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SHORT COMMUNICATION

Supraphysiological 25-hydroxy vitamin D_3 level at admission is associated with illness severity and mortality in critically ill patients

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Abstract We studied the association between admission serum 25-hydroxy vitamin D_3 level and in-hospital mortality in a prospective cohort of critically ill patients admitted to the medical intensive care unit of a tertiary care referral center. Of the 180 patients enrolled, 129 were included. Vitamin D_3 deficiency was observed in 37 % ($n = 48$) and supra-physiological levels (≥250 nmol/L) in 15.5 % ($n = 20$). Patients with supraphysiological vitamin D_3 levels were grouped as outliers. There was no difference in mortality ($p = 0.41$) between vitamin D_3 deficient (21/48) and non-deficient (36/81) patients in analysis with and without outliers. Patients with vitamin D_3 ≥250 nmol/L had a significantly higher ($p = 0.02$) Simplified Acute Physiology Score (SAPS) II and mortality ($p = 0.003$) [mean (SD) 60.1 ± 17.1 and 75 % (15/20), respectively] when compared with the rest [45.6 ± 18 and 38.5 % (42/109), respectively]. The sensitivity, specificity and SAPS II independent odds ratio to predict mortality in patients with supraphysiological vitamin D_3 levels were 26.3, 93.1 and 3.7 % (95 % confidence interval 1.2–11.4; $p = 0.03$), respectively. In conclusion, vitamin D_3 deficiency in our cohort was not associated with mortality. A patient subset with supra-physiological vitamin D levels had higher illness severity scores and mortality. Extrinsic factors interfering with test results were ruled out. A biological hypothesis to explain this observation is proposed. Further clarification of mechanisms leading to this observation is warranted.

Keywords 25-Hydroxyvitamin D · Critical illness · SAPS II score · Mortality

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Introduction

Vitamin D is a steroid hormone vital not only for skeletal health and mineral metabolism but also for processes disrupted in critical illness such as endothelial proliferation, glucose homeostasis and immunomodulation [1]. Vitamin D's role as an extra-skeletal hormone may be accentuated in critically ill patients to meet metabolic demands such as cathelicidin release by macrophages, endothelial repair and blood glucose control [1]. Thus, several studies have examined the relationship between vitamin D levels and mortality.

Some studies report a high prevalence of vitamin D deficiency (>50 %) in critically ill patients with worse outcomes (a direct causal effect is unproven) [2–4], while other studies that may have been underpowered, did not observe this [5, 6]. Variation in the definition of Vitamin D deficiency limits comparison between studies. The study of vitamin D in the critically ill is further hampered by fluid shifts and diurnal fluctuations [7].

There is paucity of information on vitamin D in critical illness from the Indian subcontinent. Indian studies suggest

that 70 % of the general population is vitamin D deficient [8]. In the context of a high background prevalence of Vitamin D deficiency, we set out to assess the relationship between vitamin D levels at intensive care unit (ICU) admission and outcome in critically ill patients.

Materials and methods

Setting and study population

This study was conducted at a 2200-bed tertiary care referral centre in South India (12°55′N 79°11′E) over 5-months, during the post monsoon season, in 2010. The study was approved by the institutional review board and the human research ethics committee (No: 7261; 11.08.2010). Written, informed consent was obtained from the relatives of patients prior to inclusion. Consecutive patients admitted to the medical ICU were recruited irrespective of organ system involvement and followed up till death or hospital discharge. Exclusion criteria were death or transfer out within 24-h, transfer from other ICUs or discharged against medical advice.

Definitions

Vitamin D deficiency was defined as serum 25-hydroxy vitamin D_3 level <50 nmol/L (<20 ng/mL). Levels between 50 and 75 nmol/L (20–29 ng/mL) indicated relative insufficiency and ≥75 nmol/L (>30 ng/mL), a physiological replete state. Levels ≥250 nmol/L (≥100 ng/mL) were considered supraphysiological [9].

Variables and outcomes

Demographics, admission diagnosis, co-morbidities (diabetes, hypertension, cardiac, respiratory, renal, hepatic disease), smoking and alcohol consumption, drug therapy, particularly Vitamin D supplementation in the past year and underlying neoplastic processes were documented. The simplified acute physiology score (SAPS II) was calculated [10]. The primary outcome was in-hospital mortality. Secondary outcomes included ventilator-free days [11] and duration of ICU and hospital stay.

Serum measurements

Blood was sampled within 4-h of ICU admission and assayed for serum 25-hydroxy vitamin D_3, total and ionised calcium and phosphate. Serum samples were stored at 4 °C, batched and analysed daily. Assay: Serum 25-hydroxyvitamin D_3 level was measured by a sandwich electrochemiluminescence immunoassay on a Roche Hitachi Modular System Analyser E170 (Roche Centralised Diagnostics, Mannheim, Germany) with a lower detection limit of 10 nmol/L and a upper detection limit of 250 nmol/L [between-run co-efficient of variation (CV%) at 57 nmol/L 8.4 %, at 145 nmol/L 6.5 % and at 210 nmol/L 4.7 %]. The total calcium (Ca^{2+}) and inorganic phosphorus (PO_4^{3-}) were measured on a Roche Hitachi Modular System Analyser P800 (Germany) by ion selective electrode and photometric method (between-run CV%: Ca^{2+} at 8.5 mg/dL 5.1 % and at 11.8 mg/dL 5.6 %; PO_4^{3-} at 4 mg/dL 5.5 % and at 7.2 mg/dL 4.4 %). The ionised calcium i (Ca^{2+}) was measured on an Instrumentation laboratory GEM premier 4000 analyzer (Instrumentation Laboratory Milan, Italy) by ion selective electrode.

Statistical methods

Sample size was calculated based on a previous study that showed an inverse relation between Vitamin D levels and SAPS II based predicted mortality [5]. Data was analysed using STATA v10.0 (StataCorp, TX, USA). All statistical tests were two tailed. A p value <0.05 was considered significant. Continuous variables were summarized as mean [standard deviation (SD)] if normally distributed or as median with interquartile ranges. Comparisons between two groups (low/normal vitamin D) were done using the independent two sample t-test or Mann–Whitney U test as appropriate for continuous variables and Chi square or Fisher's exact test for dichotomous variables. The Kruskal–Wallis test was used for analysis of vitamin D levels in occupational subgroups. Sensitivity, specificity and likelihood ratio of high vitamin D

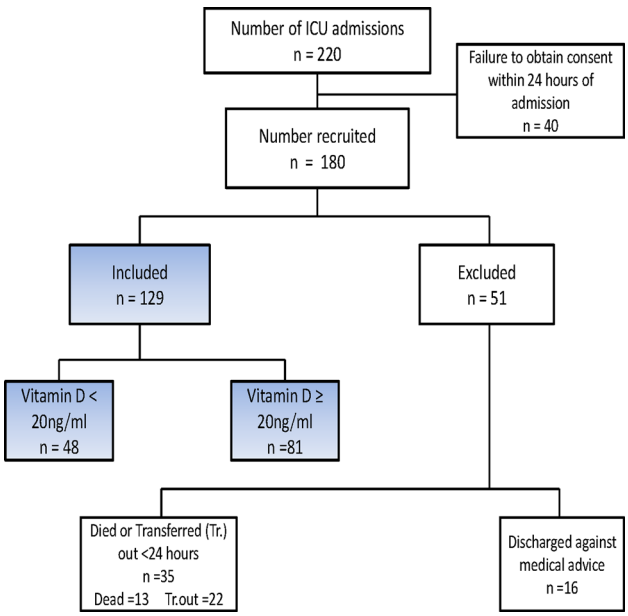

Fig. 1 Strobe diagram of progress through study phases

Table 1 Demographics

Variable	
Age in years (mean ± SD)	46.5 ± 16.2
Male:female ratio	1.4:1
Admission SAPS II score (mean ± SD)	47.9 ± 17.1
Median admission serum 25-hydroxy vitamin D (10–250 nmol/L)	53.6
Mean admission serum ionised calcium[a] (mg/dL)	4.3 ± 0.6
Mean admission serum phosphate[b] (mg/dL)	4.5 ± 3.0
Level of 25-hydroxyvitamin D, % (*n*)	
Sufficient (>75 nmol/L)	75.3 (97)
Insufficient (51–75 nmol/L)	38.7 (50)
Deficient (≤50 nmol/L)	96.8 (125)
Supraphysiological (≥250 nmol/L)	38.7 (50)
Disease profile, *n* (%)	
Infections	84 (65.1)
Primary respiratory disease	14 (10.8)
Malignancy	12 (9.3)
Primary cardiac disease	8 (6.2)
Poisoning/envenomation	5 (3.8)
Autoimmune disease	3 (2.3)
Primary neurologic disease	3 (2.3)
Co-morbidities, *n* (%)	
Diabetes mellitus	41 (31.8)
Hypertension	40 (31)
Chronic obstructive pulmonary disease	15 (1.6)
Chronic renal failure	12 (9.3)
Ischemic heart disease	8 (6.2)
Chronic liver disease	6 (4.7)
Cerebrovascular accident	2 (1.6)

[a] Ionised calcium reference range 4.5–5.6 mg/dL

[b] Serum phosphate reference range 2.5–4.6 mg/dL

levels were estimated using standard diagnostic test evaluation, with mortality as the gold standard. Independent association of vitamin D levels to mortality was estimated using logistic regression analysis.

Results

Demography

Of the 180 patients recruited, 129 patients (77 males) were included and 51 excluded (Fig. 1). The mean (SD) age of the cohort was 46.5 (16.2) years and SAPS II score, 47.9 (17.1). Ninety-five patients (74 %) were ventilated; ventilator-free days were 14.0 (12.8). Patient characteristics are summarized in Table 1. Co-morbidities and liver transaminase levels were similar in patients with Vitamin D_3 levels <50 and >50 nmol/L.

Table 2 Comparison of characteristics between patients with vitamin D levels <250 nmol/L and vitamin D ≥250 nmol/L

	Vitamin D <250 nmol/L (*n* = 109)	Vitamin D ≥250 nmol/L (*n* = 20)	*p* value
Baseline characteristics			
Age in years (mean ± SD)	46.1 (15.3)	48.6 (12.6)	0.5
Male:female (*n*)	62:47	15:5	0.1
Calcium (median, IQR), mg/dL	4.4 (4.12–4.6)	4.3 (4.1–4.5)	0.2
Phosphate (median, IQR), mg/dL	3.5 (2.2–5.1)	5.7 (4.75–7.5)	0.05
Creatinine (median, IQR)[a], mg/dL	1.3 (1–2.2)	2.45 (1.3–4.2)	0.01
CPK (median, IQR)[b], U/L	156 (66.3–460.3)	550 (85.5–1505.5)	0.14
Distribution of co-morbidities			
Diabetes	35 (32.1)	6 (30)	0.85
Hypertension	35 (32.1)	5 (25)	0.53
COPD	14 (12.8)	1 (5)	0.46
CRF	11 (5.5)	1 (5)	0.69
IHD	7 (6.4)	1 (5)	0.81
Smoking	7 (6.4)	2 (10)	0.41
CLD	4 (3.6)	2 (10)	0.23
CVA	2 (1.8)	0 (0)	0.99
Indicators of illness severity and mortality rates			
SAPS score (mean ± SD)	45.6 (18)	60.1 (17.1)	0.02
Ventilator free days at (28 days) (median, IQR)	19 (0–28)	0 (0–10)	0.03
Mortality, % (*n*)	38.5 (*n* = 42)	75 (*n* = 15)	0.003

CPK creatinine phosphokinase, *IHD* ischemic heart disease, *CVA* cerebrovascular accident, *CLD* chronic liver disease, *CRF* chronic renal failure, *COPD* chronic obstructive disease

[a] Serum creatinine reference range 0.7–1.4 mg/dL

[b] CPK reference range 45–195 U/L

Assay reliability

The serum vitamin D_3 assay tested reliable using the intraclass correlation co-efficient, ICC (ICC at vitamin D <50, 50–249 and ≥250 nmol/L: $r = 0.99$ with estimated mean difference of −1.05 between two measurements; 95 % confidence interval (CI) −2.11, −0.0001 and between-run CV% at 57 nmol/L 8.4 %, at 145 nmol/L 6.5 % and at 210 nmol/L 4.7 %).

Prevalence of vitamin D deficiency

None of the patients/patient relatives gave a history of vitamin D supplementation. The prevalence of vitamin D

deficiency (<50 nmol/L; <20 ng/mL) at ICU admission was 37 %. The vitamin D distribution curve displayed a bimodal pattern with ≥250 nmol/L observed in 15.5 % ($n = 20$). Individuals with vitamin D_3 ≥250 nmol/L were grouped and analysed as outliers. These patients had similar age and co-morbidities as individuals with levels <250 nmol/L (Table 2). Mean serum vitamin D_3 levels, including and excluding outliers were 92 nmol/L and 64 nmol/L, respectively, while inpatients who died or were transferred within 24-h (excluded group) were 87.9 nmol/L and 91 nmol/L, respectively.

Outcomes

Overall mortality was 44.2 % (57/129). Survivors had significantly ($p < 0.0001$) lower SAPS II (40 ± 14) as compared with non-survivors (58 ± 15). Though serum vitamin D_3 levels were significantly ($p = 0.05$) higher [median 77 (IQR 40–250) nmol/L] in patients who died than in survivors [median 65 (IQR 35–82.3) nmol/L], there was no significant ($p = 0.42$) difference in mortality between patients with vitamin D_3 deficiency [21/48 (43.8 %)] and those with serum vitamin D_3 ≥50 nmol/L [36/81 (44 %)]. When patients with Vitamin D_3 levels ≥250 nmol/L were excluded, the difference in vitamin D_3 levels between survivors and non-survivors ceased to be significant and the mortality rate of vitamin D <50 and ≥50 nmol/L groups continued to be insignificant.

Patients with serum vitamin D_3 levels ≥250 nmol/L had significantly ($p = 0.003$) higher mortality [15/20 (75 %)] than those with levels <250 nmol/L [42/109 (38.5 %)]. All patients with supraphysiological vitamin D levels, who expired, died of sepsis related causes.

The sensitivity, specificity and odds ratio (OR) to predict mortality with supraphysiological levels of vitamin D_3 were 26.3 % (95 % CI 0.07–0.5), 93.1 % (95 % CI 0.8–1.0) and 4.8 (95 % CI 1.5–17.9) respectively. Logistic regression analysis showed an adjusted OR of 3.7 (95 % CI 1.2–11.4; $p = 0.03$) and 4.31 (95 % CI 1.69–11.01; $p = 0.002$) for vitamin D and SAP II scores, respectively to predict mortality.

Discussion

The prevalence of vitamin D deficiency in our cohort at ICU admission was 37 %, much lower than the community population prevalence of 70 % in our region [8]. The reported prevalence of vitamin D deficiency in critical illness ranges from 17 % to 85 % [2, 4–6, 12, 13] with two studies having similar prevalence [5, 6] to ours. Of note, 15.5 % of patients in our study who had supra-physiological vitamin D levels had higher severity of illness scores and mortality.

We did not observe an association between vitamin D deficiency and mortality, consistent with one other published ICU study [14]. Four other ICU based studies, however, suggested an association between vitamin D deficiency and mortality [2, 3] [5, 6]. In a large study of 2399 patients *pre-admission* vitamin D deficiency increased odds of death (OR 1.69, 95 % CI 1.28–2.23; $p < 0.0001$) relative to vitamin D sufficient patients [15].

We observed a significant ($p = 0.003$) association between supraphysiological vitamin D levels and mortality, even after adjusting for severity of illness. This observation is not surprising given a recent publication involving 4418 cardiac surgical patients, that showed a significant association (OR 2.34, 95 % CI 1.12–4.89) between high (>100 nmol/L) *pre-operative* 25-hydroxy vitamin D levels and major cardiac and cerebrovascular events [13]. In another 9-year follow-up study of the third national health and nutrition examination survey, a reverse J-shaped association between vitamin D and mortality was observed [16]. Vitamin D levels >120 nmol/L were associated with mortality (RR 1.5, 95 % CI 1.02–2.3).

Several steps were taken to confirm the validity of the observed supraphysiological vitamin D levels. Haemolysis, the only extrinsic factor affecting the electrochemiluminescence immunoassay was minimized by careful collection and transport of samples [17]. None of the patients with supraphysiological levels had evidence of a haemolytic disorder. Vitamin D supplementation was ruled out on history and medication records. Serum 25-hydroxy vitamin D levels are low or unaffected in granulomatous diseases, characterised instead by an excess of 1α, 25-dihydroxy vitamin D [18, 19]. These diseases were, therefore, considered an unlikely explanation for the supraphysiological levels observed.

A putative model may explain the observations of a lower prevalence of vitamin D deficiency than expected and a patient subset with high vitamin D_3 levels (≥250 nmol/L) associated with a poor outcome. Renal 1-alpha hydroxylase is classically induced by parathormone. The induction of *extra-renal* 1-alpha hydroxylase is instead controlled by the absolute concentration of its substrate and not by parathormone [1]. Vitamin D metabolism in vivo, thus, follows first order reaction kinetics [20]. The K_m or substrate concentration of 25-hydroxy vitamin D required for 50 % maximal activity of 1 α-hydroxylase is ~100 nmol/L (40 ng/mL) [21]. This suggests that enzyme induction and consequent vitamin D activation only occurs when tissue 25-hydroxy vitamin D concentrations are above this threshold value.

In settings with restricted vitamin D supplies, 25-hydroxy vitamin D utilisation in physiological pathways other than calcium-phosphate homeostasis is compromised [20]. In order to meet increased extra-skeletal requirements

in vitamin D insufficient or deficient critically ill individuals, we hypothesize a release of 25-hydroxy vitamin D from adipose tissue and muscle [22, 23] in order to drive extra-renal 1-α, 25-dihydroxy vitamin D production, with a concomitant rise in serum 25-hydroxy vitamin D levels. If this is indeed true, a rise in vitamin D in insufficient or deficient critically ill individuals would only result in an apparent normalisation of serum values. This may explain the lower than expected prevalence of vitamin D deficiency in the critically ill patient. More severe illness may create a greater local need for 25-hydroxy vitamin D which, when accompanied by rhabdomyolysis (Table 2), may result in supra-physiological levels. These aspects warrant further study.

Our findings of an association between supraphysiological vitamin D levels and mortality is of significance to Asian countries where, as a consequence of high community prevalence of vitamin D deficiency, more patients may have apparently "normal" vitamin D levels during critical illness [24].

Some limitations merit mention. Serial vitamin D estimations, rather than single estimation, would have provided greater insights given the variation in vitamin D levels due to fluid shifts following large volume fluid resuscitation [7]. Critical illness associated with decline in vitamin D binding globulin (VDBG) may also affect serum vitamin D [25]. Although VDBG was not measured in this study, the potential impact of this is likely to be small given that vitamin D levels were measured within 4-h of ICU admission.

In conclusion, the prevalence of vitamin D_3 deficiency in our critically ill cohort is less than the community prevalence in the same region. Vitamin D deficiency was not associated with higher mortality. However, a subset of critical patients who had supra-physiological vitamin D levels had high mortality despite adjusting for severity of illness. The hypothesis proposed in our paper to explain the findings warrants further study.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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