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Original Research Article

PROSPECTIVE STUDY OF VITAMIN D IN CRITICALLY ILL PATIENTS AND ITS ASSOCIATION WITH MORTALITY

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ABSTRACT

A potential role of vitamin D in immune response modulation was first identified with the discovery of vitamin D receptors in activated human inflammatory cells. Both vitamin D deficiency and insufficiency are prevalent worldwide. Neither condition poses an immediate risk in otherwise healthy individuals. However, vitamin D deficiency or insufficiency may influence outcomes in critically ill patients. Analysis of samples collected from 60 patients admitted to a medical/surgical ICU in a tertiary care hospital was done. Data collected included the underlying disease, basic hematological and biochemical blood test results, APACHE II scores, SOFA scores and serum 25-hydroxyvitamin D₃ levels. A total of patients 49 (81.6%) had insufficient or deficient level of vitamin D. Mortality was higher in patients who had APACHE II scores, SOFA scores and lactate levels on admission and had more difficult clinical courses with more organ dysfunction ($p < 0.05$). Also, they had lower ionized calcium levels, lower glomerular filtration rates and higher levels of parathyroid hormone. Positive findings in prospective studies may establish 25(OH) D₃ as an important and modifiable risk factor for ICU mortality. There is no doubt that Vitamin D deficiency is commonly seen in intensive care patients. Although it is not an independent decisive factor for mortality, it might be related to worse clinical status at ICU admission. The effect of vitamin D replacement on mortality is controversial, but the APACHE II score and number of organs dysfunctional are still important parameters for increased mortality.

KEYWORDS: Vitamin D, Calcitriol, APACHE II score

Vitamin D is a fat-soluble vitamin that is synthesized in the skin in response to sunlight exposure and also obtained, to a limited extent, from dietary intake (vitamins D₂ and D₃). Vitamin D is stored in adipose cells and is converted in the liver to its circulating form, 25-hydroxyvitamin D₃ [25(OH)D₃]. 25(OH)D₃ is converted to its active metabolite, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], also known as calcitriol in kidneys. Calcitriol synthesis is enhanced by parathyroid hormone (PTH) and hypophosphatemia. The endocrine action of calcitriol on bone mineral metabolism has been known for decades. It is known that vitamin D deficiency is associated with diabetes mellitus, chronic obstructive pulmonary diseases and autoimmune diseases. A potential role of vitamin D and its active metabolite 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] in immune response modulation was first identified with the discovery of vitamin D receptors in activated human inflammatory cells. It is now known that Vitamin D affects a wide range of biological processes, including cell proliferation and differentiation, regulation of inflammation, immune modulation, muscle strength, in addition to bone formation and electrolyte homeostasis. Reduced formation of calcitriol in the tissues might lead to impaired immune responses, mucosal barriers and endothelial functions. Its action on innate immunity is stimulatory, while its action on adaptive immunity is mainly considered to be modulatory.

Vitamin D is synthesized in response to ultraviolet light, sun exposure remains important for maintaining a healthy level of vitamin D. In recent years, sun exposure has declined because people are spending more time indoors, a phenomenon particularly common in younger individuals. Since very few foods naturally contain vitamin D, natural sunlight remains the major source of vitamin D for most individuals. Accordingly, the prevalence of vitamin D deficiency remains strongly associated with factors affecting ultraviolet light intensity, including geographic location, skin tone, and time of year.

Vitamin D deficiency is known to be related with disease severity, increased systemic inflammatory markers, increased infection and mortality (Jeng *et al.*, 2009). Vitamin D receptors are found in nearly all types of immune cells. (Aranow, 2011). Various clinical studies and trials have shown correlation between vitamin D and systemic infections. Its deficiency has been associated with acute respiratory tract infections as proved in research by Ginde *et al.*, 2009, cardiovascular diseases and other chronic illnesses. Scientists are now exploring its possible association with acute life-threatening illnesses.

Vitamin D deficiency is defined as serum vitamin D levels below 10 ng/mL, and the incidence in intensive care patients varies between 17% and 82%

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(Hewison *et al.*, 2004; Arnson *et al.*, 2012) Vitamin D insufficiency is defined as serum vitamin D levels between 10 to 20 ng/ml. Both vitamin D deficiency and insufficiency are prevalent worldwide. Neither condition poses an immediate risk in otherwise healthy individuals. However, vitamin D deficiency or insufficiency may influence outcomes in critically ill patients. Low vitamin D status is reported to simulate mild acute phase response in which cause elevated concentrations of C-reactive protein (CRP), several hemostatic factors and different pro-inflammatory cytokines. Vitamin D deficiency is known to be related with disease severity, increased systemic inflammatory markers, increased infection and mortality (Higgins *et al.*, 2012).

Data about vitamin D deficiency and the role of its replenishment are from Western countries where the sources of vitamin D, especially some kinds of food, sunlight exposure, habits of vitamin supplementation, or genetic variation may differ from other countries. Several studies from Asia (Mora *et al.*, 2008; Pilz *et al.*, 2008) reported the prevalence of vitamin D deficiency; however, these studies were performed in healthy individuals. There are limited data about the prevalence and impact of vitamin D deficiency among patients with critical illness.

So, the role of vitamin D in critically ill patients has not yet been fully established. Despite the numerous disease conditions associated with vitamin D deficiency in the general population, the relationship of this deficiency to outcome in critically ill patients remains unclear. The relevance of vitamin D measurement and kinetics at ICU admission is open to debate. Furthermore, the 25(OH)D₃ levels required for proper maintenance of the pleiotropic effects of vitamin D remain unknown. Data regarding the relationship between 25-hydroxyvitamin D₃ levels and outcomes in the medical intensive care unit are sparse. The goal of the study was to evaluate the prevalence of 25-hydroxyvitamin D₃ deficiency in the intensive care units. This study also investigated the association between circulating vitamin D levels and mortality rates in critically ill patients.

MATERIALS AND METHODS

Study Design

This prospective study was conducted in Bhagat Phool Singh Government Medical College, a tertiary care hospital located in Sonapat district of Haryana which is a 450 bed teaching hospital with 19 ICU beds. Data was collected from 60 patients admitted in ICU during a period of 2 months.

Inclusion Criteria

All adult men and women, 18 years old or older, expecting to require at least 48 hours of critical care (as determined by the treating ICU team) were deemed eligible to participate. Informed consent was obtained

from the subjects if they were oriented to person, place and person. Surrogate consent was obtained when subjects did not meet these criteria. All questions were asked from the participants in a language they could easily comprehend. Each patient was given 15-20 minutes.

Exclusion Criteria

The patients who were under 18 years old, readmitted or had elective surgery were excluded from the study. Patients were also excluded if they didn't have suitable healthcare proxy (when patients were not able to provide consent), if they had recent history of vitamin D supplementation more than or equal to 4,000 IU/d or if they refused to participate.

Blood Sample Processing and Vitamin D Measurement

Following informed consent, fresh blood was acquired from an indwelling venous catheter and collected directly into a red vacutainer. The sample was allowed to clot. The serum so obtained was stored at temperature of 2 to 8 degree Celsius and assays were performed at the central research laboratory. All the reagents and specimens were allowed to come to room temperature before doing the procedure. All reagents were gently mixed without foaming.

Total 25(OH)D (combined D₂ and D₃) was be measured by enzyme-linked immunosorbent assay (ELISA), using commercially available calbiotech kit.

Clinical Data Collection

Study was carried out after getting approval from institute's ethics committee. The database contained information on demographics, medications, laboratory values, microbiology data, procedures and the records of inpatient and outpatients, length of ICU stay and mortality. The patient's APACHE II (Acute Physiology and Chronic Health Evaluation II) score will be calculated to measure the severity of disease for adult patients admitted to intensive care units. It is applied within 24 hours of admission of a patient to an intensive care unit (an integer score from 0 to 71 is computed based on several measurements). Higher scores correspond to more severe disease and a higher risk of death. It is calculated from a patient's age and 12 routine physiological measurements:

1. PaO₂ (depending on FiO₂)
2. Temperature (rectal)
3. Mean arterial pressure
4. pH arterial
5. Heart rate
6. Respiratory rate

7. Sodium (serum)
8. Potassium (serum)
9. Creatinine
10. Hematocrit
11. White blood cell count
12. Glasgow Coma Scale

The sequential organ failure assessment score (SOFA score) was also calculated. The score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems.

Statistical Analysis

Data was analyzed by using SPSS software. Descriptive statistics included frequencies and percentages for categorical variables and means and standard deviations for continuous variables. Continuous variables were analyzed using a t-test. Categorical data was described as number (percentage) and analyzed with the chi-square test. Data to be analyzed included age, diagnosis, length of stay in the hospital, number of organs dysfunctional, APACHE II score and vitamin D level. Multivariate logistic regression analysis included age, diagnosis, number of organs dysfunctional, APACHE II score and vitamin D level at ICU admission. The type 1 error level was set as 0.05. Data were presented as mean ± standard deviation.

OBSERVATIONS AND RESULTS

A total of patients 49 (81.6%) had insufficiency or deficient level of vitamin D. Among these 49 patients, 36 (73.4%) had insufficiency and remaining 13 (26.5%) had deficiency. Vitamin D levels recommended by Cal biotech Inc. recommended are shown in the Table 1. The 28-day mortality rate was 36.6%. No patient received supplemental vitamin D during his or her ICU stays. Nonsurvivors had higher APACHE II scores, SOFA scores and lactate levels on admission and had more difficult clinical courses with more organs dysfunctional (p < 0.05). Electrolytes, liver and renal function tests were assessed in 48 patients as one patient was on dialysis. Non-survivors had lower ionized calcium levels, lower glomerular filtration rates and higher levels of parathyroid hormone.

While age, gender, diagnosis, vitamin D level at ICU admission and length of ICU stay were similar in

both groups, the APACHE II score and the number of organs dysfunctional were significantly higher in non-survivor patients (p<0.05 for both). In the multivariate logistic regression model, the likelihood of mortality was increased 7.2-fold and 7.9-fold for an APACHE II score ≥24 and for the number of organs dysfunctional ≥2, respectively (p<0.001). (Table 2 to Table 5)

Table 1: Serum vitamin D level Range

Grading	Vitamin D Level (ng/mL)
Deficient	<10
Insufficient	10-20
Sufficient	>20
Intoxication	>150

Table 2: Demographic details of Vitamin D Deficient patients

Variable	No. of patients	Percentage
Sex		
Males	32	65.3
Females	17	34.69
Admission Diagnosis		
Cardiac	4	8.16
Respiratory	14	28.57
Abdominal	13	26.53
Neurology	2	4.08
Poisoning	8	16.32
Others	8	16.32
Variable	Mean ± SD	
Age	46 ± 13	
Admission APACHE	19.4 ± 3.1	
Vitamin D levels	15.1 ± 4.8	
ICU Stay	13.2 ± 11.4	
Mechanical Ventilation	8.4 ± 6.1	

Table 3: Comparison of Survivors and Non Survivors

Variable	Survivors	Non Survivors	P Value
Age	65 ± 13.1	67 ± 14.4	0.23
APACHE II Score	17.1 ± 4.38	23 ± 5.2	< 0.001
Vitamin D Levels	18.2 ± 6.9	13.1 ± 7.4	0.61
Albumin	2.5 ± 0.41	2.3 ± 0.3	0.54
ICU length	21.1 ± 18.1	15.4 ± 15.6	0.12
Length of MV	9 ± 15.1	13 ± 12.8	0.14
Creatinine	1.9 ± 5.1	2.8 ± 4.8	0.31
Lactate	18.6 ± 9.8	25.3 ± 16.4	0.04
SOFA Score	5.1 ± 2.1	7 ± 3.9	< 0.001
PTH	97.7 ± 88.1	204.2 ± 98.6	0.023

SOFA: Sequential organ failure assessment; APACHE II: Acute physiological and chronic health evaluation; MV: Mechanical Ventilation; PTH: Parathyroid hormone.

Table 4: Electrolytes and Renal function of patient not on Dialysis

Variable	Survivors	Non Survivors	P Value
Calcium	4.9 (4.6-5.1)	4.2 (3.8-4.7)	0.011
GFR	70 (49-70)	62 (25-63)	0.031
Phosphate	3.9 (3.3-5.2)	4.2 (3.7-5.3)	0.436
Magnesium	3.0 + 0.4	2.9 + 0.46	0.445

Table 5: Multivariate logistic Regression Model for Mortality

Component	Odds Ratio (Confidence Interval)	P Value
Vitamin D at admission <30ng/dl	0.61 (0.1-1.3)	0.31
Age >62	0.12 (0.1-1.4)	0.43
APACHE > 24	9.1 (4.2-18.1)	<0.001
No. of organs dysfunctional>2	8.1 (3.5-16.1)	<0.001
Creatinine	0.94 (0.81-1.12)	0.45
Albumin	0.41 (0.30-1.09)	0.43
Ventilation Days	1.1 (1.05-1.2)	0.62

DISCUSSION

The present study showed that vitamin D deficiency was commonly observed in critically ill patients at ICU admission. Moreover, serum vitamin D level was poorly and negatively correlated with APACHE II score. However, lower vitamin D levels were associated only with higher APACHE II score and more number of organs dysfunctional. Recent studies have reported the incidence of Vitamin D deficiency in intensive care unit as 17-82%. In our study the incidence of vitamin D came out to be 81.6%, though on the higher side of previous results, still consistent with the previous data.

The wide range of prevailing rates reported in various studies may result from particular characteristics of the populations studied. Our study didn't found any significant relation of sex with vitamin D deficiency. But Previous studies have demonstrated sex as an important risk for vitamin D deficiency. A possible explanation for

this greater risk in other studies might be due to the tradition of women avoiding sun exposure or greater usage of sunblock devices and sunscreen among women than men in the areas where previous studies were carried out.

Previous researches have suggested that serum vitamin D level is in inverse proportion to serum calcium and albumin level. Our results suggest inverse relation between Vitamin D and calcium but no significant association of albumin and vitamin D.

Several studies report that vitamin D deficiency in critically ill patients is associated with infection, the development of sepsis and acute respiratory distress syndrome (ARDS) and increased mortality rates. Moromizato *et al.*, 2014, found that serum vitamin D level below 16 ng/ml is associated with sepsis. Van de Berghe *et al.*, 2003, showed significantly lower serum vitamin D levels in non-survivor critically ill patients. In a COPD study, a range of 20–24 ng/mL was found to be

related to decreased mortality. In contrast to the above studies, Cecchi *et al.*, 2011, concluded that serum vitamin D levels do not have any significant effects on the outcome in septic patients. In the present study, the median vitamin D levels in survivors was 18.2 ± 6.9 ng/ml, but there is no direct association of low vitamin D levels with mortality. Although our result shows that Low Vitamin D levels are associated with higher APACHE II and more organs dysfunctional i.e. indirect association with mortality is present.

Our study could not demonstrate a relationship between length of hospital stay and duration of mechanical ventilation of patients according to vitamin D deficiency status. Previous studies have demonstrated inconsistent findings regarding the length of hospital stay. Venkatram *et al.*, 2011, similar to our study, did not find any influence from vitamin D deficiency on the length of hospital stay, while McKinney *et al.*, 2011 found a two-fold increase in length of ICU stay when patients had $25(\text{OH})\text{D}_3 < 20$ ng/mL.

Septic patients with vitamin D deficiency also have significantly higher serum levels of intact Parathyroid Hormone (PTH), hypocalcemia, inhibition of 1α -hydroxylase activity, and $1,25(\text{OH})_2\text{D}_3$ deficiency as the most common causes of PTH elevation. Vitamin D is a key participant in the calcium-PTH axis, which is responsible for maintaining calcium homeostasis (Holick, 2008). In study by Nair *et al.*, 2013, vitamin D insufficiency and deficiency affected the prognosis of critical illness as evidenced by the association of higher PTH levels with worse outcomes in patients in the ICU. In the present study, PTH responders comprised patients with hypocalcemia and patients with hypovitaminosis D. This finding is consistent with a previous report by Kritchevsky *et al.*, 2012 that hypovitaminosis D and secondary hyperparathyroidism are highly prevalent among critically ill patients. Indeed, higher PTH concentrations are reportedly associated with an increased mortality risk among older individuals in the general population (Hagström *et al.*, 2009). Plasma PTH levels have also been shown to predict cardiovascular mortality among community-dwelling individuals, even in those with normal PTH levels (Schmitz *et al.*, 2009). Elevated PTH levels can also decrease insulin sensitivity, aggravate endothelial stress, and suppress the immune system, probably making patients more susceptible to infection (Horl, 2004).

In contrast to previous reports on the relationship (Holick *et al.*, 2011) between clinical outcomes and vitamin D deficiency our study could not demonstrate vitamin D deficiency to be associated with poor clinical outcomes. A possible reason for this result might be the small number of subjects (deficient/insufficient levels in 49 patients) in the vitamin D insufficient group were not adequate to show a meaningful difference in clinical outcomes. Additionally,

the present study included all patients who were admitted to the ICU rather than those with a specific disease category; thus, the determinants of prognosis might be multifactorial. The most significant limitation of this study was the small, single-center, retrospective design without sequential vitamin D blood sampling.

As a result of this work, we can only conclude that increased mortality is related to increased APACHE II score and increased number of organs dysfunctional. The low serum vitamin D levels might only be related to increased APACHE II score, and they might be solely responsible from the worse clinical status at ICU admission. Thus, if there is low vitamin D level in patients with high APACHE II score at ICU admission, it is recommended to treat the vitamin D deficiency.

Positive findings in prospective studies may establish $25(\text{OH})\text{D}_3$ as an important and modifiable risk factor for ICU mortality. There is no doubt that Vitamin D deficiency is commonly seen in intensive care patients. Although it is not an independent decisive factor for mortality, it might be related to worse clinical status at ICU admission. The effect of vitamin D replacement on mortality is controversial, but the APACHE II score and the number of organs dysfunctional are still important parameters for increased mortality. Given the safety profile of vitamin D, positive results provide strong motivation for examining the utility of vitamin D as a novel therapeutic agent designed to improve survival in critical illness.

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