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Assessment of immune status in relation to vitamin D levels in patients with severe sepsis.

Abolfazl Jokar[1], Ghasem Mosayebi[2], Ghasem Moshar Movahed[1], Mehran Bahramian[3], Koorosh Ahmadi[3], Peyman Khademhoseini[3*]

[1]Department of Emergency Medicine, Arak University of Medical Sciences, Arak, Iran

[2]Department of Immunology and Microbiology, Arak University of Medical Sciences, Arak, Iran

[3]Department of Emergency Medicine, Alborz University of Medical Sciences, Karaj, Iran

Abstract

Introduction: Systemic sepsis is the body's response to invading microorganisms is from bacteria and fungi. In the most severely ill patients are vitamin D deficient. Since vitamin D in innate and adaptive immune responses is essential for the proper functioning of antimicrobials. The aim of this clinical trial to evaluate the safety associated with vitamin D levels in patients with severe sepsis.

Methods: In this clinical trial, 60 patients with sepsis were divided into 3 groups of 20 and 20 cases of healthy people as a control group was considered. Serum levels of IL-10 and TNF-alpha, length of stay and mortality were recorded. The data was analysed.

Results: A statistically significant difference in mean age and sex distribution between the groups was observed. The patients showed a significant difference that indicates that vitamin D deficiency in the number of people admitted they were more than the other two groups were fatal. The mean levels of vitamin D, IL-10 and TNF levels in vitamin D deficiency showed significant differences between groups (P value=001/0).

Conclusion: The results and lack of complication in the use of vitamin D muscle, as well as ease of use and low cost of these medications can these drugs in improving patient safety sepsis and reduce hospital admissions and deaths and morbidity can be as adjunctive therapy in the treatment of patients with symptoms of sepsis can be used.

Keywords: Vitamin D levels, Immune status, Sepsis, Patient, Serum.

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Introduction

It considers that sepsis is a response to an invasive microorganism such as bacteria and fungi [1] and it could frequently occur in patients with malignancy; in addition, this condition is associated with highly significant mortality [2]. In recent years, despite significant improvement in symptoms by using appropriate treatments [3], successful treatment remains a major challenge which is tightly associated with the rapid removal of microorganisms from the body and supportive measures [4,5].

Past investigations demonstrated that vitamin D deficiency [6-10] commonly occurs in most severe disorders. Numerous studies have shown that there is a correlation between lower vitamin D levels and some conditions including sepsis, increased the duration of hospitalization [11], high inflammatory responses [12], and acute kidney damage [13,14]. However, the exact mechanism of this condition is still understood. It has suggested that due to the potential role of the active metabolite of vitamin D (1 and 25 dihydroxy-vitamin D) on the host defense system [15].

Primarily vitamin D considers as a prohormone in calcium and phosphorus homeostasis for proper neuromuscular function and musculoskeletal health. This vitamin is available through the diet or by exposing ultraviolet radiation to the sun. Then the liver turns it into 25 hydroxyvitamin D and finally to an active form of 1 and 25 dihydroxy vitamin D in the kidneys, which increases the calcium intake in the intestines. Vitamin D levels also fluctuate based on seasonal variations. Recent studies have shown that vitamin D could perform actions beyond its intrinsic form [16]. Most types of cells, including immune cells, such as macrophages, B, and T lymphocytes, have an intra-nuclear receptor of vitamin D that respond to active metabolites 1 and 25 of dihydroxy vitamin D [17,18]. The active form of vitamin D inhibits the production of pro-inflammatory cytokines including interleukin 2, 6, 8 and TNF-α [19,20] and also increases the production of anti-inflammatory cytokines such as interleukin 10 [21,22], which

can reduce the damage to the kidneys. Therefore, it could be concluded that vitamin D is not only an alternative indicator for determining the severity of the disease but also an important factor in the incidence and severity of sepsis [23]. Since vitamin D is essential for proper antimicrobial function in immune responses [24,25], this clinical trial aims to investigate the effect of vitamin D on the immune response of patients with severe sepsis.

Materials and Methods

Conditions and sampling

According to the incidence of 2% sepsis and 0.3% severe sepsis in hospitalized patients, we used to survey all patients with an early diagnosis of severe sepsis in Valiasr Arak hospital. Also, we enrolled 20 healthy people of the community, which were matched for age and sex, and other criteria for inclusion with an average vitamin D level, and considered them as a control group. Also, due to changes in the seasonal pattern of vitamin D, which is the lowest during the winter and spring, and in the summer and fall seasons, the present study has been selected for the period of 6 months of autumn and winter.

Study design

In this study, patients with sepsis (two criteria: temperature between 36 and 38, heart rate more than 90, respiratory rate more than 20 or arterial CO_2 pressure more than 32, WBC between 4000 and 12000, and the presence of a new source of infection: (Blood pressure<60/90 or MAP<70 or BP>40 mmg, mottled skin or capillary filling more than or equal to 3 s or PaO_2/FIO_2<300, all of which are arterial hypoxia, DIC, acute renal failure or urine flow<0.5 ml/kg/h or <45 mmol/L for at least 2 h, heart failure, serum lactate>2, creatinine level Serum>2 mg/dl or Cr>0.5 mg/dl, platelet counts below 100.000, liver failure based on bilirubin level>2 mg/dl or INR>1.5 or PTT>60, acute pulmonary injury or ARDS, non-intestinal sounds or ileus, which are not justified by other causes of organ dysfunction) during the season fall and winter visits to Vali-e-Asr Hospital in Arak were entered into the study after receiving written consent and according to inclusion and exclusion criteria. If necessary, after initial recovery and simultaneously with the initiation of specific treatment for these patients, Serum vitamin D levels were measured in their kidneys (group A). According to the level of vitamin D, patients were divided into 3 groups (groups A1: patients with vitamin D deficiency (vitamin D levels<20 ng/dl or <50 nmol/L), A2: patients with vitamin D deficiency (level vitamin D between 20 and 9/29 ng/dl or 50 and 72 nmol/L), A3: patients with adequate levels of vitamin D (vitamin D>30 ng/dl or >75 nmol/L). Also, 20 healthy people in the community, which were matched for age and sex and other criteria for inclusion and exclusion were considered as the control group (group B). In this step, serum levels of interleukin 10 and TNF-α were measured in both groups. Sampling was done through the cubital vein. Then the samples were centrifuged at room temperature for 20 min and, according to the necessity of measuring the level of interleukins during a session, samples were stored in a refrigerator of 70°C in Immunology lab of Arak University of Medical Sciences until complete collection of samples. Then we measured the level of interleukin 10 and TNF-alpha, and the results recorded. It should be noted that the data recorded by an emergency specialist assistant.

Inclusion and exclusion criteria

Inclusion: 70>Age>18, and severe sepsis diagnostic criteria

Exclusion: Chronic kidney disease (Cr>2)

Chronic liver disease

Chronic heart failure

Previous gastrostomy or intestinal malabsorption

Calcium level ≥ 10

Receive vitamin D supplements in the last 7 days or anti-depressant drugs

History of parathyroid disease

Metabolic bone diseases, nephrolithiasis, sarcoidosis, ESRD

Imminent death

Pregnancy

Dissatisfaction with the company in the study

Statistical analysis

Data were analysed by SPSS22 software, Chi-square and t-test, and ANOVA with repeated observations.

Results

This double-blind clinical trial was performed on patients with sepsis. In this study, 60 patients with sepsis which referred to Vali-Asr Hospital in Arak were divided into three groups: vitamin D deficiency (below 20), vitamin D deficiency (20 to 30), and normal vitamin D levels (above 30) and 20 healthy people were considered as control group. The mean age in subjects with vitamin D deficiency was 62.75 ± 6.84, vitamin D was 55.75 ± 10.8, and vitamin D was 54.7 ± 10.07, respectively, and in The control group had a mean age of 42.4 ± 8.35, which according to the P value, there was no significant difference in mean age between the groups and were matched in age (P value=0.6246, Table 1).

Table 1. *Relation between vitamin D and age.*

P value	Group		Age
	SD	Mean	
0/624	6/84	62/75	Vit D (less than 20)
	10/80	55/75	Vit D (20-30)
	10/07	54/70	Vit D (over 30)

8/35	42/40	Control
11/61	53/90	Total

The distribution of sex in the vitamin D deficiency group was 9 (45%) male, and 11 (55%) were female. In the sufficient vitamin D group, ten were male (50%), and 10 were female (50%). In the normal vitamin D group, 12 (60%) were male and 8 were female. Also, in the control group, twelve (60%) men and eight were women. According to the P value, there was no significant difference in gender between the subjects (Table 2).

Table 2. *Distribution of gender including vitamin D levels.*

P value	Group		Gender	
	Percent	Frequency		
0/715	45%	9	Male	Vit D (Less than 20)
	55%	11	Female	
	50%	10	Male	Vit D (20-30)
	50%	10	Female	
	60%	12	Male	Vit D (over 30)
	40%	8	Female	
	60%	12	Male	Control
	40%	8	Female	
	53/75%	43	Male	Total
	46/25%	37	Female	

The distribution of patients in the vitamin D deficiency group was 12 cases discharged (60%) and 8 (40%) died, in the vitamin D sufficient group, 18 (90%) were discharged and 2 (10%) died, and in the normal level of vitamin D, 17 (85%) were discharged and 3 (15%) died. According to the P value, there was a significant difference between the three groups of participants in the design. This means that in the vitamin D deficiency group, the number of hospitalized patients was more than the others (Table 3).

Table 3. *Frequency distribution of patients' condition.*

P value	Group		Patients condition	
	Percent	Frequency		
0/048	60%	12	Discharged	Vit D (less than 20)
	40%	8	Died	
	90%	18	Discharged	Vit D (20-30)
	10%	2	Died	
	85%	17	Discharged	Vit D (over 30)
	15%	3	Died	
	78/33%	47	Discharged	Control
	21/66%	13	Died	

The mean vitamin D level in the vitamin D deficiency group was 11.28 ± 5.21 in the vitamin D deficiency group of 25.95 ± 2.74 and the normal vitamin D group was 37.75 ± 7.38 and in the control group was 46.06 ± 10.87 There was a significant difference between the groups in terms of P value (P value=0.001, Table 4).

Table 4. *Mean level of vitamin D.*

P value	Group		Vitamin D levels
	Mean	SD	
0/001	5/21	11/28	Vit D (Less than 20)
	2/74	25/95	Vit D (20-30)
	7/38	37/75	Vit D (over 30)
	10/87	46/06	Control
	14/94	30/26	Total

The mean level of IL-10 in the vitamin D deficiency group was 5.03 ± 8.29 in the vitamin deficient group of 15.92 ± 7.70 and the normal level of vitamin D was 21.78 ± 7.84 and in the control group it was 53.23 ± 21.15 There was a statistically significant difference between the groups (P value=0.000, Table 5).

Table 5. *Mean level of IL-10.*

P value	Group		IL-10 level
	Mean	SD	
0/001	5/03	8/29	Vit D (Less than 20)
	7/70	15/92	Vit D (20-30)
	7/84	21/87	Vit D (over 30)
	21/15	53/23	Control
	20/94	24/83	Total

The mean TNF-a level in the vitamin D deficiency group was 19.10 ± 10.92 in the vitamin D deficiency group 11.67 ± 3.53 and the normal diet group 11.61 ± 3.60 and the control group 5.48 ± 2.7. According to P value, there was a significant difference between the groups (P value=0.001, Table 6).

Table 6. *Mean level of TNF-a.*

P value	Group		TNF-a level
	Mean	SD	
0/001	10/92	19/10	Vit D (Less than 20)
	3/35	11/67	Vit D (20-30)
	3/60	11/61	Vit D (over 30)
	2/07	5/48	Control

7/69	11/96	Total

The mean duration of hospitalization in patients with hypothyroidism in the vitamin D deficiency group was 4.4 ± 2.47, in the vitamin D deficiency group was 2.31 ± 3.90 and in the normal diet group was 3.65 ± 1.59, there was no statistically significant difference between the studied groups (p value=0.540, Table 7).

Table 7. Mean level of hospitalization duration.

P value	Group		Hospitalization duration
	Mean	SD	
0/001	2/47	4/40	Vit D (Less than 20)
	2/31	3/90	Vit D (20-30)
	1/59	3/65	Vit D (over 30)
	2/15	3/98	Control

The average duration of hospitalization in patients without vitamin D deficiency was 2.30 ± 5.33, 2.40 ± 4 in vitamin D deficiency and 1.49 ± 3.88 in normal vitamin D group. There was no significant difference between the groups (P=0.414, Table 8).

Table 8. Mean duration of hospitalization in patients without died one.

P value	Group		Hospitalization duration
	Mean	SD	
0/001	2/30	5/33	Vit D (Less than 20)
	2/40	4	Vit D (20-30)
	1/49	3/88	Vit D (over 30)
	2/12	4/29	Control

Discussion

This study aimed to evaluate the immune status of vitamin D in patients with severe sepsis. Vitamin D could affect human immunity, for example, by preventing excessive release of cytokine and activating the integrated immune system. The results of past studies revealed the role of vitamin D in infectious diseases, but often limited to a small number of patients.

To investigate the effect of vitamin D on the sepsis disease, researchers reviewed the records of patients admitted to the intensive care unit in Boston between March 1, 1998, and January 2011. The 3386 adults were analysed by measuring the amount of vitamin D from 1 year before admission. Women accounted for 46% of the patients, 81% were white, and 67% had a medical diagnosis. 69% of vitamin D measurements were performed less than six months before admission to ICU [26]. According to the International Classification of Diseases, for each five ng/ml increase in vitamin D levels before admission, the ratio of sepsis decreased by 4%.

In sepsis, the cases with less than 30 ng/ml of vitamin D levels before treatment, the rate of mortality was 1.6 times higher in comparison to normal vitamin D level.

In this study, there was no significant difference in mean age between the groups [9]. Also, the frequency distribution of gender did not show a significant difference between the groups.

Haan et al.'s meta-analysis study, concluded that vitamin D levels below 50 were associated with an increase in risk of infections, sepsis, death within 30 d, and death in the hospital [27].

Leaf et al. conducted a study which shows that the primary outcome of using cathelicidine protein in the first 24 h after injection and the secondary outcome was the level of cytokines and the marker of renal injury. In their study, they concluded that administration of calcitriol did not increase the level of cathelicidine in severely painful patients with sepsis, and also had different effects on immune markers [14].

Amrein et al. indicated that most patients suffered from vitamin D deficiency, and this was different in winter compared with summer [28].

Jeng et al. concluded that there is a correlation between the severity of the disease, vitamin D deficiency and vitamin D-binding protein levels in severely ill patients in comparison to healthy. They also concluded that there is a positive relationship between vitamin D level and cathelicidine LL-37 [9].

Frequency distribution of patients showed a significant difference between the three groups in the study, which indicates that the number of hospitalized patients who died in vitamin D deficiency was more than the other two groups. Mean vitamin D levels and interleukin 10 levels also showed a significant difference between the studied groups. There was no significant difference in the mean hospitalization time in patients with dead patients, among the studied groups. Also, there was no significant difference in the mean duration of hospitalization in the patients without considering the deaths among the patients.

Conclusion

According to the results of this study, use of vitamin D is an easy and low cost; it can be used to improve the immune status in sepsis patients and reduce hospitalization and mortality in patients. It was used as an auxiliary therapy for treating patients with sepsis symptoms. It should be noted that the reduction in the hospitalization days, hospital infections are reduced, treatment costs are reduced.

Conflict of Interest

None

References

1. Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, Kurosawa S, Remick DG. The pathogenesis of sepsis. Annu Rev Pathol 2011; 6: 19-48.
2. Angus DC, Van Der Poll T. Severe sepsis and septic shock. N Engl J Med 2013; 369: 840-851.
3. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001; 345: 1368-1377.
4. Schaub N, Frei R, Muller C. Addressing unmet clinical needs in the early diagnosis of sepsis. Swiss Med Wkly 2011; 141: 13244.
5. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348: 138-150.
6. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med 2009; 360: 1912-1914.
7. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metabol 2003; 88: 4623-4632.
8. Lucidarme O, Messai E, Mazzoni T, Arcade M, du Cheyron D. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. Intens Care Med 2010; 36: 1609-1611.
9. Darvishi M. Virulence factors profile and antimicrobial resistance of acinetobacter baumannii strains isolated from various infections recovered from immunosuppressive patients. Biomed Pharmacol J 2016; 9: 1057-1062.
10. Lee P. Vitamin D metabolism and deficiency in critical illness. Best Pract Res Clin Endocrinol Metabol 2011; 25: 769-781.
11. Matthews LR, Ahmed Y, Wilson KL, Griggs DD, Danner OK. Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. Am J Surg 2012; 204: 37-43.
12. Braun A, Chang D, Mahadevappa K, Gibbons FK, Liu Y, Giovannucci E. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Crit Care Med 2011; 39: 671.
13. Braun AB, Litonjua AA, Moromizato T, Gibbons FK, Giovannucci E. Association of low serum 25-hydroxyvitamin D levels and acute kidney injury in the critically ill. Crit Care Med 2012; 40: 3170-3179.
14. Leaf DE, Raed A, Donnino MW, Ginde AA, Waikar SS. Randomized controlled trial of calcitriol in severe sepsis. Am J Resp Crit Care Med 2014; 190: 533-541.
15. Mora JR, Iwata M, Von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 2008; 8: 685.
16. Langlois K, Greene-Finestone L, Little J, Hidiroglou N, Whiting S. Vitamin D status of Canadians as measured in the 2007 to 2009 Canadian Health Measures Survey. Health Rep 2010; 21: 47.
17. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008; 29: 726-776.
18. Verstuyf A, Carmeliet G, Bouillon R, Mathieu C. Vitamin D: a pleiotropic hormone. Kidney Int 2010; 78: 140-145.
19. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metabol 2008; 4: 80.
20. Darvishi M. Antibiotic resistance pattern of uropathogenic methicillin-resistant staphylococcus aureus isolated from immunosuppressive patients with pyelonephritis. J Pure Appl Microbiol 2016; 10: 2663-2667.
21. Agrawal T, Gupta GK, Agrawal DK. Vitamin D supplementation reduces airway hyperresponsiveness and allergic airway inflammation in a murine model. Clin Exp Allerg 2013; 43: 672-683.
22. Allen AC, Kelly S, Basdeo SA, Kinsella K, Mulready KJ, Mills KH. A pilot study of the immunological effects of high-dose vitamin D in healthy volunteers. Multi Scler J 2012; 18: 1797-1800.
23. Cluse ZN, Fudge AN, Whiting MJ, McWhinney B, Parkinson I, OLoughlin PD. Evaluation of 25-hydroxy vitamin D assay on the immunodiagnostic systems iSYS analyser. Ann Clin Biochem 2012; 49: 159-165.
24. Hewison M. Vitamin D and innate and adaptive immunity. Vitam Horm 2011; 86: 23-62.
25. Hewison M. Antibacterial effects of vitamin D. Nat Rev Endocrinol 2011; 7: 337-345.
26. Moromizato T, Litonjua AA, Braun AB, Gibbons FK, Giovannucci E. Association of low serum 25-hydroxyvitamin D levels and sepsis in the critically ill. Crit Care Med 2014; 42: 97-107.
27. de Haan K, Groeneveld AJ, de Geus HR, Egal M, Struijs A. Vitamin D deficiency as a risk factor for infection, sepsis and mortality in the critically ill: systematic review and meta-analysis. Crit Care 2014; 18: 660.
28. Amrein K, Zajic P, Schnedl C, Waltensdorfer A, Fruhwald S, Holl A. Vitamin D status and its association with season, hospital and sepsis mortality in critical illness. Crit Care 2014; 18: 47.

*Correspondence to

Peyman Khademhoseini

Department of Emergency Medicine

Alborz University of Medical Sciences

Iran