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Effect of Severe Vitamin D Deficiency at Admission on Shock Reversal in Children With Septic Shock: A Prospective Observational Study

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Abstract

Objectives: To evaluate the association of severe vitamin D deficiency with clinically important outcomes in children with septic shock. **Methods:** We enrolled children ≤17 years with septic shock prospectively over a period of 6 months. We estimated 25-hydroxyvitamin D [25 (OH) D] levels at admission and 72 hours. Severe deficiency was defined as serum 25 (OH) <10 ng/mL. We performed univariate and multivariate analysis to evaluate association with clinically important outcomes. **Results:** Forty-three children were enrolled in the study. The prevalence of severe vitamin D deficiency was 72% and 69% at admission and 72 hours, respectively. On univariate analysis, severe vitamin D deficiency at admission was associated with lower rates of shock reversal, 74% (23) versus 25% (3); relative risk (95% confidence interval [CI]): 2.9 (1.09-8.08), at 24 hours and greater need for fluid boluses (75 vs 59 mL/kg). On multivariate analysis, nonresolution of shock at 24 hours was significantly associated with severe vitamin D deficiency after adjusting for other key baseline and clinical variables, adjusted odds ratio (95% CI): 12 (2.01-87.01); 0.01. **Conclusion:** The prevalence of severe vitamin D deficiency is high in children with septic shock admitted to pediatric intensive care unit. Severe vitamin D deficiency at admission seems to be associated with lower rates of shock reversal at 24 hours of ICU stay. Our study provides preliminary data for planning interventional studies in children with septic shock and severe vitamin D deficiency.

Keywords

vitamin D deficiency, 25 (OH) D, septic shock, inotrope score, prevalence, severe vitamin D deficiency

Introduction

Vitamin D deficiency is common in critically ill children.[1-6] While the primary role of this pleotropic hormone is regulation of calcium metabolism, it also plays a key role in several pathways of the innate immune response system, controlling cell growth, differentiation, and apoptosis.[6,7] Therefore, it is not surprising to find that in states of immune dysregulation such as severe sepsis, the deficiency would have important clinical implications.[8-15] A recent meta-analysis evaluating effects of vitamin D deficiency (<50 nmol/L) in critically ill adult patients on occurrence of infection, sepsis, and mortality showed a significant increase in infection rate, sepsis, and mortality.[14] However, evidence on such outcomes in children with severe sepsis or septic shock is limited.[16-18]

Few studies in children have reported high prevalence of vitamin D deficiency in children with suspected sepsis mostly from the developed world.[1,2,16,18] While in few of these studies, it has been associated with greater illness severity at presentation, increased need for inotropes, mechanical ventilation, and culture positive infection,[16,18] in others no such association was found.[17] The mortality due to severe sepsis and septic shock are much higher in resource restricted settings in comparison to units from the developed world.[19,20] However, there is a paucity of literature on the prevalence and outcome of vitamin D deficiency in children with septic shock. Only 1 study from the Indian subcontinent has been published so far in children with suspected sepsis[17] and in this study, the authors reported the prevalence to be about 50% and found no significant

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association with key clinical outcomes. Thus, even though the immunomodulatory role of vitamin D and association of hypovitaminosis D and sepsis has been shown across studies,[6-11,13-15] whether vitamin D deficiency is the cause or effect of severe sepsis is not yet clear. Due to the limited data available in the pediatric population[16-18] and lack of interventional studies to show that administration of vitamin D may improve clinical outcomes, opinion is still divided as to whether it is just an innocent bystander or a marker of severe disease in patients with sepsis. Further evidence from the pediatric population and interventional studies is therefore needed to address this dilemma.

Prevalence of severe vitamin D deficiency (<10 ng/mL) was 61% in critically ill children in a previously published study from our intensive care unit (ICU).[5] The prevalence may be higher in children with septic shock owing to its role in immunomodulation and the levels may fall further during the course. The mortality due to fluid refractory septic shock is as high as up to 60% in the Indian pediatric intensive care unit (PICU)[21] and any intervention that would improve the outcomes would be helpful. However, until more evidence is available on the association of vitamin D deficiency in septic shock and clinical outcomes, it would be difficult to plan clinical trials with vitamin D in septic shock.

The most commonly and widely used definition of vitamin D deficiency has been a cutoff value of 25-hydroxyvitamin D [25 (OH) D] of <20 ng/mL or <50 nmol/L and is also recommended by the American Academy of Pediatrics committee on nutrition, US Endocrine Society and Institute of Medicine[22-25] for starting therapy in symptomatic cases. A level of 25 (OH) D of <10 ng/mL has been referred to as "very low level" or "severe deficiency" in various studies and has been shown to be associated with increased risk of respiratory infections, rickets, osteomalacia, and affects the immunomodulatory response in sepsis.[26-31] Severe deficiency like mild to moderate deficiency has been shown to be associated with increased disease severity, duration of stay, as well as mortality in few while no association has been found in few other studies.[14,32-34] In a study of 492 critically ill adults with vitamin D deficiency, the authors observed that the subset of patients with 25 (OH) D levels of <12 ng/mL had significantly higher risk of 28-day mortality.[35]

With this background, we hypothesized that prevalence of severe vitamin D deficiency may be high in children with septic shock and that this deficiency would be associated with clinically important outcomes such as shock resolution and mortality.

Materials and Methods

Design and Setting

This was a prospective cohort study conducted over a period of 7 months (January 2014 to July 2014) in children ≤17 years of age admitted to PICU of a tertiary care hospital of a developing country.

Table 1. Baseline Demographic and Clinical Characteristics of Study Population.

Variable	N = 43
Age (median, IQR)	4 (0.5, 10)
Male (n, %)	24 (56)
PIM-2 score (median, IQR)	22 (11.6, 33)
Weight (median, IQR), kg	12 (5, 19)
Duration of sun exposure in hours (median, IQR)	2.5 (1, 4)
Admission season: January–February (n, %)	18 (42)
Admitting diagnosis (n, %)	
Pneumonia	9 (22)
Gastrointestinal illness	9 (22)
Meningitis	6 (14)
Skin and soft tissue infection	3 (8)
Tuberculosis	3 (8)
Malaria	3 (5)
Dengue	8 (19)
No focus at admission	2 (5)
Symptomatic hyocalcemia at admission (n, %)	7 (16)
Laboratory investigations (mean [SD] or median [IQR])	
Total calcium, mg/dL	8.2 (1)
Phosphate, mg/dL	3.1 (0.3)
Ionized calcium, mmol/L	0.67 (0.22)
Albumin, g/dL	2.8 (0.2)
SGOT, U/L	53 (31, 182)
SGPT, U/L	35 (21, 108)

Abbreviations: CI, confidence interval; IQR, interquartile range; PIM, pediatric index of mortality; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

Participants

Consecutive children with features of septic shock admitted to the PICU (as per definitions; Supplemental Table 1) were screened for eligibility. We excluded children who were already on vitamin D supplementation, had received large doses for rickets, or documented vitamin D deficiency in the past 1 year or steroids for at least 10 days before admission, or had preexisting or acute liver disease, recent kidney stones, or chronic kidney disease. Eligible children were enrolled in the study after obtaining informed written consent from parents. The study was approved by the institutional ethics committee.

Objectives and Outcome Measures

Our primary objective was to evaluate the association between severe vitamin D deficiency, defined as serum 25 (OH) D <10 ng/mL, and resolution of shock (proportion attaining therapeutic end points) in the first 24 hours. Our secondary objectives were to estimate the prevalence of severe vitamin D deficiency in children with septic shock and to evaluate its association with other clinically important outcomes such as need for fluid boluses in the first 6 hours, duration of PICU stay, and mortality. The definitions used for the purpose of the study are provided in Supplemental Table 1.

Methods

Eligible children were enrolled and data were recorded on specified data collection form. The children were managed as per preexisting protocol for management of septic shock as per the surviving sepsis campaign guidelines 2012.[35] Data collected included diagnosis, demographic variables, illness severity score (pediatric index of mortality-2 [PIM-2]) at admission, duration of sun exposure (determined by questioning the parents as to the number of hours the child stayed outdoors on an average per day), and clinical details on a daily basis till death or discharge from the hospital. For examining association of severe deficiency at admission and clinically important outcomes, we recorded information for outcome variables such as pediatric logistic organ dysfunction (PELOD) score at 24 hours, need for fluid boluses during the first 6 hours, need for mechanical ventilation, inotrope score at 24 hours, duration of ICU stay, and mortality. Relevant laboratory tests were performed on all patients at admission. Arterial lactate, ionized calcium, and parathyroid hormone were measured at inclusion. Samples for estimation of serum 25 (OH) D levels were drawn at admission *before administration of fluid boluses* alongside other blood tests. Samples were cold centrifuged at 4°C and the plasma aliquoted and stored at −20°C till sufficient samples were collected to run the test. Serum 25 (OH) D was measured with automated chemiluminescent immunoassay technology (VITROS eci, Johnson and Johnson Ortho Clinical Diagnostics, UK). The analytical sensitivity of this test is 4 ng/mL for 25 (OH) D with a reportable range of 4 to 512 ng/mL.

Statistical Analysis

Data were entered into Microsoft Excel 2007 and analyzed using Stata 11.2 (Stata Corp, College Station, Texas). Results are presented as mean (SD) or median (interquartile range) as appropriate for continuous variables and as absolute numbers (%) for categorical variables. For determining association between severe vitamin D deficiency and outcomes, we performed univariate analysis using Student *t* test/ Wilcoxon rank-sum test and χ^2 test for continuous and categorical variables, respectively. As our primary objective was to study the association between severe vitamin D deficiency at admission and rate of shock resolution, we performed multivariate regression analysis with nonresolution of shock resolution as the dependent variable after adjusting for important variables such as age, gender, PIM-2, diagnosis, and need for fluid boluses in the first 6 hours. The selection of baseline variables was before the start of the study. We used clinically important variables irrespective of *P* values for the multivariate analysis. The results of the multivariate analysis are reported as odds ratio (OR)/mean difference with 95% confidence intervals (CI).

Results

A total of 51 children were admitted with septic shock during the study period. Of these, 7 were excluded as per prespecified exclusion criteria, and 1 excluded as parents refused to give consent for blood sampling (Figure 1). A total of 43 children were enrolled in the study.

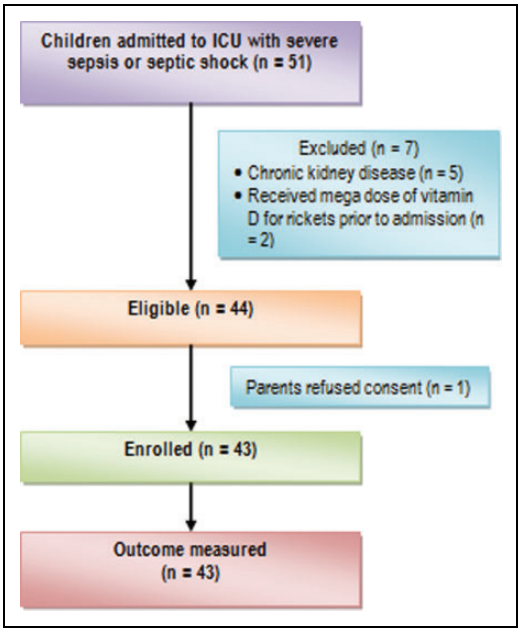

Figure 1. Study flow. ICU indicates intensive care unit.

Baseline demographic and clinical data are described in Table 1. The median age was 4 (0.5, 10) years and there was a slight preponderance of boys (59%, n = 22). At admission, the PIM-2 score was 22 (11.6, 33) and the PELOD score was 21 (21, 31). The duration of sun exposure (only exposed parts) was 2.5 (1, 4) h/d. The most common diagnoses at admission were pneumonia (22%), gastrointestinal illness (22%), and dengue (19%). The common underlying illness was genetic/neurometabolic disorders, autoimmune/immunodeficiency disorders, and nephrotic syndrome. Seven children (19%) had features of hypocalcemia at admission.

The prevalence of severe vitamin D deficiency was 72% (n = 31/43; 95% CI: 53-81) and 69% (n = 25/36; 51-79) at admission and after 72 hours of ICU stay, respectively (Figure 2). The corresponding mean vitamin D levels were 6.7 (2.3) ng/mL at admission and 4.5 (2.8) ng/mL after 72 hours. The fall in vitamin D levels from admission values was statistically significant ($P = .0003$).

Severe vitamin D deficiency was associated with nonresolution of shock in the first 24 hours in 74% (23) of patients as compared to 25% (3) of patients without severe vitamin D deficiency, relative risk [RR] (95% CI): 2.9 (1.09-8.08); $P = .005$. Children with severe vitamin D deficiency also received larger amount of fluid as boluses in the first 6 hours (75 vs 59 mL/kg; $P = .02$), and were more likely to have catecholamine refractory shock [21 of 31 (67%) vs 4 of 12 (33%); $P = .04$] as compared to others. In addition, children with severe vitamin D deficiency were found to be older (median age, 4 vs 1.5 years), were more likely to be undernourished, have higher median PIM-2 and PELOD scores, and had longer duration of mechanical ventilation, ICU stay, and mortality (Table 2).

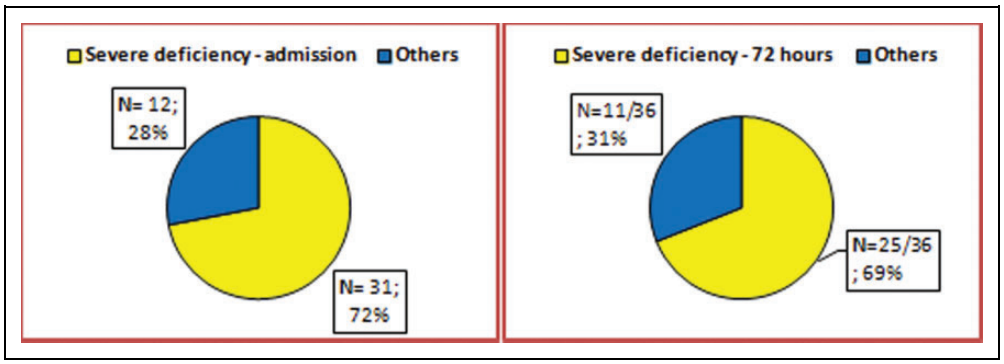

Figure 2. Prevalence of severe vitamin D deficiency at admission and 72 hours.

Table 2. Association of Severe Vitamin D Deficiency With Demographic, Clinical, and Outcome Variables in Children With Septic Shock.

Outcome Variables	Severe Vitamin D Deficiency (n = 31)	Others (n = 12)	RR (95% CI)	*P* Value
Age in years, median (IQR)	4 (0.5, 10)	1.5 (0.45, 8.5)	–	.32
Gender, n (%)	16 (52)	5 (42)	1.23 (0.58-2.62)	.40
Presence of underlying disease, n (%)	13 (42)	6 (50)	0.83 (0.41-1.69)	.44
Weight for height (<−3SD), n (%)	27 (87)	8 (67)	1.30 (0.85-1.99)	.13
PIM-2 probability, median (IQR)	22 (13, 38)	20 (8, 32)	–	.17
Nonresolution of shock in the first 24 hours, n (%)	23 (74)	3 (25)	**2.9 (1.09-8.08)**	**.005**
Fluid boluses in mL/kg in the first 6 hours, mean (SD)	75 (20)	59 (13)	**–**	**.02**
Catecholamine refractory shock, n (%)	21 (67)	4 (33)	2.03 (0.88-4.68)	.04
PELOD score, mean (SD)	27 (8)	25 (6)	–	.4
Parathyroid levels pg/mL, mean (SD)	15.5 (5)	12 (3)	3.5 (−2.4-9.4)	.19
Incidence of hypocalcemia, n (%)	20 (64)	10 (83)	0.77 (0.53-1.11)	.25
Need for mechanical ventilation, n (%)	26 (83)	11 (92)	0.91 (0.73-1.15)	.45
Duration of PICU stay, median (IQR)	10 (7, 14)	7 (7, 15)	–	.44
Mortality, n (%)	19 (61)	7 (58)	1.05 (0.60-1.82)	.86

Abbreviations: CI, confidence interval; IQR, interquartile range; PELOD, pediatric logistic organ dysfunction; PICU, pediatric intensive care unit; PIM, pediatric index of mortality; RR, relative risk; SD, standard deviation.

Table 3. Univariate and Multivariate Analysis of Factors Affecting Reversal of Shock in First 24 Hours.

Variable	Nonreversal of Shock (n = 26)	Reversal of Shock (n = 17)	Unadjusted OR (95% CI); *P* Value	Adjusted Odds Ratio/MD (95% CI); *P* Value
Severe vitamin D deficiency at admission, n (%)	23 (88)	8 (47)	1.90 (1.11-3.17); 0.005	12 (2.01-87.01); 0.01
Age in years	4 (0.6, 10)	7 (1, 11)	–0.25	0.98 (0.96-1.003); 0.12
Gender (male), n (%)	12 (46)	9 (53)	0.87 (0.47-1.60); 0.66	0.87 (0.18-4.22); 0.87
Fluid boluses in mL/kg in first 6 hours, mean (SD)	68 (21)	64 (16)	–0.46	1.00 (0.96-1.04); 0.88
Diagnosis	7 (27)	9 (53)	0.50 (0.23-1.10); 0.10	3.54 (0.65-19.4); 0.14
PIM-2 probability, median (IQR)	97 (2.2)	96 (2)	–0.49	0.99 (0.95-1.03); 0.6

Abbreviations: CI, confidence interval; IQR, interquartile range; MD, mean difference; PIM, pediatric index of mortality; SD, standard deviation.

None of these associations were, however, statistically significant (Table 2). In the multivariate analysis, nonresolution of shock at 24hours was significantly associated with severe vitamin D deficiency after adjusting for other key baseline and clinical variables (adjOR [95% CI]: 12 [2.01-87.01]; 0.01; Table 3).

Discussion

In the present study, we found a high prevalence of severe vitamin D deficiency (72%) at admission in children with septic shock. The vitamin D levels fell further during the course with lower levels recorded on day 3. Severe vitamin D deficiency was associated with lower rates of shock resolution in the first 24 hours of ICU stay on multivariate analysis with a RR of 12 (2.01-87.017). On evaluating association with other variables, children with severe vitamin D deficiency had longer duration of mechanical ventilation and ICU stay. However, none of these associations were statistically significant.

The association of vitamin D deficiency and sepsis has been well reported in adults and in a few pediatric studies.[1,14,16-18]

In a pooled analysis of 14 studies including 1967 patients, the authors reported the risk of infection to be 1.49 (1.12-1.99), $P = .007$ and of sepsis to be 1.46 (1.27-1.68), $P < .001$ in patients with vitamin D deficiency.[14] In the pediatric studies, the prevalence of vitamin D deficiency has ranged from 10% to 60% in various studies.[1,16-18] Though the immunomodulatory role of vitamin D and association of hypovitaminosis D and sepsis has been proven across pediatric studies as well[14,16-18] whether vitamin D deficiency in septic patients is the cause or effect of the illness is not yet clear.

During an infectious process, pathogen-associated molecules are recognized by Toll-like receptors on cells of immune system, especially macrophages which in turn trigger activation of 1-α hydroxylase (CYP27B1) resulting in synthesis of 1,25-dihydroxyvitamin D by the immune cells.[6-8] This in turn binds to vitamin D receptors and vitamin D response elements and induces genes for expression of various antimicrobial peptides, cathelicidins, β-defensins, and LL-37 (or CAP-18 for cathelicidin antimicrobial peptide, 18 kDa) which are potent mediators of antibacterial and antiviral activity. Other antimicrobial factors such as reactive oxygen species, nitric oxide synthase, and active form of interleukin-1β (IL-1β) are also stimulated by 1,25-dihydroxyvitamin D.[9,10] Moreover, vitamin D also modulates adaptive immunity by inducing differentiation of naïve T cells to regulatory T cells and development and differentiation of T helper cells favoring T helper type 2 thus fine tuning the inflammatory response and liming the tissue damage.[11,13] Experimental data have also demonstrated that vitamin D administration improves the blood coagulation parameters in sepsis-associated disseminated intravascular coagulation,[36,37] inhibits lipopolysaccharide (LPS)-induced activation of vascular endothelium, and further it modulates inflammatory cytokines such as tumor necrosis factor α and IL-6.[38] Among human studies, Jeng et al[39] had demonstrated a positive association of 25 (OH) D and LL-37 (cathelicidin) among critically ill patients and levels of both these mediators were low. To summarize, all of these data suggest that vitamin D deficiency is probably associated with the development of sepsis in an infected individual and also has important role in modulating further course of the illness.

If such causal association is true, vitamin D administration in deficient patients should result in favorable immunomodulatory effects and in turn favorably impact on clinical outcomes. However, interventional studies evaluating outcomes of vitamin D supplementation in critically ill patients did not result in any positive outcomes.[34,40] There are no pediatric studies till date evaluating the effect of vitamin D administration on outcomes in children with septic shock or critically ill children in general and therefore, even if a causal association is reported, whether supplementation will indeed improve outcomes remains to be seen.

We found severe vitamin D deficiency to be associated with lower rates of shock reversal at 24 hours in comparison to others. There was greater use of fluids and vasopressors in this group as well. This was expected in view of the lower rates of shock reversal. Our findings are similar to what has been reported previously in critically ill children with vitamin D deficiency.[41] In a study of 90 children, vitamin D deficiency was associated with higher rates of septic shock (1.9; 1.3-2.9, $P < .001$), vasopressor use (1.6; 1.2-2.3; $P < .01$), and fluid bolus requirement of > 40 mL/kg in the first 24 hours of admission (1.5; 1.1-2.1, $P < .05$) as compared to others.[41] In the study by Madden et al,[1] 51 children (10%) admitted with diagnosis of severe septic shock (cardiovascular sequential organ failure score ≥3 plus confirmed or suspected infection) had significantly low levels of 25 (OH) D ($P = .0008$) as compared to others. There was significant association of vitamin D deficiency with illness severity in these children. In another study of 120 patients with suspected sepsis, vitamin D deficiency was associated with greater PIM score ($P = .026$) and duration of mechanical ventilation ($P = .008$).[18] Although we found an association between vitamin D deficiency and nonresolution of shock, this did not change the overall clinical outcomes such as duration of stay or mortality as our study was underpowered due to the small numbers enrolled. Similar to the findings in our study, in a previously published study from the Indian subcontinent in children with suspected sepsis,[17] the authors reported a high prevalence of vitamin D deficiency (about 50%) but found no significant association with key clinical outcomes.

We observed that the mean vitamin D levels declined from 6.7 ng/mL at admission to 4.5 ng/mL after 72 hours. In contrast to our study, Alves et al[42] reported improvement in vitamin D deficiency status on day 7 as compared to at admission in selected patients with sepsis. For example, this finding was noted in only 6 patients in who the sequential organ failure assessment (SOFA) score had improved as well. While in more than 73% patients with sepsis, there was worsening of the vitamin D status on day 7 accompanied by worsening of SOFA scores as well in this study. Therefore, it appears that worsening of sepsis or sepsis-related multi-organ dysfunction *may* worsen vitamin D status and aggravate deficiency. Also a dilutional effect of fluid boluses on vitamin D levels has been documented to be responsible for this phenomenon which could have been responsible for the decline in levels in our study as well.[43]

The strengths of our study are that it is the first study to evaluate association of severe vitamin D deficiency with clinical outcomes in children with septic shock and provides preliminary data to plan larger studies to evaluate effect on mortality or other clinical outcomes and for planning interventional studies. Our study has several limitations. The major limitation of our study is the small number of patients enrolled and therefore a cause and effect relationship cannot be established. However, despite this limitation, our study provides preliminary data for future observational and interventional trials in children with septic shock. Other limitations are: we did not estimate 1, 25 (OH) D levels which is considered to be superior to 25 (OH) D for identifying patients with infections[44,45] due to logistic reasons. However, the task force committee of the Endocrine Society recommends using 25 (OH) D for initial screening of patients at risk of vitamin D deficiency. We also did not evaluate the correlation between vitamin D

deficiency at 72 hours and organ dysfunction scores. This could have thrown light on why the levels fell further. Thus, our findings are preliminary but important in planning future investigations concerning vitamin D and septic shock.

Conclusion

The prevalence of severe vitamin D deficiency is high in children with septic shock admitted to PICU. Severe vitamin D deficiency at admission may be associated with lower rates of shock reversal at 24 hours of ICU stay. Large multicentre studies are needed to confirm the findings and give direction to further research in this area.

Authors' Note

J.S. designed the study, supervised data collection, analyzed, and revised the manuscript. J.I. and R.R.D. reviewed the literature, helped with data analysis and writing of the manuscript. ND helped with designing the study, collection of data, data entry, and with literature review. A.C. helped with study design, 25 (OH) D estimation, and helped with writing the manuscript. M.J.S. helped in designing the study, statistical analysis, and in revising the manuscript. All authors approved the final version of the manuscript.

Declaration of Conflicting Interests

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Supplemental Material

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