ASSOCIATION OF SERUM VITAMIN D LEVELS AND SEVERITY OF SEPSIS

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ABSTRACT**BACKGROUND**

Sepsis is a leading cause of death among critically ill patients in non-coronary intensive care units. Vitamin D is a key regulator of innate and adaptive immune systems that may influence host susceptibility to infection. It enhances the body's first line defence against microbes, which cause sepsis.

The aim of the study was to investigate the association between Vitamin D level and severity of sepsis and its outcome.

MATERIALS AND METHODS

A case-control study was conducted in the Department of Medicine of SCB Medical College, Cuttack. Sixty adult patients of sepsis and sixty age and sex matched healthy controls were included in the study. Patients were categorised into sepsis, severe sepsis and septic shock. 25(OH) D level was estimated in all the patients and controls. The outcome of the patients, either discharged or death was recorded and the level of Vitamin D was correlated with the severity and outcome of the patients.

RESULTS

Out of 59 cases who completed the study, 33 (56%) had sepsis, 15 (25%) had severe sepsis and 11 (15%) were in septic shock at presentation. Vitamin D insufficiency was seen in 88.14% of cases and 70% of healthy controls. Mean 25(OH) vitamin D level was found to be 13.87 ± 11.4 ng/mL in cases and 20.7 ± 12.4 ng/mL in controls, which was statistically significant ($p=0.002$). 25(OH) D insufficiency was found in all the 11 (100%) cases of septic shock, 93% of severe sepsis and 82% of sepsis patients at presentation. Eleven cases died during treatment and all of them had Vitamin D insufficiency. No mortality was found in patients with sufficient vitamin D level.

CONCLUSION

Patients of sepsis with low vitamin D level was associated with severe sepsis, septic shock, higher mortality rate and poor outcome.

KEYWORDS

Vitamin D, Severe Sepsis, Septic Shock, ICU, Outcome.

HOW TO CITE THIS ARTICLE: Mohapatra N, Sahu AN, Mishra SK, et al. Association of serum vitamin D levels and severity of sepsis. J. Evolution Med. Dent. Sci. 2018;7(05):646-649, DOI: 10.14260/jemds/2018/146

BACKGROUND

"Sepsis" is a systemic inflammatory response syndrome (SIRS) that has a proven or suspected microbial aetiology. When sepsis is associated with one or more signs of organ dysfunction, it is called "severe sepsis." Severe sepsis when accompanied by hypotension or evidence of hypoperfusion that cannot be corrected by infusing fluids it is "septic shock."⁽¹⁾ Sepsis and severe sepsis are leading causes of death in the United States and the most common cause of death among critically ill patients in non-coronary intensive care units. Severe sepsis is a contributing factor in causing 2 lakh deaths per year in the United States. The incidence of severe sepsis and septic shock has increased over the past 30 years.⁽²⁾

'Financial or Other Competing Interest': None.
Submission 21-11-2017, Peer Review 12-01-2018,
Acceptance 18-01-2018, Published 29-01-2018.

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DOI: 10.14260/jemds/2018/146

Despite more than two decades of research, the pathogenesis of sepsis and immune mechanisms remain incompletely understood. The innate immune system is the first line of defence against infection and is responsible for responding rapidly by recognising and eliminating invading pathogens to prevent exacerbation of infections. It involves activation of toll-like receptors (TLRs) in monocytes leading to the induction of antimicrobial peptides including cathelicidins and the subsequent killing of bacteria. The only known human cathelicidin hCAP-18 (LL-37) is encoded by human cathelicidin antimicrobial peptide (CAMP) gene. CAMP is expressed in neutrophils, monocytes, lymphocytes, dendritic cells, epithelial cells lining the skin, respiratory and gastrointestinal tract and has a broad antibacterial activity against both gram positive and gram negative bacteria, fungi and mycobacteria.⁽³⁾

Vitamin D plays an important part in promoting the innate antimicrobial response. It is a major steroid hormone and its active form $1,25(\text{OH})_2\text{D}_3$ mediates its biological activities by binding to a member of nuclear receptor family, the Vitamin D receptors (VDR) that are present in all types of immune cells including neutrophils, macrophages, dendritic cells, activated CD4^+ , CD8^+ T cells and B cells. $1,25(\text{OH})_2\text{D}_3$ has been reported to be a major regulator of CAMP in the monocyte, intestinal and bronchial epithelial cells.⁽⁴⁾ It also

causes down regulation of TLR2 and TLR4 (key factors in the pathogenesis of sepsis) expression on human monocyte, thereby imparting an important immunomodulatory effect.⁽⁵⁾ In regards to adaptive immunity, vitamin D affects the proliferation and differentiation of T and B cells and modulates immunoglobulin production.^(6,7) 1,25(OH)₂D₃ suppresses the production of inflammatory cytokines IL-2 and interferon gamma (IFN γ) produced by the Type 1 T helper (Th1) cells. It also decreases TNF α and tissue factor, both end product of LPS activation and important inflammatory molecules in sepsis.⁽⁵⁾ A number of observational studies have found an association between low vitamin D levels and risk of sepsis. Therefore, this study was conducted to find out a possible correlation between serum vitamin D level and severity of sepsis and outcome of patients.

MATERIALS AND METHODS

This is a case-control study carried out in the Department of Medicine of a tertiary care centre in the eastern part of India during a period of one year from September 2012 to August 2013. A total of 60 sepsis patients admitted in the Medical Emergency or Intensive Care Unit with features of sepsis, severe sepsis or septic shock during the study period were included in the study. They were compared with 60 other healthy matched adults taken as controls conventionally.

Inclusion Criteria

Adult (age ≥ 15 years) admitted patients fulfilling the criteria for sepsis, severe sepsis or septic shock were taken as study subjects.

Exclusion Criteria

Those with chronic liver disease, chronic kidney disease, pregnant and lactating women, chronic diarrhoea, malabsorption syndrome and on vitamin D replacement therapy were excluded from the study.

Healthy controls were age and sex matched adults without known acute or chronic diseases. No hospitalisations for any illness in the previous 12 months, not taking any medications or vitamin supplements. Their physical examination was normal and laboratory examinations including complete blood count, biochemical tests revealed no abnormality. The study was approved by our Institutional Ethics Committee, and written consent was obtained from the patients or their family members after explaining to them about the study.

Methodology

Patients suspected to have sepsis were evaluated with detail history, clinical examination and routine haematological and biochemical investigations including complete blood count, serum bilirubin, serum transaminases, serum urea, serum creatinine, blood glucose, serum sodium and potassium. Urine and blood culture, Arterial Blood Gas analysis were also done in all patients. They were categorised into sepsis, severe sepsis, septic shock as per American College of Chest Physician (ACCP)/ Society of Critical Care Medicine (SCCM) consensus panel 2001. Serum 25(OH) D level, which is the circulating form of vitamin D was measured in all these patients at the time of admission or within 24 hours of hospitalisation, and also in the age and sex matched healthy controls. Serum 25(OH) D was assayed by competitive ELISA kit manufactured by Euro-Immune (Germany). 25(OH) D

from the patient's sample is bound by anti-25(OH) D antibodies on the microplate. Free antibody binding sites are occupied by labelled 25 (OH) D. The intensity of the colour formed after addition of the chromogen/ substrate solution is measured using a photometer. The colour intensity is inversely proportional to 25(OH) D concentration in the serum or plasma. All patients were followed up till discharge or death as the final outcome. All the cases were divided into two groups on the basis of 25(OH) D level, those with sufficiency and those with insufficiency. Serum 25(OH) D level ≥ 30 ng/mL was considered as sufficient and < 30 ng/mL was considered as insufficient. The data were analysed by using both descriptive and inferential statistics in SPSS version 16.0. For statistical comparison of cases with controls, Chi-square test (for categorical data) and unpaired t test (for continuous data) were used. P less than 0.05 was taken as statistically significant.

RESULTS

Data were collected from 120 patients, out of which 60 were cases and 60 controls. One sepsis case left the hospital after 6 days and was lost for follow-up. Hence, 59 sepsis cases completed the study. The mean age group for cases was 51.76 ± 18.87 years and for controls 47.63 ± 16.09 years. Males were more than females in both cases (63%) and controls (60%) (Table 1).

Age Group (Years)	Control		Cases	
	Number	%	Number	%
15-29	12	20.00	10	16.95
30-44	10	16.67	6	10.17
45-59	20	33.33	22	37.29
≥ 60	18	30.00	21	35.59
Total	60	100	59	100
Mean $\pm$ S.D.	47.633 $\pm$ 16.093		51.762 $\pm$ 18.871	
Sex				
Male	36	60.00	37	62.71
Female	24	40.00	22	37.29

Table 1. Distribution of Study Subjects by Age and Sex

Most of the patients were above 45 years of age (73%).

Vitamin D {25(OH) D}	Subject Groups				Total		P value*
	Control		Cases				
	No.	%	No.	%	No.	%	
Sufficient (≥30 ng/mL)	18	30.00	7	11.86	25	21.01	0.015
Insufficient (<30 ng/mL)	42	70.00	52	88.14	94	78.99	
Total	60	100.00	59	100.00	119	100.00	
Table 2. Status of 25 (OH)D Level in Subject Groups							

Table 2. Status of 25 (OH)D Level in Subject Groups

*Chi-square test, 25(OH) D insufficiency (< 30 ng/ml) was observed in 52 (88%) of cases and 42 (70%) of healthy controls. The difference was statistically significant ($p=0.015$) (Table 2). The Odds Ratio (OR) for sepsis and Vitamin D insufficiency was 3: 1

Subject Groups	No.	Mean 25(OH) D (ng/mL)	Std. Deviation	Std. Err Mean	t	P value*
Controls	60	20.702	12.478	1.611	3.113	0.002
Cases	59	13.871	11.423	1.487		

Table 3. Comparison of Mean 25(OH) D Level between Controls and Cases

*Unpaired 't' test. Mean 25(OH) D level was found to be 13.87 ± 11.42 in cases and 20.70 ± 12.47 in controls. The difference was statistically significant ($p = 0.002$) (Table 3).

Severity of Sepsis in Cases			Sufficient 25(OH) D (≥ 30 ng/mL)		Insufficient 25(OH) D (< 30 ng/mL)	
Cases	No.	%	No.	%	No.	%
Sepsis	33	55.93	6	85.71	27	51.92
Severe Sepsis	15	25.43	1	14.29	14	26.92
Septic Shock	11	18.64	0	0.00	11	21.16
Total	59	100	7	100	52	100

Table 4. Relationship of 25(OH) D Level with Severity of Sepsis at Time of Presentation

The severity of sepsis observed among the 59 cases were as follows: Sepsis- 33 (55.93%), Severe sepsis- 15 (25.42%) and Septic shock- 11 (18.64%). Out of the 52 cases of 25(OH)D insufficiency 27 (51.92%) were in sepsis, 14 (26.92%) in severe sepsis and 11 (21.15%) in septic shock at presentation. Out of the 7 cases having sufficient vitamin D level, 6 (85.71%) were in sepsis and 1 (14.28%) was in severe sepsis and none in septic shock at presentation. All the 11 patients admitted in septic shock had 25(OH) D insufficiency (Table 4).

25(OH) D Level	Outcome		Total
	Discharged N (%)	Death N (%)	N (%)
< 30 ng/mL (Insufficient)	41 (85.42)	11 (100)	52 88.14
≥ 30 ng/mL (Sufficient)	7 (14.58)	0 (0)	7 11.86
Total	48 (100)	11 (100)	59 100

Table 5. Association of 25(OH) D Level and Outcome of Sepsis

On follow-up, it was found that death occurred in 11 (18.64%) and the rest 48 (81.36%) were discharged. Out of the 48 cases that were discharged, 41 (85.42%) had insufficient and 7 (14.58%) had sufficient 25(OH) D level. All the 11 (100%) cases who died had vitamin D insufficiency. No mortality was found in the vitamin D sufficient group of patients (Table 5).

DISCUSSION

A total of 60 cases of sepsis and 60 healthy controls were enrolled in this study. One case was excluded from the study due to unknown outcome, so data was analysed from 59 cases. 73% cases were above the age of 45 years indicating that sepsis is more common in older age group. The mean age of our study population was 51.76 years and males outnumbered females (63% vs. 37%) comprised of 63%. Studies by previous workers also indicated a higher incidence of sepsis in the older age group and male preponderance. S. Todi et al reported from a multicentre trial done at 12 centres in India that the mean age of sepsis patients was 54.9 years, of which 67% were males.⁽⁸⁾ Sudhir et al observed that the incidence of sepsis was more in patients aged above 50 years (60%) with a male preponderance.⁽⁹⁾ The rise in the incidence of sepsis with increasing age could be due to the

compromised immune system, existing comorbidities like diabetes and multiple medications.

In this study, Vitamin D insufficiency [25(OH) D < 30 ng/mL] was observed in significantly greater number of sepsis cases (88.4%) than healthy controls (70%). Also, the mean 25(OH) D level was significantly lower in the sepsis group than the healthy control group ($p = 0.002$). It was also observed that a larger proportion of patients with 25(OH) D insufficiency were in severe sepsis (26.92%) and septic shock (21.15%) at presentation, while none with sufficient vitamin D level had septic shock during admission. All patients (100%) who were admitted in septic shock had vitamin D insufficiency. Several studies have provided similar data on the relationship between vitamin D and patients of sepsis. Jeng et al showed that 25(OH) D insufficiency was present in 100% of critically ill patients with sepsis, 92% of critically ill patients without sepsis and 66.5% of healthy controls.⁽¹⁰⁾ Ginde et al more specifically studied the effects of low vitamin D levels on the severity of sepsis, following up 81 patients suspected of having an infection in the Emergency Department. They found that serum levels of 25(OH) D less than 30 ng/mL were more likely at enrolment to have severe sepsis and SOFA (Sepsis-related Organ Failure Assessment) scores ≥ 2 and more likely at 24 h to have severe sepsis, SOFA scores ≥ 2 , APACHE II (Acute Physiology and Chronic Health Evaluation II) scores ≥ 25 and two or more organ dysfunction.⁽¹¹⁾ A retrospective study by McKinney et al conducted on 136 veterans admitted to the ICU who had a serum 25(OH) D drawn within a month before or after admission demonstrated that 98% of the veterans had low serum 25(OH) D concentrations.⁽¹²⁾ A recent systematic review and meta-analysis by Upala S et al assessing the association between serum 25(OH) D level and sepsis concluded that vitamin D deficiency is associated with higher chance of sepsis.⁽¹³⁾

In the present study, it was found that all the 11 (100%) cases who expired had insufficient 25(OH) D (< 30 ng/mL) level. No mortality was found in patients with sufficient 25(OH) D level (≥ 30 ng/mL). This is similar to the findings by Ginde et al, who also reported that all the sepsis patients who died during hospitalisation had 25(OH) D insufficiency.⁽¹¹⁾ In a retrospective study of more than 3000 patients, Moromizato T et al indicated that the risk for sepsis was 1.6 times higher in critically ill patients with serum 25(OH) D less than 15 ng/mL compared with those with a level of 30 ng/mL or higher. Finally, in the cohort with sepsis ($n = 568$), the multivariable-adjusted risk for 90 days mortality was 1.6-fold higher in the patients with 25(OH) D level less than 30 ng/mL than those with preadmission level more than 30 ng/mL.⁽¹⁴⁾

Our study has potential limitations. Firstly, it was a single centre study with a small sample size. So, our study results may not be generalisable. Secondly, 25(OH) D levels were measured within 24 hours of hospitalisation, which probably could be a reflection of pre-hospital insufficiency. However, larger multicentre prospective observational studies are required that measure Vitamin D level before critical illness to determine the causal relationship of sepsis.

CONCLUSION

Patients with sepsis had greater prevalence of Vitamin D insufficiency and lower mean Vitamin D level than healthy

adults. There was a greater association of severe sepsis and septic shock in patients with insufficient Vitamin D level than those with sufficient vitamin D level. Mortality was observed in patients with insufficient vitamin D level only.

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