



Original Article


A Study to Evaluate Levels of Serum Vitamin-D and Testosterone in Patients Admitted to ICU with Sepsis

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ABSTRACT

Background: Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, leading to organ dysfunction and high mortality. Despite advances in critical care, sepsis continues to pose a major healthcare burden. Emerging evidence suggests that hormonal and micronutrient imbalances, particularly involving serum testosterone and vitamin D, may influence disease severity and outcomes in critically ill patients.

Objectives: To evaluate the levels of serum vitamin D and testosterone in patients admitted to the intensive care unit (ICU) with sepsis and to determine their association with clinical outcomes.

Methods: This prospective observational study was conducted at JSS Medical College and Hospital, Mysuru. A total of 50 patients aged 18–65 years diagnosed with sepsis as per SEPSIS-3 criteria were included. Blood samples were collected within 24–48 hours of ICU admission to measure serum testosterone and vitamin D levels. Patients were followed until ICU discharge, and outcomes were categorized as survivors or non-survivors. Statistical analysis included Pearson's correlation to assess associations between biochemical parameters and outcomes.

Results: Among the 50 patients, 72% were non-survivors and 28% survived. Mean serum testosterone levels were significantly lower in non-survivors compared to survivors in both males (163.85 ± 88.57 vs. 320.30 ± 151.89 ng/mL; $p=0.018$) and females (14.78 ± 8.06 vs. 43.22 ± 23.29 ng/mL; $p=0.002$). Similarly, serum vitamin D levels were significantly lower in non-survivors (20.34 ± 8.93 ng/mL) compared to survivors (33.82 ± 18.01 ng/mL; $p=0.001$). No significant correlation was observed between testosterone and vitamin D levels ($p=0.066$).

Conclusion: Both serum testosterone and vitamin D levels are reduced in patients with sepsis and are significantly associated with poor clinical outcomes. These parameters may serve as potential prognostic biomarkers, although they appear to act independently.

Keywords: Vitamin-D, Testosterone, ICU, Sepsis, dysregulated .

INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. In the early stages, the clinical manifestations of this process are nonspecific and it is often underappreciated in clinical practice. However, early recognition of this syndrome is important to reducing mortality in sepsis. ¹

From clinical studies sepsis can be seen as a continuum of severity that begins with an infection, followed in some cases by sepsis, severe sepsis – with organ dysfunction – and septic shock. There has been a substantial increase in the incidence of sepsis during the last decades, and it appears to be rising over time, with an increasing number of deaths occurring despite a decline in overall in-hospital mortality. ²

Organ dysfunction in sepsis can be diagnosed as acute rise in SOFA (Sequential organ failure assessment) score of ≥ 2 at presentation to hospital in the background of infection.²

Patients with no previously known organ dysfunction can be taken as 0. An acute rise in SOFA score of ≥ 2 reflects a risk of approximately 10% mortality in general population. Septic shock can be defined as a subset of sepsis with organ dysfunction and persistent hypotension requiring vasopressors to maintain MAP ≥ 65 mm of Hg and serum lactate of > 2 mmol/L despite adequate volume resuscitation, such subset of patients with sepsis with septic shock have overall hospital mortality of $> 40\%$.^{2,3}

This SOFA score is used only to clinically characterize a patient with prognostication and cannot be used as a tool for in patient management.²

Sepsis can arise secondary to both hospital-acquired and community-acquired infections, most common source of infection leading to sepsis is pneumonia accounting for almost 50% of cases followed by intraabdominal and Urinary tract/genitourinary infections. Blood cultures are positive only in a third of total cases, out of the isolates, most common was staph. Aureus and strep. pneumonia in gram positive and E coli, klebsiella and pseudomonas aeruginosa in gram negative isolates.^{4,5}

Sepsis has been thought to be a result of excessive inflammatory host response, as time progressed it has been understood that the inciting infection causes a much more complex, variable and prolonged host response that causes solitary or multiple organ dysfunction leading to sepsis. Sepsis can be considered a complex clinical syndrome when host response gets inappropriately amplified. Dysregulation of host response to infection becomes a harmful host response.¹

OBJECTIVE

To evaluate levels of serum Vitamin-D and testosterone in patients admitted to ICU with sepsis.

MATERIALS AND METHODS

The present prospective study was done by the Department of Medicine at JSS Medical College and Hospital, JSSAHER at Mysore for a period of one and half year. Based on the results of previous studies⁶ the prevalence was found to be 13.1% and using the formula $z^2 p(1-p)/d^2$ where z is the statistic corresponding to confidence interval which is 95% and p is the prevalence of the condition according to previous studies which is 13.1% and d value is taken as 0.1. Which when calculated gives a value of 43.7 and with 10% of dropout rate added (48.07) and rounded off to nearest number gives a sample size of 50. Patients admitted to JSSMC & Hospital ICU during the study period diagnosed with sepsis were recruited consecutively. Samples were collected from medial cubital vein in a vacutainer. Patients between age groups of 18-65 years and both sexes will be recruited for the study.

Inclusion criteria:

1. Patients who are admitted to JSSMC&H, Mysore diagnosed with sepsis according the SEPSIS 3 guidelines, with an acute rise in SOFA score more than or equal to 2.
2. Patients between 18-65 years of age.

Exclusion criteria

1. Patients who were known or suspected to have low serum testosterone and/or Vitamin D independent of the disease under study like
 - Patients who were known case of primary hypogonadism (XX and XY anomalies, SRY gene abnormality-Turner's, Noonan's and Klinefelter's etc.)
 - Patients who were known case of Secondary hypogonadism (PCOD, Allman's, Hypogonadotropic hypogonadism)
 - Patients who were on long term anabolic steroid treatment/ Steroid abuse
 - Patients who were already on testosterone/ Vitamin D therapy
 - Known h/o adrenalectomy/orchiectomy/pituitary surgery or tumor
 - K/C/O hemochromatosis
 - Known opioid abuse/ chronic opioid usage for pain management
 - Patients who were on radio/chemotherapy
 - H/O viral orchitis, testicular torsion or any kind of testicular surgery.
 - H/O established (history wise/ treatment history) CLD, CKD, Renal failure and Nephrotic syndrome, Rickets and hypoparathyroidism before admission

Data was collected from the patients in form of blood samples in between 24-48 hours of admission to ICU among patients diagnosed with sepsis. And the outcome was noted at the time of discharge from ICU. The expected end points by the end of the study were improvement/discharge, further deterioration as evidenced by rise in SOFA score or Death. These end points were assessed and analyzed with relation to the variables in the study.

RESULTS

A total of 50 study subjects were evaluated and analyzed in the course of this study.

Table 1: Social Profile of the study subjects

		Frequency	Percentage
Gender	Male	33	66.0
	Female	17	34.0
Age Group	18-35	3	2
	36-50	4	4
	51-65	26	11

In the present study 33 of 50 (66%) were males and 7(34%) were females. Out of 50, total of 5 were in the age group of 18-35, 8 were in age group of 36-50 and the rest (37) were in age group of 51-65 years. Majority of patients recruited for the study were in age group of 51-65 with 26 among them being males and 11 being females. The mean age of the study subjects was 53.10 ± 11.62 years of age.

Table 2: Distribution of Study subjects based on the outcome of the patients

		Frequency	Percent
Outcome	Expired	42	72.0
	Improved	14	28.0
	Total	50	100.0

Out of the study sample, patients were divided based on outcome into non survivors (patients who expired plus patients who worsened during study period and was discharged against medical advice) and survivors (who survived current illness and was discharged). Out of 50 samples, 36 patients (72%) were non-survivors and rest were survivors (28%).

Table 3: Distribution of Subjects based on duration of hospital and ICU Stay

	Outcome			
	Expired		Improved	
	Mean	SD	Mean	SD
Duration of hospital stay	4.46	5.20	15.29	10.95
Duration of ICU stay	3.57	3.67	10.29	9.39

Mean duration of hospital stay and ICU stay was analyzed and a mean duration of hospital stay in survivors as 15.29 ± 10.95 days and mean duration of ICU stay 10.29 ± 9.39 days was observed in survivors. Even in non-survivors, mean duration of hospital and ICU stay were 4.4 ± 5.2 and 3.57 ± 3.6 days respectively. This indicates a prolonged course if ICU care probably also involving prolonged ventilator assistance.

Table 4: Distribution of serum testosterone and Vitamin-D based on outcome

			Outcome				Pearson Correlation	p value
			Expired		Improved			
			Mean	SD	Mean	SD		
Gender	S. Testosterone (ng/mL)	Male	163.85	88.57	320.30	151.89	0.410	0.018*
		Female	14.78	8.06	43.22	23.29	0.705	0.002**
S. Vitamin D (ng/mL)			20.34	8.93	33.82	18.01	0.455	0.001**

Serum testosterone and vitamin D levels were analyzed in serum of patients recruited for the study and mean values were calculated separately in case of testosterone for males and females and vitamin D was calculated in toto. Mean testosterone serum concentration is found to be 163 ± 88.57 ng/mL in males and 14.78 ± 8.06 in females who were non-survivors (reference range for males 247-821 ng/mL and for females 14-76mg/nL). In survivors, the testosterone serum concentration values were observed as 320.30 ± 151.89 in males and 43.22 ± 23.29 in females.

Serum vitamin D levels in patients with sepsis was found to be 20.34 ± 8.93 ng/mL in non-survivors versus 33.82 ± 18.01 ng/mL in survivors (reference range for Vitamin D-30-74 ng/mL).

These values were correlated with outcome at the end of study with Pearson's r bivariate analysis and there was significant association between serum testosterone levels and outcome in both males ($p=0.018$) and females ($p=0.002$) and also vitamin D and outcome ($p=0.001$).

Table 5: Correlation between S. Testosterone and Vitamin D levels in sepsis:

	Mean	SD	Pearson correlation	p value
S. Vitamin D (ng/mL)	24.12	13.43	0.262	0.066
S. Testosterone (ng/mL)	144.17	136.14		

As the secondary objective, overall mean serum vitamin D and testosterone was calculated and was correlated with each other using Pearson's r bivariate statistical analysis. Mean serum testosterone in sepsis was found to be 144.17±136.14 ng/mL and Vitamin D was observed to be 24.12±13.43 ng/mL

The correlation between vitamin D and serum testosterone was analyzed and was found to be insignificant (p=0.066). This shows that the serum vitamin D and testosterone are independent of each other and were also reduced in patients with sepsis.

DISCUSSION:

Sepsis is a dysregulated life-threatening host response to infection which still carries a high mortality despite advances in critical care. It is one of the leading causes of hospital associated mortality and also forms a significant percentage of healthcare expenditure every year. Studies and trials regarding sepsis are actively being pursued all over the world by government agencies and researchers alike. WHO also is running a surviving sepsis campaign for newer and advanced treatment modalities and hourly based guidelines to tackle the problem more effectively. However, sepsis still poses a major healthcare problem and continues to have high mortality rates.

Serum testosterone and vitamin D are also extensively studied regarding their role in acute and chronic illnesses and they are found to be significantly associated with different aspects of disease including the outcome of the illness. It has been consistently found that hypovitaminosis D and also Hypogonadism as evidenced by low serum testosterone are observed during acute phase of illness and several randomized controlled trials have been done to replace these hormones to alter the progression of disease.

This study was conducted in JSS medical college and hospital, a tertiary care referral center for analyzing serum testosterone and vitamin D levels in patients with sepsis. Patients have been selected from ICU randomly with admission diagnosis of sepsis as per sepsis 3 guidelines and was assessed for the relation with the illness and its outcome.

In our study, total number of patients who fulfill inclusion and exclusion criteria were selected, and total sample size was 50, of which 33(66%) were males and 17(34%) were females.

The mean age of study samples was 53.1±11.62 with minimum age of 24 and maximum age of 65 years. Of this, majority of patients were in 51-65 years of age category which accounted for about 74% of total samples. Younger patients of age less than 50 were only 13(26%) of study population.

Epidemiological data for age and sex distribution is lacking in Indian population. However, multiple large population studies were done worldwide. A study by Madsen et al.,⁷ study in 2014 done on a sample of 814 patients showed a mean age of patients as 66 with 44.8% being females. Another meta-analysis in 2017 by Papatransoglou et al.⁸, in Canada in cases of surgical sepsis with total sample size of 25,619 patients showed that 55.8% of the patients were males whereas 44.2% were females. Another prospective observational study in 2017 by Van Vught et al.,⁹ showed sepsis patients admitted to ICU were more frequently males with 61% of study population being males versus 39% of females. Another retrospective study from healthcare data from USA by Rhee et al.,¹⁰ in 2017 showed mean age of incidence of sepsis as 66.5 with SD of 15.5 with 42.4% of study population being females.

Older landmark studies by Schroder J et al.¹¹, showed mean age of 55.4 for females and 53.1 years for men in a prospective observational study. Another study by Martin GS et al.³, showed increased incidence among men compared to women with average age of 60.8 years with 48.1% incidence in men although with increased relative risk.

Although all the studies above showed varied results in incidence and frequency in age distribution, it has been shown that about 40-45% were females and rest were males, with a greater number of male patients compared to female patients. However, the discrepancies in data could be due to difference in reporting or due to bias. Older studies might have shown different results probably due to different criteria being used before 2016 for diagnosis of sepsis.

In our study, out of 50 patients in the study, total of 36(72%) expired and 14(28%) survived the illness. Another landmark retrospective study by Martin et al.,³ showed overall mortality nationally from sepsis as 27.8% (which declined to the later part of study period (2000) to 17.9%. Another retrospective observational study of national health records by Rhee et al.,¹⁰ showed 15.0% patients of the study population expired and 6.2% discharged to hospice (worsened), a total of 21.2%. Another study by Gaieski et al.,¹² in 2013 analyzed the outcomes of sepsis over a 6-month period and reported

to be ranging from 14.7% to 29.9% while using various abstraction methods. Latest study by Van der Woude et al., in 2018 which used only qSOFA for diagnosis of sepsis showed a mortality percentage of 29.4%¹³. Another retrospective study by Zhou et al., in 2017 conducted in Beijing, china showed overall hospital mortality rate of 20.6%¹⁴.

In our study, mean durations of hospital stay for survivors and non survivors were compared. The mean duration of hospital stay and ICU stay in non survivors was 4.46±5.2 days and 3.57±3.57 respectively and 10.95±0.5 and 10.29±9.39 in survivors. This is significant in the view that even survivors of sepsis will have prolonged hospital stay and also long-term morbidity and patients who survived also needs longer ICU care and ventilator care.

A study by Vishnupriya et al., showed mean duration of hospital and ICU stay as 9.68±9.2 and 4.78±6.24 respectively¹⁵. Another study by Sakr et al., showed median duration of ICU stay for patients with severe sepsis with septic shock as 5 days with slightly higher duration (7days) with higher SOFA score¹⁶. Another study from Nyugen et al., had showed that median length of hospital stay in sepsis was 7 days with slightly higher mean duration (7.65)¹⁷. Another study by Marik et al., showed mean duration of hospital and ICU stay as 5.1 and 9.1 days, respectively.¹⁸

In our study, the study parameters were serum testosterone and vitamin d levels and relation to sepsis. The testosterone levels were assessed by chemiluminescent immunoassay with samples being taken within 48 hours of ICU admission. Mean testosterone value in patients diagnosed with sepsis were 144.17±136.14 ng/mL. Later, these values were correlated with outcome in sepsis with pearsons r bivariate analysis. The mean value of serum testosterone in male non survivors was 163.85±88.57 ng/mL compared to 320±151.89 ng/mL in male survivors (p= 0.018). and female non survivors had serum testosterone mean concentration of 14.78±8.06 ng/dL as compared to 43.22±23.29 ng/mL in female survivors (p=0.002). (Reference values for testosterone in men were 247-821ng/mL and women were 14-76 ng/mL).

A study by Schröder et al., published in JAMA surgery on patients with surgical sepsis showed low serum levels of testosterone in men at about 25ng/mL¹⁹. Another study by Christeff et al., 48.99±8.64 ng/mL in male patients with septic shock²⁰. However, these studies did not correlate the outcome of the patients with serum levels of testosterone. Moreover, many more studies were done in experimental animals and there were consistent results that reduced testosterone was found in animals with induced sepsis²¹ but overall, our study data closely correlates with studies done elsewhere and shows that low serum levels of testosterone are seen in sepsis and correlates inversely with severity of sepsis with an impact on outcome.

It is also observed in many of the above studies that, serum estradiol levels were increased in sepsis along with decrease in testosterone which might imply that the enzymes in synthesis of testosterone were suppressed and the common precursor, androstenedione was utilized to produce estradiol. Another probable cause would be direct suppression of adrenals and testes by sepsis and host response.

In our study, we estimated the serum concentration of vitamin D in patients admitted to ICU with diagnosis of sepsis at admission. The mean serum concentration of vitamin D was found to be 24.12±13.43 ng/mL, moreover, when compared with outcomes by using Pearson's r bivariate analysis, mean values of vitamin D in survivors was found to be 33.82±18.01ng/mL compared to 20.34±8.93 ng/mL in non survivors of sepsis (p= 0.001). (Reference value 30-74 ng/mL)

Many studies were conducted in sepsis with regard to role of vitamin D in sepsis and trials are even underway for potential therapeutic role in sepsis. A latest meta-analysis by Zhou et al., which analyzed 24 independent studies conclusively showed that significantly low levels of vitamin D were observed in patients with sepsis as compared to patients with non-sepsis patients. However, this study has also concluded that there is no impact of vitamin D levels on mortality.²² Another prospective observational study by Vipul et al., has showed mean vitamin D level was significantly lower among patients with unfavorable outcome(expired)²³ and it was even found that vitamin D levels were inversely related to duration of hospital stay. A large-scale meta-analysis by Upala et al., which analyzed 21 studies on vitamin D and sepsis concluded that Vitamin D deficiency was associated with increased incidence of sepsis²⁴. Another review article published by Kempker et al., has found that many studies showed significantly low vitamin D values in critical illness and poorer outcomes with vitamin D deficiency²⁵.

Another case-control study by Parekh et al., on 61 patients showed serum vitamin D levels of 6.29 ng/mL and 19.3 ng/mL in patients with severe sepsis and sepsis respectively, compared to 26.64 ng/mL in controls (p=0.0001)²⁶. Further, this study has observed that there was significant difference between median vitamin D serum concentrations between survivors and non survivors (10.37 ng/mL vs 5.80 ng/mL respectively, p=0.025) and it was a strong predictor for 30-day mortality in sepsis.

A study done by Moromizato et al., measuring Vitamin D levels in patients prior to hospitalization in critically and analysis showed that vitamin D deficiency initially is a significant predictor of that patient developing sepsis during course of hospital stay²⁷.

These studies mentioned above and many other studies which were done conclusively shows that vitamin D deficiency is associated with sepsis, leads to sepsis and also is associated with worse outcome compared to patients with normal vitamin D levels. However, the contention remains that whether vitamin D deficiency is a risk factor for sepsis or the vitamin D deficiency is a consequence of sepsis leading to worsened outcome. However, our study data is significantly correlating with several large-scale studies and meta-analyses done worldwide.

CONCLUSION:

Serum testosterone levels are lower than normal in patients with sepsis and lower levels are associated with poorer outcome. Serum Vitamin D levels are lower than normal in patients with sepsis and lower levels are associated with poorer outcome. Serum testosterone and vitamin D levels are independent of each other in sepsis and probably also in healthy subjects.

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