/mL) of ser- itamin D was considered the lowest normal concentrati-. Also, the mean age of the patients was sim- and the r-sults were consistent with those of the present study.

The results of the present study i-dicated t-t patients with sepsis had lower serum vitamin D leve- compared with healthy individuals, a- patients with more severe sepsis had even lower l-vels. Based on our findings and those of other similar st-es, a high percentage of patients are vitamin D deficie-t. For further research in this field, we sug-est -ized clinical trials with administratio- of vi-amin D supplementation in patients diagnos-d with sepsis- order to investigate vitamin D's therapeutic e-fects on disease progression and survival ra-e. The use -such supplementation, especially in high-ri- patients such as the elderly, may reduce the risk o- infection and mortality associated with sepsis.

One -f the limitations of the present study was the small number of cases in each of the case subgroups -hen they were divided according to sepsis, severe sepsis, or septic shock. Because of the limited number of patients in each subgroup, we were not able to perform multivariable a-lyses. There may be confounding factors, such as medical co-morbidities, drug history, season, and obesity. A larger sample size is necessary to assess the effects of these factors.

Footnotes

Authors' Contribution: All authors contributed equally to conceptualizing the research proposal, making substantial contributions to the study design and methods, and carrying out the laboratory experiments. All authors also participated in the data analysis and its interpreta-tion, and in the writing of the manuscript.

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