Vitamin D deficiency in critically ill patients, does its deficiency matter?

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ABSTRACT

Introduction: Vitamin D supplementation during the critical illness for patients with vitamin D deficiency has been studied yet not fully. Daily supplementation of the recommended dietary allowance (RDA) of vitamin D slightly improve the 25(OH) D serum concentrations in those patients with vitamin D deficiency.

Methodology: A prospective observational study included 56 consecutive patients who admitted to the critical care department ICU, Cairo University. Patients were analyzed for serum level of 25-hydroxy vitamin D using ELISA technique and calculating APACHE II score on admission and SOFA score on admission (SOFA 0), after 48 hour (SOFA 1) and at discharge (SOFA 2).

Results: The mean age of the studied patients was 63.2 ± 17.4 years,42 (75%) were males and 14 (25%) were females. The mean duration of hospital stay was 13.6 ± 7 days with mean APACHE II score and SOFA on admission, 48 hours and 96 hours post admission. The mean value of vitamin D in the whole studied population was 19.01 ± 13.95 ng/ml. The levels showed insignificant statistical difference between male and female genders (18.9 versus 19.2 ng/ml,p value 0.9). The mortality rate was 7.14% (out of the studied 56 patients 4/56 patients. By comparing the length of hospital stay (LOS), APACHE II, SOFA scores and vitamin D level between survivors and non-survivors; there was a statistically significant higher APACHE II score, SOFA scores (SOFA 0, SOFA 1and SOFA2) and a lower vitamin D level in non-survivors.

Conclusion: vitamin D insufficiency is commonly observed in the critically ill patients. Vitamin D levels were found to be significantly lower in the critically ill non-survived patients and correlated significantly to APACHE II score on admission and SOFA scores. A cut off value of 9.9 ng/ml was adequately predictive for 28 days' survival with sensitivity of 72.5% and specificity of 75%..

Key words: Vitamin D, RDA, SOFA, Cairo University, APACHE.

INTRODUCTION

Some data in literature showed an association between vitamin D deficiency and poor outcome of patients in the intensive care units. Large studies showed an increased mortality in patients with vitamin D insufficiency and deficiency when compared to those with sufficient vitamin D concentrations. There is some evidence that the serum level of vitamin D decreases during the acute illness.

Vitamin D supplementation during the critical illness for patients with vitamin D deficiency has been studied yet not fully. Daily supplementation of the recommended dietary allowance (RDA) of vitamin D slightly improve the 25(OH) D serum concentrations in those patients with vitamin D deficiency. Data demonstrated that high dose therapy of vitamin D corrects the decreased serum levels of this vitamin; however, there are no available clinical data till now. [1].

Adherence between insufficient or deficient vitamin D levels and the outcome (mortality and morbidity) in the critically ill patients appears to be important. Vitamin D Supplementation will increase the serum concentrations of this vitamin; however, the optimal dose needs to be identified along with an assessment of clinical outcome [1]. The Recommended Dietary Allowance (RDA) for vitamin D in the first year of life is 400 IU daily. From 1 to 70 years of age the daily RDA is 600 IU and it increases to 800 IU for those adults over 70 [1]. It is well known that vitamin D is essential for bone health and calcium and phosphorus balance. Vitamin D deficiency is a

frequently reported medical problem and around 1 billion people on earth are estimated to have some degree of vitamin D deficiency [2,3]. Recent literature demonstrated that vitamin D has functions more than regulating calcium and phosphorus homeostasis [4–5].

Of note that vitamin D is responsible of upregulation and downregulation of more than 2000 genes [6]. Clinicians consider the optimal serum vitamin D level to be between 20 and 40 ng/mL (50 to 100 nmol/L) or between 30 and 50 ng/mL (75 to 125 nmol/L).

The association between low levels of vitamin D and critically ill patient needs more evaluation and whether this can be correlated with the outcome of those patients especially in those critically ill patients who had organ insufficiency namely liver and kidney disease that will halt activation of vitamin D.

The prolonged ICU stay and poor ultraviolet rays' exposure, malnutrition and high catabolic rates aggravates the risk of deficiency in this vitamin.

Aim of work:

The aim of this study was to evaluate the vitamin D serum levels in critically ill patients, and to study the correlation between its deficiency or insufficiency on the outcome.

PATIENTS AND METHODS

A prospective observational study included 56 consecutive patients who admitted to the critical care department ICU, Cairo University. Patients were analyzed for serum level of 25-hydroxy vitamin D using ELISA technique and calculating APACHE II score on admission and SOFA score on admission (SOFA 0), after 48 hour (SOFA 1) and at discharge (SOFA 2).

Inclusion criteria:

All patients with critical illness who need ICU admission and their APACHE II score more than 15 and their hospital length of stay not less than 6 days.

Exclusion criteria:

1) Chronic kidney disease.
2) End stage malignancy.
3) Malnutrition or malabsorption syndromes before ICU admission.
4) Age less than 18 years.

Evaluation of End point:

Mortality and morbidity described by SOFA score as indicator of organ failure assessment and length of ICU stay.

Methodology:

- Patients were selected according to the previously mentioned inclusion criteria and were thoroughly examined. Daily laboratory investigations including (serum creatinine, bilirubin total, bilirubin direct, WBCs, hematocrit, platelet count and arterial blood gases).

- APACHE II score was calculated on admission.
- SOFA score was calculated on admission, 48 hours later and at discharge.
- Serum Vitamin D assay (25-hydroxy vitamin D) using ELISA. Measured levels >30 ng/mL is considered sufficient, 15–30 ng/mL is considered insufficient, and <15 ng/mL is considered deficient.
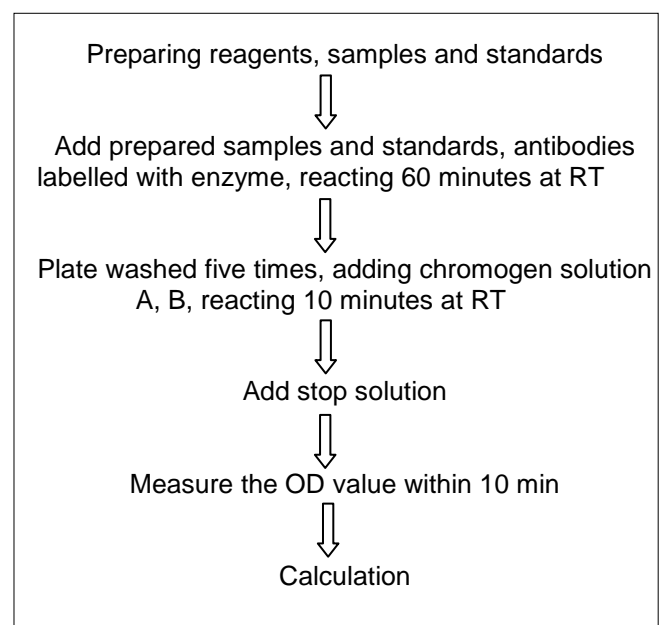
Test principle:

The kit uses a double-antibody sandwich enzyme-linked immune-sorbent assay (ELISA) to assay the level of Human Vitamin D3 (VD3) in samples. Add Vitamin D3(VD3) to monoclonal antibody Enzyme well which is pre-coated with Human Vitamin D3(VD3) monoclonal antibody, incubation; then, add Vitamin D3(VD3) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The Chroma of color and the concentration of the Human Substance Vitamin D3 (VD3) of sample were positively correlated (7)
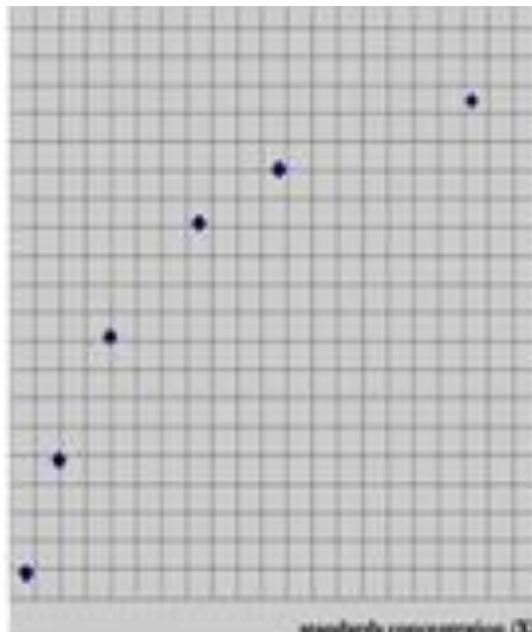
Specimen requirements:

1. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active
2. Extract as soon as possible after specimen collection, and according to the relevant literature, should be performed as soon as possible after the extraction. If it can't, specimen can be kept in -20℃ to preserve and avoid repeated freeze-thaw cycles.
3. Serum coagulation at room temperature 10-20 min, centrifugation 20-min at the speed of 2000-3000 r.p.m. removes supernatant and if precipitation appeared, Centrifugal should be done again.

Figure-1. Laboratory Procedure

Preparing reagents, samples and standards
⇩
Add prepared samples and standards, antibodies labelled with enzyme, reacting 60 minutes at RT
⇩
Plate washed five times, adding chromogen solution A, B, reacting 10 minutes at RT
⇩
Add stop solution
⇩
Measure the OD value within 10 min
⇩
Calculation

Figure-2. Results interpretation

Vitamin D level determination:

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, find out the corresponding density according to the sample OD value by the sample curve (the result is the sample density) or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density. (7)

Sensitivity, Assay range:

Sensitivity: 0.328ng/ml

(The sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by subtracting two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.)

Assay range:0.5ng/ml→150ng/ml

Intra-assay Precision: 3 samples with low, middle and high level Human VD3 were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level Human VD3 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100, Intra-Assay: CV<10% and Inter-Assay: CV<12%.

Statistical methods:

Data from patients was collected and analyzed statistically using Statistical Package for Social Sciences (SPSS/version 20) software. Arithmetic mean, standard deviation, for categorized parameters, chai square test was used, while for numerical data to compare between more than two groups ANOVA test (F-test) was used. The level of significant was 0.05.

RESULTS

Our study was conducted as a prospective study that enrolled 56 patients who were admitted to the critical care department ICU, Cairo University. and had APACHE II score more than 15, with minimal length of ICU stay > 7 days Various admission diagnoses were recorded.

The mean age of the studied patients was 63.2±17.4 years,42 (75%) were males and 14 (25%) were females.

The mean duration of hospital stay was 13.6±7 days with mean APACHE II score and SOFA on admission, 48 hours and 96 hours post admission as shown in table-1:

Table-1. Calculated APACHE II and SOFA among the studied patients.

	N	Min	Max	Mean	Std. Deviation
APACHE II	56	15.00	37.00	19.25	4.54
SOFA on admission	56	2.00	14.00	5.83	2.65
SOFA 48 hours	56	2.00	13.00	4.78	2.44
SOFA 96 hours	56	2.00	12.00	4.34	2.54

Table-2. Associated Co-morbidities among the studied patients

Co morbidities	No	%
No comorbidities	7	12.5
DM	21	37.5
HTN	31	55.4
Hepatic impairment	7	12.5
CVS	14	25
Ischemic heart disease	14	25
Congestive heart failure	3	5.3

The mean value of vitamin D in the whole studied population was 19.01±13.95 ng/ml. The levels showed insignificant statistical difference between male and female genders (18.9 versus 19.2 ng/ml,p value 0.9). The mortality rate was 7.14% (out of the studied 56 patients 4/56 patients) as shown in figure (3).

By comparing the length of hospital stay (LOS), APACHE II, SOFA scores and vitamin D level between survivors and non-survivors; there was a statistically significant higher APACHE II score, SOFA scores (SOFA 0, SOFA 1and SOFA2) and a lower vitamin D level in non-survivors as shown in table (3) and figure (4).

By analysis of ROC curve for a cut off value of vitamin D levels that would be considered safe to predict survival among studied population; a cut off value of 9.9 ng/ml was significantly predictive for survival with AUC of

0.79, sensitivity of 72.5% and specificity of 75% with p value of 0.05 as shown in figure (5).

There was a weakly significant inverse correlation between Vitamin D levels and APACHE II scores (R was -0.228 and p value was 0.05) & SOFA at 48 hours (R was -0.248 and p value was 0.05) as shown in the scatter plot figures (6&7).

Figure-3. Mortality rate.
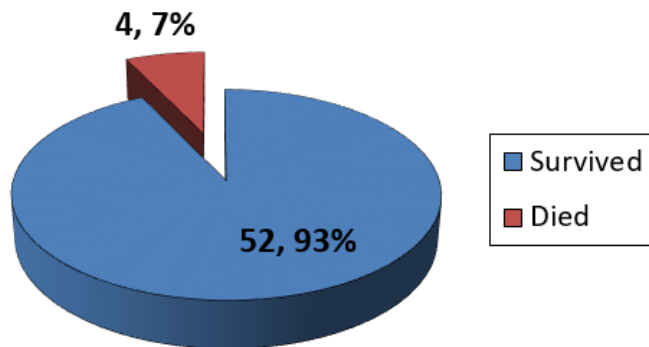
Table-3. APACHE II, SOFA scores and vitamin D level in survivors and non-survivors

|	Group	Mean	Std. Deviation	P
LOS	Survived	13.529	7.22317	0.9
	Died	13.250	5.85235	
APACHE	Survived	18.400	2.77010	0.0001
	Died	26.000	9.55685	
SOFA 0	Survived	5.5000	2.19694	0.009
	Died	9.0000	5.22813	
SOFA 1	Survived	4.3400	1.88019	0.0001
	Died	9.5000	3.69685	
SOFA 2	Survived	4.0200	2.14276	0.0001
	Died	8.5000	4.04145	
Vitamin D	Survived	20.039	14.25938	0.0001
	Died	9.1750	.91788	

DISCUSSION

Vitamin D(25(OH)D) levels varied between studies as the level to be considered deficient or insufficient ranged between 15 or 20 ng/ml, also it differed according the studied population either ward or ICU patients, male or female, young or elderly even varied between season at which samples were taken. Vitamin D sufficiency may be broadly defined as a circulating 25(OH) D level that satisfies physiologic needs.

Figure-4. APACHE II, SOFA scores and vitamin D level in survivors and non-survivors
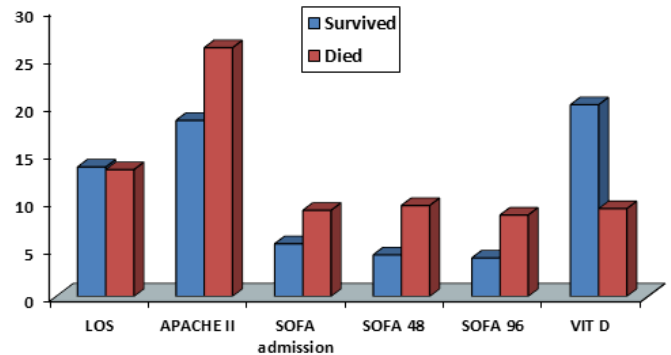
**Figure-5. ROC curve for a cut off value of vitamin D levels**
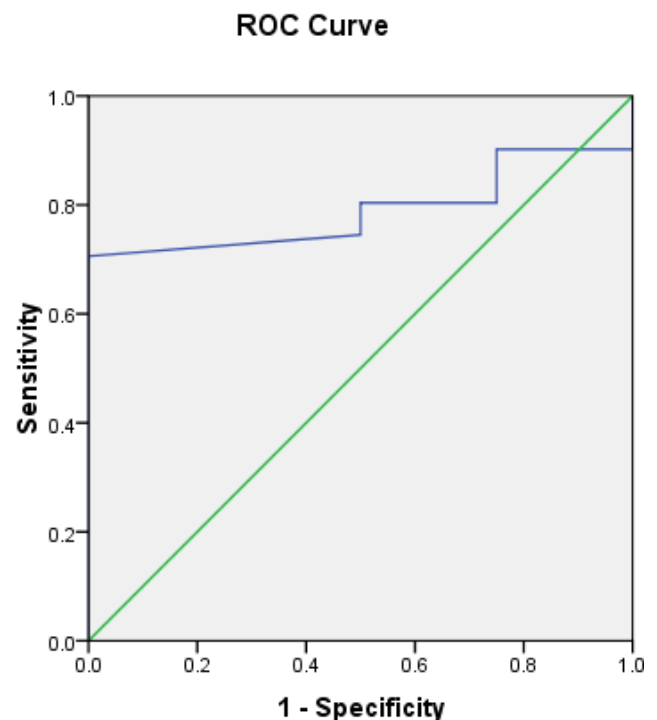

Diagonal segments are produced by ties.

According to the 14th International Workshop Consensus Conference on Vitamin D, in the general population, a minimum 25(OH)D level of 20 ng/mL (50 nmol/L) is necessary to support bone and mineral health, and 30–40 ng/mL (75–100 nmol/L) is necessary to maintain muscle strength and immune functions [8]. But the question persists does these levels can be satisfactory in ICU patients in whom many other factors vary regarding vitamin activation and high catabolic rates?

The most published studies to evaluate vitamin D insufficiency in critically ill patients have applied definitions used in the general population [4–6]. Therefore, we used a minimum 25(OH)D level (20 ng/mL) as a cutoff to define insufficiency in our ICU population.

Figure-6. Correlation between vitamin D levels and APACHE II score
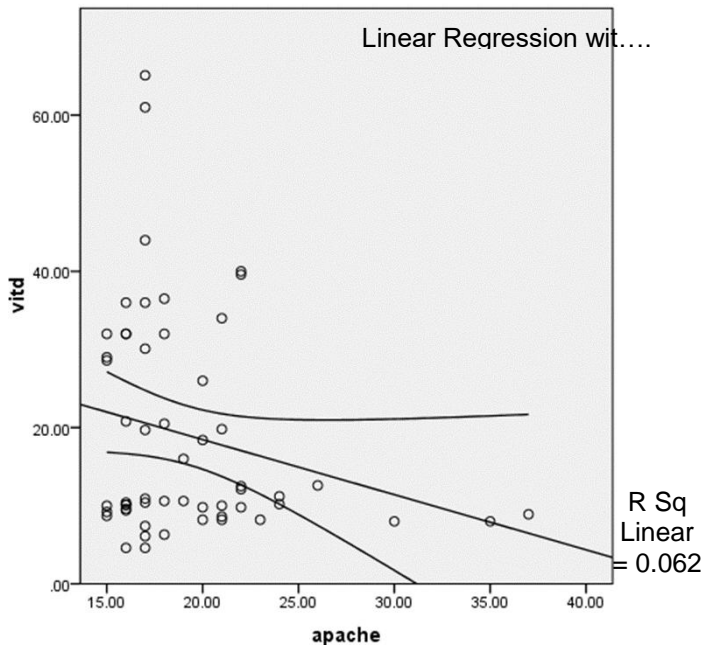
Figure-7. Correlation between vitamin D levels and SOFA scores.
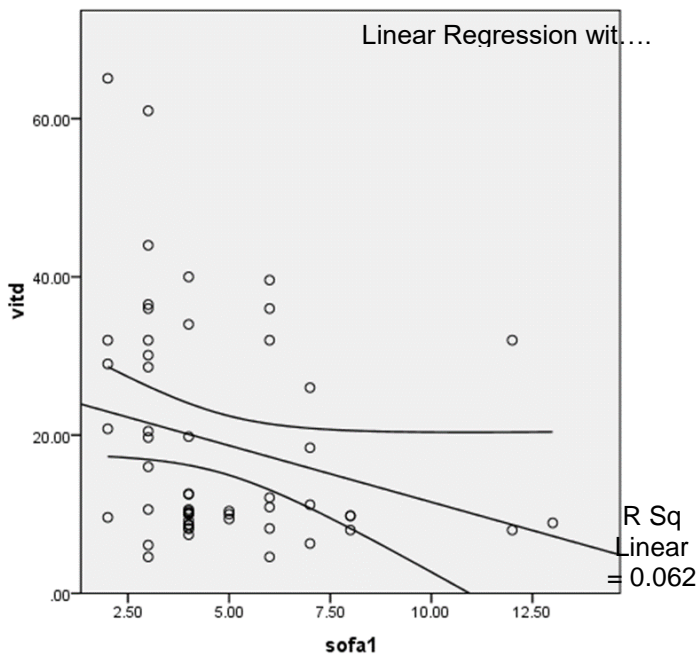
Although a lot of criteria were applied across the studies, reported prevalence of vitamin D insufficiency ranged from 26% to 82% in ICU patients [5, 6]. This reported prevalence is nearly 50% higher than that of patients in general medical wards [5, 6]. The current prospective observational study showed vitamin D deficiency in 75 % of the studied population admitted to our general ICU with the mean 25(OH)D level of 19.1 ng/dl.

This finding was in accordance with Amrein et al in 2014 in their retrospective study done on 655 surgical and non-surgical ICU patients and showed that vitamin D25 (OH) was deficient and insufficient in almost 86% of the study group, in their study vitamin D deficiency was considered if the levels were below 20 ng/ml and insufficient if 20-30 ng/ml. (9).

Most published studies show a higher prevalence of vitamin D insufficiency in women and the elderly in the general population [2]. We did not find any correlation between vitamin D insufficiency and age or gender in the current study in contrast to the study by McKinney et al., we did not find any correlation between 25(OH)D insufficiency and increased length of ICU stay (LOS) [10].

Some studies suggest an association between vitamin D insufficiency and mortality in critically ill patients. [11–14] In the study by van den Berghe et al., vitamin D levels were lower among non survivors in critically ill patients [15]. A study by Lee et al. revealed a threefold mortality rate in vitamin D insufficient patients compared to those who were sufficient (25(OH)D >24 ng/mL) [16]. Another study by McKinney et al. revealed that vitamin D insufficiency was nearly twice as prevalent among non survivors in ICU [10]. The reported higher mortality in critically ill patients with vitamin D insufficiency might be due to changes in glucose and calcium metabolism and/or immune and endothelial cell dysfunction [17,18]. In our study, we found a correlation between high APACHE II, SOFA scores as surrogate indicator of organ failure, mortality and vitamin D insufficiency in univariate analysis; however, vitamin D insufficiency was not found as an independent risk factor for mortality in multivariate analysis.

We thought it is so difficult to segregate causes of mortality in ICU patients and correlate it to solely to vitamin D deficiency alone ,the critically ill patients usually have many deficiencies not limited only to vitamins, in a large randomized controlled double blind single centered study done by Amrein et al over 2 years on ICU patient with vitamin D levels less than 20 ng/ml enrolled to either receive high dose of vitamin D 3 or placebo, in their study, they found no statistical significant difference regarding length of stay or mortality rate between study or control groups, this finding could indicate that mortality was related to the severity of illness on ICU admission measured objectively by APACHE II score and that was associated with organ dysfunction and multiple deficiencies but not correlated only to vitamin D deficiency (9).

In a study by Moraes et al, analysis of ROC curve showed 12 ng/mL as the best cutoff level correlated with mortality. Even if 25(OH)D is only a marker of disease severity, they suggested that vitamin D levels <10-12 ng/mL could be used to identify those at a higher risk of

poor outcome and hence higher mortality and mandated higher level of monitoring of these patients. they suggested that, ICU patients with severe vitamin D deficiency (Dietary vitamin D intake is reduced in critically ill patients) show the most unfavorable consequences from the lack of vitamin D; from this finding, they inferred that these patients are most likely to make benefit from supplementation of vitamin D during their stay in the ICU. This hypothesis should be confirmed by large clinical trials. (19)

Large multicenter randomized controlled study is actually needed to define the levels at which we should consider vitamin D deficiency which is diagnosis dilemma.

The current study had some limitations. First, it was a single center study with a small sample size; therefore, may not be generalizable yet it represents an Egyptian snap shot look on magnitude of vitamin D deficiency among ICU patients. Second,25(OH)D levels obtained within 24 hours on admission are probably a reflection of preadmission insufficiency. Third, we did not follow the 25(OH)D levels sequentially.

In conclusion, vitamin D insufficiency is commonly observed in the critically ill patients. Vitamin D levels were found to be significantly lower in the critically ill non survived patients and correlated significantly to APACHE II score on admission and SOFA scores. A cut off value of 9.9 ng/ml was adequately predictive for 28 days' survival with sensitivity of 72.5% and specificity of 75%. Further larger studies should be considered for possible benefit of adding vitamin D level for the commonly used clinical and laboratory scores that are used for prediction of morbidity and mortality in critically ill patients.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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