Applied nutritional investigation

Vitamin D deficiency and mild to moderate anemia in young North Indian children: A secondary data analysis

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ABSTRACT

Objectives: The aim of this study was to examine the association between vitamin D deficiency and anemia status among young children in the resource-poor setting of northern urban India.
Methods: We used data from a randomized controlled trial of daily supplementation with folic acid, vitamin B_{12}, or both for 6 mo in children 6 to 30 mo of age conducted in Delhi, India. We measured serum vitamin D status, hemoglobin, plasma vitamin B_{12}, folate, soluble transferrin receptor, and homocysteine levels at baseline. Children with severe anemia (hemoglobin [Hgb] <7 g/dL) were excluded from enrollment. Multivariable logistic and multinomial logistic regressions were used to examine the association between vitamin D and anemia status at baseline.
Results: 25-Hydroxyvitamin-D (25 OHD) concentration was measured for 960 (96%) children. Of the children, 331 (34.5%) were vitamin-D deficient (<10 ng/mL). Approximately 70% of the enrolled children were anemic, with ~46% having moderate (Hgb 7–9.9 g/dL) and 24% mild (Hgb 10–10.9 g/dL) anemia. There was no association between vitamin D and anemia status after adjusting for confounders; however, the risk for moderate anemia was significantly higher among vitamin D-deficient children than those who were vitamin-D replete (relative risk, 1.58; 95% confidence interval, 1.09–2.31).
Conclusions: Vitamin D deficiency was associated with moderate anemia among young children and the effect was independent of iron deficiency. The causal association of vitamin D deficiency with anemia risk remains debatable. The role of vitamin D in risk for anemia needs to be examined in further studies.
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Introduction

Vitamin D deficiency is one of the most common nutritional deficiencies and undiagnosed medical conditions in the world [1]. The prevalence of vitamin D deficiency in young children is ~50%

to 90% in the Indian subcontinent [2]. Vitamin D is primarily produced in the skin after exposure to ultraviolet radiation and <10% is derived from dietary sources [3].

25-Hydroxyvitamin D [25(OH)D], the main circulating form of vitamin D, is now gradually more accepted for its role in immune function, cell proliferation, and differentiation in addition to bone and mineral metabolism [4,5]. This extraskeletal action of vitamin D usually has been grouped into three major effects: hormone secretion control, immune function modulation, and control of cellular proliferation and differentiation [4].

Recent studies suggest that 25(OH)D deficiency is associated with increased risk for anemia, an important public health problem experienced by as many as 50% of Indian children [4,6]. Lower 25(OH)D levels have been independently associated with anemia in adults with chronic diseases such as heart failure, diabetes, and

chronic kidney disease (CKD) [7–11], even among healthy adults [12]. However, there is a scarcity of data on the association between vitamin D and anemia status in young children.

We conducted a randomized controlled trial (RCT) with children 6 to 30 mo of age. The children were supplemented daily with folic acid, vitamin B_{12}, or both for 6 mo. Using data from this study, we examined the association between vitamin D and anemia status in young children from a resource-poor setting in northern urban India.

Materials and methods

Participants

The study was conducted from January 2010 to February 2012 in the low to middle socioeconomic settings of Tigri and Dakshinpuri in New Delhi, India. The total population of this site was ~300 000. Details of the population have been described previously [13]. This randomized, double-blind, placebo-controlled trial with a factorial design enrolled 1000 children, and evaluated the effects of supplementation with folic acid, vitamin B_{12}, or both on childhood infections [13]. Children with severe systemic illness requiring hospitalization, severe malnutrition (weight-for-height Z-score < −3), or severe anemia (Hgb < 7 g/dL), those on folic acid or vitamin B_{12} supplements, and those not consenting or considering migration were excluded from enrollment. A blood specimen was obtained in EDTA-containing vacutainers (BD, Franklin Lakes, NJ, USA) for all children at baseline.

Definitions

Anemia was defined on the basis of World Health Organization criteria [14] as follows:

- Mild anemia = Hgb 10 to 10.9 g/dL
- Moderate anemia = Hgb 7 to 9.9 g/dL
- Severe anemia = Hgb < 7 g/dL

Iron deficiency was defined as soluble transferrin receptor (sTfR) concentrations > 4.7 nmol/L [15]. We defined vitamin B_{12} deficiency as plasma vitamin B_{12} level < 200 pmol/L and folate deficiency as a plasma folate level of < 7.5 nmol/L [16].

We defined high homocysteine (Hcy) level as plasma Hcy level ≥ 10 μmol/L [17]. Vitamin D deficiency was defined as < 10 ng/mL (25 nmol/L) [18]. We also conducted a sensitivity analysis classifying baseline vitamin D status as < 10, 11 to 20, 21 to 29, and ≥ 30 ng/mL.

Analytical procedures

The blood specimen was centrifuged (Remi Sales & Engineering Ltd, Mumbai, India) at ~450g at room temperature for 10 min in field settings. Plasma was separated, transferred into storage vials, and stored at −20°C at the central laboratory until analysis. HemoCue AB (HemoCue Hb Angelholm, Sweden) was used to analyze Hgb concentration [19,20]. Plasma concentrations of folate and vitamin B_{12} were estimated by microbiologic assays [21,22], and plasma sTfR was analyzed using an immunoturbidimetric assay [23]. Plasma total homocysteine (tHcy) was analyzed using commercial kits (Abbott Laboratories, Abbott Park, IL, USA) [24]. Plasma concentration of vitamin D was measured by quantitative electrochemiluminescence binding assay, with detection of 25(OH) D_2, the hydroxylated forms of vitamin D_2 (Roche Diagnostics, Mannheim, Germany) [25].

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures were approved by the ethics committees of the Society for Applied Studies, New Delhi, Christian Medical College Vellore and Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST). The consent form for the main trial also sought permission from parents to store their children's blood specimen for use in future research. All parents consented for the same.

Statistics

Proportions, means (SD), or medians (interquartile range [IQR]) were calculated for categorical and continuous variables by anemia status at baseline. We had conducted a dominance analysis to show the relative importance of different erythropoietic nutrients (folate, vitamin B_{12}, sTfR) on Hgb status.

We used multiple regression and a "purposeful selection of covariates method" to identify variables that were associated with vitamin D deficiency and different types of anemia [26,27]. These variables were used as adjustment variables in the multiple models where vitamin D deficiency was the exposure variable. We also examined whether the predefined associations were modified by other

Table 1
Baseline characteristics of anemic and non-anemic children 6 to 30 mo of age included in the analysis (N = 1000)*

Characteristics	No anemia (Hgb ≥11 g/dL) n = 304	Anemia (Hgb <11 g/dL) n = 696
Proportion of children		
No anemia	304 (30.4)	
Anemia	696 (69.6)	
Infant characteristics		
Proportion of children		
<12 mo	131 (43.1)	190 (27.3)
12–23 mo	115 (37.8)	369 (53)
24–30 mo	58 (19.1)	137 (19.7)
Boys	150 (49.3)	357 (51.3)
Ever breastfed	252 (83.2)	546 (78.9)
Anthropometric status		
Wasted (<−2 WHZ)	29 (9.54)	76 (10.9)
Stunted (<−2 HAZ)	80 (26.3)	285 (40.9)
Sociodemographic characteristics		
Mother's schooling, y (median, IQR)	8 (5,12)	6 (0,9)
Annual family income, rupees (median, IQR)	104 000 (60 000–180 000)	72 000 (48 000–120 000)
Biochemical status		
Vitamin D level (<10 ng/mL)	N = 292	N = 668
	87 (29.8)	244 (36.5)
Plasma vitamin B_{12} level (<200 pmol/L)	N = 304	N = 695
	87 (28.6)	241 (34.7)
Plasma folate level (<7.5 nmol/L)	N = 304	N = 695
	66 (21.7)	237 (34.1)
Plasma soluble transferrin receptor concentration (>4.7 nmol/L)	N = 303	N = 694
	21 (6.93)	288 (41.5)
Plasma homocysteine level (≥10 μmol/L)	N = 302	N = 692
	175 (57.9)	454 (65.6)

HAZ, height-for age Z-score; Hgb, hemoglobin; IQR, interquartile range; WHZ, weight-for-height Z-score.
*Values are number (percentage) unless stated otherwise.

variables using interaction terms (on a multiplicative scale) in the multiple regression models.

Multiple logistic regression analyses were used to compare the anemia status (anemia and no anemia) between the vitamin D-deficient and the non-deficient groups at baseline. In these models, we adjusted for age of the child, family income, mothers' years of schooling, stunted, season, baseline plasma folate, plasma sTfR, and plasma Hcy level.

We used multinomial logistic regression analyses to measure the association between vitamin D deficiency and different categories of anemia (mild, moderate) compared with no anemia at baseline. In these models, we adjusted for age of the child, family income, mothers' years of schooling, stunted, season, baseline plasma folate, plasma sTfR, and plasma Hcy level. Statistical analyses were performed using STATA version 15 (Stata Corporation, College Station, TX, USA).

We used generalized additive models in the statistical software R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) to explore non-linear associations between the vitamin D status and Hgb level at baseline after adjustment for potential confounders [28].

Results

We included 1000 children in the main trial. Vitamin D concentration was available in 960 baseline samples. Of the children 331 (34.5%) were vitamin D deficient (<10 ng/mL). The baseline characteristics of the population by anemia status are presented in Table 1. Approximately 70% of the enrolled children were anemic, with ~46% having moderate anemia and 24% having mild anemia. Approximately 40% of the anemic children were stunted (height-for-age Z-score <−2) and had higher plasma sTfR concentration (>4.7 nmol/L).

The prevalence of iron deficiency (elevated sTfR i.e., >4.7 nmol/L) was 31% (n = 309) and elevated sTfR showed highest dominance (standardized dominance statistics 83%) among folate, vitamin B_{12}, and sTfR.

The anemia status among vitamin D-deficient and non-deficient children is shown in Table 2. There was no association between vitamin D status and anemia after adjusting for confounders. However, the risk for moderate anemia was significantly higher among vitamin D-deficient children compared with those who were vitamin D replete (relative risk [RR], 1.58; 95% confidence interval [CI], 1.09−2.31). The prevalence of mild anemia was not significantly associated with vitamin D status (RR, 1.14; 95% CI, 0.77−1.69).

We also conducted a sensitivity analysis by classifying baseline vitamin D status as <10, 11 to 20, 21 to 29, and ≥30 ng/mL. Of the children, 34.6% had <10 ng/mL, 42.4% had 11 to 20 ng/mL, 17% had 21 to 29 ng/mL, and 6% had ≥30 ng/mL levels of vitamin D. Anemia overall and the mild and moderate anemia subgroups were not significantly associated with any of the vitamin D category.

The association between vitamin D and Hgb level at baseline is shown in Figure 1. We restricted the analysis to vitamin D level ≤40 ng/mL as there were very few children above that level. There was a non-linear association between vitamin D and Hgb level at baseline.
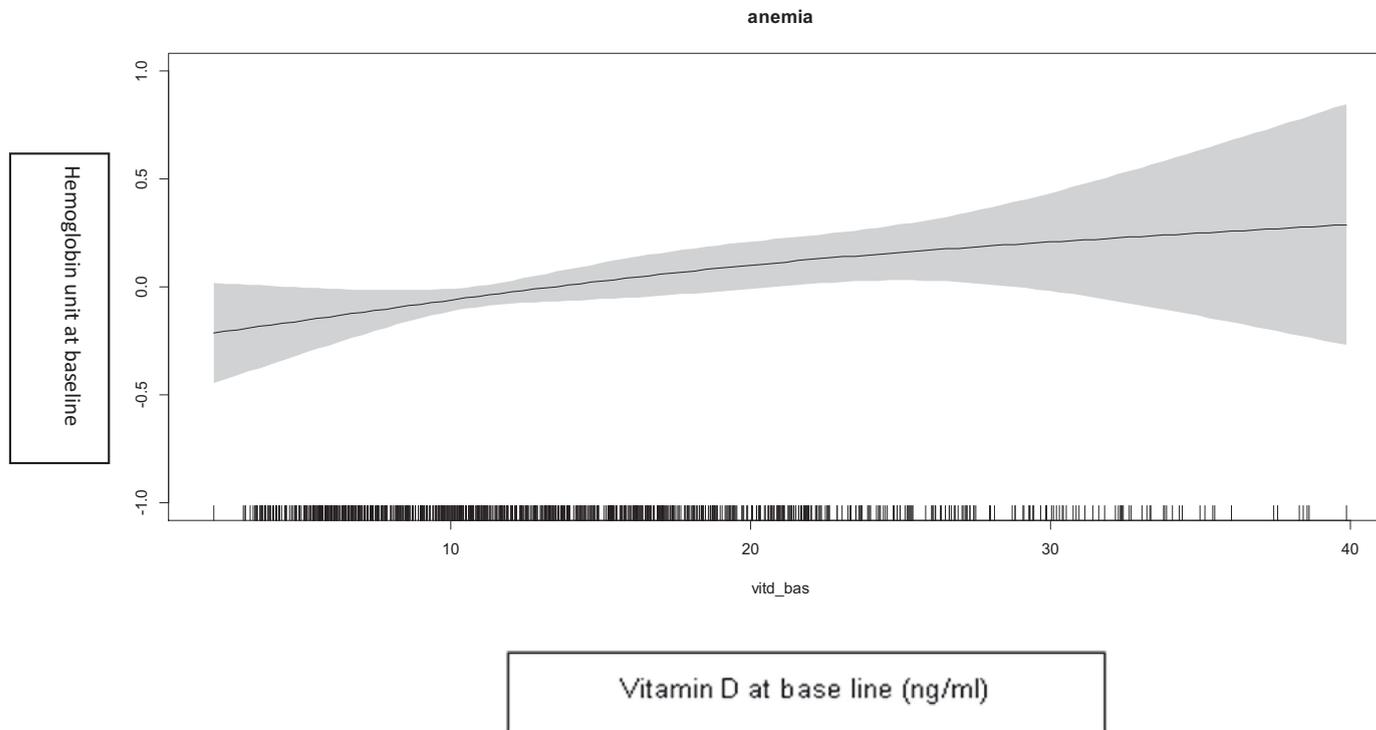
Discussion

The present study demonstrated that in a population-based cohort of young Indian children, lower 25(OH)D levels were associated with increased risk for moderate anemia. The observed association between vitamin D status and moderate anemia was independent of other factors that may contribute to anemia risk, including socioeconomic status and nutritional status (including stunting and iron deficiency).

In recent years, vitamin D has received interest as a regulator of a variety of biological functions including immune function and cellular proliferation [29−31].

Recent literature showed increased risk for anemia among vitamin D-deficient children and adolescents; however, most of the studies were conducted in developed countries. A cross-sectional study conducted among children and adolescents in the United States, showed increased odds (odds ratio [OR], 1.9; 95% CI, 1.3−2.7) of anemia among the vitamin D-deficient population compared with a vitamin D-sufficient population. The effect was independent of other confounding factors that could have contributed to anemia risk, such as obesity, inflammation, socioeconomic status, and nutritional status such as vitamin B12, folic acid, and iron deficiency [32]. In another study in South Korea, the authors showed vitamin D deficiency in a higher proportion of infants with iron deficiency anemia compared with a vitamin D-replete group and a significant correlation between Hgb and 25(OH)D levels [33]. In China, Chang et al. showed increased risk for anemia among vitamin D-deficient children between 6 mo and 14 y of age [34]. None of the studies excluded severe anemic (Hgb <7 g/dL) children/adolescent. We did not find any association with vitamin D deficiency and overall anemia status, possibly because of excluding severe anemic children from the main trial.

Similar findings were shown in studies done with adult population. Lower 25(OH)D levels had been associated with anemia in adults with non-dialysis CKD, end-stage kidney disease, end-stage heart failure, and type 2 diabetes [32]. Vitamin D supplementation among adults with CKD had demonstrated to improve anemia management and decrease dose requirements for erythropoiesis-stimulating agents, suggesting that vitamin D plays a role in erythropoiesis [35,36].

There are several possible mechanisms that could explain our findings. Vitamin D and its metabolites are present in many tissues, as are the vitamin D receptors (VDR) for the active form of vitamin D, calcitriol. Although calcitriol production for the regulation of bone mineral metabolism takes place via the action of the 1-α-hydroxylase enzyme in renal tissue, there are multiple extrarenal sites where locally produced calcitriol regulates host-cell DNA, and from which the extraskeletal actions of vitamin D are controlled [4,37]. Inadequate levels of 25(OH)D leading to decreased

Table 2
Prevalence of anemia in vitamin-D deficient and non-deficient children

	Deficient (vitamin D level <10 ng/mL)(n = 331)	Non-deficient (vitamin D level >10 ng/mL)*(n = 629)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[†]
Anemia (Hgb <11 g/dL)	244 (73.7)	424 (67.4)	1.35 (1.01−1.82)	1.35 (0.96−1.88)
Subgroup			Unadjusted OR (95% CI)	Adjusted OR (95% CI)[‡]
Moderate anemia (Hgb 7−9.9 g/dL)	173 (52.3)	267 (42.4)	1.53 (1.11−2.09)	1.58 (1.09−2.31)
Mild anemia (Hgb 10−10.9 g/dL)	71 (21.5)	157 (25.0)	1.06 (0.73−1.55)	1.14 (0.77−1.69)

Hgb, hemoglobin.
*Reference category: Non-deficient (vitamin D level >10 ng/mL).
[†]ORs were calculated by using logistic regression and adjusted for age, family income, mothers' years of schooling, stunted, season, baseline plasma folate, plasma soluble transferring receptor saturation, plasma homocysteine level.
[‡]ORs were calculated by using multinomial logistic regression and adjusted age, family income, mothers' years of schooling, stunted, season, baseline plasma folate, plasma soluble transferring receptor saturation, plasma homocysteine level.

anemia

Hemoglobin unit at baseline

vitd_bas

Vitamin D at base line (ng/ml)

Fig. 1. Association between baseline vitamin D and hemoglobin levels. The graph was constructed using generalized additive models in R; the solid line depicts the association of vitamin- D and hemoglobin levels at baseline. The shaded area spans the 95% confidence interval of this association.

local calcitriol production in the bone marrow may limit erythro-poiesis; calcitriol has a direct proliferative effect on erythroid burst-forming units, which is synergistic with endogenously produced erythropoietin, and also upregulates expression of the erythropoietin receptor on erythroid progenitor cells [34,38,39]. Calcitriol also plays a key role in the regulation of immune function by inhibiting the expression of proinflammatory cytokines by a variety of immune cells, thus providing negative feedback to prevent excessive inflammation [4]. The immunomodulatory effects of vitamin D may be central to its role in preventing anemia via modulation of systemic cytokine production, which may in turn suppress specific inflammatory pathways that contribute to the development of anemia. The role of inflammation in the etiology of anemia has been further clarified through study of the iron regulatory protein hepcidin, an inflammation-induced negative regulator of erythropoiesis [40,41].

To our knowledge, this is the first study conducted in India among young children to show the association between vitamin D deficiency and anemia status. We also assessed the relevant and important erythropoietic nutrients and its metabolites like plasma vitamin B_{12}, folate, Hcy (active metabolite of vitamin B_{12} and folate) and sTfR (markers of iron deficiency). These assessments helped us demonstrate the effect of vitamin D deficiency on anemia status independent of important erythropoietic nutrient. As a marker of iron deficiency status, we assessed sTfR, a reliable marker for the diagnosis of iron deficiency, especially when iron metabolism is influenced by inflammatory disorders such as infection and chronic inflammation, and thus provide a robust estimate of iron deficiency status.

The present study had several strengths, including a population-based sample of children, standardized data collection, and quality control procedures. Results were adjusted for several relevant confounders including nutritional status of children and the season of enrollment.

The present study had some limitations, including type II errors and weaknesses with the immunologic vitamin D assay. Type II errors can also be due to low sample size. For the subgroups of vitamin D categories, there was not sufficient power ($<80\%$). We used an immunologic method to measure vitamin D concentration. It should be noted that immunoassays can overestimate 25(OH)D [42] as it is lipophilic and is therefore vulnerable to matrix effects in the protein binding assays [43]. However, this is a cross-sectional study, and thus the association between vitamin D deficiency and anemia cannot be assumed to be causal.

Conclusion

Although the causal association of vitamin D deficiency with anemia risk (especially iron deficiency anemia) remains debatable, our analysis showed increased risk for moderate anemia among vitamin D-deficient children, which was independent of iron deficiency status. RCTs measuring the effect of vitamin D supplementation on anemia in these setting should be prioritized.

Acknowledgments

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