Vitamin D status and child health, growth, and neurodevelopment

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Abbreviations

1, 25 $(OH)_2$ D	1, 25-dihydroxyvitamin D
25(OH) D	25-hydroxyvitamin D
7-DHC	7- de hydro-cholesterol
ALRIs	Acute lower respiratory tract infections
AR1	First-order auto regression
ASQ-3	The Ages and Stages Questionnaire 3rd ed.
BRIEF 2	The Behavior Rating Inventory of Executive Function 2nd edition
CaT1	Calcium channel protein
CI	Confidence Interval
CVS	The Crichton Vocabulary Scales
DC	Dendritic cell
DHS	Demographic and health survey
ECM	Extracellular matrix
GAM	Generalized Additive Model
GEE	Generalized estimating equation
GH	Growth hormone
GLM	Generalized linear model
Hb	Hemoglobin
hCAP18	human cathelicidin antimicrobial peptide
HIV	Human immunodeficiency viruses
IFNγ	Interferon gamma
IGF-1	Insulin-like growth factor-1
IGF-2	Insulin-like growth factor-2
IOM	Institute of Medicine
LAZ/HAZ	Length/height-for-age Z scores
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LMIC	Low and Middle Income Country
L-VGCCs	L type of voltage dependent calcium channel
MDI	Mental development index
MICS	Multiple indicator cluster survey
MMP9	Matrix metalloproteinase-9
mRNA	Messenger Ribonucleic Acid
NDD	Neurodevelopmental disorders
NEPSY-II	A Developmental Neuropsychological Assessment-second edition

NFAT	Nuclear factor of activated T-cells
nNOS	neural nitric oxide synthase
OR	Odds ratio
p75NTR	p75 neurotrophin receptor
PDI	Psychomotor development index
PNNs	Perineuronal networks
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RDA	Recommended Dietary Allowance
RR	Relative Risk
RXR	Retinoid X receptor
SD	Standard deviation
sTfR	Soluble transferrin receptor
Th 1	Type 1 T helper
Th 2	Type 2 T helper
tHcy	Total homocysteine
Treg	Regulatory T cell
UVB	Ultraviolet B
VDR	Vitamin D receptor
VDBP	Vitamin D binding protein
VDREs	Vitamin D responsive elements
WAZ	Weight-for-age Z scores
WHO	World Health Organization
WISC-IV ^{INDIA}	The Wechsler Intelligence Scale for Children 4th edition (India)
WLZ/WHZ	Weight-for-length/height Z scores

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The only limit to our realization of tomorrow will be our doubts of today.
-Franklin D. Roosevelt

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Summary

Background: Vitamin D deficiency is one of the most commonly under diagnosed micronutrient deficiencies in the world. Both animal and human studies suggest that vitamin D plays an important role in innate and adaptive immunity, erythropoiesis and neurodevelopment, in addition to its osteoblastic activities.

Objectives: To estimate the associations between vitamin D status in early childhood and i) acute lower respiratory tract infections, clinical pneumonia, diarrhea, anemia, neurodevelopment and physical growth in early childhood; ii) neurodevelopment and physical growth in school age.

Methods: We used data from a randomized placebo controlled trial among 1000 North Indian children 6-30 months of age and a follow up study conducted when the children were between 6-9 years. A quantitative electrochemiluminescence-binding assay was used to measure the plasma concentration of vitamin D. We used multivariable regressions to examine the association between vitamin D status and episodes of acute lower respiratory tract infections (ALRIs), clinical pneumonia, diarrhea, hemoglobin, anemia, neurodevelopment and growth.

Results: The prevalence of vitamin D deficiency (<10 ng/mL) was 34.5% when the children were 6-30 months old. The risk of ALRIs (OR 1.26; 95% CI: 1.03 to 1.55) and moderate anemia (RR 1.58; 95% CI: 1.09 to 2.31) was significantly higher among vitamin D deficient children than among non-deficient children. There were no significant differences in any of the cognitive outcomes and linear growth between vitamin D deficient and non-deficient children either during early childhood or at school age.

Conclusions: Our results imply that vitamin D is one of the critical micronutrients for the prevention of ALRIs and anemia during early childhood, but it has a negligible role in neurodevelopment and growth in this population.

Consequences: The protective role of vitamin D on ALRIs and anemia needs to be confirmed in randomized controlled trials targeting vitamin D deficient children.

List of articles

Paper-I

Chowdhury R, Taneja S, Bhandari N, Sinha B, Upadhyay RP, Bhan M, Strand TA. Vitamin D deficiency predicts infections in Young North Indian Children: a secondary data analysis. PLOS ONE 2017; 12(3): e0170509

Paper-II

Chowdhury R, Taneja S, Bhandari N, Strand TA, Bhan MK. Vitamin D deficiency, and mild to moderate anemia in young North Indian children: a secondary data analysis. Nutrition 2019; 57:63-68.

Paper-III

Chowdhury R, Taneja S, Bhandari N, Kvestad I, Strand TA, Bhan MK. Vitamin D status, and neurodevelopment, and growth in young North Indian children: a secondary data analysis. Nutrition Journal 2017; 16: 59

Paper-IV

Chowdhury R, Taneja S, Kvestad I, Hysing M, Bhandari N, Strand TA. Vitamin D status in early childhood is not associated with cognitive development, and linear growth at 6-9 years of age in North Indian Children: a cohort study. Nutrition Journal 2020;19:14

Introduction

Vitamin D belongs to the group of fat-soluble vitamins. Vitamin D3 and vitamin D2 are essential compounds in the vitamin D group. Vitamin D is primarily produced in the skin after exposure to ultraviolet B (UVB) radiation. Thus, a significant cause of vitamin D deficiency is inadequate exposure to sunlight. Other factors contributing to vitamin D deficiency are atmospheric pollution, dark skin, and low vitamin D from dietary intake (1). Vitamin D deficiency is common among children in the Indian subcontinent (2). Vitamin D increases intestinal absorption of calcium, magnesium, and phosphate (3). The Vitamin D receptor (VDR), a member of the steroid hormone receptor superfamily, is present in the intestines, bones, kidney, respiratory epithelium, and brain (4). The VDR primarily plays a role in the phosphorylation of 1, 25-dihydroxyvitamin D (1, 25 $(OH)_2$ D). Vitamin D exerts many effects on innate and adaptive immunity, hematopoiesis, and development through the VDR (5). Animal models and observational studies have suggested that vitamin D deficiency causes respiratory tract infections, anemia, and impaired neurocognitive development (6-8). Thus, delineating skeletal and extra-skeletal roles of vitamin D is essential. Currently, vitamin D supplementation is recommended in infants and children to improve their bone health (9). However, it is not recommended for prevention of common infections, such as respiratory tract infections, or anemia or impaired neurodevelopment.

Vitamin D metabolism

Vitamin D exists in two primary nutritionally relevant chemical forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (10). Vitamin D3 is produced in the skin of vertebrates on exposure to UVB radiation, whereas vitamin D2 is produced from the precursor ergosterol by phytoplankton, yeast, invertebrates, and fungi in response to UV radiation (10).

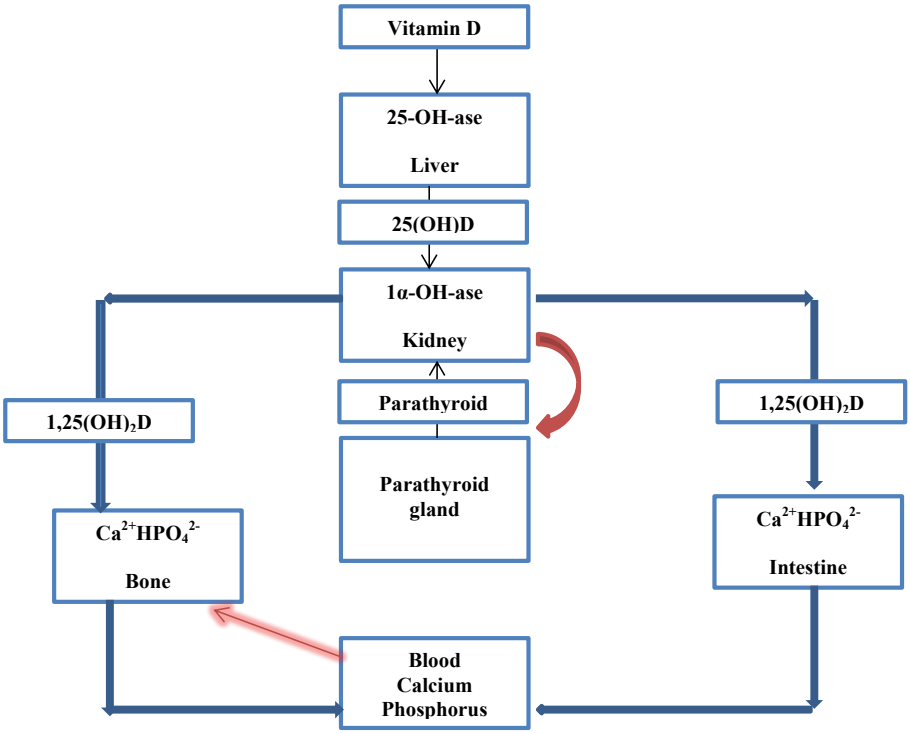
Vitamin D synthesis begins in the stratum basale and stratum spinosum, the innermost layers of the skin. In the skin, 7-dehydrocholesterol (7-DHC), a precursor of cholesterol,

absorbs UVB radiation on exposure to sunlight. It transforms into previtamin D3, which undergoes thermal isomerization to form vitamin D3 at average body temperature (11). This thermal isomerization facilitates the translocation of vitamin D3 from the skin to the blood circulation. In the blood circulation, vitamin D3 is bound to vitamin D binding protein (VDBP) (12). The VDBP bound vitamin D3 is transported to the liver and hydroxylated to form 25-hydroxycholecalciferol (25(OH)D) by hepatic 25-hydroxylase. Hepatic 25-hydroxylase is a mitochondrial and microsomal enzyme encoded by the *CYP2R1* gene (11). Further, mitochondrial 25-hydroxyvitamin D-1α-hydroxylase, encoded by *CYP27B1* gene in the kidney, converts 25(OH)D to 1, 25 $(OH)_2$ D (11). The parathyroid hormone (PTH) stimulates the expression of the *CYP27B1* gene (13).

Biological actions of vitamin D

$1,25(OH)_2D$ is the biologically active form of vitamin D (14). One of the critical functions of $1,25(OH)_2D$ is to maintain plasma concentration of calcium within a physiological range by increasing calcium absorption from the intestine (11). It also increases intestinal phosphorus absorption and induces the formation and activation of osteoclasts and reabsorption of calcium from the distal renal tubule using PTH. Thus, $1,25(OH)_2D$ acts on the intestines, bones, and kidneys to elevate plasma calcium (Figure 1) for mineralization of the bone and many other biological actions (11).

Figure 1 Metabolism of vitamin D and its biological actions

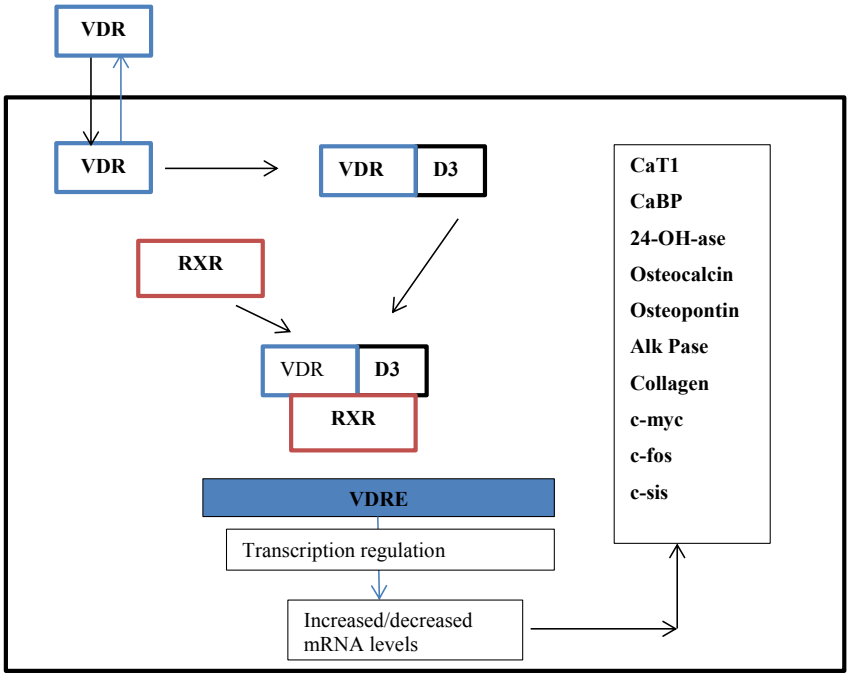


25-OH-ase: 25-hydroxylase; 25(OH)D: 25-hydroxycholecalciferol; 1α-OH-ase: 25-hydroxyvitamin D 1α-hydroxylase; $1,25(OH)_2D$: 1,25-di-hydroxycholecalciferol; $Ca^{2+}HPO_4^{2-}$: Calcium hypophosphate

Genomic actions of vitamin D

The VDR is a member of the steroid hormone receptor superfamily (4). VDRs are present in the tissues of the intestine, bone, kidney, respiratory epithelium, and brain (15). They exhibit high affinity for $1,25(OH)_2D$. $1,25(OH)_2D$ is phosphorylated after binding to VDR and then it is taken by one of the three 9-*cis* retinoid X receptors (RXRα, RXRβ, and RXRγ) (16). The combination of VDR, RXR and $1,25(OH)_2D$ interacts with coregulatory proteins and regulates the transcription of vitamin D responsive elements (VDREs). VDREs are present in the genes involved in a diverse range of processes, including the regulation of cell proliferation, differentiation, and apoptosis (17). Osteocalcin, osteopontin, and alkaline phosphatase are few of the best characterized gene products that are induced by $1,25(OH)_2D$ in osteoblasts (18). The epithelial calcium channel protein (CaT1) is the dominant gene product induced by $1,25\ (OH)_2D$ in the small intestine. CaT1 directly affects the entry of calcium into intestinal cells. $1,25(OH)_2D$ induces the expression of the regulator gene and proto-oncogene products (c-myc, c-fos, and c-sis), which regulate cell proliferation and differentiation (Figure 2) (19).

Figure 2 Genomic actions of vitamin D



VDR: Vitamin D receptor; RXR: *cis*-retinoid; VDRE: vitamin D–responsive elements; CaT1: calcium channel protein; CaBP: Calcium binding protein; 24-OH-ase: 24-hydroxylase; Alk Pase: Alkaline Phosphatase; c-myc, c-fos, c-sis (proto-oncogene)

Nongenomic actions of vitamin D

The nongenomic actions of $1,25(OH)_2D$ are independent of receptor-mediated transcription (20). One of the critical nongenomic actions of $1,25(OH)_2D$ is the induction of rapid intestinal absorption of calcium called transcaltachia (20). Transcaltachia leads to the activation of multiple signaling cascades, such as the phosphorylation of protein kinase C, the opening of cellular calcium channels increasing the intracellular calcium uptake, and further activation of mitogen-activated protein kinase pathways (20).

Role of vitamin D in immunity

Vitamin D modulates various immune functions of different cell types, such as monocytes, lymphocytes, and epithelial cells, through its biologically active form $1,25(OH)_2D$ (Figure 3).

Innate immunity

Innate immunity is the first line of defense against microbes in the body. Innate immune response to microbial invasion involves the complement system and antibacterial responses by neutrophils and macrophages (21). The role of vitamin D in innate immunity can be traced back to reports regarding tuberculosis treatment with cod liver oil, which is a source of vitamin D (22). Vitamin D levels have been reported to be significantly lower in septic shock patients and are associated with lower concentration of antimicrobial protein cathelicidin (23). It supports the hypothesis that the vitamin D status regulates antimicrobial protein levels.

Vitamin D enhances chemotaxis and phagocytic capabilities of innate immune cells (24). Vitamin D, VDR, and RXR activate the transcription of antimicrobial peptides, such as defensin β2 and human cathelicidin antimicrobial peptide (hCAP18) (25). The 1α-hydroxylase and VDR recognize pathogens through toll-like receptors and modulate the gene expression for cathelicidin production. (25) hCAP18 is cleaved from LL-37 (a 37-residue active cationic peptide) and destabilizes microbial membranes (26).

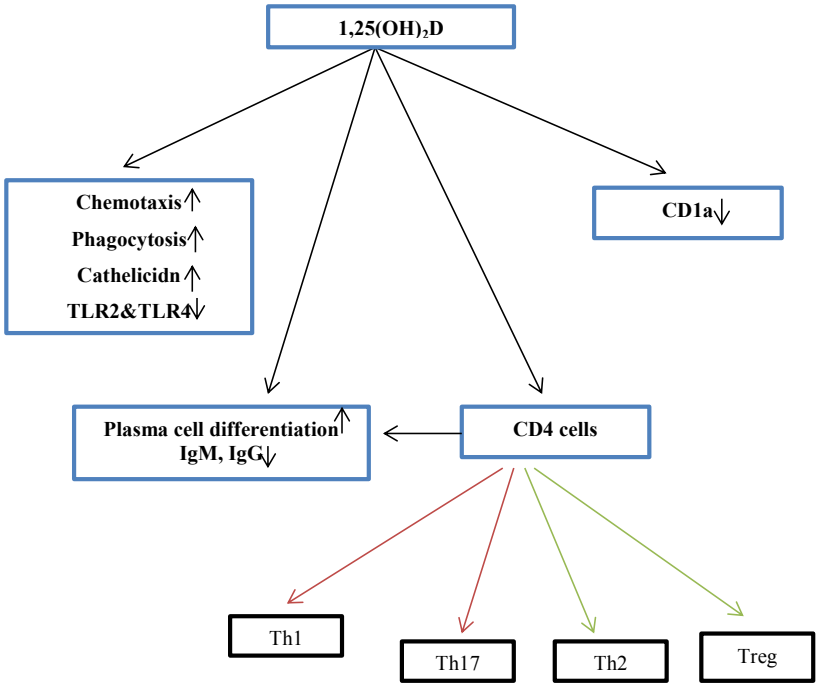
Vitamin D exerts immune-modulatory effects on monocytes and other innate antigen-presenting cells, particularly dendritic cells (DC). It can alter the function and morphology of DC to induce a more tolerogenic immature state (27). Vitamin D also inhibits T-cell cytokines, such as IL-2 and IL-17, and toll-like receptors on monocytes (22).

Adaptive immunity

Vitamin D suppresses adaptive immunity (28). It suppresses immune responses mediated by Type 1 T helper (Th1) cells. Th1 cells produce inflammatory cytokines, such as IL-2 and interferon gamma (IFNγ) (29). Suppressive actions on IL-2 result in the blocking of NFAT/AP-1 complex formation and sequestrating of Runx1 (The Runx1 gene translates to the protein runt-related transcription factor 1) (30). $1,25(OH)_2D$ directly binds the VDR/RXR to negative VDREs in the IFNγ promoter (17). Vitamin D enhances cytokines associated with Type 2 T helper (Th2) cells and shifts the expression of a Th1 based response toward a Th2 based response (31).

Vitamin D induces regulatory T (Treg) cells (cells that are important for the inhibition of inflammation) through the induction of Foxp3 (the transcription factor involved in the development and function of Treg cells) and downregulates Th17-producing cells (32). The effects of vitamin D on IL-17 include the following mechanisms: blocking Nuclear factor of activated T-cells (NFAT), Runx1 binding to the IL-17 promoter, and the induction of Foxp3 (30). Vitamin D suppresses IL-17 through the inhibition of the transcription factor RORγt, which leads to Th17 cell differentiation (33); furthermore, it inhibits the DC differentiation, suppressing the proinflammatory cytokine IL-12 and increasing IL-10, an anti-inflammatory cytokine produced by Th2 cells (34).

Figure 3 Role of vitamin D in innate and adaptive Immunity



TLR2: Toll-like receptor 2; TLR4: Toll-like receptor 2; IgM: Immunoglobulin M; IgG: Immunoglobulin G; CD4: Cluster of differentiation 4; CD1a: Cluster of differentiation 1a; Th1: T helper 1; Th17: T helper 17; Th2: T helper 2; Treg: Regulatory T cells

Role of vitamin D in anemia

Existing evidence suggests that vitamin D deficiency increases the risk of anemia by upregulating proinflammatory cytokines, decreasing iron availability, and decreasing erythropoiesis (Figure 4).

Inflammation and Hepcidin

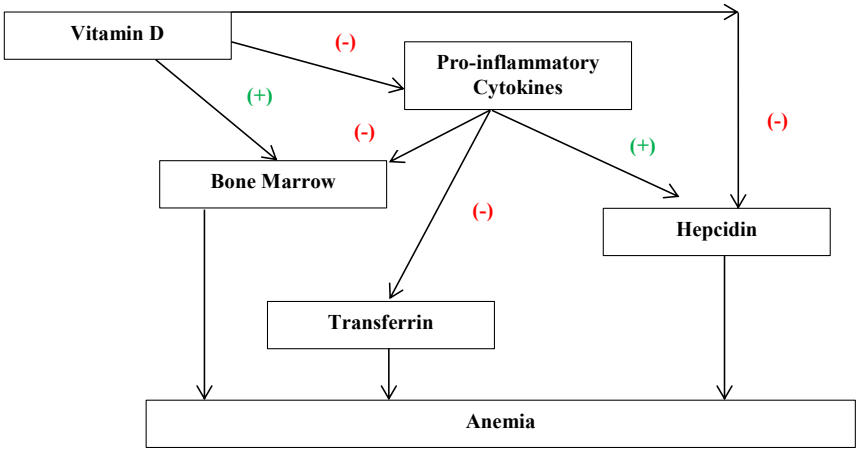
Vitamin D concentration and anemia are associated through the direct suppression of hepcidin messenger Ribonucleic Acid (mRNA) expression by vitamin D and a reduction of hepcidin-stimulatory proinflammatory cytokines (8).

Hepcidin is a peptide hormone secreted predominantly by hepatocytes. It binds to and causes degradation of the iron-efflux transporter ferroportin. This process leads to iron retention within these cells, resulting in decreased intestinal iron absorption and decreased iron recycling from macrophages; this aberration can result in iron deficiency anemia (35). Hepcidin synthesis is induced not only through iron stores but also through inflammation, thus acting as a component of innate immunity (8). VDRE is present in the promoter region of the hepcidin antimicrobial peptide gene, which provides a strong basis for the direct action of vitamin D on hepcidin (8). In subjects with persistent low-grade systemic inflammation, hepcidin-induced iron trapping occurs in macrophages. Evidence suggests that there exists an anti-inflammatory mechanism of vitamin D on the hepcidin–ferroportin axis (36). A dose-dependent decrease was observed in the release of IL-6 and IL-1β from a cultured human monocyte cell line in the presence of vitamin D (36).

Erythropoiesis

Vitamin D enhances erythropoiesis by inhibiting proinflammatory cytokines, which may impair erythropoiesis by inhibiting the production of erythropoietin and proliferation of erythroid progenitor cells (37). It sustains erythropoiesis by increasing burst forming unit-erythroid proliferation. Moreover, it exerts a synergistic effect with erythropoietin to enhance the erythroid progenitor cell (38, 39).

Figure 4 Role of vitamin D in inflammation induced anemia



Role of vitamin D in neurodevelopment

Vitamin D mediates various functions in the brain (6). The vitamin D activating enzyme *CYP*27B1 and catabolic enzyme *CYP*24A1 are present in neural cells of the cerebral cortex and cerebellar Purkinje cells, suggesting that the brain metabolizes vitamin D (40). Vitamin D deficiency in pregnant rat causes extreme alterations in the brains of their pups. These alternations include a longer cortex, proportionately thinner and enlarged lateral ventricle at birth, along with reduced nerve growth factor, glial cell line-derived neurotrophic factor, and altered p75 neurotrophin receptor (p75NTR) (41, 42). Vitamin D deficiency in utero resulted in rat embryos having significantly fewer numbers of apoptotic cells and more mitotic cells. Animal models have also revealed that vitamin D deficiency during pregnancy is associated with a reduced fetal crown-rump length, head size, and lateral ventricle volume (43). Vitamin D deficiency causes alternation of brain-derived neurotrophic factor, transforming growth factor-β1, and forkhead box protein gene expression in the developing cortex (43). Forkhead box proteins belong to a

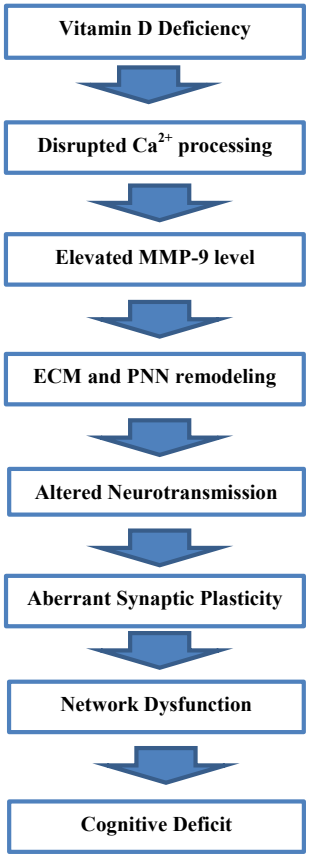
transcription factor family and play critical roles in regulating the expression of genes involved in cell growth, differentiation, and proliferation (43). There was a reduction of brain-specific tyrosine hydroxylase gene expression in the substantia nigra following vitamin D depletion in mice (43). This change may have significant implication for neurodevelopment.

$1,25(OH)_2D$ may affect brain development by downregulating the production of cytokines, affecting neurotransmission and synaptic plasticity, which may affect neurocognitive development (44). It also affects dopamine activity in the brain, which is attributed to the presence of the VDR in brain areas responsive to dopamine (6).

Vitamin D deficiency may alter the calcium flow through the L-type voltage-dependent calcium channels (L-VGCCs) by moderating the transcription of L-VGCCs and protein kinases (calcium/calmodulin-dependent protein kinase II, protein kinase A, and phosphoinositide 3-kinase). These alterations may contribute to changes in neural nitric oxide (NO) synthase (nNOS), which may result in the abnormal secretion of NO into the extracellular space. The abnormal NO secretion may increase matrix metalloproteinase-9 (MMP-9) levels, which may affect both extracellular matrix (ECM) and aggrecan-rich perineuronal networks (PNNs), decreasing PNN-positive cells. The decrease in PNN-positive cells may destabilize the neuronal circuits, resulting in network dysfunctions leading to cognitive deficits (Figure 5) (44).

Despite evidence from animal studies, human studies have reported mixed results on the association between vitamin D status and neurodevelopment (45-50). Most of the studies have reported an association between pregnancy vitamin D status and neurodevelopmental outcomes in children before 4 years, except for a few studies that demonstrated a marginal association with language scores at 10 years (46, 48, 50, 51).

Figure 5 Schematic diagram of vitamin D and the integrity of perineuronal nets



ECM: Extracellular matrix; MMP-9: matrix metalloproteinase-9; PNN: perineuronal net

Evaluation of vitamin D status

Source of vitamin D

Exposure of the skin to sunlight, usually between 10 am and 3 pm in the spring, summer, and fall, acts as the most crucial source of vitamin D for humans (52). Several factors reduce the skin's production of vitamin D3, including increased skin pigmentation and the topical application of sunscreen (1). A change in the zenith angle of the sun due to a change in the latitude, season of the year, or time of the day influences the skin's production of vitamin D3 (1). With latitude of approximately 33-35° above and below the Earth's equatorial plane, vitamin D3 synthesis in the skin is very low or absent during most of the winter (53, 54).

Dietary sources of vitamin D are primarily limited to oily fish, eggs, and fortified foods (55).

Vitamin D assessment in population-based studies

Measuring serum 25-hydroxyvitamin D (25(OH)D) is the most accurate method for assessing the vitamin D status in humans (2, 56). However, serum 25(OH)D measurements used in population based research have limitations. Firstly, a single assessment of serum 25(OH)D may not accurately reflect an individual's vitamin D status over a year. Serum 25(OH)D levels vary with season; highest concentration are observed in the summer and lowest in the winter due to differences in sun exposure (57). For this reason, analyses of serum 25(OH)D should be adjusted for the season of blood collection to assess the long-term effects of vitamin D. Secondly, there is debate on the cut-offs for defining vitamin D deficiency (57). Thirdly, serum 25(OH)D concentration should be considered in the context of the genetic variation in proteins involved in the vitamin D transport, function, and metabolisms such as VDBP, VDR, 25-hydroxylase (*CYP27A1*), 1-alpha-hydroxylase (*CYP27B1*), and 24-hydroxylase (58). Fourthly, intra-individual variation in serum 25(OH)D concentration are not considered while measuring the vitamin D status. As a result, serum 25(OH)D measurements at entry into a study may

not reflect a person's long term vitamin D status. Therefore, in the absence of repeated longitudinal serum 25(OH)D measurements, the only method available to assess the vitamin D status over lifetime is repeated, longitudinal, or retrospective assessments of vitamin D intake and sunlight exposure (57).

Definitions of adequate and optimal vitamin D status

The World Health Organization (WHO) and the Nordic Nutrition Recommendations (NNR 2012, Nordic Council of Ministers 2014) have defined vitamin D "insufficiency" and "deficiency" as a serum 25(OH)D concentration below 20 ng/mL and 10 ng/mL, respectively (59). These cut-off values were set with regard to prevention of rickets and/or symptomatic osteomalacia.

According to the US Institute of Medicine's recommendations, serum 25(OH)D < 12 ng/mL is "deficient"; serum 25(OH)D of 12-20 ng/mL is "inadequate"; and serum 25(OH)D > 20 ng/mL is "sufficient"(60).

The Endocrine Society (www.endocrine.org) suggests that a 25(OH)D concentration of at least 30 ng/mL is essential for optimal health outcomes and <20 ng/mL as vitamin D deficiency (56, 61). The Endocrine Society considered the following factors to define the vitamin D cut off 1) elevated PTH is consistently lowered to a plateau when serum 25(OH)D is at 30 ng/mL or higher; 2) there is reduction in the risk of falls among older persons at serum 25(OH)D levels of 30 ng/mL or higher; and 3) calcium absorption is maximal at serum 25(OH)D levels of 30 ng/mL (62).

Inconsistency in vitamin D cut-off levels used along with interchangeable use of terminology, makes accurate comparisons of reported prevalence difficult (59).

Vitamin D deficiency prevalence in low- and middle-income countries and India

The prevalence of vitamin D deficiency is reported worldwide, both in sunshine-deficient and sunshine-sufficient countries, irrespective of age, sex, and geography. It has been

argued that vitamin D deficiency is the most underdiagnosed and undertreated nutritional deficiency in the world (63). Table 1 presents the overview of studies on the vitamin D status among children in India.

Table 1 Vitamin D Status among children in India from observational Studies

Author Name and Year	Place of the study	Study Population	Sample Size	Mean (SD)/ Median (IQR) of 25(OH)D (ng/mL)	Criteria for defining vitamin D deficiency based on serum 25(OH)D levels	Proportion Deficient
Agarwal et al. 2002	Delhi	Urban children, mean (SD) age: 16 (4.1) months	26	12.4 (7)	-	-
		Urban children, mean (SD) age: 15.9 (3.8) months	31	27.1 (7)	-	-
Tiwari et al. 2004	Delhi	Urban children, age 9-30 months				
		Sundernagari area, winter	47	38.52 (10.28)	14 ng/mL	2%
		Rajiv Colony area, winter	49	9.5 (10.8)		82.9%
		Rajiv Colony area, summer	48	7.12 (8.96)		84%
		Gurgaon area, summer	52	7.68 (8.08)		82%
Sachan et al. 2005	Delhi	Neonates/cord blood	207	8.4 (5.7)	20 ng/ mL	95.7%
Bhalala et al. 2007	Mumbai	Exclusively breastfed infants, 3 months	35	18.19 (9.74)	20 ng/ mL	80%
Seth et al. 2009	Delhi	Exclusively breastfed infants, 2-24 weeks	180	11.56 (8.3)	10 ng/ mL	43.2%

Author Name and Year	Place of the study	Study Population	Sample Size	Mean (SD)/ Median (IQR) of 25(OH)D (ng/mL)	Criteria for defining vitamin D deficiency based on serum 25(OH)D levels	Proportion Deficient
Mehrotra et al. 2010	Delhi	Breastfed infants, mean (SD) age: 3.0 (0.14) months	60	9.03 (4.63)	10 ng/ mL	90%
Agarwal et al. 2010	Delhi	Exclusively breastfed infants, age: 10 weeks	97	12.59 (8.37)	11 ng/ mL	55.67%
Ekbote et al. 2011	Pune	Urban children, mean (SD) age: 2.8 (0.6) years	110	11.94 (12.61)	20 ng/ mL	84%
Jain et al. 2011	Delhi	Breastfed infants, mean (SD) age: 13.6 (2.2) weeks	98	Median: 10.1 (2.5–17.1)	20 ng/ mL	86.5%
Agarwal et al. 2012	Delhi	Infants, low birth-weight, at birth	220	Median 6.5 (4.0–54.5)	20 ng/ mL	93%
		Infants, low birth-weight, at 3 months	127	Median 11.1 (4.0–78.0)		72.4%
		Infants, normal birth-weight, at birth	116	Median 5.8 (4.0–26.6)		94.8%
		Infants, normal birth-weight, at 3 months	77	Median 8.2 (4–29.7)		83.1%
Marwaha et al. 2011	Delhi	Exclusively breastfed infants	342	8.92 (4.2)	20 ng/ mL	98.8%

Prevalence of pneumonia, diarrhea, anemia and stunting among under five children in India

Globally, pneumonia and diarrhea are two of the primary causes of morbidity and mortality among children under 5 years of age. The incidence of clinical pneumonia is 0.37 episodes per child per year and India accounts for 36% of the total WHO Southeast Asia regional burden (64, 65). In 2000 and 2015, India had 32% of all episodes of clinical pneumonia among developing countries, and the incidence of pneumonia decreased by only 3% during these 15 years (66).

Diarrhea attributes to 9% of all under-5 deaths, most of which occur in developing countries (67). Although a reduction in the incidence of diarrhea in resource-limited settings was observed from 2000 to 2015, the disease burden associated with enteric illnesses remains a public health issue.

Anemia affects a quarter of the global population; nearly 293 million (47%) children under 5 years of age are anemic (68). Approximately 85% of the anemia burden in children under 5 years of age is from Asia and Africa (69). According to the recent National Family Health Survey-4 (NFHS-4, 2015-2016), 59% of children aged 6–59 months are anemic in India, which is alarming and of immense concern (70, 71).

Global estimates of undernutrition among children under 5 years of age suggest that 150.8 million children are stunted, 50.5 million children are wasted. India along with Nigeria and Pakistan contribute to almost half (47.2%) of all stunted children (72). While stunting and underweight prevalence have reduced, trends in wasting indicate an overall increase in the past decade in India (73). There was one percentage point decrease of stunting per year from NFHS-3 (2005-2006) to NFHS-4 (2015-2016) (73). Similarly, underweight prevalence has been reduced by 0.68 percentage points from NFHS-3 (2005-2006) to NFHS-4 (2015-2016) over a decade. Recent data are promising and suggest acceleration in the reduction of stunting and underweight. However, the pace of reduction remains low and calls for focused interventions for optimal results (73).

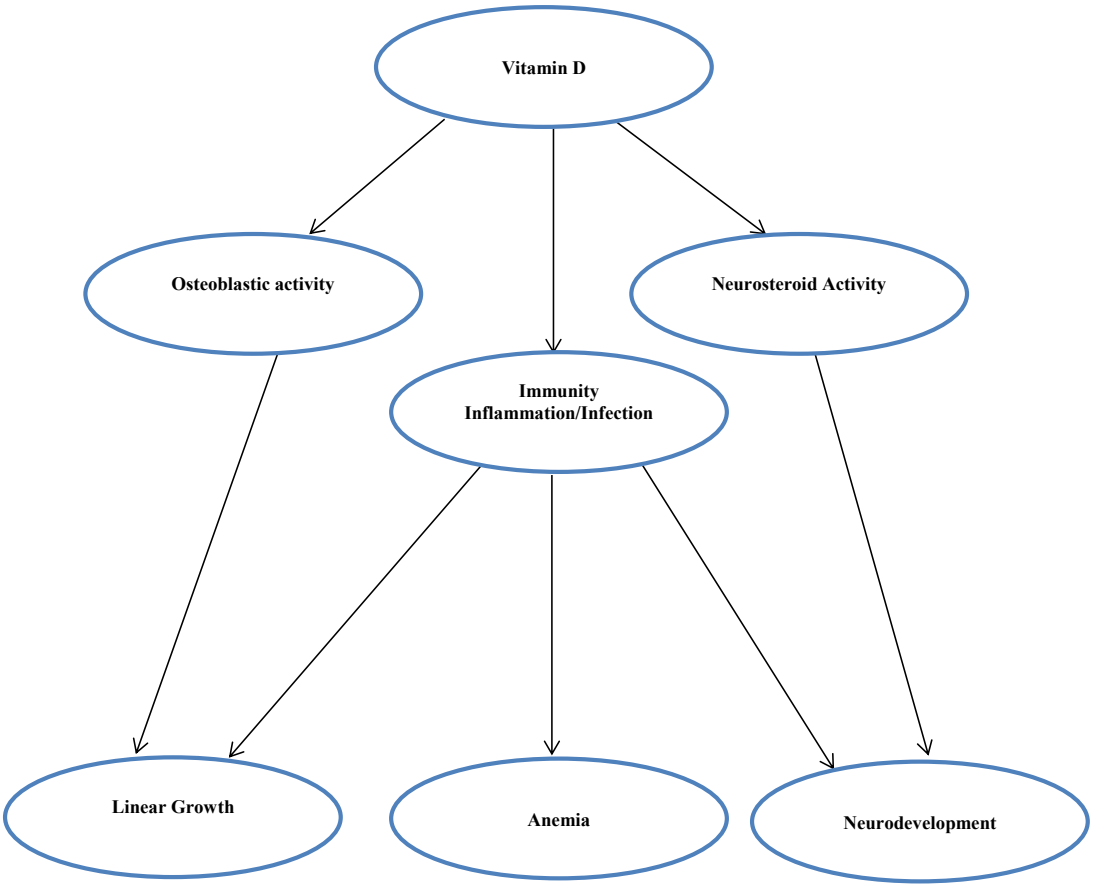
Neurodevelopment status among children: globally and in India

More than 250 million children under 5 years of age in low- and middle-income countries (LMICs) are at a risk of not attaining their complete developmental potential (74). The early years of life are critical for the development of cognitive and socio-emotional functions (75). Children develop a set of age-appropriate core cognitive skills between birth and 5 years that allow them to maintain attention, understand and follow directions, communicate with others, and solve progressively complex problems (76). Children with developmental deficits lose an estimated 19.8% of adult income yearly (77).

According to the results from the multiple indicator cluster survey and the demographic and health survey, a total of 80.8 million children aged 3-4 years in LMICs had poor cognitive or socioemotional development in 2010 (76). Specifically, 14.6% had low early childhood development index scores in the cognitive domain, 26.2% had low socioemotional scores, and 36.8% performed poorly in either or both areas.

Recently, a study was conducted in five states of India to assess the population-level prevalence of childhood neurodevelopmental disorders (NDDs) among children aged 2-9 years. The study assessed vision impairment, epilepsy, neuromotor impairments, including cerebral palsy, hearing impairment, speech and language disorders, autism spectrum disorders, intellectual disability, attention deficit hyperactivity disorder, and learning disorders. The total burden of any of the nine NDDs in the 6-9 year old children ranged from 6.5% to 18.5% (78).

Figure 6 Conceptual Framework for association between vitamin D status and child health, growth and development



Rationale for assessing vitamin D status and child health, growth and development

Animal studies have suggested that vitamin D plays a critical role in innate and adaptive immunity, inflammation, anemia, growth, and neurodevelopment (Figure 6). Primarily, vitamin D exerts its nonosteoblastic activities through the VDR, which is widely present in different parts of the human body, such as the intestine, bone, kidney, respiratory epithelium and the brain. However, few studies have measured the associations between the vitamin D status and functions of the respiratory system, hematopoietic cells, bone, and brain in a single cohort. In previous cohorts, the time of exposure to vitamin D deficiency, use of different cut-offs for vitamin D deficiency, age of the child at the time of outcome assessments, and handling of the confounders have varied. These could be the reasons for inconsistent findings on the associations between the vitamin D status, and different extra skeletal functions in these studies.

The cohort of young Indian children described in this thesis has provided a unique opportunity to measure the associations between vitamin D status in early childhood on common childhood infections, such as diarrhea and pneumonia, anemia, physical growth, and neurodevelopment at different time points in childhood. The present study consists of a cohort of 1000 children included during their early childhood (6-30 months), where we were able to follow 791 of them until they were of school going age (6-9 years). Vitamin D concentration of 960 children was available during early childhood. Information on diarrhea and pneumonia incidence was obtained through biweekly 6 months follow-up, hemoglobin (Hb) concentration was measured in early childhood, and physical growth and neurodevelopment were measured in early childhood and at school age. Detailed information on the exposure of interest (vitamin D concentration) in early life and different clinical (infections), biochemical (anemia), physical (growth), and neurodevelopmental outcomes at different time points during childhood provides an excellent opportunity to test different hypotheses surrounding the extra-skeletal roles of vitamin D.

Objectives

To estimate the association between vitamin D deficiency in early childhood, and

1. Respiratory infections (ALRIs, clinical pneumonia), and diarrhea in early childhood.
2. Anemia status in early childhood.
3. Neurodevelopment and physical growth in early childhood.
4. Neurodevelopment and physical growth in school age.

Methods

The original study on vitamin B12 and/or folic acid supplementation

The original study was conducted between January 2010 and February 2012 in low-to-middle socioeconomic settings in New Delhi, India. The total population of the study site was approximately 300,000. One thousand children aged between 6-30 months were enrolled in this randomized, double-blind placebo-controlled factorial trial (NCT00717730 at www.clinicaltrials.gov) to evaluate the impact of supplementation with folic acid, vitamin B-12, or both on childhood infections. The children were randomized to one of four treatment groups: *1)* placebo (n = 249), *2)* 2 times the Recommended Dietary Allowance (RDA) of folic acid (n = 249), *3)* 2 times the RDA of vitamin B-12 (n = 252), and *4)* a combination of both vitamin B-12 and folic acid (n = 250) (79).

Inclusion and exclusion Criteria

Caregivers of children aged 6-30 months who consented to participate and with no plans to move away over the next 6 months of supplementation were considered for enrollment. We excluded children with severe systemic illness requiring hospitalization, severe acute malnutrition (weight-for-height Z score <-3 SD), severe anemia (Hb <7 g/dL), and those who were using folic acid and/ or vitamin B-12 supplements (79).

Interventions

The intervention was a lipid-based nutritional supplement prepared by NUTRISET Ltd. Children aged 6-11 months received one spoon (5 g), and children older than 12 months received two spoons (10 g). The supplement was administered daily by a fieldworker for six months at the participants' homes, except on Sundays and public holidays when the mother administered the supplement herself. Each 10 g (corresponding to the daily dose for children older than 12 m) contained 54.1 kcal energy, 0.7 g protein, and 3.3 g fat. The

supplement contained 150 mcg folic acid for the folic acid groups and 1.8 mcg vitamin B-12 for the vitamin B-12 groups (79).

Blood sample collection and processing

We obtained 3 mL blood samples from all children at enrollment in a tube containing EDTA (Becton Dickinson). The plasma was centrifuged at approximately 450 × *g* at room temperature for 10 min, separated, and transferred into storage vials and stored at -20 ^{0} C until analyzed (79).

Assessment of vitamin D

We used the quantitative electrochemiluminescence-binding assay to measure the plasma concentration of vitamin D (Roche Diagnostics, Mannheim, Germany) (80).

Hemoglobin, vitamin B-12, folate, and homocysteine assessment

HemoCue AB (HemoCue Hb Angelholm, Sweden) was used to measure Hb concentration (81, 82). Microbiological assays estimated plasma concentration of vitamin B-12 and folate (83, 84). Plasma soluble transferrin receptor (sTfR) was analyzed using an immunoturbidimetric assay (85). Total plasma homocysteine (tHcy) was analyzed using kits (Abbott Laboratories, Abbott Park, IL, USA) (86).

Assessment of infection

Field workers visited households twice weekly for six months post enrollment. During these visits, they asked the mothers or primary caregivers about symptoms of respiratory illness (cough, difficult breathing, fast breathing), diarrheal disease (number and consistency of stools), fever on any day since the last visit, and care-seeking behaviors for the illnesses. The workers counted respiratory rates twice at each visit, measured

temperature, and examined for signs of dehydration if the child had diarrhea or vomiting (87).

Neurodevelopment assessments in the original study

Neurodevelopment was assessed after six months of supplementation using the Ages and Stages Questionnaire 3rd edition (ASQ-3). The ASQ-3 consists of age-appropriate questionnaires divided into five subscales: Communication, Gross motor, Fine motor, Problem-solving, and Personal-social. The items are summed to reveal subscale scores ranging from 0 to 60, and these subscale scores are further summed to obtain a total score ranging from 0 to 300 (88).

The ASQ-3 forms were translated to Hindi, the spoken language in the study area (89). The field supervisors performed the forward translations. The translations were then back-translated to English by another person who was otherwise not involved in the study (89). Four items in the ASQ-3 were identified not to be appropriate for the study population and were changed; these were an item involving a fork, an item involving a zipper, an item involving a mirror, and one requiring the child's knowledge of his/her first and last name (89).

We asked questions to assess the stimulation and learning environment of the children. Questions included number of toys in the home, number of hours of play during the week and numbers of books. We also used two questions from the Home Observation for the Measurement of the Environment (HOME) to assess the caregiver's encouragement of developmental advances and her/his promotion of child development (90).

The follow up study on vitamin B12 and/or folic acid supplementation

In September 2016, approximately six years after the end of the original study, the team contacted the 1000 children enrolled in the original study, of whom 798 (79.8%) children were available. Of these, 791 consented for participation in the follow up study (91). The children were then 6-9 years old.

Neurodevelopment assessments in the follow-up study

In the follow-up we performed a comprehensive assessment of cognitive functioning (91). The Wechsler Intelligence Scale for Children 4th edition (India) (WISC-IV^{INDIA}) is an assessment tool for intellectual ability in children that has been validated for the Indian population and has Indian norms (92). For the current study, we used seven subtests, which were summed to obtain three index scores; perceptual reasoning, processing speed, and working memory.

The Crichton Vocabulary Scale (CVS) is a tool for the assessment of language skills for children 4 to 18 years old (93). The CVS has been translated to Hindi and provides a standard total score according to Indian norms (94).

The NEPSY-II is a neuropsychological test battery for children aged 3 to 16 years with American norms (95). We administered seven age-appropriate subtests: inhibition, design fluency, word generation, visuomotor precision, manual motor sequences, affect recognition, and geometric puzzles. No modifications or cultural adjustments were necessary to perform the tests in this setting.

The Behavior Rating Inventory of Executive Function 2nd edition (BRIEF 2) is a parent-reported questionnaire for children 5 to 18 years of age assessing executive functions in everyday life according to American norms (96). The scale comprises of three clinical indexes; the behavior, emotion and regulation index; and an overall global executive composite score. We translated the questionnaire to Hindi in close collaboration with the developers.

Anthropometry

Weight (Digitron scales, to the nearest 50 g), length (locally manufactured infantometers, to the nearest 0.1 cm), and height (Seca 213, to the nearest 0.1 cm) were measured at 6-30 months age in the original study, and at 6-9 years in the follow up study.

Definitions used in the analysis

Exposure

Vitamin D deficiency was defined as vitamin D concentration <10 ng/mL (59). We also categorized vitamin D status according to the US Institute of Medicine`s recommendations; serum 25(OH)D <12 ng/mL as 'deficient'; 12-20 ng/mL as 'inadequate'; and >20 ng/mL as 'sufficient'. We also used vitamin D concentration as continuous variable in generalized additive models (GAM) (60).

Outcomes

We defined diarrhea as the passage of 3 or more loose or watery stools in a 24 hours period. Two different episodes of diarrhea were separated by ≥3 days (72 h) without diarrhea.

We defined ALRIs as cough or difficult breathing with an increased respiratory rate above the age-specific cutoff values (≥ 50 breaths/min and ≥ 40 breaths/min in infants and older children, respectively) or cough or difficult breathing and lower chest indrawing (87).

We defined clinical pneumonia either by a combination of cough with crepitations or bronchial breathing by auscultation or as an episode of ALRIs associated with at least one of the following features: lower chest indrawing, convulsions, inability to drink or feed, extreme lethargy, restlessness or irritability, nasal flaring, or abnormal sleep and difficulty in waking (87).

We defined anemia as Hb concentration <11 gm/ dL, mild anemia as Hb concentration 10 to 10.9 gm/ dL, moderate anemia as Hb concentration 7 to 9.9 gm/ dL, and severe anemia

as Hb concentration <7 gm/dL based on WHO criteria (97). Iron deficiency was defined as soluble transferrin receptor (sTfR) concentration >4.7 nmol/L (98). We defined vitamin B-12 deficiency as plasma vitamin B-12 concentration of <200 pmol/L, folate deficiency as plasma folate concentration of <7.5 nmol/L, and high tHcy level as total homocysteine concentration ≥ 10 μmol/L. (99, 100)

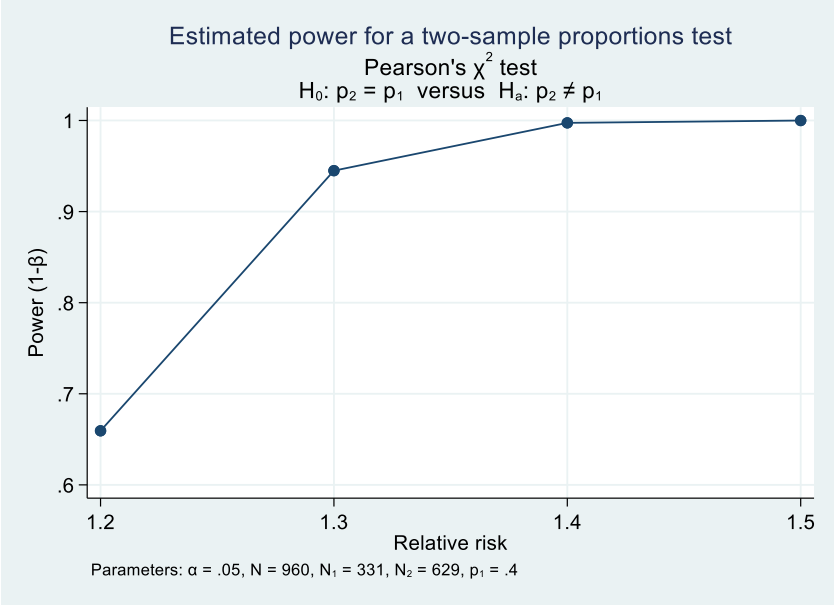
Wasting was defined as weight-for-height/length Z scores (WHZ/WLZ) < −2SD, stunting as height/ length-for-age Z scores (HAZ/LAZ) <−2SD, and underweight as weight-for-age Z scores (WAZ) < −2SD according to WHO standards (101).

Power calculations

Objective 1

To estimate the association between vitamin D deficiency and ALRIs, clinical pneumonia and diarrhea, 960 children were available. With this number we had more than 90% power to detect a relative risk of 1.3 between vitamin D deficiency and ALRIs, assuming a 40% prevalence of at least one episode of ALRIs in the vitamin D non-deficient group at a 5% significance level. With this number we had at least 80% power to detect a relative risk of 1.3 between vitamin D deficiency and clinical pneumonia, assuming a 30% prevalence of at least one episode of clinical pneumonia in the vitamin D non-deficient group at a 5% significance level. With this number we had more than 80% power to detect a relative risk of 1.3 between vitamin D deficiency and diarrhea, assuming a 35% prevalence of at least one episode of diarrhea in the vitamin D non-deficient group at a 5% significance level.

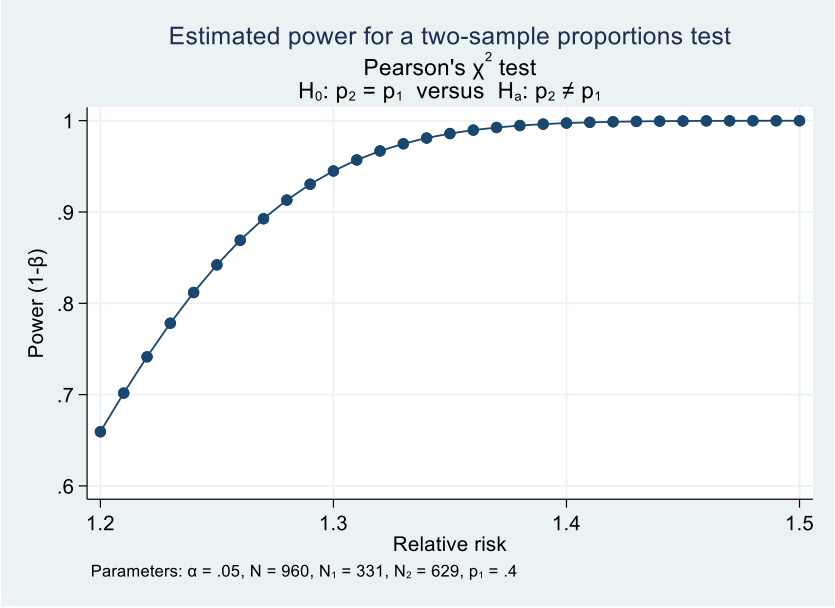
Figure 6 Estimated power for objective 1 with different relative risks



Objective 2

To estimate the association between vitamin D deficiency and anemia, 960 children were available. With this number we had more than 90% power to detect a relative risk of 1.3 between vitamin D deficiency and moderate anemia, assuming a 40% prevalence of moderate anemia in the vitamin D non-deficient group at a 5% significance level.

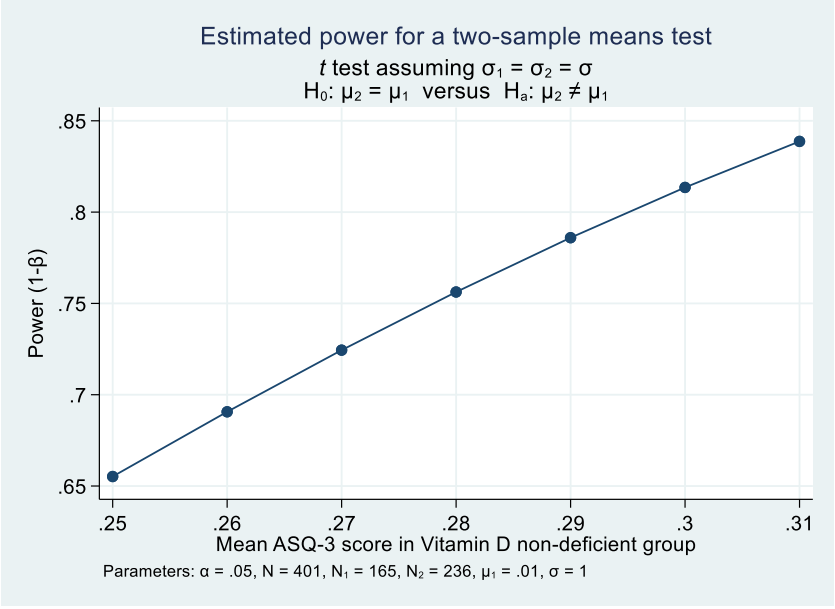
Figure 7 Estimated power for objective 2 with different relative risks



Objective 3

To estimate the association between vitamin D deficiency and neurodevelopment and growth in early childhood, 401 and 960 children were available, respectively. With these numbers we had more than 80% power to detect at least a 0.3 SD difference in the total ASQ-3 scores between the vitamin D deficient children and vitamin D non-deficient children at a 5% significance level. We also had more than 80% power to detect at least a 0.2 SD difference in the mean LAZ scores between vitamin D deficient children and vitamin D non-deficient children at a 5% significance level.

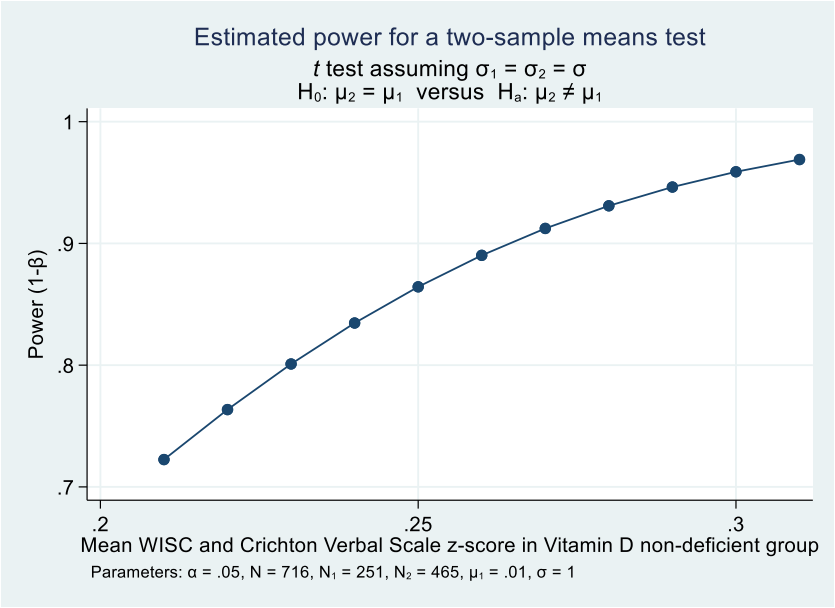
Figure 8 Estimated power for objective 3 with different standardized mean differences



Objective 4

To estimate the association between vitamin D deficiency and neurodevelopment and growth at school age, 716 children were available. With this number we had more than 90% power to detect a 0.3 SD difference in WISC-IV^{INDIA} and CVS Z scores between the vitamin D deficient children and the vitamin D non-deficient children at a 5% significance level. We also had more than 80% power to detect at least a 0.21 SD difference in mean LAZ scores between vitamin D deficient children and vitamin D non-deficient children at a 5% significance level.

Figure 9 Estimated power for objective 4 with different standardized mean differences



Statistical Analyses

We summarized baseline characteristics with measures of dispersion such as the mean with standard deviation, median with interquartile range, and the percentage by vitamin D status at baseline, as appropriate. We used a method of purposeful selection of covariates to identify variables to be included in the multivariable models (102). Generalized additive models (GAM) were used to explore nonlinear associations between vitamin D concentration at baseline and different outcomes, such as the incidence of ALRIs, Hb concentration, LAZ/HAZ scores, and different neurodevelopmental scores (103).

We created a wealth index that determined the wealth of an individual using principal component analysis based on assets owned by the household (104). The study population

was categorized into five equal wealth quintiles, i.e., 'poorest', 'very poor', 'poor', 'less poor', and 'least poor', based on principal component analysis.
We used STATA version 15 (Stata Corporation, College Station, TX) and R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) for all statistical analyses.

Objective 1

To estimate the association between vitamin D deficiency and ALRIs, clinical pneumonia and diarrhea, we used generalized estimating equations (GEEs) with a logit link, binomial variance first-order autoregressive (AR1) covariance-variance matrix and robust standard error, taking time into account. We divided the six month follow up period of each child into 26 periods of 7 days. For a period to be included in the analyses, we required information on 4 or more days of the given 7 days' period. We adjusted for the age, sex, breastfeeding status, wasting, stunting, underweight, anemia status of the children, season, and intervention groups. The GAM was used to explore nonlinear associations between baseline vitamin D concentration and season.

Objective 2

To estimate the association between vitamin D deficiency and anemia, we used a multivariable logistic regression model. We adjusted for family income, age of the child, mothers' years of schooling, stunting, season, baseline plasma folate, plasma soluble transferrin receptor saturation, and plasma homocysteine level.
We used multinomial logistic regression analysis to measure the association between vitamin D deficiency and different categories of anemia (mild, moderate) compared to no anemia at baseline.
We performed a dominance analysis to show the relative importance of different erythropoietic nutrients (folate, vitamin B-12, sTfR) on Hb status.

Objective 3

To estimate the association between vitamin D deficiency and neurodevelopment we used multivariable linear and logistic regression models. In linear regression models, we used the ASQ-3 scores as continuous variables. In logistic regression models, we categorized the total and subscale ASQ-3 scores at the 25th percentile. We adjusted for the child's age, mother's years of schooling, father's years of schooling, log-transformed annual family income, family structure, hours of play with other children during the week, family owns books, number of children in the family, mother's encouragement of developmental advances, mother's belief that child's behavior can be modified, WLZ/WHZ, WAZ and intervention group.

To estimate the association between vitamin D deficiency and growth, we used multivariable linear and logistic regression models. We used wasting, stunting, and underweight as dependent variables in the logistic regression models and WHZ/WLZ, LAZ/HAZ, and WAZ in the linear regression models. In these models, we adjusted for family structure, age of the child, sex of the child, breastfeeding status, log-transformed annual family income, mother's and father's years of schooling, baseline levels of vitamin B-12, folate, Hb and intervention groups.

Objective 4

To estimate the association between vitamin D deficiency and neurodevelopment and growth at school age, we used generalized linear model (GLM). In these models, we used the Gaussian distribution family and identity link function to estimate beta coefficients for the cognitive outcomes and HAZ (28). We used the poisson distribution family and log link to calculate the relative risk (RR) for stunting (105).

We calculated a combined WISC-IV ^{INDIA} and CVS Z-scores based on converted Z-scores for the three index scores in the WISC-IV INDIA (perceptual reasoning, processing speed, and working memory) and the total CVS scores. We also calculated a combined NEPSY-II Z score based on the converted Z-scores in the seven subtests (IN-naming vs. inhibition

contrast scaled scores, design fluency total scaled scores, word generation-semantic vs. initial letter contrast scaled scores, visuomotor precision combined scaled scores, manual motor sequences total scores - raw scores, affect recognition total scaled scores, and geometric puzzles total scaled scores). For BRIEF 2, we used the overall global executive composite scores in the analyses.

In these models, we adjusted for log folate level, log soluble transferrin receptor level and log total homocysteine level at baseline and the wealth index, father's occupational status, maternal education at follow-up and intervention groups.

Ethics

The ethics committees of the Society for Applied Studies, New Delhi, and the Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST) approved all procedures in the original and follow up studies. The consent form for the original study also sought permission from parents to store their children's blood samples for use in future research. All parents provided consent.

Results

Blood samples for vitamin D estimation were available for 960 (96%) children at baseline. Of these, 331 (34.5%) children had vitamin D concentration <10 ng/mL and were classified as vitamin D deficient. The baseline characteristics of the enrolled children and their families by vitamin D deficiency status are shown in Table 2. More than a third (36.4%) of the children were stunted, 31% were underweight, and 10.7% were wasted. Approximately 70% of the children were anemic.

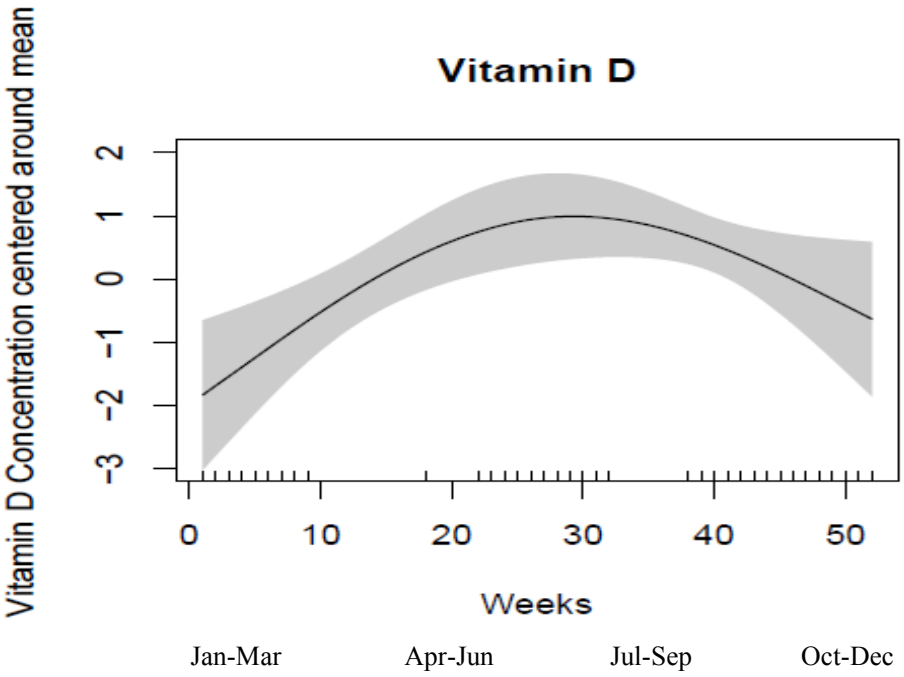
Table 2 Baseline characteristics of vitamin D deficient and non-deficient children aged 6-30 months included in the analysis

Baseline characteristics	Deficient n = 331	Non-deficient n = 629
Infant characteristics		
Age at enrolment in months, mean (SD)	16.9 (7.1)	15.8 (7.0)
Children		
<12 months	91 (27.5%)	210 (33.4)
12 to 23 months	166 (50.1)	306 (48.7)
24 to 30 months	74 (22.4)	113 (18.0)
Boys	162 (48.9)	328 (52.2)
Ever breastfed	325 (98.2)	622 (98.9)
Illness in previous 24 hours		
Diarrhea	17 (5.1)	32 (5.1)
Cough or difficult breathing or fast breathing	114 (34.4)	192 (30.5)
Nutritional status		
WHZ/WLZ, mean (SD)	-0.86 (0.92)	-0.89 (0.94)
HAZ/LAZ, mean (SD)	-1.56 (1.24)	-1.63 (1.16)
WAZ, mean (SD)	-1.46 (1.06)	-1.52 (1.05)
Wasted	35 (10.6)	68 (10.8)
Stunted	117 (35.3)	233 (37.0)
Underweight	101 (30.5)	197 (31.3)
Anemia (Hb<11 g/dL)	244 (73.7)	424 (67.4)
Socio-demographic characteristics		
Mother's age in years, mean (SD)	26.3(5.8)	25.6(4.1)
Mother's schooling in years, median (IQR)	8 (5,10)	7 (0,10)
Father's schooling in years, median (IQR)	10 (7,12)	9 (6,12)
Annual family income in rupees, median (IQR)	72000 (60000 - 144000)	84000 (60000 - 138000)

Figures are number (percentages) unless stated otherwise

Figure 10 depicts how the vitamin D concentration varies throughout the year. The baseline vitamin D concentration was higher in children who were enrolled from May to July (weeks 24 to 32) and lower in the period from December through February (weeks 48 to 8).

Figure 10 Association between baseline vitamin D concentration and weeks of the year



The solid line depicts the association of vitamin-D concentration at baseline and weeks of the year. The shaded area spans the 95% confidence interval of this association.

Synopsis of Paper 1

Specific Objective

To estimate whether vitamin D deficiency (<10 ng/ mL) at 6-30 months of age determines ALRIs, clinical pneumonia, and diarrhea incidence during a six-month follow-up period.

Results

The risk of ALRIs was significantly higher among vitamin D deficient children than among non-deficient children (OR: 1.26; 95% CI: 1.03 to 1.55), while clinical pneumonia was not associated (OR: 1.05; 95% CI: 0.79 to 1.38) with vitamin D status (Table 3).

We found no association between vitamin D status and episodes of diarrhea overall or episodes lasting for six days or more.

Table 3 Incidence of ALRIs and clinical pneumonia in vitamin D deficient and non-deficient children

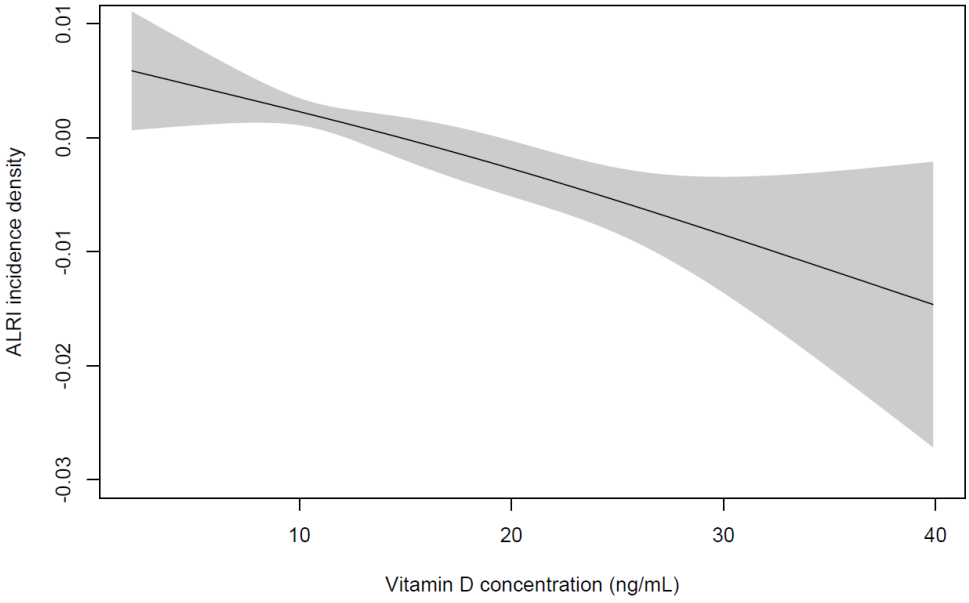
	Deficient (Vitamin D level < 10 ng/mL) (n=331)	**Non-Deficient (Vitamin D level ≥ 10 ng/mL)[a] (n=629)**	**Adjusted OR (95% CI)[b]**
Child-years of follow-up	162.3	308.5	
Episodes of ALRIs	244	418	
Incidence density of ALRI per child year (95% CI)	1.50 (1.32 to 1.70)	1.35 (1.23 to 1.49)	1.26 (1.03 to 1.55)
Episodes of clinical pneumonia	144	294	
Incidence density of clinical pneumonia per child year (95% CI)	0.89 (0.75 to 1.04)	0.95 (0.85 to 1.07)	1.05 (0.79 to 1.38)

[a] Reference category: Non-deficient (vitamin D concentration ≥ 10 ng/mL)

[b] ORs were calculated by using generalized estimating equations with a logit link, binomial variance, autoregressive correlation, and robust standard error and adjusted for age, sex, breastfeeding status, wasted, stunted, underweight, anemia status, season, and intervention groups

The relationship between vitamin D concentration and incidence of ALRIs appeared to be almost linear, as indicated by the GAM plot (Figure 11). In the GAM, we removed the highest 1.5% (vitamin D concentration >40 ng/mL) from the analyses as the estimated GAM curve beyond this cut-off was highly uncertain.

Figure 11 Association between baseline vitamin D concentration and ALRIs incidence density



The solid line depicts the association of vitamin D concentration at baseline and ALRI incidence density. The shaded area spans the 95% confidence interval of this association.

Synopsis of Paper 2

Objective

To examine the association between vitamin D deficiency and anemia status among children 6-30 months of age

Results

The prevalence of anemia among vitamin D deficient and non-deficient children is shown in Table 4. We did not find any association between vitamin D deficiency and overall anemia after adjusting for confounders. However, anemia was categorized into mild and moderate, we found an association between vitamin D deficiency and moderate anemia (OR: 1.58; 95% CI: 1.09 to 2.31).

Table 4 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)[a] (n=629)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)[b]**
Anemia (Hb <11 gm/dL)	244 (73.7)	424 (67.4)	1.35 (1.01 to 1.82)	1.35 (0.96 to 1.88)
Moderate Anemia (Hb 7 to 9.9 gm/dL)	173 (52.3)	267 (42.4)	1.53 (1.11 to 2.09)	1.58 (1.09 to 2.31)
Mild Anemia (Hb 10 to 10.9 gm/dL)	71 (21.5)	157 (25.0)	1.06 (0.73 to 1.55)	1.14 (0.77 to 1.69)

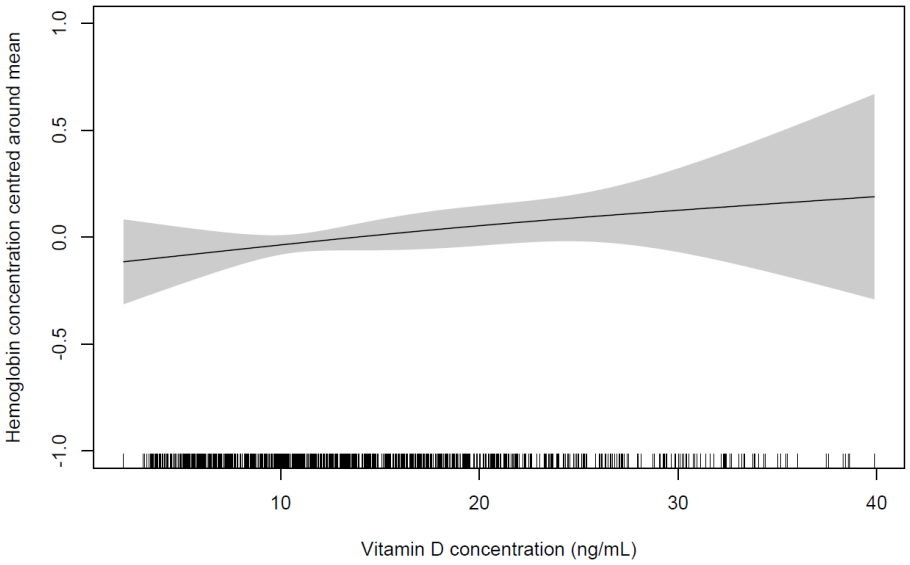
[a] Reference category: Non-deficient (vitamin D concentration ≥ 10 ng/mL)

[b] ORs were calculated by using logistic regression, and adjusted for age, family income, mothers years of schooling, stunted, season, baseline plasma folate, plasma soluble transferrin receptor saturation, plasma homocysteine

[c] ORs were calculated by using multinomial logistic regression, and adjusted for age, family income, mothers years of schooling, stunted, season, baseline plasma folate, plasma soluble transferrin receptor saturation, plasma homocysteine level

The GAM plot revealed that here was no association between vitamin D and haemoglobin levels at baseline (Figure 12). We removed the highest 1.5% (vitamin D >40 ng/mL) from the analyses as the estimated GAM curve beyond this cut-off was highly uncertain.

Figure 12 Association between baseline vitamin D and hemoglobin concentration



The solid line depicts the association of vitamin D and hemoglobin concentration at baseline. The shaded area spans the 95% confidence interval of this association.

Synopsis of Paper 3

Objective

To estimate whether vitamin D deficiency (<10 ng/ mL) is associated with neurodevelopment and physical growth in young children

Results

The overall ASQ-3 scores were not significantly different between the vitamin D-deficient children and the vitamin D non-deficient children (mean difference -6.54; 95% CI: -16.15 to 3.08). The Personal-social subscale score was significantly lower in the vitamin D deficient children (mean difference -2.63; 95% CI: -5.00 to -0.25), while the other subscale scores were not different between the groups. We also categorized the total ASQ-3 scores into two categories: scores <25th percentile and scores ≥25th percentile. After adjusting for potential confounders, we found a greater odds of having scores in the lowest 25th percentile of the Personal-social score in the vitamin D deficient children compared to the non-deficient children (OR: 1.63; 95% CI: 1.03 to 2.58). We did not detect such differences for the other subscale scores or for the overall total ASQ-3 score (Table 5).

Table 5 Association between baseline vitamin D status and ASQ-3 scores

	Non-deficient (Vitamin D level≥10 ng/mL) OR	Deficient (Vitamin D level < 10 ng/mL) Adjusted OR [a,b,c]	95% CI
Total ASQ-3	1	1.36	0.79 to 2.31
Subscale			
Communication	1	1.58	0.97 to 2.59
Gross motor	1	1.27	0.80 to 2.03
Fine motor	1	1.31	0.81 to 2.11
Problem-solving	1	1.33	0.84 to 2.11
Personal-Social	1	1.63	1.03 to 2.58

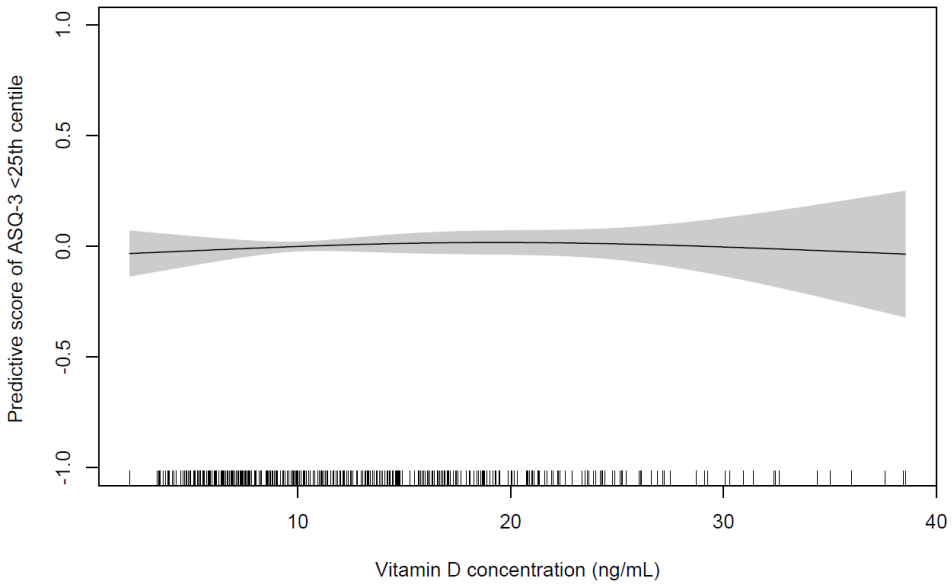
[a] Reference category: Non-deficient (Vitamin D concentration ≥ 10 ng/mL)

[b] ORs for the lower quartile of total ASQ-3 and subscale scores compared with non-deficient vitamin D concentration (≥ 10 ng/mL)

[c] Adjusted for age of child, mother's years of schooling, father's years of schooling, log-transformed annual family income, family structure, number of toys available, family owns books, number of children in the family, hours of play with other children during the week, mother's belief that a child`s behaviour can be modified, mother's encouragement of developmental advances, WLZ/WHZ scores, WAZ scores, and intervention groups

We were not able to identify any non-linear associations between the vitamin D concentration and the ASQ-3 scores (Figure 13). In the GAM, we removed the highest 1.5% (vitamin D concentration >40 ng/mL) from the analyses as the estimated GAM curve beyond this cut-off was highly uncertain.

Figure 13 Association between baseline vitamin D concentration and total ASQ-3 scores



The solid line depicts the association of vitamin-D concentration at baseline and total ASQ-3 score. The shaded area spans the 95% confidence interval of this association

Vitamin D deficiency was not associated with stunting, wasting, or underweight in this population.

Synopsis of Paper 4

Objective

To examine whether vitamin D deficiency in early childhood is associated with cognitive development and linear growth measured during school age.

Results

The demographic characteristics of the children and their families in the follow up study are presented in Table 6. The mean (SD) age of the children was 7.9 (0.6) years. Approximately 98% of children were going to school at the time of the assessments. Twenty-five percent of the mothers had no schooling and 5% of the fathers were unemployed.

Table 6 Baseline characteristics of vitamin D deficient and non-deficient children at follow up

	Vitamin D sufficient (n=154)	Vitamin D inadequate (n=234)	Vitamin D deficient (n=328)
Child characteristics			
Age at follow up in years, mean (SD)	7.9 (0.6)	7.8 (0.6)	7.9 (0.6)
Schooling			
None	3 (1.9)	6 (2.6)	4 (1.2)
Hindi medium	61 (39.6)	88 (37.6)	129 (39.3)
English medium	90 (58.4)	140 (59.8)	195 (59.4)
Family characteristics			
Mothers years of schooling			
Never been to school	54 (35.5)	65 (28)	76 (23.3)
Primary (1- 5 years)	23 (15.1)	18 (7.8)	49 (15)
Middle (6-12 years)	65 (42.8)	123 (53)	153 (46.9)
Higher (> 12 years)	10 (6.6)	26 (11.2)	48 (14.7)
Fathers occupation			
Government job or private services	88 (57.1)	135 (57.9)	165 (50.8)
Self-employed	31 (20.1)	56 (24)	95 (29.2)
Daily wager/farming	29 (18.8)	31 (13.3)	52 (16)
Unemployed	6 (3.9)	11 (4.7)	13 (4)
Wealth Quintile			
Poorest	39 (25.3)	47 (20.1)	60 (18.3)
Very Poor	36 (23.4)	38 (16.2)	65 (19.8)
Poor	31 (20.1)	57 (24.4)	60 (18.3)
Less Poor	32 (20.8)	43 (18.4)	69 (21)
Least Poor	16 (10.4)	49 (20.9)	74 (22.6)

Figures are number (percentages) unless stated otherwise

The association between vitamin D deficiency and the WISC-IV^{INDIA} and CVS z-scores, NEPSY-II z-scores and the Global BRIEF-2 scores are shown in Table 7. There were no significant differences between the vitamin D sufficient children and those with inadequate or deficient vitamin D status on any of the cognitive outcomes. Furthermore, vitamin D status was not associated with HAZ scores or the proportion of children stunted at follow up.

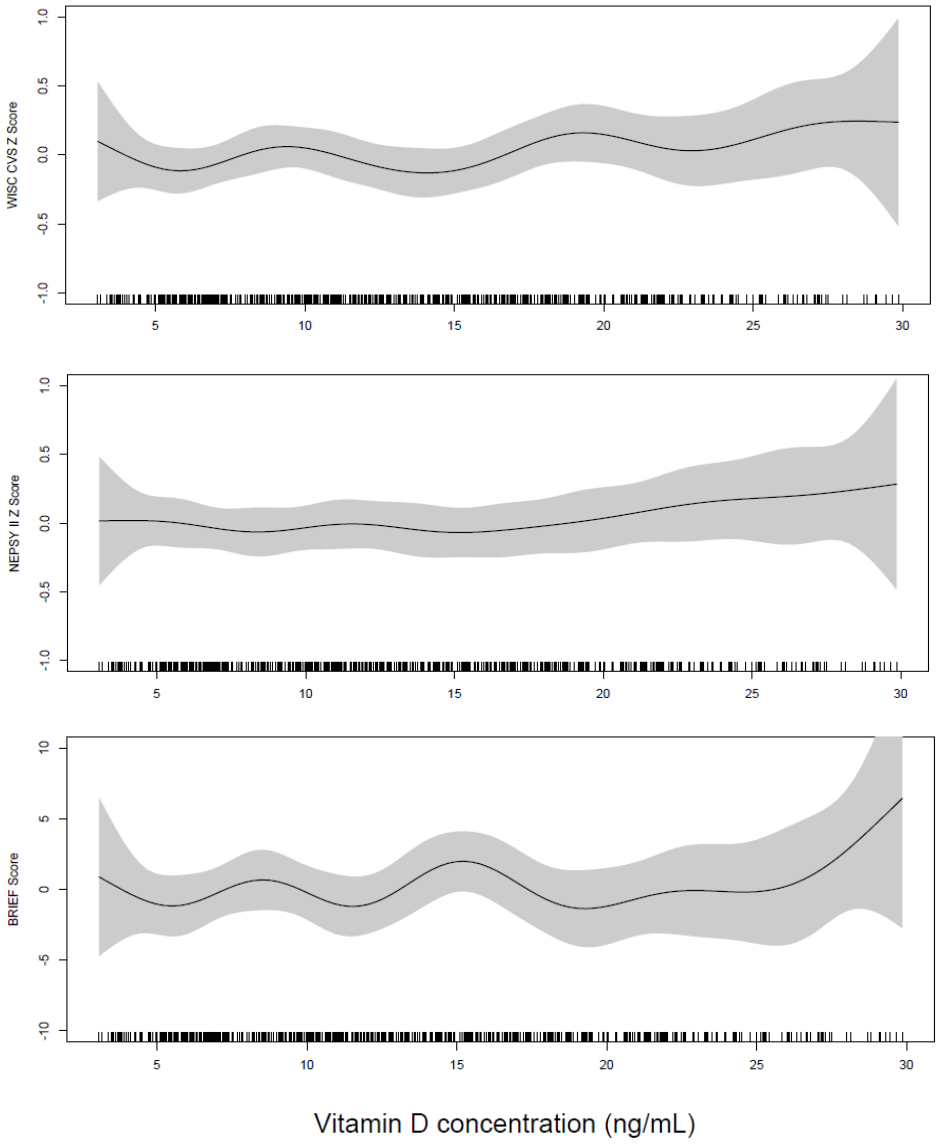
Table 7 Association between baseline vitamin D status and cognitive scores at follow-up

	WISC-IV^{INDIA} and CVS z-score		NEPSY z-score		Global BRIEF score	
	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)[a]	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)[a]	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)[a]
Vitamin D sufficient	Reference		Reference		Reference	
Vitamin D inadequate	0.03 (-0.17 to 0.23)	-0.12 (-0.30 to 0.05)	-0.01 (-0.21 to 0.19)	-0.15 (-0.33 to 0.04)	0.21 (-2.10 to 2.48)	0.87 (-1.40 to 3.14)
Vitamin D deficient	0.02 (-0.16 to 0.22)	-0.13 (-0.29 to 0.04)	-0.02 (-0.21 to 0.17)	-0.16 (-0.34 to 0.02)	-1.10 (-3.21 to 1.05)	-0.38 (-2.53 to 1.77)

[a]adjusted for log folate, log soluble transferrin receptor and log homocysteine levels at baseline, and the wealth index, paternal occupational status, and maternal education at follow-up and intervention groups.

The GAM models did not reveal any non-linear associations between the vitamin D levels at baseline and the combined WISC-IV^{INDIA} and CVS z-score, the NEPSY-II z-score or the Global BRIEF-2 score at follow-up (Figure 14). We removed the highest 5% (vitamin D concentration >30 ng/mL) from the analyses as the estimated GAM curve beyond this cut-off was highly uncertain.

Figure 14 Association between baseline vitamin D concentration and the combined WISC-IV^{INDIA} and Crichton Vocabulary Scale (CVS) z-score, the combined NEPSY II z score, and the BRIEF Global Executive composite score at follow up



The solid line depicts the association of vitamin D concentration at baseline and WISC-IV^{INDIA} and Crichton Vocabulary Scale (CVS) z-score, the combined NEPSY II z score, and the BRIEF P Global Executive composite score at follow up. The shaded area spans the 95% confidence interval of these associations.

Discussion

Our key findings are that approximately 35% of the children had vitamin D deficiency (<10 ng/mL) in early childhood, and this was associated with a higher risk of ALRIs and moderate anemia (Hb 7 to 9.9 g/dL). Vitamin D deficiency was not associated with neurodevelopment and growth during early childhood (6-30 months) or at school age (6 to 9 years).

Vitamin D deficiency

The high prevalence of vitamin D deficiency during early childhood is similar to the results from other studies in India and south east Asia (106-109). This can have two primary causes; inadequate sun exposure or low dietary intake of vitamin D. Inadequate sun exposure may be due to the relatively high solar zenith angle, atmospheric pollution, the type V skin of the population, and restricted outdoor activities (110, 111). If there is a higher solar zenith angle, more UVB photons are absorbed in the stratospheric ozone layer. Consequently, fewer UVB photons can penetrate the earth's surface to produce cutaneous pre-vitamins (110). Air pollution can cause vitamin D deficiency in two ways; directly by blocking UVB photons and indirectly by restricting outdoor activities, thus allowing less exposure to sunlight (112). Individuals with type V skin have significant constitutive pigmentation (melanin), which protects the underlying skin against damage from UVR and thus reduces vitamin D synthesis (113). We conducted the current study in an urban slum where houses are clustered with small open spaces. Additionally, the lack of space offers limited options for outdoor activities. Dietary sources of vitamin D are primarily limited to oily fish, eggs, and fortified foods (55). As most of the population in northern India is vegetarians, the predominant dietary source of vitamin D among the study children would be milk (114) . In India, milk is rarely fortified with vitamin D and the vitamin D content of unfortified milk is very low (2 IU/100 mL) (63). The prevalence of lactose intolerance also contributes to vitamin D deficiency in this setting (63). The complementary foods given to Indian children are primarily cereal based and these are

low in vitamin D content. This is another contributing factor of the widespread vitamin D deficiency among children in this setting (115).

Vitamin D and common childhood infections

We found a higher incidence of ALRIs among vitamin D deficient children than among those that were non deficient. We did not find any association between clinical pneumonia, a severe form of ALRIs, and vitamin D status.

Similar to our findings, a systematic review of observational studies has shown that vitamin D deficiency was more prevalent (OR: 3.29, 95% CI: 1.27–8.56) in children with lower respiratory tract infections (LRTIs) than in those without lower respiratory tract infections (116). Moreover, mean vitamin D concentration in children with LRTIs were significantly lower than those in children with no LRTIs, with a mean difference of 8.75 (95% CI:1.80 to 15.70) nmol/L (116). The Cochrane review on vitamin D supplementation for preventing infections in children under five years of age, showed that vitamin D supplementation did not reduce episodes of 'radiologically confirmed' first or only episode of pneumonia (RR: 1.06; 95% CI: 0.89 to 1.26; two trials, 3134 participants) (117).

The protective role of vitamin D against ALRIs may be through its modulatory effect on innate and adaptive immunity and the inflammatory cascade (7, 118-120). Vitamin D induces TLR activation and antibacterial responses, which in turn enhance the production of cathelicidin (LL-37), an antimicrobial peptide that is present at different natural barrier sites, such as the lungs (121). Vitamin D has effects on both innate and adaptive immune responses, which may suggest a different role according to pathogens and severity (122). Vitamin D might not have a role once an infection sets in, other individual and environmental factors may determine its natural course. This could be the reason we found a significant association between vitamin D deficiency and ALRIs but not with clinical pneumonia.

Vitamin D deficiency was not associated with the incidence of diarrhea or its severity in our study, which is similar to findings from other observational studies (123). A randomized controlled trial (RCT) of 3-monthly bolus vitamin D3 supplementation (100,000 IU) among children 1 to 29 months of age, showed no effect on diarrheal illnesses (124). Constant exposure to pathogenic organisms and subsequent clinical and subclinical enteric infections are frequent in these children and could reduce the potential beneficial role of vitamin D on diarrheal illnesses in the study setting (125).

Vitamin D and anemia

We found an increased risk of moderate anemia among children with vitamin D deficiency. This finding is consistent with an increased risk of anemia among vitamin D deficient children and adolescents both in developed and developing countries (126). Several mechanisms could explain our findings. Vitamin D enhances erythropoiesis by inhibiting pro-inflammatory cytokines, which may impair erythropoiesis by inhibiting the production of erythropoietin and proliferation of erythroid progenitor cells (8). Vitamin D also causes direct suppression of hepcidin mRNA transcription which leads to less production of hepcidin. This process leads to the availability of more iron for intestinal absorption (8).

Vitamin D and neurodevelopment

Our study did not show a significant association between vitamin D deficiency and neurodevelopment during early childhood measured by the ASQ-3 except for in the Personal-social sub scale. The association was not strong and could very well be a chance finding. There were no significant differences in any of the cognitive outcomes; the combined WISC-IV^{INDIA} and CVS z-score, the combined NEPSY-II z-score and the Global BRIEF-2 composite score between categories of vitamin D status in school age children.

Our findings are similar to those from a recent cohort study in India, where vitamin D status was not associated with gross motor functioning among children aged 5 years (127). In contrast, another study in a similar setting suggested an inverted-U–shaped relationship between neonatal vitamin D status and neurocognitive development in children aged 16-18 months (128). Children in the lowest quintile of cord blood 25(OH)D concentration exhibited a deficit of 7.60 (95% CI: 2.82 to 12.4) and 8.04 (95% CI: 3.11 to 12.9) points in the Mental development index (MDI) and psychomotor development index (PDI) scores of Bayley Scales of Infant Development second edition, respectively, compared with the reference category. In this study, children in the highest quintile of cord blood 25(OH)D concentration, also showed a significant deficit of 12.3 (95% CI: 6.67 to 17.9) points in PDI scores compared with the reference category. However, higher levels of vitamin D (≥ 20 ng/mL) showed a positive association with reported temperament (activity and soothability), reported receptive language and directly observed behavior but not with cognitive and motor development among infants aged 6-8 months in Bangladesh (129). Studies that have examined the association between cord blood vitamin D concentration or vitamin D deficiency during pregnancy with neurodevelopment measured in early childhood and school age, have shown inconsistent results (91, 128, 130-137). These are likely due to differences in the populations, the timing of vitamin D assessment during pregnancy, the use of different cut-offs for vitamin D deficiency, age of the child at the neurodevelopmental assessments, and the way potential confounders were adjusted for.

Available evidence from cellular mechanisms of vitamin D suggests that vitamin D may play an essential role in neurodevelopment. Vitamin D may have an effect on brain development through its anti-inflammatory, anti-autoimmune effects, increasing Treg cells, enhancing DNA repair mechanisms, upregulating glutathione and protecting mitochondria (138). However, our findings suggest that these mechanisms may not be relevant for the impact of vitamin D on cognition neither during early childhood nor at school age. Multiple factors interplay for impaired neurodevelopment during childhood in low resource settings, such as inadequate early stimulation, inflammation, infection,

and micronutrient deficiencies (75). The influence of vitamin D may be of limited importance when viewed with other important factors that influence neurodevelopment in these children.

Vitamin D and linear growth

Vitamin D deficiency was not associated with linear growth during early childhood or at school age. Similar findings were seen in infants in Bangladesh, preschool children in Nepal and HIV-exposed but uninfected infants in Africa (129, 139, 140). In a study in Indian low-birth-weight (1.8 to 2.5 kg) infants however, vitamin D supplementation of 1 RDA resulted in significantly higher length and weight at six months of age compared to those who received placebo (141).

Linear growth during early childhood and school age is regulated by different endocrinal factors. While the early childhood phase is primarily regulated by insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2) and insulin, the school age period is primarily dependent on growth hormone (GH) and thyroxin (142). Recent studies have shown that vitamin D, GH and IGF-1 may have an impact on epiphyseal chondrocytes. In addition vitamin D helps the growth plate cells to be more sensitive to GH and IGF-1 action (143). Vitamin D also maintains bone health ensuring normal calcium and phosphate levels in the blood. 1,25 $(OH)_2D$) along with PTH and calcitonin acts to maintain endocrine control of calcium and phosphorus concentration in blood (11). This tight regulation of calcium and phosphorus flux (extracellular [bones, blood], intracellular) is critical for the development and maintenance of bones, which prevents growth faltering (144). Our findings of no association between vitamin D deficiency and linear growth may hence be unexpected. There may be deficiencies of other growth-limiting macro and micronutrients, such as calcium, zinc, and vitamin B12 that may account for the variance of growth in these children. Poor quality of food, i.e., a lower proportion of animal-sourced protein, may also contribute to impaired growth in children

in this population (145). The role of vitamin D may accordingly be negligible in the light of other growth-limiting factors in this population.

Methodological Issues

The accuracy of the results from an epidemiological study depends on the validity and precision of the measurements and effect estimates. The validity of a study can be categorized into internal validity and external validity. Most violations of internal validity can be classified into three groups, selection bias, measurement bias, and confounding. The precision of the effect estimates depends on the sample size, variability, and random errors (146).

Selection bias

Selection bias occurs when the study population is systematically different from those who are not included in the study. Specific definitions for inclusion and exclusion criteria and low attrition rates may reduce the chance of selection bias (146). In the original study, children with severe systemic illness requiring hospitalization, severe acute malnutrition (WLZ/WHZ <-3 SD), severe anemia (Hb <7 g/dL), and those who were using folic acid and/or vitamin B-12 supplements were excluded. The attrition rate was <1% during the six months follow up period (79). We were able to include ~80% (791/1000) of the children from the original cohort after more than 5 years of study completion. The prevalence of vitamin D deficiency may be different in those who were excluded and lost to follow-up. There were, however, no differences between the children who were included in the analyses and those who were not. We therefore believe that there is limited selection bias in this study.

Measurement errors

Measurement errors can be of two types; systematic and random. Random measurement error occurs when measurements fluctuate unpredictably around their true values and is

caused by imprecise measurement tools, true biological variability, or both (147). For example, a single assessment of serum 25(OH)D may not accurately reflect an individual's vitamin D status over a year. The serum 25(OH)D levels vary with season; highest concentrations are observed in the summer and lowest in the winter due to differences in sun exposure (57). Random measurement error can bias the effect estimates (for example the regression slope coefficient) towards the null, a phenomenon known as attenuation or regression dilution bias (148). This bias occurs when the random measurement error is in the exposure variable.

Systematic error is when the measurement error, does not average out to zero, after multiple measurements. The measurements are consistently wrong in a particular direction (148). We expect minimal systematic measurement errors because of extensive standardization and re standardization exercises of the teams who assessed infections and cognitive development. All laboratory instruments, weighing scales, and infantometers were calibrated frequently to minimize both random and systematic errors.

Confounding

Confounding occurs when an apparently causal relationship between an exposure and an outcome is distorted by the effect of a third variable (the confounder) (149). Multivariable regression method is one of the few ways to minimize confounding (149). The common approach to build a statistical model is minimization of variables till the most parsimonious model is achieved. We used the purposeful selection of covariate method for selecting potential confounders in the multivariable regression models. The main advantage of this method is that it offers a systematic approach for selecting potential confounding variables (150). However, this method was designed to identify significant predictor variables of a dependent variable and not to adjust for confounding for one specific exposure. Moreover, the purposeful selection process was not designed to include all dummy variables in the model (for example, one variable that has three nominal levels which corresponds to two dummy variables which need to be considered

as a unit in model inclusion), if one is significant (150). It is important to keep in mind that regardless of the variable selection method employed, positive or negative residual confounding can't be ruled out.

We did not have information on the status of other nutrients that are associated with increased risk of diarrhea and pneumonia among the infants, and young children. Moreover, there are several other non-nutritional factors linked to vitamin D status that may confound the observed associations. The residual confounding can both be because we did not measure these variables or because they were measured with poor precision (151).

Precision

Although an important problem, poor precision is usually less of concern than systematic errors because it is less likely to distort the findings or reverse the overall direction. However, poor precision decreases the probability of finding a real association by reducing the statistical power of a study (148). We calculated that we had more than 80% power to detect at least 25% increased incidence of ALRIs during the six months follow-up in the vitamin D deficient children compared to the vitamin D non-deficient children with the available sample, at a 5% significance level. It should be noted that the power to detect an association between vitamin D deficiency and diarrhea and clinical pneumonia was lower than the power to detect its association with ALRIs. When we estimated the statistical power, we assumed minimal random errors in both the exposure and outcome variables. This assumption might not have been correct and led to loss of power both through attenuated effect estimates and poorer precision.

External validity

The thesis consists of secondary data analyses from a well-conducted randomized controlled trial (RCT) and its follow up study. The high prevalence of vitamin D deficiency among the infants and young children could be generalizable to any urban

slum population of India as well as to other LMICs in the tropics where atmospheric pollution is high, and there are restrictions to outside activities, especially in winters. Our results should provide a valuable addition to the existing evidence of the role of vitamin D status during infancy and early childhood on common childhood infection, anemia, growth, and neurodevelopment. The study setting was characterized by a wide range of factors known to be associated with compromised child well-being such as low socioeconomic status, high prevalence of undernutrition, widespread deficiency of micronutrients critical for growth and neurodevelopment, and high incidence of childhood illnesses. The findings from this study may not be generalizable to any larger context of children in this age group.

Strengths

The main strength of the thesis is that it is a secondary data analysis from a well-conducted RCT with a low attrition rate. We measured vitamin D concentration during a critical window of child growth and development. All outcomes were clearly defined and measured by trained and supervised workers and psychologists. There were multiple follow up visits (twice weekly for 26 weeks) for assessing ALRIs, clinical pneumonia, and diarrhea through active surveillance which allowed a minimal recall bias. In the follow up, some of the neurodevelopment outcomes were assessed using validated tools with Indian norms at an age when cognitive outcomes are considered to be more stable, and with greater predictive value (152, 153). All effect sizes were estimated after adjustment for several relevant confounders.

Limitations

An immunoassay method was used to assess vitamin D concentration. The immunoassay can underestimate serum 25(OH) D concentration compared to the gold standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) (154). Approximately, 85% of 25(OH)D is bound to VDBP, 15% to albumin, and 0.03% is free (155). Automated

immunoassays are based on non-denaturing conditions to free 25(OH)D from VDBP, and other serum binding components to allow its binding either to the kit antibodies or VDBP. This process may yield varying results because of matrix effects. Thus, the immunoassay method might overestimate the prevalence of vitamin D deficiency.
We translated the ASQ-3 questionnaires for this study, but the alpha values indicated questionable internal consistency in some subscales. We could have improved the alpha values by pilot testing. However, more than 30% of the variability of ASQ-3 scores was explained by the incidence of diarrhea and pneumonia, linear and ponderal growth, socioeconomic status and stimulation and learning during early childhood which indicate that the translated and adjusted ASQ-3 test had good convergent validity (156). In the follow up, the WISC and CVS have been validated for the Indian setting, but this is not the case for BRIEF and NEPSY. Type II errors can be due to inadequate sample sizes for the various outcomes.

Ethical considerations

The declaration of Helsinki of ethical principles for medical research involving human subjects, guides all medical research in human subjects worldwide (157). The basic principle for this declaration is the fundamental respect for human beings, as well as the investigators' sole duty to promote and safeguard the health of the participant. While there is always a need for new research, respect for the individual's well-being should precede the interest of science and society.
The research questions for the current thesis are from a well-conducted RCT, and its follow up study on the association between vitamin D status, and common childhood infection, anemia, growth, and development. The consent form for the original study and follow up study also sought permission from parents to store these children's blood samples for use for future research. All parents consented for the same.
Principle number 19 in the Helsinki declaration states that: *Medical research is only justified if there is a reasonable likelihood that the populations in which the research is*

carried out stand to benefit from the results of the research. Currently, India does not have any national program for vitamin D supplementation for infants, young children, and school-age children. We need more evidence to show the adverse effects of vitamin D deficiency in the context of urban slum children who are a vulnerable segment of society. The current study is of interest to the individual participating child, as well as for children on a societal level.

Conclusions

We have shown that vitamin D deficiency is common in young children in an urban slum of New Delhi. Vitamin D deficiency is associated with a higher risk of ALRIs and moderate anemia, but not with linear growth and neurodevelopment during early childhood and at school age. Our results imply that vitamin D is one of the critical micronutrients for common infections and anemia during early childhood. However, we believe that vitamin D may have a negligible role in growth and neurodevelopment in the light of other growth and development limiting factors in this population. Thus, a comprehensive approach should be taken to improve the growth and neurodevelopment of young children in South Asia and vitamin D status may not be a critical factor in this effort.

Future perspectives

The protective role of vitamin D on ALRIs and anemia needs to be confirmed in randomized controlled trials especially in vitamin D deficient children.This will help to understand more comprehensively the extra-skeletal roles of vitamin D. The biological interactions of vitamin D with other growth and development relevant nutrients should also be explored in the context of LMICs.

References

1. Nair R, Maseeh A. Vitamin D: The "sunshine" vitamin. J Pharmacol Pharmacother. 2012;3(2):118-26.
2. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73-8.
3. Christakos S, Dhawan P, Porta A, Mady LJ, Seth T. Vitamin D and intestinal calcium absorption. Mol Cell Endocrinol. 2011;347(1-2):25-9.
4. Kato S. The function of vitamin D receptor in vitamin D action. Journal of biochemistry. 2000;127(5):717-22.
5. Medrano M, Carrillo-Cruz E, Montero I, Perez-Simon JA. Vitamin D: Effect on Haematopoiesis and Immune System and Clinical Applications. Int J Mol Sci. 2018;19(9):2663.
6. Anjum I, Jaffery SS, Fayyaz M, Samoo Z, Anjum S. The Role of Vitamin D in Brain Health: A Mini Literature Review. Cureus. 2018;10(7):e2960-e.
7. Esposito S, Lelii M. Vitamin D and respiratory tract infections in childhood. BMC infectious diseases. 2015;15:487.
8. Smith EM, Tangpricha V. Vitamin D and anemia: insights into an emerging association. Curr Opin Endocrinol Diabetes Obes. 2015;22(6):432-8.
9. Khadilkar A, Khadilkar V, Chinnappa J, Rathi N, Khadgawat R, Balasubramanian S, et al. Prevention and Treatment of Vitamin D and Calcium Deficiency in Children and Adolescents: Indian Academy of Pediatrics (IAP) Guidelines. Indian pediatrics. 2017;54(7):567-73.
10. Goring H. Vitamin D in Nature: A Product of Synthesis and/or Degradation of Cell Membrane Components. Biochemistry Biokhimiia. 2018;83(11):1350-7.
11. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014;21(3):319-29.
12. Bikle DD, Schwartz J. Vitamin D Binding Protein, Total and Free Vitamin D Levels in Different Physiological and Pathophysiological Conditions. 2019;10(317).
13. Bikle DD, Patzek S, Wang Y. Physiologic and pathophysiologic roles of extra renal CYP27b1: Case report and review. Bone Rep. 2018;8:255-67.
14. Khammissa RAG, Fourie J, Motswaledi MH, Ballyram R, Lemmer J, Feller L. The Biological Activities of Vitamin D and Its Receptor in Relation to Calcium and Bone Homeostasis, Cancer, Immune and Cardiovascular Systems, Skin Biology, and Oral Health. BioMed research international. 2018;2018:9276380.
15. Gil A, Plaza-Diaz J, Mesa MD. Vitamin D: Classic and Novel Actions. Annals of Nutrition and Metabolism. 2018;72(2):87-95.
16. Dawson MI, Xia Z. The retinoid X receptors and their ligands. Biochim Biophys Acta. 2012;1821(1):21-56.
17. Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). Endocrinol Metab Clin North Am. 2010;39(2):255-69.
18. Rutkovskiy A, Stensløkken K-O, Vaage IJ. Osteoblast Differentiation at a Glance. Med Sci Monit Basic Res. 2016;22:95-106.
19. Pérez AV, Picotto G, Carpentieri AR, Rivoira MA, Peralta López ME, Tolosa de Talamoni NG. Minireview on Regulation of Intestinal Calcium Absorption. Digestion. 2008;77(1):22-34.
20. Hii CS, Ferrante A. The Non-Genomic Actions of Vitamin D. Nutrients. 2016;8(3):135-.
21. Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S24-S32.
22. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013;5(7):2502-21.
23. Jeng L, Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR, et al. Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. J Transl Med. 2009;7:28-.
24. Iruretagoyena M, Hirigoyen D, Naves R, Burgos PI. Immune Response Modulation by Vitamin D: Role in Systemic Lupus Erythematosus. 2015;6(513).
25. Lin R. Crosstalk between Vitamin D Metabolism, VDR Signalling, and Innate Immunity. BioMed research international. 2016;2016:1375858-.
26. Kuroda K, Okumura K, Isogai H, Isogai E. The Human Cathelicidin Antimicrobial Peptide LL-37 and Mimics are Potential Anticancer Drugs. Front Oncol. 2015;5:144-.
27. Barragan M, Good M, Kolls JK. Regulation of Dendritic Cell Function by Vitamin D. Nutrients. 2015;7(9):8127-51.

28. Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. 2014;5(151).
29. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Endocrinol Metab Clin North Am. 2010;39(2):365-79.
30. Joshi S, Pantalena L-C, Liu XK, Gaffen SL, Liu H, Rohowsky-Kochan C, et al. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. Mol Cell Biol. 2011;31(17):3653-69.
31. Yousefzadeh H, Jabbari Azad F, Rastin M, Banihashemi M, Mahmoudi M. Expression of Th1 and Th2 Cytokine and Associated Transcription Factors in Peripheral Blood Mononuclear Cells and Correlation with Disease Severity. Rep Biochem Mol Biol. 2017;6(1):102-11.
32. Nedoszytko B, Lange M, Sokołowska-Wojdyło M, Renke J, Trzonkowski P, Sobjanek M, et al. The role of regulatory T cells and genes involved in their differentiation in pathogenesis of selected inflammatory and neoplastic skin diseases. Part I: Treg properties and functions. Postepy Dermatol Alergol. 2017;34(4):285-94.
33. Zhang H, Shih DQ, Zhang X. Mechanisms underlying effects of 1,25-Dihydroxyvitamin D3 on the Th17 cells. Eur J Microbiol Immunol (Bp). 2013;3(4):237-40.
34. Schülke S. Induction of Interleukin-10 Producing Dendritic Cells As a Tool to Suppress Allergen-Specific T Helper 2 Responses. Front Immunol. 2018;9:455-.
35. Nairz M, Haschka D, Demetz E, Weiss G. Iron at the interface of immunity and infection. Front Pharmacol. 2014;5:152-.
36. Zughaier SM, Alvarez JA, Sloan JH, Konrad RJ, Tangpricha V. The role of vitamin D in regulating the iron-hepcidin-ferroportin axis in monocytes. Journal of clinical & translational endocrinology. 2014;1(1):19-25.
37. Weiss G, Goodnough LT. Anemia of chronic disease. The New England journal of medicine. 2005;352(10):1011-23.
38. Alon DB, Chaimovitz C, Dvilansky A, Lugassy G, Douvdevani A, Shany S, et al. Novel role of 1,25(OH)(2)D(3) in induction of erythroid progenitor cell proliferation. Experimental hematology. 2002;30(5):403-9.
39. Aucella F, Scalzulli RP, Gatta G, Vigilante M, Carella AM, Stallone C. Calcitriol increases burst-forming unit-erythroid proliferation in chronic renal failure. A synergistic effect with r-HuEpo. Nephron Clinical practice. 2003;95(4):c121-7.
40. Jang H, Choi Y, Yoo I, Han J, Hong JS, Kim YY, et al. Vitamin D-metabolic enzymes and related molecules: Expression at the maternal-conceptus interface and the role of vitamin D in endometrial gene expression in pigs. PloS one. 2017;12(10):e0187221.
41. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. Neuroscience. 2003;118(3):641-53.
42. O'Loan J, Eyles DW, Kesby J, Ko P, McGrath JJ, Burne TH. Vitamin D deficiency during various stages of pregnancy in the rat; its impact on development and behaviour in adult offspring. Psychoneuroendocrinology. 2007;32(3):227-34.
43. Hawes JE, Tesic D, Whitehouse AJ, Zosky GR, Smith JT, Wyrwoll CS. Maternal vitamin D deficiency alters fetal brain development in the BALB/c mouse. Behavioural brain research. 2015;286:192-200.
44. Mayne PE, Burne THJ. Vitamin D in Synaptic Plasticity, Cognitive Function, and Neuropsychiatric Illness. Trends in neurosciences. 2019;42(4):293-306.
45. Brouwer-Brolsma EM, Vrijkotte TGM, Feskens EJM. Maternal vitamin D concentrations are associated with faster childhood reaction time and response speed, but not with motor fluency and flexibility, at the age of 5-6 years: the Amsterdam Born Children and their Development (ABCD) Study. The British journal of nutrition. 2018;120(3):345-52.
46. Hanieh S, Ha TT, Simpson JA, Thuy TT, Khuong NC, Thoang DD, et al. Maternal vitamin D status and infant outcomes in rural Vietnam: a prospective cohort study. PloS one. 2014;9(6):e99005.
47. Keim SA, Bodnar LM, Klebanoff MA. Maternal and cord blood 25(OH)-vitamin D concentrations in relation to child development and behaviour. Paediatric and perinatal epidemiology. 2014;28(5):434-44.
48. Morales E, Guxens M, Llop S, Rodriguez-Bernal CL, Tardon A, Riano I, et al. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics. 2012;130(4):e913-20.
49. Strom M, Halldorsson TI, Hansen S, Granstrom C, Maslova E, Petersen SB, et al. Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. Annals of nutrition & metabolism. 2014;64(3-4):254-61.
50. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Kusel MM, Hart PH. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics. 2012;129(3):485-93.

51. Gould JF, Anderson AJ, Yelland LN, Smithers LG, Skeaff CM, Zhou SJ, et al. Association of cord blood vitamin D with early childhood growth and neurodevelopment. Journal of paediatrics and child health. 2017;53(1):75-83.
52. Holick MF. Vitamin D deficiency. The New England journal of medicine. 2007;357(3):266-81.
53. Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. Dermatoendocrinol. 2013;5(1):51-108.
54. Roth DE, Abrams SA, Aloia J, Bergeron G, Bourassa MW, Brown KH, et al. Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries. Ann N Y Acad Sci. 2018;1430(1):44-79.
55. Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Mawer EB, Puliyel JM. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. Arch Dis Child. 2002;87(2):111-3.
56. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2011;96(7):1911-30.
57. Millen AE, Bodnar LM. Vitamin D assessment in population-based studies: a review of the issues. The American journal of clinical nutrition. 2008;87(4):1102s-5s.
58. Knight JA, Lesosky M, Barnett H, Raboud JM, Vieth R. Vitamin D and reduced risk of breast cancer: a population-based case-control study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007;16(3):422-9.
59. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutrition bulletin. 2014;39(4):322-50.
60. Institute of Medicine FaNB. Dietary Reference Intakes for Calcium and Vitamin D2010. Available from: https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/.
61. Endocrine Society. Available from: https://www.endocrine.org/
62. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, et al. IOM committee members respond to Endocrine Society vitamin D guideline. The Journal of clinical endocrinology and metabolism. 2012;97(4):1146-52.
63. G R, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014;6(2):729-75.
64. Mathew JL, Patwari AK, Gupta P, Shah D, Gera T, Gogia S, et al. Acute respiratory infection and pneumonia in India: a systematic review of literature for advocacy and action: UNICEF-PHFI series on newborn and child health, India. Indian pediatrics. 2011;48(3):191-218.
65. Rudan I, O'Brien KL, Nair H, Liu L, Theodoratou E, Qazi S, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. Journal of global health. 2013;3(1):010401.
66. McAllister DA, Liu L, Shi T, Chu Y, Reed C, Burrows J, et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health. 2019;7(1):e47-e57.
67. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388(10063):3027-35.
68. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. Lancet. 2011;378(9809):2123-35.
69. Gebreweld A, Ali N, Ali R, Fisha T. Prevalence of anemia and its associated factors among children under five years of age attending at Guguftu health center, South Wollo, Northeast Ethiopia. PloS one. 2019;14(7):e0218961-e.
70. Nguyen PH, Scott S, Avula R, Tran LM, Menon P. Trends and drivers of change in the prevalence of anaemia among 1 million women and children in India, 2006 to 2016. 2018;3(5):e001010.
71. National Family Health Survey (NFHS-4), 2015-16. [Internet]. International Institute for Population Sciences (IIPS) and Macro International. Mumbai. 2015.
72. Global Nutrition Report 2018. Available from: https://globalnutritionreport.org/reports/global-nutrition-report-2018/burden-malnutrition/#note-b2a67844-3.
73. Bhandari N CR. Infant and Young Child Feeding. Proc Indian Natn Sci Acad. 2016;82(3):1507-17.
74. Black MM, Walker SP, Fernald LCH, Andersen CT, DiGirolamo AM, Lu C, et al. Early childhood development coming of age: science through the life course. Lancet. 2017;389(10064):77-90.

75. John CC, Black MM, Nelson CA, 3rd. Neurodevelopment: The Impact of Nutrition and Inflammation During Early to Middle Childhood in Low-Resource Settings. Pediatrics. 2017;139(Suppl 1):S59-s71.
76. McCoy DC, Peet ED, Ezzati M, Danaei G, Black MM, Sudfeld CR, et al. Early Childhood Developmental Status in Low- and Middle-Income Countries: National, Regional, and Global Prevalence Estimates Using Predictive Modeling. PLoS Med. 2016;13(6):e1002034-e.
77. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B, et al. Developmental potential in the first 5 years for children in developing countries. Lancet. 2007;369(9555):60-70.
78. Arora NK, Nair MKC, Gulati S, Deshmukh V, Mohapatra A, Mishra D, et al. Neurodevelopmental disorders in children aged 2-9 years: Population-based burden estimates across five regions in India. PLoS Med. 2018;15(7):e1002615-e.
79. Taneja S, Strand TA, Kumar T, Mahesh M, Mohan S, Manger MS, et al. Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: a randomized placebo-controlled trial. The American journal of clinical nutrition. 2013;98(3):731-7.
80. Abdel-Wareth L, Haq A, Turner A, Khan S, Salem A, Mustafa F, et al. Total vitamin D assay comparison of the Roche Diagnostics "Vitamin D total" electrochemiluminescence protein binding assay with the Chromsystems HPLC method in a population with both D2 and D3 forms of vitamin D. Nutrients. 2013;5(3):971-80.
81. Bäck S-E, Magnusson CGM, Norlund LK, von Schenck HH, Menschik ME, Lindberg PES. Multiple-Site Analytic Evaluation of a New Portable Analyzer, HemoCue Hb 201+, for Point-of-Care Testing. 2004;3(2):60-5.
82. Gehring H, Hornberger C, Dibbelt L, Rothsigkeit A, Gerlach K, Schumacher J, et al. Accuracy of point-of-care-testing (POCT) for determining hemoglobin concentrations. Acta anaesthesiologica Scandinavica. 2002;46(8):980-6.
83. Kelleher BP, Walshe KG, Scott JM, O'Broin SD. Microbiological assay for vitamin B12 with use of a colistin-sulfate-resistant organism. Clinical chemistry. 1987;33(1):52-4.
84. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol. 1992;45(4):344-7.
85. Cotton F, Thiry P, Boeynaems J. Measurement of soluble transferrin receptor by immunoturbidimetry and immunonephelometry. Clinical biochemistry. 2000;33(4):263-7.
86. Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst(e)ine with the Abbott IMx analyzer. Clinical chemistry. 1995;41(7):991-4.
87. Organization WH. Integrated Management of Childhood Illness: distance learning course 2014.
88. Squires J BD. Ages & stages questionnaires [R], (ASQ-3 [TM]): a parentcompleted child-monitoring system: Brookes Publishing Company; 2009.
89. Kvestad I, Taneja S, Kumar T, Bhandari N, Strand TA, Hysing M. The assessment of developmental status using the Ages and Stages questionnaire-3 in nutritional research in north Indian young children. Nutrition Journal. 2013;12(1):50.
90. Bradley R CR. Caring for children around the world: A view from HOME. . International Journal of Behavioral Development. 2005;29(6):468-78.
91. Winje BA, Kvestad I, Krishnamachari S, Manji K, Taneja S, Bellinger DC, et al. Does early vitamin B₁₂ supplementation improve neurodevelopment and cognitive function in childhood and into school age: a study protocol for extended follow-ups from randomised controlled trials in India and Tanzania. BMJ Open. 2018;8(2):e018962.
92. D W. WISC-IV India. Wechsler Intelligence Scale for Children - fourth (India edition): Pearson; 2016.
93. Raven J RJ, Squire A. Manual for Coroured Progressive Matrices and Crichton Vocabulary Scale: NCS Pearson Inc; 2008.
94. Raven J RJ, Squire A. RAVEN´S Coloured Progressive Matrices and Crichton Vocabulary Scale NCS Pearson Inc; 2015.
95. Brooks BL, Sherman EMS, Strauss E. NEPSY-II: A Developmental Neuropsychological Assessment, Second Edition. Child Neuropsychology. 2009;16(1):80-101.
96. Roth RM, Isquith PK, Gioia GA. Assessment of executive functioning using the Behavior Rating Inventory of Executive Function (BRIEF). Handbook of executive functioning. New York, NY, US: Springer Science + Business Media; 2014. p. 301-31.
97. Organization WH. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity 2011. Available from: https://www.who.int/vmnis/indicators/haemoglobin/en/.
98. Kolbe-Busch S, Lotz J, Hafner G, Blanckaert NJ, Claeys G, Togni G, et al. Multicenter evaluation of a fully mechanized soluble transferrin receptor assay on the Hitachi and cobas integra analyzers. the determination of reference ranges. Clinical chemistry and laboratory medicine. 2002;40(5):529-36.

99. Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. The American journal of clinical nutrition. 2007;86(5):1302-9.
100. Dielis AWJH, Cate Ht. Homocysteine: Plasma Levels and Genetic Basis. In: Lang F, editor. Encyclopedia of Molecular Mechanisms of Disease. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. p. 852-4.
101. Organization WH. WHO Child Growth Standards: Methods and development. Geneva: World Health Organization 2006. Available from: https://www.who.int/childgrowth/standards/Technical_report.pdf.
102. Hosmer DW LS, Sturdivant RX. Applied logistic regression. Third Edition ed: New York: Wiley; 2013.
103. Wood SN. Modelling and Smoothing Parameter Estimation with Multiple Quadratic Penalties. Journal of the Royal Statistical Society Series B (Statistical Methodology). 2000;62(2):413-28.
104. Vyas S, Kumaranayake L. Constructing socio-economic status indices: how to use principal components analysis. Health Policy and Planning. 2006;21(6):459-68.
105. Zou G. A modified poisson regression approach to prospective studies with binary data. American journal of epidemiology. 2004;159(7):702-6.
106. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. European journal of clinical nutrition. 2009;63(4):473-7.
107. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. Acta paediatrica (Oslo, Norway : 1992). 2010;99(3):389-93.
108. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. European journal of clinical nutrition. 2004;58(4):563-7.
109. Roth DE, Shah MR, Black RE, Baqui AH. Vitamin D status of infants in northeastern rural Bangladesh: preliminary observations and a review of potential determinants. J Health Popul Nutr. 2010;28(5):458-69.
110. Harinarayan CV, Joshi SR. Vitamin D status in India--its implications and remedial measures. The Journal of the Association of Physicians of India. 2009;57:40-8.
111. Puri S, Marwaha RK, Agarwal N, Tandon N, Agarwal R, Grewal K, et al. Vitamin D status of apparently healthy schoolgirls from two different socioeconomic strata in Delhi: relation to nutrition and lifestyle. The British journal of nutrition. 2008;99(4):876-82.
112. Mousavi SE, Amini H, Heydarpour P, Amini Chermahini F, Godderis L. Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms. Environment International. 2019;122:67-90.
113. Del Bino S, Duval C, Bernerd F. Clinical and Biological Characterization of Skin Pigmentation Diversity and Its Consequences on UV Impact. Int J Mol Sci. 2018;19(9):2668.
114. (India) OotRGaCC. India Sample Registration System Baseline Survey 2014. 2014.
115. Kuriyan R, Kurpad AV. Complementary feeding patterns in India. Nutr Metab Cardiovasc Dis. 2012;22(10):799-805.
116. Jat KR. Vitamin D deficiency and lower respiratory tract infections in children: a systematic review and meta-analysis of observational studies. Tropical Doctor. 2016;47(1):77-84.
117. Yakoob MY, Salam RA, Khan FR, Bhutta ZA. Vitamin D supplementation for preventing infections in children under five years of age. Cochrane Database of Systematic Reviews. 2016(11).
118. Sun J. Vitamin D and mucosal immune function. Current opinion in gastroenterology. 2010;26(6):591-5.
119. Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. Current opinion in nephrology and hypertension. 2008;17(4):348-52.
120. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science (New York, NY). 2006;311(5768):1770-3.
121. Quraishi SA, Bittner EA, Christopher KB, Camargo CA, Jr. Vitamin D status and community-acquired pneumonia: results from the third National Health and Nutrition Examination Survey. PloS one. 2013;8(11):e81120.
122. Wei R, Christakos S. Mechanisms Underlying the Regulation of Innate and Adaptive Immunity by Vitamin D. Nutrients. 2015;7(10):8251-60.
123. Ahmed AMS, Magalhaes RJS, Ahmed T, Long KZ, Hossain M, Islam MM, et al. Vitamin-D status is not a confounder of the relationship between zinc and diarrhoea: a study in 6–24-month-old underweight and normal-weight children of urban Bangladesh. European journal of clinical nutrition. 2016;70(5):620-8.
124. Aluisio AR, Maroof Z, Chandramohan D, Bruce J, Mughal MZ, Bhutta Z, et al. Vitamin D_3supplementation and childhood diarrhea: a randomized controlled trial. Pediatrics. 2013;132(4):e832-e40.

125. Abba K, Sinfield R, Hart CA, Garner P. Pathogens associated with persistent diarrhoea in children in low and middle income countries: systematic review. BMC infectious diseases. 2009;9(1):88.
126. Uwaezuoke SN. Vitamin D deficiency and anemia risk in children: a review of emerging evidence. Pediatric Health Med Ther. 2017;8:47-55.
127. Filteau S, Rehman AM, Yousafzai A, Chugh R, Kaur M, Sachdev HPS, et al. Associations of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term with low birth weight. BMJ Open. 2016;6(1):e009268.
128. Zhu P, Tong S-L, Hao J-H, Tao R-X, Huang K, Hu W-B, et al. Cord blood vitamin D and neurocognitive development are nonlinearly related in toddlers. The Journal of nutrition. 2015;145(6):1232-8.
129. Tofail F, Islam MM, Mahfuz M, Ashraful Alam M, Aktar S, Haque R, et al. Association of vitamin D nutrition with neuro-developmental outcome of infants of slums in Bangladesh. PloS one. 2019;14(9):e0221805-e.
130. Brouwer-Brolsma EM, Vrijkotte TGM, Feskens EJM. Maternal vitamin D concentrations are associated with faster childhood reaction time and response speed, but not with motor fluency and flexibility, at the age of 5-6 years: the Amsterdam Born Children and their Development (ABCD) Study. The British journal of nutrition. 2018;120(3):345-52.
131. Darling AL, Rayman MP, Steer CD, Golding J, Lanham-New SA, Bath SC. Association between maternal vitamin D status in pregnancy and neurodevelopmental outcomes in childhood: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). The British journal of nutrition. 2017;117(12):1682-92.
132. Gould JF, Anderson AJ, Yelland LN, Smithers LG, Skeaff CM, Zhou SJ, et al. Association of cord blood vitamin D with early childhood growth and neurodevelopment. Journal of paediatrics and child health. 2017;53(1):75-83.
133. Hanieh S, Ha TT, Simpson JA, Thuy TT, Khuong NC, Thoang DD, et al. Maternal vitamin D status and infant outcomes in rural Vietnam: a prospective cohort study. PloS one. 2014;9(6):e99005-e.
134. Keim SA, Bodnar LM, Klebanoff MA. Maternal and cord blood 25(OH)-vitamin D concentrations in relation to child development and behaviour. Paediatric and perinatal epidemiology. 2014;28(5):434-44.
135. Morales E, Guxens M, Llop S, Rodríguez-Bernal CL, Tardón A, Riaño I, et al. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics. 2012;130(4):e913-e20.
136. Strøm M, Halldorsson TI, Hansen S, Granström C, Maslova E, Petersen SB, et al. Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. Annals of nutrition & metabolism. 2014;64(3-4):254-61.
137. Whitehouse AJO, Holt BJ, Serralha M, Holt PG, Kusel MMH, Hart PH. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics. 2012;129(3):485-93.
138. Cannell JJ, Grant WB. What is the role of vitamin D in autism? Dermatoendocrinol. 2013;5(1):199-204.
139. Avagyan D, Neupane SP, Gundersen TE, Madar AA. Vitamin D status in pre-school children in rural Nepal. Public Health Nutrition. 2016;19(3):470-6.
140. Sudfeld CR, Duggan C, Aboud S, Kupka R, Manji KP, Kisenge R, et al. Vitamin D Status Is Associated with Mortality, Morbidity, and Growth Failure among a Prospective Cohort of HIV-Infected and HIV-Exposed Tanzanian Infants. The Journal of Nutrition. 2014;145(1):121-7.
141. Kumar GT, Sachdev HS, Chellani H, Rehman AM, Singh V, Arora H, et al. Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. BMJ (Clinical research ed). 2011;342:d2975.
142. Esposito S, Leonardi A, Lanciotti L, Cofini M, Muzi G, Penta L. Vitamin D and growth hormone in children: a review of the current scientific knowledge. J Transl Med. 2019;17(1):87-.
143. Ameri P, Giusti A, Boschetti M, Murialdo G, Minuto F, Ferone D. Interactions between vitamin D and IGF-I: from physiology to clinical practice. Clin Endocrinol (Oxf). 2013;79(4):457-63.
144. Goretti Penido M, Alon US. Phosphate homeostasis and its role in bone health. Pediatr Nephrol. 2012;27(11):2039-48.
145. Eaton JC, Rothpletz-Puglia P, Dreker MR, Kaganda J, Iannotti L, Lutter C, et al. Effectiveness of provision of animal-source foods for supporting optimal growth and development in children 6 to 59 months of age. Cochrane Database of Systematic Reviews. 2017(10).
146. Rothman KJG, S; Lash, T.L. Modern Epidemiology. Third ed: Lippincott Williams & Wilkins; 2010. 733 p.
147. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. BMJ (Clinical research ed). 2010;340:c2289.
148. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. 2010;340:c2289.

149. McNamee R. Regression modelling and other methods to control confounding. Occupational and Environmental Medicine. 2005;62(7):500.
150. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med. 2008;3:17-.
151. Fewell Z, Davey Smith G, Sterne JA. The impact of residual and unmeasured confounding in epidemiologic studies: a simulation study. American journal of epidemiology. 2007;166(6):646-55.
152. Anderson PJ, Burnett A. Assessing developmental delay in early childhood - concerns with the Bayley-III scales. Clin Neuropsychol. 2017;31(2):371-81.
153. Canivez GL, Watkins MW. Long-term stability of the Wechsler Intelligence Scale for Children—Third Edition. Psychological Assessment. 1998;10(3):285-91.
154. Li L, Zeng Q, Yuan J, Xie Z. Performance evaluation of two immunoassays for 25-hydroxyvitamin D. J Clin Biochem Nutr. 2016;58(3):186-92.
155. Bikle DD, Schwartz J. Vitamin D Binding Protein, Total and Free Vitamin D Levels in Different Physiological and Pathophysiological Conditions. Frontiers in endocrinology. 2019;10:317.
156. Kvestad I, Taneja S, Hysing M, Kumar T, Bhandari N, Strand TA. Diarrhea, stimulation and growth predict neurodevelopment in young North Indian children. PloS one. 2015;10(3):e0121743-e.
157. World Medical A. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ. 2001;79(4):373-4.

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RESEARCH ARTICLE

Vitamin-D deficiency predicts infections in young north Indian children: A secondary data analysis

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Abstract

Background

Recent studies have demonstrated a relationship between poor vitamin D status and respiratory infections and diarrhea among young children. Acute lower respiratory infections (ALRI) and diarrhea are among the two most important causes of death in under-5 children. In this paper, we examined the extent to which vitamin-D deficiency (<10 ng/ml) predicts ALRI, clinical pneumonia and diarrhea among 6 to 30 months old children.

Methods

We used data from a randomized controlled trial (RCT) of daily folic acid and/or vitamin B12 supplementation for six months in 6 to 30 months old children conducted in Delhi, India. Generalized estimating equations (GEE) were used to examine the associations between vitamin-D deficiency and episodes of ALRI, clinical pneumonia and diarrhea.

Results

Of the 960 subjects who had vitamin-D concentrations measured, 331(34.5%) were vitamin-D deficient. We found, after controlling for relevant potential confounders (age, sex, breastfeeding status, wasting, stunting, underweight, anemia status and season), that the risk of ALRI was significantly higher among vitamin-D deficient (OR 1.26; 95% CI: 1.03 to 1.55) compared to vitamin-D-replete children in the six months follow-up period. Vitamin-D status was not associated with episodes of diarrhea or clinical pneumonia.

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Conclusion

Vitamin-D deficiency is common in young children in New Delhi and is associated with a higher risk of ALRI. The role of vitamin D in Indian children needs to be elucidated in further studies.

Introduction

Vitamin D deficiency is considered to be the most common nutritional deficiency and often one of the most commonly undiagnosed medical conditions in the world [1]. The prevalence of vitamin D deficiency in young children is around 50–90% in the Indian subcontinent [2]. Vitamin D is primarily produced in the skin after exposure to ultraviolet radiation and less than 10% is derived from dietary sources [3].

Vitamin D is a potent immune-modulator of adaptive and innate immune responses [4]. In vitro studies have shown that 1,25-dihydroxyvitaminD3, the active metabolite of vitamin D, is important for promoting and regulating immune responses [5,6]. Observational studies suggest a link between low vitamin D concentrations and an increased risk of lower and upper respiratory tract infections in infants and young children [7]. A recent prospective cohort study found that vitamin-D deficiency was associated with increased rates of diarrheal illnesses among school-aged children [8]. However, the extent to which vitamin D deficiency predicts these infections in young children is less clear.

The estimated incidence of pneumonia in children under 5 years is 0.29 episodes per child-year in developing countries, resulting in 151 million new episodes each year, of which 7–13% of cases are severe enough to be life-threatening and necessitate hospital admission [9]. In 2013, 25.3% of deaths in children aged 1–59 months in India were due to pneumonia, totaling 150,169 deaths [10]. Globally, diarrhea causes 9% of all under-5 deaths, most of these in developing countries [10]. Although a reduction has been observed in the incidence of diarrhea in resource-limited settings, the disease burden associated with recurrent enteric illnesses still remains a public health problem [11–12] that results in excess childhood mortality [13–15].

Several micronutrients are important for innate and adaptive immunity in young children [16]. Recent meta-analyses have demonstrated a 15% reduction in childhood diarrheal incidence following vitamin-A supplementation [17] and 13% reduction in diarrheal and pneumonia incidence following zinc supplementation [18, 19]. The role of Vitamin-D as an immune-modulator has led to an increased interest in investigating its function in infectious diseases [20].

We conducted a randomized controlled trial (RCT) where children aged 6 to 30 months were supplemented daily with folic acid and/or vitamin B12 for six months. The main outcomes were the incidence of respiratory infections (ALRI, clinical pneumonia) and diarrhea. Enrolled participants were followed biweekly for respiratory and diarrheal morbidity [21]. Using data from this study we examined the extent to which vitamin-D deficiency (<10 ng/ml) at baseline predicted these outcomes during the 6 months follow-up period.

Materials and methods

Subject

The study was conducted from January 2010 to February 2012 in the low-to-middle socioeconomic settings of Tigri and Dakshinpuri in New Delhi. The total population of this site was

about 300,000. Details of the population have been described previously [21]. This randomized double-blind placebo-controlled trial (NCT00717730 at www.clinicaltrials.gov) with a factorial design enrolled 1000 children, and evaluated the impact of supplementation with folic acid, vitamin B12, or both on childhood infections [21]. The analyses in the current manuscript are restricted to the group of 960 children whose baseline vitamin-D levels were available.

Definitions

Diarrhea was defined as the passage of 3 or more loose or watery stools in a 24-h period. Two episodes of diarrhea were separated by a 72-hour or more diarrhea free period. ALRI was defined as cough or difficult breathing with an elevated respiratory rate above the age-specific cutoff values ($\geq$ 50 breaths/min in infants and $\geq$ 40 breaths/min in older children) according to WHO criteria, or cough or difficult breathing and lower chest in drawing [22]. Clinical pneumonia was defined either by a combination of cough with crepitations or bronchial breathing by auscultation or as an episode of ALRI associated with at least one of the following features: lower chest indrawing, convulsions, inability to drink or feed, extreme lethargy, restlessness or irritability, nasal flaring, or abnormal sleeping and difficulty in waking.

Analytical procedures

Blood samples were obtained at baseline from all the children; 3 mL blood was collected in an evacuated tube containing EDTA (Becton Dickinson). The plasma was centrifuged at ~ 450 × g at room temperature for 10 min, separated, and transferred into storage vials and stored at– 20˚C until analyzed. Plasma concentration of vitamin-D was measured by quantitative electro-chemiluminescence binding assay, with detection of 25(OH) D_2, the hydroxylated forms of vitamin D2 (Roche Diagnostics, Mannheim, Germany) [23] at Christian Medical College, Vellore biochemistry laboratory.

Ethics

This study was conducted according 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 these children's blood specimen for use in future research. All parents consented for the same.

Statistical analysis

Proportions and means (SD) or median (IQR) were calculated for categorical and continuous variables by Vitamin-D status at baseline. Vitamin D deficiency was defined at <10ng/mL (25 nmol/L) [24]. The 6 months' follow-up period was divided into 26 periods of 7 d for every child. For a period to be included in the analyses, we required information on 4 d or more days of the given 7 d period. To account for interdependence of multiple observation periods in the same child, we used generalized estimating equations (GEE) with an autoregressive covariance-variance matrix taking time into account. In these models, occurrence of a new episode of diarrhea, ALRI, or clinical pneumonia in a child period was modeled as dependent variables and baseline vitamin D status as an independent variable. We included types of intervention received and other baseline variables as independent variables (age, sex, breastfeeding status, wasting, stunting, underweight, anemia status and season) in the model to adjust for potential confounding. The model used a logit link, binomial variance, autoregressive

correlation and robust standard error to yield odds ratio (OR). We used STATA version 14 (Stata Corporation, College Station, TX) for most statistical analyses. We used generalized additive models in the statistical software R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) to explore nonlinear associations between the vitamin D level at baseline and ALRI incidence after adjustment for potential confounders [25]. We also used generalized additive models to explore nonlinear associations between vitamin D level at baseline and season defined by period of enrollment in weeks. We considered an association to be statistically significant when the P value was <0.05. Post-hoc calculations of statistical power showed that we had more than 90% power to detect at least 25% more episodes of ALRI during 6 months follow-up in the vitamin- D deficient group compared to vitamin-D non deficient group with the available sample, at 5% significance level.

Results

A total of 1000 children were included in the main trial. Blood samples for vitamin D were collected at baseline for 960 (96%) children. Of these, 331 (34.5%) children were Vitamin D deficient (<10 ng/ml). The baseline characteristics of the population by deficiency status are presented in Table 1. Approximately half of the enrolled children were boys and almost all (98%) were ever breast fed. Over 36.4% of the children were stunted, 31% underweight, and 10.7% wasted. Approximately 70% of the children were anemic.

Fig 1 shows the relationship between vitamin-D according to weeks of enrolment. As distinct seasons are difficult to define in India, we divided the period of enrollments into weeks. The vitamin D concentrations were higher in the 24^{th} to 32^{nd} weeks which correspond to months (May- July) with more daylight. The baseline vitamin D levels were lower for children who were enrolled in the initial weeks of the year which correspond to months (January-February) and have less daylight.

The diarrheal episodes in vitamin-D deficient and non-deficient children are shown in Table 2. There was no association between vitamin-D status and episodes of diarrhea overall and according to episodes of diarrhea lasting 6 days or more. The association between vitamin-D status and ALRI and clinical pneumonia are shown in Table 3. The incidence of ALRI was significantly higher among vitamin-D deficient children than in vitamin-D replete children (OR: 1.26; 95% CI: 1.03–1.55). However, the incidence of clinical pneumonia was not significantly associated with vitamin D status (OR: 1.05; 95% CI: 0.79–1.38).

The association between baseline vitamin-D levels and incidence density of ALRI is depicted in Fig 2. The ALRI incidence density increases with decreasing baseline vitamin-D concentrations.

Discussion

We report the prevalence of vitamin D deficiency and its association with common infections in young children. We found a high prevalence of vitamin D deficiency which is consistent with other studies in India [2]. However, a recent study from Nepal found that only <5% of breastfed infants were vitamin-D deficient, even when a higher cut off (<20 ng/l) was used [26]. High prevalence of vitamin-D deficiency observed in our study setting, in spite of abundant sunlight may be because of relatively high solar zenith angle, in combination with atmospheric pollution, type- V skin types of the population and restricted outside activities [27, 28]. More Ultraviolet B (UVB) photons are absorbed by the stratospheric zone, and therefore fewer UVB photons penetrate to earth's surface to produce cutaneous pre-vitamin D3 with a relatively high solar zenith angle. [29]. A recent study indicated that infants may get enough vitamin D from breast milk if their mothers take high-dose vitamin D supplements [30]. The

Table 1. Baseline characteristics of Vitamin D deficient and non deficient children aged 6–30 months included in the analysis.

Characteristics	n = 960	
Proportion of children		
Deficient (< 10 ng/ml)	331 (34.5)	
Non deficient (≥ 10 ng/ml)	629 (65.5)	
	Deficient	**Non deficient**
	n = 331	**n = 629**
Infant characteristics		
Age at enrollment in months, mean (SD)	16.9 (7.1)	15.8 (7.0)
Proportion of children		
<12 months	91 (27.5)	210 (33.4)
12 to 23 months	166 (50.1)	306 (48.7)
24 to 30 months	74 (22.4)	113 (18.0)
Boys	162 (48.9)	328 (52.2)
Ever breastfed	325 (98.2)	622 (98.9)
Prevalence of illness in previous 24 hours		
Diarrhea	17 (5.1)	32 (5.1)
Cough or difficult breathing or fast breathing	114 (34.4)	192 (30.5)
Anthropometric status		
Mean(SD): Z score		
Weight for Height Z score(WHZ)	-0.86 (0.92)	-0.89 (0.94)
Height for Age Z score (HAZ)	-1.56 (1.24)	-1.63 (1.16)
Weight for Age Z score (WAZ)	-1.46 (1.06)	-1.52 (1.05)
Wasted (<-2 WHZ)	35 (10.6)	68 (10.8)
Stunted (<-2 HAZ)	117 (35.3)	233 (37.0)
Underweight (<-2 WAZ)	101 (30.5)	197 (31.3)
Anemia (Hb<11g/dl)	244 (73.7)	424 (67.4)
Socio-demographic characteristics		
Mother's age in years, mean (SD)	26.3(5.8)	25.6(4.1)
Mother's schooling in years, median (IQR)	8(5,10)	7(0,10)
Father's schooling in years, median (IQR)	10 (7,12)	9 (6,12)
Annual family income in rupees, median (IQR)	72000 (60000–144000)	84000 (60000–138000)

Figures are number (percentage) unless stated otherwise

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complementary foods in the diets of Indian infants and children are primarily cereal based and low in vitamin D [31]. This is probably another contributing factor of the widespread vitamin-D deficiency among children in this setting.

We found a significantly higher incidence of ALRI among vitamin-D deficient children when compared to vitamin-D replete children. Similar findings have been shown in previous observational studies [32–34]. Vitamin-D induces TLR activation and antibacterial responses which in turns enhances production of cathelicidin (LL-37), an endogenous antimicrobial peptide which is highly expressed at natural barrier sites e.g. lungs [35]. The protective role of vitamin-D against ALRI can be explained through its modulatory effect of both innate and adaptive immunity and regulatory function of inflammatory cascade [36–39].

We did not find any association with clinical pneumonia, a severe form of ALRI, and vitamin D status. Other observational studies have shown mixed results; while some studies found associations between vitamin-D status and clinical pneumonia, others did not [40–43].

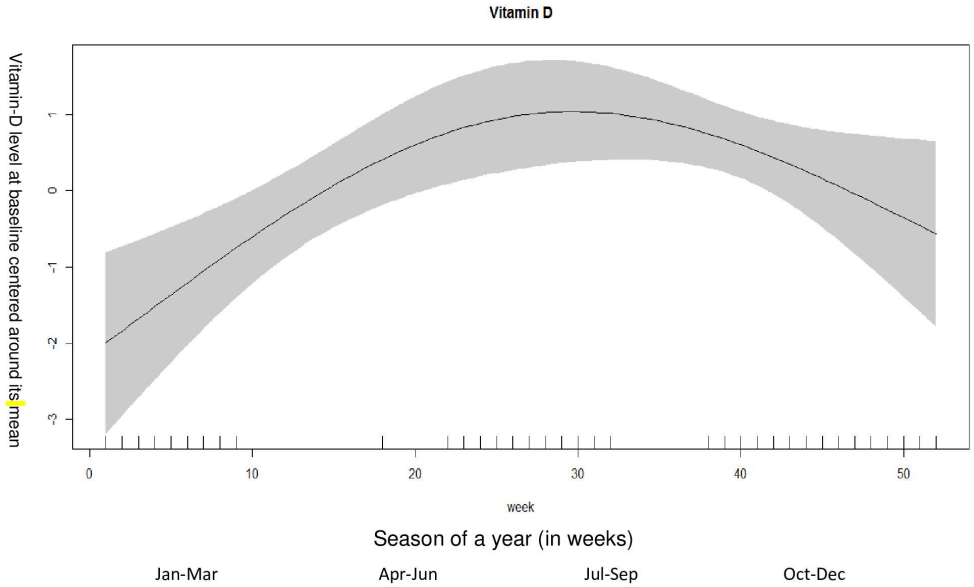

Fig 1. Associations between vitamin-D level at baseline and weeks of a year (among 960 children). The graph was constructed using generalized additive models in R, the solid line depicts the association of vitamin-D level at baseline and season. The shaded area spans the 95% confidence interval of this association.

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Vitamin D has distinct effects on the innate and adaptive immune responses that may explain different roles in pathogen-specific infection severity [44]. Furthermore, a recent systematic review concluded that there was no evidence of vitamin D supplementation among under-5 children in the management of clinical pneumonia [45]. Even if vitamin D has a role in the defense against infections it might not have a therapeutic role as once an infection has taken

Table 2. Incidence of diarrheal episodes in vitamin-D deficient and non deficient children.

	Deficient (Vitamin D level < 10 ng/ml)	Non Deficient (Vitamin D level ≥ 10 ng/ml)[a]	OR (95% CI)[b]
Total child-years of follow-up	162.3	308.5	
Episodes of diarrhea	775	1385	
Incidence density of diarrhea per child year (95% CI)	4.78 (4.44 to 5.12)	4.49 (4.26 to 4.73)	1.07 (0.95 to 1.20)
Episodes of diarrhea lasting			
> = 3 d	328	568	1.07 (0.96 to 1.19)
> = 5 d	182	339	0.98 (0.83 to 1.15)
> = 7 d	114	218	0.95 (0.76 to 1.20)
> = 14 d	34	63	1.01 (0.63 to 1.61)
Episodes of diarrhea with > = 6 stools/on any day	200	409	1.01 (0.83 to 1.22)

[a] Reference category: Non Deficient (Vitamin D level ≥ 10 ng/ml)

[b] ORs were calculated by using generalized estimating equations with a logit link, binomial variance, autoregressive correlation and robust standard error and adjusted for age, sex, breastfeeding status, wasted, stunted, underweight, anemia status, season and type of interventions

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Table 3. Incidence of ALRI and clinical pneumonia in vitamin-D deficient and non deficient children.

	Deficient (Vitamin D level < 10 ng/ml)	Non Deficient (Vitamin D level ≥ 10 ng/ml)[a]	OR (95% CI)[b]
Total child-years of follow-up	162.3	308.5	
Episodes of ALRI	244	418	
Incidence density of ALRI per child year (95% CI)	1.50 (1.32 to 1.70)	1.35 (1.23 to 1.49)	1.26 (1.03 to 1.55)
Episodes of clinical pneumonia	144	294	
Incidence density of clinical pneumonia per child year (95% CI)	0.89 (0.75 to 1.04)	0.95 (0.85 to 1.07)	1.05 (0.79 to 1.38)

[a] Reference category: Non Deficient (Vitamin D level ≥ 10 ng/ml)

[b] ORs were calculated by using generalized estimating equations with a logit link, binomial variance, autoregressive correlation and robust standard error and adjusted for age, sex, breastfeeding status, wasted, stunted, underweight, anemia status, season and type of interventions

ALRI, acute lower respiratory infection

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place, other factors determine its course and how quickly it will resolve. Given the complexity of interaction of vitamin-D with the immune system and inflammatory cascade, more research is needed to further define the specific role of vitamin D in enhancing immune function and reducing the severity of infections.

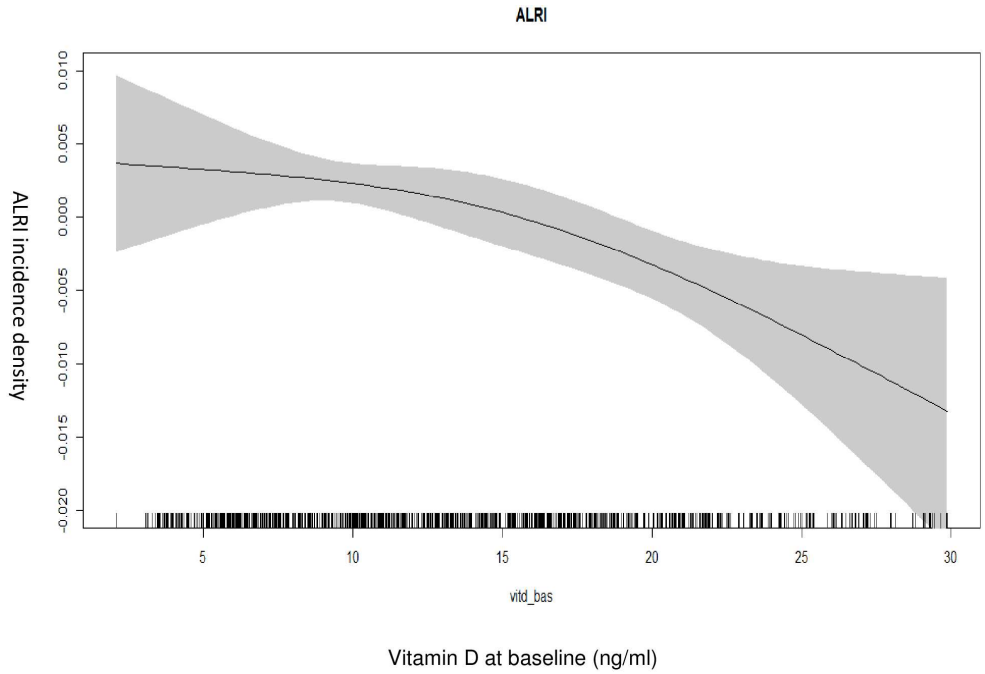

Fig 2. Associations between vitamin-D level at baseline and ALRI incidence density (among 960 children). The graph was constructed using generalized additive models in R, the solid line depicts the association of vitamin-D level at baseline and ALRI incidence density. The shaded area spans the 95% confidence interval of this association.

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Poor vitamin D status was not associated with an increased incidence and severity of diarrhea in our study, which is in line with findings from another observational study [46]. A randomized controlled trial with 3-monthly bolus supplementation with 100,000 IU of vitamin D3 among children aged 1 to 29 months, showed no effect on diarrheal illnesses [47]. Our study was done in urban slum where constant exposure to pathogenic organisms and subsequent enteric infection is common in children [48]. This could masquerade the potential beneficial role of vitamin-D in this population.

There may be deficiencies of other limiting micronutrients. Zinc deficiency increases the risk of diarrhea and pneumonia. A previous study in this population showed that zinc deficiency is common and zinc supplementation reduces the burden of diarrhea and lower respiratory tract infections [49,50]. It has been shown that vitamin-D-depenent genes in the cell are influenced by the intracellular zinc concentration [51]. Because the sources of vitamin D and zinc are different, we do not believe that vitamin D status is confounded by zinc status. However, there is a possibility that these nutrients may interact with each other.

The strengths of our study are that the data are from a well conducted study with very low attrition rates. We undertook multiple follow up visits for assessing ALRI, clinical pneumonia and diarrhea to ensure that virtually all episodes were documented. Outcomes were clearly defined and assessed by highly trained field staff. Results were adjusted for several relevant confounders including nutritional status of children and the season of enrollment.

We used an immunological method to measure vitamin-D concentration. It should be noted that immunoassays can overestimate 25OHD [52] because it is lipophilic which makes it vulnerable to matrix effects in the protein binding assays [53].

The results of this study could have important public health implications. As fortified foods have been recognized as an important source of vitamin D [54] such as oils, cereal powders and even salt supplementation and fortification may help in preventing vitamin D deficiency. Vitamin D supplementation is recommended in many countries, such public health interventions need serious consideration in the Indian context.

Conclusion

The present study demonstrates that vitamin D deficiency is common in New Delhi children aged 6–30 months and that it is associated with increased risk of ALRI. Randomized controlled trials measuring the effect of vitamin D supplementation in these setting should be prioritized.

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Writing – original draft: RC TS.

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References

1. Holick MF. Vitamin D: extraskeletal health. Rheumatic diseases clinics of North America. 2012; 38 (1):141–60. doi: 10.1016/j.rdc.2012.03.013 PMID: 22525849
2. Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014; 6(2):729–75. doi: 10.3390/nu6020729 PMID: 24566435
3. Norris JM. Can the sunshine vitamin shed light on type 1 diabetes. Lancet. 2001; 358(9292):1476–1478. doi: 10.1016/S0140-6736(01)06570-9 PMID: 11705556
4. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. Journal of molecular medicine (Berlin, Germany). 2010; 88(5):441–50.
5. Cantorna MT. Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY). 2000; 223(3):230–3
6. Pichler J, Gerstmayr M, Szepfalusi Z, Urbanek R, Peterlik M, Willheim M. 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. Pediatric research. 2002; 52 (1):12–8. doi: 10.1203/00006450-200207000-00005 PMID: 12084841
7. Esposito S, Lelii M. Vitamin D and respiratory tract infections in childhood. BMC infectious diseases. 2015; 15:487. doi: 10.1186/s12879-015-1196-1 PMID: 26521023
8. Thornton KA, Marin C, Mora-Plazas M, Villamor E. Vitamin D deficiency associated with increased incidence of gastrointestinal and ear infections in school-age children. The Pediatric infectious disease journal. 2013; 32(6):585–93. doi: 10.1097/INF.0b013e3182868989 PMID: 23340562
9. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bulletin of the World Health Organization. 2008; 86(5):408–16. doi: 10.2471/BLT.07.048769 PMID: 18545744
10. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015; 385(9966):430–40. doi: 10.1016/S0140-6736(14)61698-6 PMID: 25280870

11. Fischer Walker CL, Perin J, Aryee MJ, Boschi-Pinto C, Black RE. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC public health. 2012; 12:220. doi: 10.1186/1471-2458-12-220 PMID: 22436130

12. Bhutta ZA, Das JK. Global burden of childhood diarrhea and pneumonia: what can and should be done? Pediatrics. 2013; 131(4):634–6. doi: 10.1542/peds.2012-3737 PMID: 23509166

13. Guerrant RL, Oria RB, Moore SR, Oria MO, Lima AA. Malnutrition as an enteric infectious disease with long-term effects on child development. Nutrition reviews. 2008; 66(9):487–505. doi: 10.1111/j.1753-4887.2008.00082.x PMID: 18752473

14. Rodriguez L, Cervantes E, Ortiz R. Malnutrition and gastrointestinal and respiratory infections in children: a public health problem. International journal of environmental research and public health. 2011; 8 (4):1174–205. doi: 10.3390/ijerph8041174 PMID: 21695035

15. Checkley W, Buckley G, Gilman RH, Assis AM, Guerrant RL, Morris SS, et al. Multi-country analysis of the effects of diarrhoea on childhood stunting. International journal of epidemiology. 2008; 37(4):816–30. doi: 10.1093/ije/dyn099 PMID: 18567626

16. Katona P, Katona-Apte J. The Interaction between Nutrition and Infection. Clinical Infectious Diseases. 2008; 46: 1582–8 doi: 10.1086/587658 PMID: 18419494

17. Mayo-Wilson E, Imdad A, Herzer K, Yakoob MY, Bhutta ZA. Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis. BMJ 2011; 343: d5094 doi: 10.1136/bmj.d5094 PMID: 21868478

18. Mayo-Wilson E, Junior JA, Imdad A, Dean S, Chan XH, Chan ES et al. Zinc supplementation for preventing mortality, morbidity, and growth failure in children aged 6 months to 12 years of age. Cochrane Database Syst Rev 2014; 5: Cd009384

19. Lassi ZS, Moin A, Bhutta ZA. Zinc supplementation for the prevention of pneumonia in children aged 2 months to 59 months. Cochrane Database of Systematic Reviews 2016, Issue 12. Art. No.: CD005978.

20. Hughes DA, Norton R. Vitamin D and respiratory health. Clinical and Experimental Immunology. 2009; 158:20–25 doi: 10.1111/j.1365-2249.2009.04001.x PMID: 19737226

21. Taneja S, Strand TA, Kumar T, Mahesh M, Mohan S, Manger MS, et al. Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: a randomized placebo-controlled trial. The American journal of clinical nutrition. 2013; 98(3):731–7 doi: 10.3945/ajcn.113.059592 PMID: 23902779

22. World Health Organization. Integrated management of childhood illness. Geneva, Switzerland: WHO, 2003.

23. Cobas E411 Vitamin D Total Reagent Insert (06268668001V1). Roche Diagnostics. URL: http://www.captodayonline.com/productguides/instruments/automated-immuno-assay-analyzers-july-2012/roche-diagnostics-cobas-e411-immunoassay-analyzers-june-2011.html (accessed on 13/09/2016)

24. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutrition bulletin. 2014; 39(4):322–50 doi: 10.1111/nbu.12108 PMID: 25635171

25. Wood SN. Modelling and smoothing parameter estimation with multiple quadratic penalties. JR Stat Soc B. 2000; 62:413–428. Stable URL: http://www.jstor.org/stable/3088868

26. Ulak M, Chandyo RK, Thorne-Lyman AL, Henjum S, Ueland PM, Midttun O, et al. Vitamin Status among Breastfed Infants in Bhaktapur, Nepal. Nutrients. 2016; 8(3):149 doi: 10.3390/nu8030149 PMID: 27005657

27. Harinarayan CV, Joshi SR. Vitamin D status in India—its implications and remedial measures. The Journal of the Association of Physicians of India. 2009; 57:40–48. PMID: 19753759

28. Puri S, Marwaha RK, Agarwal N, Tandon N, Agarwal R, Grewal K et al. Vitamin D status apparently healthy school girls from two different socioeconomic strata in Delhi: relation to nutrition and lifestyle. British Journal of Nutrition. 2008; 99: 876–882 doi: 10.1017/S0007114507831758 PMID: 17903343

29. Harinarayan CV, Holick MF, Prasad UV, Vani PS, Himabindu G. Vitamin D status and sun exposure in India. Dermato-endocrinology. 2013; 5(1):130–41 doi: 10.4161/derm.23873 PMID: 24494046

30. Hollis BW, Wagner CL, Howard CR, Ebeling M, Shary JR, Smith PG, et al. Maternal Versus Infant Vitamin D Supplementation During Lactation: A Randomized Controlled Trial. Pediatrics. 2015; 136 (4):625–34 doi: 10.1542/peds.2015-1669 PMID: 26416936

31. National Institute of Nutrition, India. Food composition tables. In Nutritive Value of Indian Foods, eds Gopalan C, Sastri BVR, Balasubramanian SC. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research. 1996:45–95

32. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. European journal of clinical nutrition. 2004; 58(4):563–7 doi: 10.1038/sj.ejcn.1601845 PMID: 15042122

33. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. European journal of clinical nutrition. 2009; 63(4):473–7. doi: 10.1038/sj.ejcn.1602960 PMID: 18030309

34. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. Acta paediatrica (Oslo, Norway: 1992). 2010; 99(3):389–93

35. Quraishi SA, Bittner EA, Christopher KB, Camargo CA Jr. Vitamin D status and community-acquired pneumonia: results from the third National Health and Nutrition Examination Survey. PloS one. 2013; 8 (11):e81120. doi: 10.1371/journal.pone.0081120 PMID: 24260547

36. Sun J. Vitamin D and mucosal immune function. Current opinion in gastroenterology. 2010; 26(6):591–5 doi: 10.1097/MOG.0b013e32833d4b9f PMID: 20639756

37. Esposito S, Lelii M. Vitamin D and respiratory tract infections in childhood. BMC infectious diseases. 2015; 15:487. doi: 10.1186/s12879-015-1196-1 PMID: 26521023

38. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science (New York, NY). 2006; 311(5768):1770–3

39. Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. Current opinion in nephrology and hypertension. 2008; 17(4):348–52 doi: 10.1097/MNH.0b013e3282ff64a3 PMID: 18660668

40. Muhe L, Lulseged S, Mason KE, Simoes EA. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. Lancet. 1997; 349(9068):1801–4. doi: 10.1016/S0140-6736(96)12098-5 PMID: 9269215

41. Ren J, Sun B, Miao P, Feng X. [Correlation between serum vitamin D level and severity of community acquired pneumonia in young children]. Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics. 2013; 15(7):519–21 PMID: 23866270

42. Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. European journal of clinical nutrition. 2009; 63(2):297–9 doi: 10.1038/sj.ejcn.1602946 PMID: 17971825

43. Sakka ASE, Iman SS, Amer HA, Moustafa SA. Vitamin D deficiency and low hemoglobin level as risk factors for severity of acute lower respiratory tract infections in Egyptian children: A case-control study. Egyptian Pediatric Association Gazette. 2014; 62:1–7 http://dx.doi.org/10.1016/j.epag.2013.12.001

44. Wei R, Christakos S. Mechanisms Underlying the Regulation of Innate and Adaptive Immunity by Vitamin D. Nutrients. 2015; 7(10):8251–60. doi: 10.3390/nu7105392 PMID: 26404359

45. Yakoob MY, Salam RA, Khan FR, Bhutta ZA. Vitamin D supplementation for preventing infections in children under five years of age. Cochrane Database of Systematic Reviews 2016; 11: CD008824. doi: 10.1002/14651858.CD008824.pub2 PMID: 27826955

46. Ahmed AMS, Magalhaes RJS, Ahmed T, Long KZ, Hossain MDI, Islam M et al. Vitamin-D status is not a confounder of the relationship between zinc and diarrhoea: a study in 6–24-month-old underweight and normal-weight children of urban Bangladesh. European Journal of Clinical Nutrition. 2016; 70: 620–628. doi: 10.1038/ejcn.2016.7 PMID: 26956127

47. Aluisio AR, Maroof Z, Chandramohan D, Bruce J, Mughal MZ, Bhutta Z, et al. Vitamin D(3) supplementation and childhood diarrhea: a randomized controlled trial. Pediatrics. 2013; 132(4): e832–40.

48. Abba K, Sinfield R, Hart CA, Garner P. Pathogens associated with persistent diarrhea in children in low and middle income countries: systemic review. BMC Infectious Diseases 2009; 9:88 doi: 10.1186/1471-2334-9-88 PMID: 19515227

49. Bhandari N, Bahl R, Taneja S, Strand TA, Molbak K, Ulvik RJ, Sommerfelt H, Bhan MK. Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young North Indian children. Pediatrics 2002; 109:e86. PMID: 12042580

50. Bhandari N, Bahl R, Taneja S, Strand T, Molbak K, Ulvik RJ, Sommerfelt H, Bhan MK. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomized controlled trial in an urban slum. BMJ 2002; 324:1358. PMID: 12052800

51. Craig TA, Benson LM, Naylor S, Kumar R. Modulation effects of zinc on the formation of vitamin D receptor and retinoid X receptor alpha-DNA transcription complexes: analysis by microelectrospray mass spectrometry. Rapid Commun Mass Spectrom 2001; 15: 1011–1016 doi: 10.1002/rcm.332 PMID: 11400211

52. Costelloe SJ, Woolman E, Rainbow S, Stratiotis L, O'Garro G, Whiting S, et al. Is high-throughput measurement of 25-hydroxyvitamin D3 without 25-hydroxyvitamin D2 appropriate for routine clinical use? Annals of clinical biochemistry. 2009; 46(Pt 1):86–7

53. Hollis BW. Editorial: The determination of circulating 25-hydroxyvitamin D: no easy task. The Journal of clinical endocrinology and metabolism. 2004; 89(7):3149–51. doi: 10.1210/jc.2004-0682 PMID: 15240585

54. Lamberg-Allardt C. Vitamin D in foods and as supplements. Prog Biophys Mol Biol. 2006; 92:33–38. doi: 10.1016/j.pbiomolbio.2006.02.017 PMID: 16618499

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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.

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

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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 ~450*g* 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		
Proportion of children		
No anemia	304 (30.4)	
Anemia	696 (69.6)	
	No anemia (Hgb ≥11 g/dL) n = 304	Anemia(Hgb <11 g/dL) n = 696
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	N = 303	N = 694
(>4.7 nmol/L)	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.

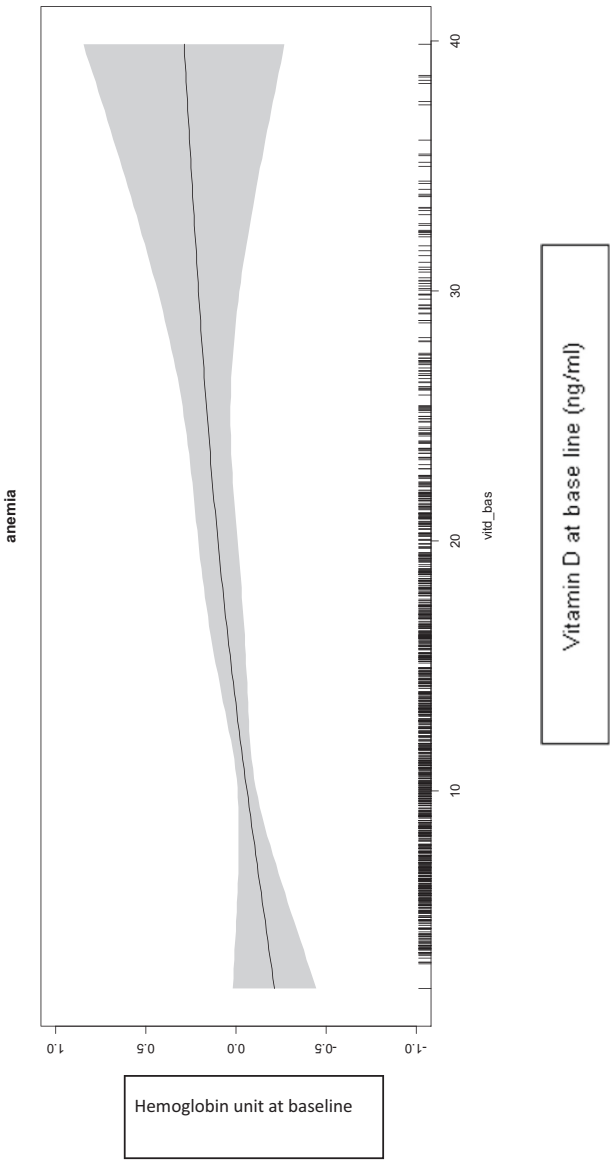

Fig. 1. Association between baseline vitamin D and hemoglobin levels. The graph was constructed using generalized additive models in R; the solid line depicts the association of vitamin- D and hemoglobin levels at baseline. The shaded area spans the 95% confidence interval of this association.

local calcitriol production in the bone marrow may limit erythropoiesis; 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.

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References

[1] Holick MF. Vitamin D: extraskeletal health. Rheum Dis Clin North Am 2012;38:141–60.
[2] Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients 2014;6:729–75.
[3] Norris JM. Can the sunshine vitamin shed light on type 1 diabetes? Lancet 2001;358:1476–8.
[4] Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009;94:26–34.
[5] Shroff R, Knott C, Rees L. The virtues of vitamin D—but how much is too much? Pediatr Nephrol 2010;25:1607–20.
[6] International Institute for Population Sciences (IIPS) and Macro International. National Family Health Survey (NFHS-4), 2015–16: India fact sheet. Mumbai: Author.
[7] Kendrick J, Targher G, Smits G, Chonchol M. 25-hydroxyvitamin D deficiency and inflammation and their association with hemoglobin levels in chronic kidney disease. Am J Nephrol 2009;30:64–72.
[8] Kiss Z, Ambrus C, Almasi C, Berta K, Deak G, Horonyi P, et al. Serum 25(OH)-cholecalciferol concentration is associated with hemoglobin level and erythropoietin resistance in patients on maintenance hemodialysis. Nephron Clin Prac 2011;117:c373–8.
[9] Patel NM, Gutierrez OM, Andress DL, Coyne DW, Levin A, Wolf M. Vitamin D deficiency and anemia in early chronic kidney disease. Kidney Int 2010;77:715–20.
[10] Meguro S, Tomita M, Katsuki T, Kato K, Oh H, Ainai A, et al. Plasma 25-hydroxyvitamin D is independently associated with hemoglobin concentration in male subjects with type 2 diabetes mellitus. Int J Endocrinol 2011;2011:362981.
[11] Zittermann A, Jungvogel A, Prokop S, Kuhn J, Dreier FuchsU, et al. Vitamin D deficiency is an independent predictor of anemia in end-stage heart failure. Clin Res Cardiol 2011;100:781–8.
[12] Sim JJ, Lac PT, Liu IL, Meguerditchian SO, Kumar VA, Kujubu DA, et al. Vitamin D deficiency and anemia: a cross-sectional study. Ann Hematol 2010;89:447–52.
[13] Taneja S, Strand TA, Kumar T, Mahesh M, Mohan S, Manger MS, et al. Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: a randomized placebo-controlled trial. Am J Clin Nutr 2013;98:731–7.
[14] World Health Organization. Hemoglobin concentrations for the diagnosis of anemia and assessment severity. Geneva, Switzerland: Author; 2011.
[15] Kolbe-Busch S, Lotz J, Hafner G, Blanckaert NJ, Claeys G, Togni G, et al. Multicenter evaluation of a fully mechanized soluble transferrin receptor assay on the Hitachi and cobas integra analyzers: the determination of reference ranges. Clin Chem Laboratory Med 2002;4029:e36.
[16] Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr 2007;86:1302. e9.
[17] Lang F. Homocysteine: plasma levels and genetic basis. In Encyclopedia of Molecular Mechanisms of Disease. 2009: 853.
[18] Spiro A, Buttriss JL. Vitamin D: an overview of vitamin D status and intake in Europe. Nutr Bull 2014;39:322–50.
[19] Gehring H, Hornberger C, Dibbelt L, Roth-Isigkeit A, Gerlach K, Schumacher J, et al. Accuracy of point-of-care-testing (POCT) for determining hemoglobin concentrations. Acta Anaesthesiol Scand 2002;46. e6.
[20] Bäck SE, Magnusson CG, Norlund LK, von Schenck HH, Menschik ME, Lindberg PE. Multiple-site analytic evaluation of a new portable analyzer, HemoCue Hb 201+, for point-of-care testing. Point Care 2004;3(60):e5.
[21] O'broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathology 1992;45:344. e7.
[22] Kelleher BP, Walshe KG, Scott JM, O'Broin SD. Microbiological assay for vitamin B12 with use of a colistin-sulfate-resistant organism. Clin Chem 1987;33:52. e4.
[23] Cotton F, Thiry P, Boeynaems J. Measurement of soluble transferrin receptor by immunoturbidimetry and immunonephelometry. Clin Biochem 2000;33:263. e7.
[24] Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst (e) ine with the Abbott IMx analyzer. Clin Chem 1995;41:991. e4.
[25] Cobas E411 Vitamin D Total Reagent Insert (06268668001 V1). Roche Diagnostics. Available at: www.cobas.com/home/product/clinical-and-immunochemistry-testing/elecsys-vitamin-d-total-assay.html; Accessed September 14, 2017.
[26] Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med 2008;3:17.
[27] Hosmer DW, Lemeshow S, Sturdivant RX. Applied logistic regression. New York, NY: Wiley; 2013.

[28] Wood SN. Modelling and smoothing parameter estimation with multiple quadratic penalties. JR Stat Soc B 2000;62:413–28.
[29] Reis JP, von Muhlen D, Miller 3rd ER, Michos ED, Appel LJ. Vitamin D status and cardiometabolic risk factors in the united states adolescent population. Pediatrics 2009;124. e371–9.
[30] Baeke F, Gysemans C, Korf H, Mathieu C. Vitamin D insufficiency: implications for the immune system. Pediatr Nephrol 2010;25:1597–606.
[31] Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 2008;4:80–90.
[32] Atkinson MA, Melamed ML, Kumar J, Roy CN, Miller 3rd ER, Furth SL, et al. Vitamin D, race, and risk for anemia in children. J Pediatr 2014;164:153–8.
[33] Jin HJ, Lee JH, Kim MK. The prevalence of vitamin D deficiency in iron-deficient and normal children under the age of 24 months. Blood Res 2013;48:40–5.
[34] Chang L, Liu X, Shi H, Dai W, Wang H, Jiang Y. Association of 25-hydroxyvitamin D with Hb and lead in children: a Chinese population-based study. Public Health Nutr 2013;17:827–32.
[35] Lac PT, Choi K, Liu IA, Meguerditchian S, Rasgon SA, Sim JJ. The effects of changing vitamin D levels on anemia in chronic kidney disease patients: a retrospective cohort review. Clin Nephrol 2010;74:25–32.
[36] Saab G, Young DO, Gincherman Y, Giles K, Norwood K, Coyne DW. Prevalence of vitamin D deficiency and the safety and effectiveness of monthly ergocalciferol in hemodialysis patients. Nephron Clin Pract 2007;105:c132–8.
[37] Armas LA, Heaney RP. Vitamin D: the iceberg nutrient. J Ren Nutr 2011;21:134–9.
[38] Alon DB, Chaimovitz C, Dvilansky A, Lugassy G, Douvdevani A, Shany S, et al. Novel role of 1,25(OH)(2)D(3) in induction of erythroid progenitor cell proliferation. Exp Hematol 2002;30:403–9.
[39] Aucella F, Scalzulli RP, Gatta G, Vigilante M, Carella AM, Stallone C. Calcitriol increases burstforming unit-erythroid proliferation in chronic renal failure. A synergistic effect with r-HuEpo. Nephron Clin Pract 2003;95:c121–7.
[40] Nemeth E. Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias. Adv Hematol 2010;2010:750643.
[41] Roy CN, Andrews NC. Anemia of inflammation: the hepcidin link. Curr Opin Hematol 2005;12:107–11.
[42] Costelloe SJ, Woolman E, Rainbow S, Stratiotis L, O'Garro G, Whiting S, et al. Is high-throughput measurement of 25-hydroxyvitamin D3 without 25-hydroxyvitamin D2 appropriate for routine clinical use? Ann Clin Biochem 2009;46:86–7.
[43] Editorial HollisBW. the determination of circulating 25-hydroxyvitamin D: no easy task. J Clin Endocrinol Metab 2004;89:3149–51.

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Vitamin-D status and neurodevelopment and growth in young north Indian children: a secondary data analysis

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Abstract

Background: Vitamin-D deficiency has been linked with impaired development in animal studies; however, the evidence from human studies is scanty. Evidence as to whether vitamin-D deficiency during early childhood affects growth is also limited and conflicting. We examined the extent to which vitamin-D deficiency (<10 ng/ml) is associated with neurodevelopment and physical growth in young children.

Methods: We used data from a randomized controlled trial (RCT) of daily folic acid and/ or vitamin B12 supplementation for six months in children aged 6 to 30 months conducted in Delhi, India. We measured vitamin-D status and neurodevelopment by the Ages and Stages Questionnaire-3 (ASQ-3) at 12 to 36 months of age. Multiple logistic and linear regressions were used to examine the association between vitamin-D deficiency at baseline and neurodevelopment and growth 6 months follow-up.

Results: 25-hydroxy-vitamin-D (25OHD) concentration was measured at baseline for 960 (96%) children. Of these, 331 (34.5%) children were vitamin-D deficient. The total and subscale (except for the Personal social scale) ASQ-3 scores, were not different between the vitamin-D deficient and non-deficient children. Vitamin-D deficiency was also not associated with physical growth at baseline and at follow -up.

Conclusion: Our data do not support the hypothesis that vitamin-D deficiency is associated with poor growth and neurodevelopment.

Trial registration: NCT00717730 and CTRI/2010/091/001090. Date of registration: 08 October, 2010

Keywords: Vitamin-D, ASQ-3, Neurodevelopment, Physical growth, Young north Indian children

Background

Vitamin-D deficiency is considered to be one of the most common nutritional deficiencies and a commonly undiagnosed medical condition in the world. [1] The prevalence of vitamin-D deficiency is 50–90% in the Indian subcontinent. [2] Vitamin-D is primarily produced in the skin after exposure to ultraviolet radiation and less than 10% is derived from dietary sources. [3]

Poor maternal vitamin-D status during pregnancy has been linked to impaired neurodevelopment among adult offspring and to structural changes in the brain such as enlarged lateral ventricles, thinner cortex, and more cell proliferation in animal studies. [4, 5] Human studies have shown that poor vitamin-D status prenatally is associated with adverse neuropsychiatric outcomes including schizophrenia and child autism. [6, 7] Two recent longitudinal studies showed a link between maternal vitamin-D status in early pregnancy and delayed neurocognitive development including language impairment, mental development, and psychomotor development in early childhood. [8, 9] To our knowledge, there are few studies on the association between vitamin-D status in children and neurodevelopment.

Vitamin-D is required for normal calcification of the growth plate and bone mineralization. [10] The major role of vitamin-D in maintaining bone health is to ensure normal calcium and phosphate levels in the blood. Children have higher calcium demands than adults; they

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require a positive calcium balance to assure adequate calcium for the mineralization of growing bone. [11] It is therefore important to ensure adequate vitamin-D status in order to enhance normal calcification of the growth plate and bone mineralization.

We undertook a randomized controlled trial (RCT) of daily supplementation with folic acid and/or vitamin B12 or placebo for six months in 6 to 30 months old children and measured the 25OHD cocentration in the blood specimens at baseline and at 6 months follow-up. We took the opportunity to estimate the association between baseline and at 6 months follow -up vitamin-D status and neurodevelopment measured by the Ages and Stages Questionnaire-3 (ASQ-3) after 6 months as well as the association between vitamin-D deficiency and physical growth (wasting, stunting and underweight) at baseline and 6 months later.

Methods

The study was conducted from January 2010 to February 2012 in the low-to-middle socioeconomic neighborhoods of Tigri and Dakshinpuri in New Delhi, India. The total population was around 300,000; details of the population have been described previously [12]. The current analysis has been done within the framework of a randomized double-blind placebo-controlled trial (NCT00717730 at https://clinicaltrials.gov/ct2/show/NCT00717730) involving supplementation with folic acid and/or vitamin B12 or placebo for six months to 1000 children who were 6 to 30 months old at enrollment [12]. Vitamin-D status was available for 960 children at baseline and in 243 at 6 months follow-up. For baseline, our analysis for neurodevelopment is restricted to the 401 children in whom ASQ-3 [13] was administered, for physical growth to 960 children in whom anthropometry data were available at baseline and to 919 children in whom anthropometry data were available 6 months later. Of the 243 children, neurodevelopment data were available for 92 children and anthropometry data were available for all.

Assessment

Neurodevelopment was assessed 6 months after enrollment using the ASQ-3 which is a developmental screening tool constructed in the USA. [13] The ASQ-3 consists of age-appropriate questionnaires, all containing 30 items divided into five subscales: Communication, Gross motor, Fine motor, Problem-solving and Personal-social, summing up to five subscale scores (range 0 to 60) and a total score (range 0 to 300). The construct and convergent validity of the translated ASQ-3 forms for the current setting were excellent, and our multiple models were able to explain more than 30% of the variability of the ASQ-3 scores. [14]

Details of the Hindi translation and process of validation of ASQ-3, training and standardization methods have been described previously. [15] Three trained field supervisors administered the ASQ-3 directly to the child at the study clinic in the presence of caregivers. The examiners elicited the relevant skills from the child during sessions using standardized materials. The caregiver served as an important contributor in supporting the child, eliciting behaviors and gave relevant information of the child's development when necessary. During the 11 days of training, the field supervisors were standardized in performing the procedure, and they reached a high inter-observer agreement both during training and in the 10% quality control checks throughout the study. To assess the caregiver's promotion of child development two questions were selected from the standardized assessment tool Home Observation for Measurement of the Environment (HOME) that were asked the caregivers during the session. [16] One question was on "Mother's belief that child's behavior can be modified" and one was on "Mother's encouragement of developmental advances".

Trained field supervisors measured weight and length at baseline and after six months of supplementation. Weight was measured to the nearest 50 g using electronic scale (Digitron scale). Length was measured using locally manufactured infantometers reading to the nearest 0.1 cm.

Analytical procedures

Blood samples were obtained at baseline from all children; 3 mL blood was collected in an evacuated tube containing EDTA (Becton Dickinson). The plasma was centrifuged at ~450×g at room temperature for 10 min, separated, and transferred into storage vials and stored at $-20\ ^{0}$ C until analyzed. Plasma concentration of vitamin-D was measured by quantitative electro-chemiluminescence binding assay, with detection of 25 OHD, the hydroxylated forms of vitamin-D2 (Roche Diagnostics, Mannheim, Germany) [17] at the Department of Biochemistry, Christian Medical College, Vellore, India.

Statistical analysis

Proportions, means (SD) or medians (IQR) were calculated for categorical and continuous variables by vitamin-D status at baseline. Though The Institute of medicine concluded that for maximum bone health a blood level should be at least 20 ng/mL and the Endocrine Society's Practice Guidelines recommended for maximum bone health a level should be above 30 ng/mL, we considered vitamin-D deficiency was defined at <10 ng/mL (25 nmol/L). [18] We also ran a sensitivity analysis classifying baseline vitamin-D status as <10, 11–20, 21–29 and > = 30 ng/mL.

We used multiple regression and a "purposeful selection of covariates method" to identify variables that were associated with vitamin-D deficiency and our predefined outcomes. [19, 20] 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 variables using interaction terms (on a multiplicative scale) in the multiple regression models.

Multiple linear and logistic regression analyses were used to compare the total ASQ-3 and subscale-scores between the vitamin-D deficient and the vitamin-D non-deficient groups at baseline and at 6 months followup. In logistic regression models the total and subscale ASQ-3 scores were categorized at the 25th percentile. In these models, we adjusted for age of child, mother's years of schooling, father's years of schooling, log transformed annual family income, family structure, number of toys in the family, whether or not the family owns books, number of children in the family, hours of play with other children during the week, mother's belief that child's behavior can be modified, mother's encouragement of developmental advances, weight-for-height Z score, weight for-age Z scores and intervention group.

We used multiple linear and logistic regression analyses to measure the association between vitamin-D deficiency and childhood physical growth at baseline and at 6 months follow-up. In logistic regression models, physical growth was categorized as wasting (< −2 Z scores weight-for-height/length), stunting (<−2 z score height/length-for-age) and underweight (< −2 Z scores weight-for-age). In the linear regression models, we used the Z scores of weight-for-height/length (WHZ), height/length-for-age (HAZ), weight-for-age (WAZ) as dependent variables. In these models, we adjusted for age, sex, breastfeeding status, family structure, log transformed annual family income, mother's years of schooling, father's year of schooling, baseline level of vitamin B12, folate and anemia status for baseline physical growth as well as, intervention group (placebo, folic acid, vitamin B12, or both) for 6 months later physical growth.

Statistical analyses were performed using STATA version 14 (Stata Corporation, College Station, TX).

We used generalized additive models in the statistical software R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) to explore nonlinear associations between the vitamin-D status at baseline and HAZ score at baseline after adjustment for potential confounders [21]. We also used generalized additive models to explore nonlinear associations between vitamin-D status at baseline and total ASQ-3 score after 6 months of follow - up.

Results

A total of 1000 children were included in the main trial. Vitamin-D level was analyzed in baseline samples for 960 (96%) children. Of these, 331 (34.5%) were vitamin-D deficient (<10 ng/ml). The mean (SD) and median (IQR) of vitamin-D level were 14.82 (8.7) ng/ml and 13.15 (8.31, 19.2) ng/ml. The baseline characteristics of the population by vitamin-D status are presented in Table 1. Approximately half of the enrolled children were boys and almost all (98%) were ever breast fed. Approximately 70% of the children were anemic (Hb < 11 g/dl).

The mean (SD) of the total ASQ-3 and subscales scores by vitamin-D status are shown in Table 2. The overall ASQ-3 score was not significantly lower in the vitamin-D deficient group [mean difference – 6.54 (95% CI: -16.15 to 3.08)] compared to the vitamin-D non-deficient group. The Personal social subscale score was significantly lower in the vitamin-D deficient group [mean difference – 2.63 (95% CI: -5.00 to −0.25)], whereas the other subscale scores were not. There was no interaction on a multiplicative scale between intervention (folic acid and/or vitamin B12 or placebo) and vitamin-D deficiency on the total ASQ-3 score or for the subscales scores.

We repeated the analyses using logistic regression after dichotomizing the outcomes at the 25th percentiles. After adjusting for potential confounders, we found a significant difference (OR: 1.63; 95% CI: 1.03 to 2.58) in the Personal-social subscale. The overall ASQ-3 score and the remaining subscales were not associated with vitamin-D deficiency. (Table 3).

Tables 4 and 5 show the distribution of the proportion of children stunted, wasted and underweight according to vitamin-D status at baseline and 6 months later respectively. At baseline in the vitamin-D deficient group, the proportion of stunted, wasted and underweight children was 35.4%, 10.6% and 30.5% respectively. 6 months later, in the vitamin-D deficient group the proportion of stunted, wasted and underweight children was 37.8%, 13.9% and 32.8% respectively. Vitamin-D deficiency was not associated with stunting nor wasting nor underweight at baseline or 6 months later in this population.

No substantial or significant differences were found when we did these analyses using HAZ, WHZ and WAZ scores as outcome variables both at baseline and 6 months later i.e. vitamin-D deficiency is not associated with any of the physical growth parameters on a continuous scale.

We estimated vitamin D status at end study in only 243 children, and the prevalence of deficiency was 38.3%. Of the 243 children neurodevelopment data were available for 92 children. None of the neurodevelopment

Table 1 Baseline characteristics of vitamin-D deficient and non-deficient children aged 6–30 months included in the analysis

Characteristics	*n* = 1000	
Number of children for whom samples for vitamin-D were available at baseline	960 (96.0%)	
Proportion of children		
Deficient (< 10 ng/ml)	331 (34.5)	
Non-deficient (≥ 10 ng/ml)	629 (65.5)	
	Deficient	Non-deficient
	n = 331	*n* = 629
Infant characteristics		
Age at enrollment, months (mean, SD)	16.9 (7.1)	15.8 (7.0)
Boys	162 (48.9)	328 (52.2)
Ever breastfed	325 (98.2)	622 (98.9)
Anemia (Hb < 11 g/dl)	244 (73.7)	424 (67.4)
Folate, nmol/L (Median, IQR)	9.1 (6.1 to 16.7)	12.5 (6.9 to 21.8)
Vitamin B12, pmol/L (Median, IQR)	266 (181 to 406)	266 (172 to 410)
Socio-demographic characteristics		
Mother's age, years (Mean, SD)	26.3 (5.8)	25.6 (4.1)
Mother's schooling, years (Median, IQR)	8 (5,10)	7 (0,10)
Literate mother	257 (77.6)	466 (74.1)
Father's schooling, years (Median, IQR)	10 (7,12)	9 (6,12)
Annual family income, US dollar(Median, IQR)[a]	1108 (923to 2215)	1292 (923 to2123)

Figures are number (percentages) unless stated otherwise
[a]1 US Dollar = INR 65

outcomes (Total ASQ-3, sub scales of ASQ-3 i.e. Communication, Gross motor, Fine motor, Problem - solving, Personal- social), nor any of the grwoth outcomes were significantly associated with vitamin D status after 6 months (Not shown in table).

We also did a sensitivity analysis by classifying baseline vitamin-D status as <10, 11–20, 21–29 and > = 30 ng/mL. 34.6% children were <10, 42.4% were 11–20, 17% were 21–29 and 6% were above 30 ng/ml. None of the neurodevelopment outcomes (Total ASQ-3, subscales of ASQ-3 i.e. Communication, Gross motor, Fine motor, Problem solving, Personal- social) and growth outcomes (wasting, stunting and underweight at baseline and end study) were significantly associated with any of these quartiles.

The association between vitamin-D status at baseline and HAZ score at baseline and total ASQ-3 score after 6 months are depicted in Figs 1 and 2. There was no association.

Table 2 The association between vitamin-D and total ASQ-3 and subscale scores

	Deficient (Vitamin-D status < 10 ng/ml)	Non-Deficient (Vitamin-D status ≥ 10 ng/ml)	
	Mean (SD) *n* = 165	Mean (SD) *n* = 236	Unadjusted Mean Diff (95% CI)
Total ASQ-3	229.98 (49.76)	236.52 (47.06)	−6.54 (−16.15 to 3.08)
Subscale			
Communication	49.04 (15.19)	49.26 (15.11)	- 0.22 (−3.22 to 2.8)
Gross motor	46.36 (14.39)	48.29 (13.41)	−1.93 (−4.69 to 0.82)
Fine motor	46.73 (14.90)	48.89 (12.09)	−2.15 (−4.81 to 0.50)
Problem-solving	46.42 (13.97)	46.03 (13.45)	0.39 (−2.33 to 3.12)
Personal social	46.42 (12.04)	49.05 (11.84)	−2.63 (−5 to −0.25)*

*p < 0.05

Table 3 Odds Ratios for the lower quartile of total ASQ-3 and subscale scores compared with Non-deficient vitamin-D status (≥ 10 ng/ml) adjusting for confounders[a]

	Non-Deficient (Vitamin-D status ≥ 10 ng/ml)	Deficient (Vitamin-D status < 10 ng/ml)	
	OR	OR	95% CI
Total ASQ-3	1	1.36	0.79 to2.31
Subscale			
Communication	1	1.58	0.97to 2.59
Gross motor	1	1.27	0.80 to 2.03
Fine motor	1	1.31	0.81to 2.11
Problem-solving	1	1.33	0.84to 2.11
Personal - social	1	1.63	1.03to 2.58*

*$p < 0.05$

[a]Adjusted for: age of child, mother's years of schooling, father's years of schooling, log transformed annual family income, family structure, number of toys in the family, whether or not the family owns books, number of children in the family, hours of play with other children during the week, mother's belief that child's behavior can be modified, mother's encouragement of developmental advances, weight-for-height Z score, weight for-age Z scores, anemia status at baseline and intervention group

Discussion

Except for the Personal - social scale, we did not find any significant differences in the total ASQ-3 and subscale scores between vitamin-D deficient and non-deficient children. Nor did we find any association between growth indices and vitamin D deficiency.

Our findings are consistent with those from a recently conductedcohort study in India, where vitamin-D status was not associated with the gross motor subscale of ASQ-3 among school- aged children [22]. A recent prospective study also suggests an inverted-U–shaped relation between neonatal vitamin-D status and neurocognitive development in toddlers [23]. Recent studies have shown an association between prenatal vitamin-D deficiency with delayed language development in early childhood [24, 25]. However, the association between vitamin-D deficiency and the Personal social subscale may very well be a chance finding considering the number of outcomes examined. It should also be noted that the personal social scores is the domain with poorest psychometric properties. [15]

The ASQ-3 is a screening tool for the assessment of developmental delay constructed in the US with binary cut-offs. It has however been used to measure developmental status on a continuous scale in the present study as in several other studies. [13] Alpha values indicated questionable internal consistency in a few subscales and age categories. Poor internal consistency can be due to constant items (lack of variability of the responses) or random error which may result in false negative results (type II error). However, in another publication from this study we also found that the ASQ-3 scores were associated with linear and ponderal growth, the incidence of diarrhea and pneumonia, as well as socioeconomic status and stimulation and learning opportunities. In fact, these variables explained more than 30% of the variability of the ASQ scores which demonstrates that the translated ASQ-3 test had good convergent validity. [14] Thus, the lack of findings of an association between vitamin-D and

Table 4 The association between baseline Vitamin-D status and growth (at baseline) among children

	Deficient (Vitamin-D status < 10 ng/ml) n = 331	Non-Deficient (Vitamin-D status ≥ 10 ng/ml) n = 629	Adjusted β coefficient (95% CI)[a]
Z scores: Mean(SD)			
HAZ	−1.56 (1.24)	−1.63 (1.16)	.05 (−0.10 to 0.20)
WHZ	−0.86 (0.92)	−0.89 (0.94)	.02 (−0.10 to 0.14)
WAZ	−1.46 (1.06)	−1.52 (1.05)	.03 (−0.10 to 0.17)
			Adjusted OR (95% CI)[a]
Stunted, n (%)	117 (35.4)	233 (37.0)	0.84 (0.63 to 1.13)
Wasted, n (%)	35 (10.6)	68 (10.8)	0.99 (0.64 to 1.54)
Underweight, n (%)	101 (30.5)	197 (31.3)	0.91 (0.67 to 1.23)

[a]Adjusted for: age, sex, breastfeeding status, log transformed annual family income, family structure, mother's years of schooling, father's years of schooling, baseline levels of vitamin B12, folate, anemia status at baseline

Table 5 The association between baseline Vitamin-D status and growth (6 months later) among children

	Deficient (Vitamin-D status < 10 ng/ml) *n* = 323	Non-Deficient (Vitamin-D status ≥ 10 ng/ml) *n* = 596	Adjusted β coefficient (95% CI)[a]
Z scores: Mean(SD)			
HAZ	−1.69 (1.17)	−1.81 (1.12)	0.11 (−0.20 to 0.43)
WHZ	−0.93 (0.97)	−0.95 (0.92)	0.20 (−0.05 to 0.46)
WAZ	−1.55 (1.08)	−1.62 (1.00)	0.20 (−0.06 to 0.47)
			Adjusted OR (95% CI)[a]
Stunted, n (%)	122 (37.8)	265 (44.5)	0.81 (0.61 to 1.07)
Wasted, n (%)	45 (13.9)	67 (11.2)	1.35 (0.90 to 2.04)
Underweight, n (%)	106 (32.8)	209 (35.1)	0.97 (0.72 to 1.29)

[a]Adjusted for: age, sex, breastfeeding status, log transformed annual family income, family structure, mother's years of schooling, father's years of schooling, baseline level of vitamin B12, folate, anemia status at baseline, intervention group

ASQ-3 scores is probably not due to poor psychometric properties of the test. In the translated ASQ-3 version, the standardized alphas for the total ASQ-3 scores were strong, indicating an overall acceptable internal consistency.

Type II errors can also be due to low sample size, negative confounding, and/or weaknesses with the immunological vitamin –D assay. We used an immunological method to measure vitamin-D concentration. It should be noted that immunoassays can overestimate 25OHD [26] because it is lipophilic which makes it vulnerable to matrix effects in the protein binding assays. [27]

We found no association between vitamin-D deficiency and physical growth at baseline and 6 months later. Similar findings have been described in pre-school children in Nepal and HIV exposed but uninfected infants in Africa, where vitamin-D deficiency was not associated with stunting and underweight, although wasted children have been found to be more commonly vitamin- D deficient. [28, 29] However, a recent trial in India showed that weekly administration of 1 RDA of vitamin-D supplementation for six months among LBW infants significantly increased weight and length, and decreased the proportion of children with stunted growth. [30] In addition, an observational study of Canadian infants found higher vitamin - D concentrations during infancy to 3 years of age were associated with leaner body composition. [31]

Although the major role of vitamin-D in maintaining bone health is to ensure normal calcium and phosphate levels in the blood, [32] we did not find any association between vitamin-D deficiency and ponderal or linear growth.

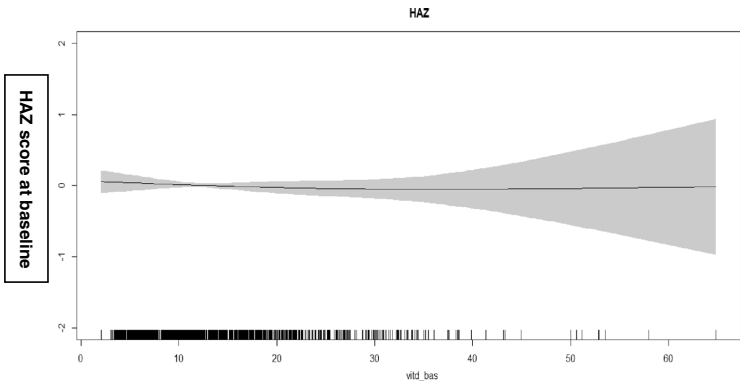

Fig. 1 Associations between vitamin-D level at baseline and HAZ score at baseline. The graphs were constructed using generalized additive models in R, the solid line depicts the association of vitamin-D level at baseline and HAZ score at baseline. The shaded area spans the 95% confidence interval of this association

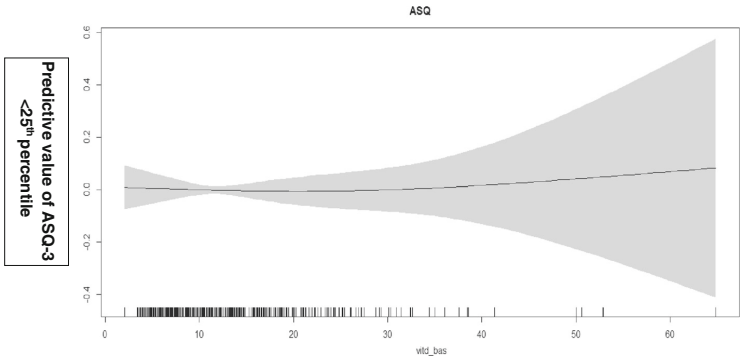

Fig. 2 Associations between vitamin-D level at baseline and total ASQ-3 score after 6 months of follow up. The graphs were constructed using generalized additive models in R, the solid line depicts the association of vitamin-D level at baseline and total ASQ-3 score. The shaded area spans the 95% confidence interval of this association

There may be deficiencies other growth-limiting macro and micronutrients such as calcium, zinc, vitamin −B12 etc. Poor quality of food i.e. lower proportion of animal source protein may also contribute to poor growth. The influence of vitamin D might be small in the light of other growth limiting factors.

To our knowledge this is the first analysis to examine the association between vitamin-D status and several domains of the ASQ-3. The strengths of our study are that the data are from a well conducted study with very low attrition rates. [33] More comprehensive assessment tools, such as the Bayley scales, or tools for social emotional functioning, could have added a broader picture of the children's skills and abilities yielding different results. There was a need for follow up assessments since 6 months may not be sufficient to assess the associations between vitamin- D status and development and growth. Finally, more advanced neuroimaging techniques may have identified unique changes to the developing brain in early childhood that could have been linked to vitamin-D deficiency.

Conclusion

The results from this analysis do not support that vitamin-D deficiency in early childhood is important for growth and neurodevelopment.

Abbreviations

25OHD: 25-hydroxy-vitamin-D; ASQ-3: Ages and Stages Questionnaire; CI: Confidence Interval; HAZ: Height/length-for-age Z score; Hb: Hemoglobin; HOME: Home Observation for Measurement of the Environment; IQR: Inter quartile range; OR: Odds ratio; RCT: Randomized controlled trial; RDA: Recommended Daily Allowance; SD: Standard Deviation; WAZ: Weight-for-age Z score; WHZ: Weight-for-height/length Z score

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Availability of data and materials

We have signed the agreement regarding "Access to data for the Healthy Birth, Growth and Development Knowledge Integration (HBGDki) Initiative" with BMGF and are in the process of uploading the data. Meanwhile request for data pertaining to the current analysis may be sent to Dr. Sunita Taneja (Email id: sunita.taneja@sas.org.in).

Authors' contributions

Obainted funding for the study: NB, ST, TS. Conceived and designed the study: RC ST TS IK. Analyzed the data: RC ST TS. Wrote the first draft of the manuscript: RC TS IK Reviewed the manuscript: RC ST TS IK NB MKB. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted according 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 participating children's blood specimen for use in future research. All parents consented for the same.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

1. Holick MF. Vitamin-D: extraskeletal health. Rheum Dis Clin N Am. 2012;38:141–60.
2. Ritu G, Gupta A. Vitamin-D status in India: prevalence, causalities and interventions. Nutrients. 2014;6:729–75.
3. Norris JM. Can the sunshine vitamin shed light on type 1 diabetes. Lancet. 2001;358:1476–8.
4. O'Loan J, Eyles DW, Kesby J, Ko P, McGrath JJ, Burne TH. Vitamin-D status during various stages of pregnancy in the rat; its impact on development and behaviour in adult offspring. Psychoneuroendocrinology. 2007;32:227–34.
5. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin-D3 and brain development. Neuroscience. 2003;118:641–53.
6. McGrath J, Eyles D, Mowry B, Yolken R, Buka S. Low maternal vitamin-D as a risk factor for schizophrenia:a pilot study using banked sera. Schizophr Res. 2003;63:73–8.
7. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Hart PH, Kusel MM. Maternal vitamin-D levels and the autism phenotype among offspring. J Autism Dev Disord. 2013;43:1495–504.
8. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Kusel MM, Hart PH. Maternal serum vitamin-D levels during pregnancy and offspring neurocognitive development. Pediatrics. 2012;129:485–93.
9. Morales E, Guxens M, Llop S, Rodr'ıguez-Bernal CL, Tard ´on A, Rian˜o I, Ibarluzea J, Lertxundi N, Espada M, Rodriguez A, et al. Circulating 25-hydroxyvitamin-D3 in pregnancy and infant neuropsychological development. Pediatrics 2012;130:e913–e920.
10. Boot AM, Krenning EP, de Muinck Keizer-Schrama SM. The relation between 25-hydroxyvitamin-D with peak bone mineral density and body composition in healthy young adults. J Pediatr Endocrinol Metab. 2011;24:355–60.
11. Shin YH, Shin HJ, Lee YJ. Vitamin-D status and childhood health. Korean J Pediatr. 2013;56(10):417–23.
12. Taneja S, Strand TA, Kumar T, Mahesh M, Mohan S, Manger MS, Refsum H, Yajnik CS, Bhandari N. Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: a randomized placebo-controlled trial. Am J Clin Nutr. 2013;98(3):731–7.
13. Squires J, Bricker D. Ages & stages questionnaires [R], (ASQ-3 [TM]): a parent-completed child-monitoring system: Brookes Publishing Company; 2009.
14. Kvestad I, Taneja S, Hysing M, Kumar T, Bhandari N, Strand TA. Diarrhea, stimulation and growth predict neurodevelopment in young north Indian children. PLoS One. 2015;10(3):e0121743. https://doi.org/10.1371/journal.pone.0121743.
15. Kvestad I, Taneja S, Kumar T, Bhandari N, Strand TA, Hysing M. The assessment of developmental status using the ages and stages questionnaire-3 in nutritional research in north Indian young children. Nutr J. 2013;12:1–11.
16. Bradley RH, Corwyn RF. Caring for children around the world: a view from HOME. Int J Behav Dev. 2005;29:468–78.
17. Cobas E411 Vitamin-D Total Reagent Insert (06268668001V1). Roche Diagnostics Web site. Available online: http://www.cobas.com/home/product/clinical-and-immunochemistry-testing/elecsys-vitamin-d-total-assay.html . Accessed 14 Sept 2017.
18. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull 2014;39(4):322–350 DOI:https://doi.org/10.1111/nbu.12108.
19. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code for Biology and Medicine. 2008;3:17.
20. Hosmer DW, Lemeshow S, Sturdivant RX. Applied logistic regression. New York: Wiley; 2013.
21. Wood SN. Modelling and smoothing parameter estimation with multiple quadratic penalties. JR Stat Soc B. 2000;62:413–28.
22. Filteau S, Rehman AM, Yousafzi A, Chugh R, Kaur M, Sachdev HPS, et al. Association of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term low birth weight. BMJ Open. 2016;6:e009268.
23. Zhu P, Tong SL, Hao JH, Tao RX, Huang K, Hu WB, et al. Cord blood vitamin D and neurocognitive development are nonlinearly related in toddlers. J Nutr. 2015 Jun;145(6):1232–8.
24. Hanieh S, Ha TT, Simpson JA, Thuy TT, Khuong NC, Thoang DD. Maternal vitamin-D status and infant outcomes in rural Vietnam: a prospective cohort study. PLoS One. 2014;9(6):e99005.
25. Gould JF, Anderson AJ, Yelland LN, Smithers LG, Skeaff CM, Zhou SJ, et al. Association of cord blood vitamin-D with early childhood growth and neurodevelopment. J Paediatr Child Health. 2016 Aug 27; https://doi.org/10.1111/jpc.13308.
26. Costelloe SJ, Woolman E, Rainbow S, Stratiotis L, O'Garro G, Whiting S, et al. Is high-throughput measurement of 25-hydroxyvitamin D3 without 25-hydroxyvitamin D2 appropriate for routine clinical use? Ann Clin Biochem. 2009;46(Pt 1):86–7. https://doi.org/10.1258/acb.2008.008157.
27. Hollis BW. Editorial: the determination of circulating 25-hydroxyvitamin D: no easy task. J Clin Endocrinol Metab. 2004;89(7):3149–51. https://doi.org/10.1210/jc.2004-0682.
28. Avagyan D, Neupane SP, Gundersen TE, Madar AA. Vitamin D status in pre-school children in rural Nepal. Public Health Nutr. 2016;19(3):470–6.
29. Sudfeld CR, Duggan C, Abound S, Kupka R, Manji KP, Kisenge R, et al. Vitamin D status is associated with mortality, morbidity, and growth failure among a prospective cohort of HIV-infected and HIV-exposed Tanzanian infants. J Nutr. 2015;145(1):121–7.
30. Kumar GT, Sachdev HS, Chellani H, et al. Effect of weekly vitamin-D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. The BMJ. 2011;342:d2975. https://doi.org/10.1136/bmj.d2975.
31. Hazell TJ, Gallo S, Vanstone CA, Agellon S, Rodd C, Weiler HA. Vitamin D supplementation trial in infancy: body composition effects at 3 years of age in a prospective follow-up study from Montréal. Pediatr Obes. 2017;12(1):38–47. https://doi.org/10.1111/ijpo.
32. http://www.fao.org/docrep/004/y2809e/y2809e0e.htm. Accessed 2 Feb 2017.
33. Kvestad I, Taneja S, Kumar T, Hysing M, Refsum H, Yajnik CS, et al. Vitamin B12 and folic acid improve gross motor and problem-solving skills in young north Indian children: a randomized placebo-controlled trial. PLoS One. 2015;10(6):e0129915. https://doi.org/10.1371/journal.pone.0129915.

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RESEARCH Open Access

Vitamin D status in early childhood is not associated with cognitive development and linear growth at 6–9 years of age in North Indian children: a cohort study

Ranadip Chowdhury[1,2*], Sunita Taneja[1], Ingrid Kvestad[3], Mari Hysing[4], Nita Bhandari[1] and Tor A. Strand[2,5]

Abstract

Background: Vitamin D is important for brain function and linear growth. Vitamin D deficiency during pregnancy has been linked with impaired neurodevelopment during early childhood. However, there is limited evidence from population-based studies on the long-term impact of vitamin D deficiency on cognitive development and linear growth. The objective of the current analysis is to examine whether vitamin D deficiency during infancy and early childhood is associated with cognitive development and linear growth measured in school age.

Methods: This is a follow-up study of a placebo-controlled trial among 1000 North Indian children 6–30 months of age. We measured growth and neurodevelopment in 791 of these children when they were 6–9 years old. Neurodevelopment was measured using the Wechsler Intelligence Scale for Children, 4th edition ^{INDIA}, the Crichton Verbal Scale, NEPSY-II subtests, and the BRIEF 2. We categorized vitamin D concentrations during infancy and early childhood according to the US Institute of Medicine's recommendations; serum 25(OH)D < 12 ng/ml as deficient; 12–20 ng/ml as inadequate; > 20 ng/ml as sufficient. In multivariable regression models, adjusting for relevant confounders, we estimated the association between vitamin D status, growth and neurodevelopmental outcomes.

Results: Among the 791 children, baseline vitamin D status was available for 716. Of these, 45.8% were vitamin D deficient, 32.7% were inadequate, and 21.5% were sufficient. Vitamin D status was not associated with any of the cognitive outcomes or linear growth [Adjusted β coefficient for height for age z-score between deficient and sufficient children was − 0.06 (95% CI − 0.24 to 0.11)] at follow up.

Conclusion: Our findings do not support the notion that poor vitamin D status in early childhood is an important limitation for cognitive development and linear growth.

Trial Registration: The trial was first registered at www.clinicaltrials.gov as NCT00717730 in July, 2008, and at CTRI/2010/091/001090 in August, 2010 and then as CTRI/2016/11/007494 in November 2016.

Keywords: Vitamin D, Wechsler intelligence scale for children, 4th edition ^{INDIA}, Crichton verbal scale, A developmental neuropsychological assessment II, The behavior rating inventory of executive function 2, Linear growth, School age

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Background

Vitamin D deficiency is one of the most common micronutrient deficiencies worldwide [1]. In the Indian subcontinent, the prevalence of vitamin D deficiency is estimated to be from 50 to 90% using an internationally accepted reference value [2]. Vitamin D acts by binding to the nuclear vitamin D receptors (VDR), which are widely distributed throughout the human brain in most neurons and some glial cells [3, 4]. Animal studies have shown that vitamin D deficiency during pregnancy causes extreme alterations in the brain at birth [5, 6]. This provides a biological plausibility for a link between vitamin D status and neurodevelopment.

There is evidence from observational studies on the association between vitamin D status during pregnancy or cord blood vitamin D at birth with cognitive, language, and behavioral development in different periods of childhood [7–12]. Previously, we have shown that vitamin D status was not associated with neurodevelopment as measured by a brief screening tool, the Ages and Stages Questionnaire 3rd edition (ASQ-3) during early childhood [13]. The consequences of vitamin D deficiency in early life on neurodevelopmental may not become evident until later in childhood. Furthermore, the predictive ability of early neurodevelopmental assessments is poor, and cognitive assessments in school-aged children have shown to be stable over time [14, 15]. We measured vitamin D status in 1000 young North Indian children and conducted a comprehensive assessment of cognitive performance and growth approximately 6 years later [16, 17]. This study gave us a unique opportunity to explore the extent to which vitamin D deficiency during early childhood is associated with impaired cognitive development and linear growth at school age.

Methods

Study design and participants

We followed up children who previously participated in a randomized double-blind placebo-controlled trial (n = 1000) on the effect of two recommended daily allowances (RDA) of vitamin B12 and/or folic acid daily for 6 months in Delhi, North India [18]. The main outcome of the study was incidence of infections. In September 2016, we approached all these children, and were able to get in contact with 798 of whom 791 consented to participate in the follow-up study. (Fig. 1) All families were initially contacted by phone to be invited to participate in the study. A physical visit was made to the family's address if no contact could be made. We requested the families who had moved out of the study area to come to the study clinic for 1 day. On the day of the assessment, consent was taken from the children's caregiver

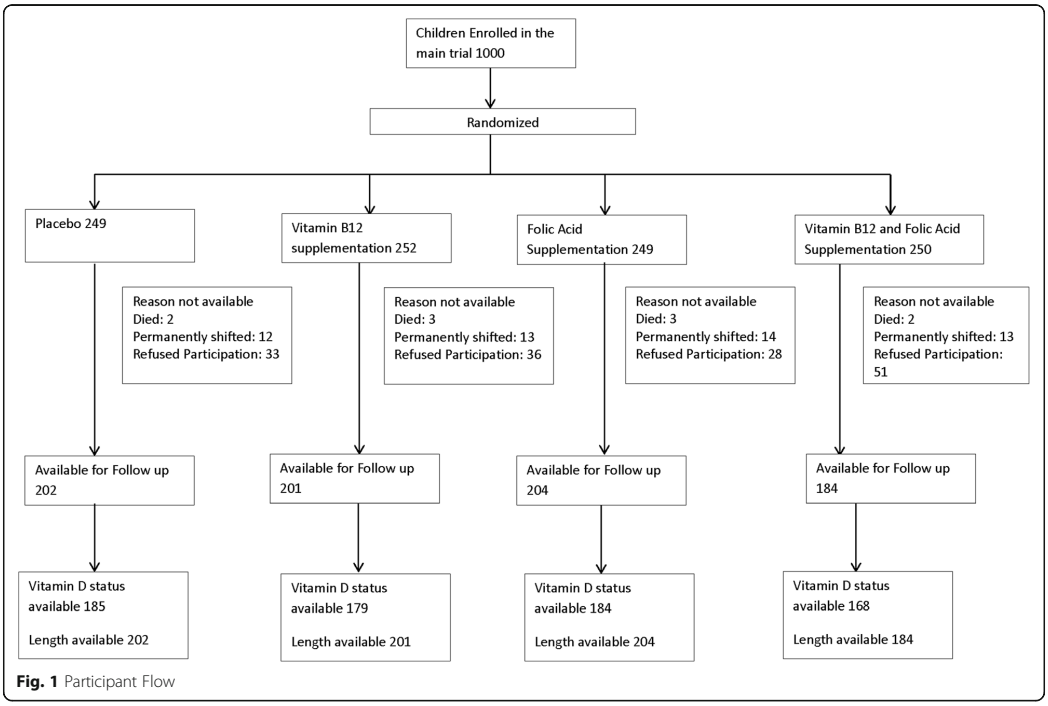

Fig. 1 Participant Flow

for the participation in the study and we gathered information on socio-economic situation of the family such as parental education and occupation and various household assets.

Assessment

Cognition

We assessed cognitive development with the Wechsler Intelligence Scale for Children, 4th edition ^{INDIA}, the Crichton Verbal Scale, NEPSY-II subtests and the BRIEF 2.

The Wechsler Intelligence Scale for Children 4th edition (India) (WISC-IV^{INDIA}) is an assessment tool of intellectual ability in children validated for the Indian population with Indian norms [19]. We assessed seven subtests (listed in the parenthesis) that were summed up to three index scores; the Perceptual Reasoning (Block design, Picture concept, Matrix reasoning), Processing Speed (Symbol search, Letter-number sequences) and Working Memory (Digit span, Coding).

The Crichton Vocabulary Scales (CVS) assess verbal skills in children 4 to 18 years through knowledge of words [20]. The CVS has been translated to Hindi and provides a standard score with Indian norms [21].

NEPSY-II is a neuropsychological test battery for children aged 3 to 16 years with American norms [22]. Seven age-appropriate subtests were administered; Inhibition and Design Fluency, Word Generation, Visuomotor Precision and Manual Motor Sequences, Affect Recognition and Geometric Puzzles. No modifications and cultural adjustments were necessary to perform the tests in this setting.

The Behavior Rating Inventory of Executive Function 2nd edition (BRIEF 2) is a parent report questionnaire for children 5 to 18 years assessing executive functions in everyday life with American norms [23]. The scale comprises three clinical indexes; the Behavior, Emotion and Regulation Index, and an overall Global Executive Composite. The questionnaire was translated and validated to Hindi in close collaboration with the developers (PARiConnect).

Growth

Trained field supervisors measured weight and height at follow up. Weight was measured to the nearest 50 g using a Digitron scales. Height was measured with Seca 213 to the nearest 0.1 cm. Inter- and intra-observer standardization exercises for weight and height assessments were conducted before study initiation for outcome ascertainment team; these are repeated every 3 months.

Analytical procedures

At baseline, 3 ml blood was collected into an evacuated tube containing EDTA (BD, Franklin Lakes, NJ, USA) from all children. Plasma was separated from the whole blood by centrifugation at room temperature (450 x g × 10 min), transferred into storage vials and stored at − 20 °C until analysis. Plasma concentration of vitamin-D was measured by quantitative electrochemiluminescence binding assay (Roche Diagnostics, Mannheim, Germany) at the Department of Biochemistry, Christian Medical College, Vellore, India [24]. Plasma homocysteine (tHcy) was analyzed using commercial kits (Abbott Park, IL, USA) [25]. Plasma concentrations of vitamin B12 and folate were determined by microbiological assays using a chloramphenicol-resistant strain of *Lactobacillus casei* and colistin sulfate-resistant strain of *Lactobacillus leichmannii*, respectivel y[26, 27]. Plasma soluble transferrin receptor (sTfR) was analyzed using an immunoturbidimetric assay [28].

Statistical analysis

Proportions, means (SD) or medians (IQR) were calculated for categorical and continuous variables by vitamin-D status at baseline. We categorized vitamin D status according to the US Institute of Medicine's recommendations; serum 25(OH)D < 12 ng/ml as 'deficient'; 12–20 ng/ml as 'inadequate'; > 20 ng/ml as 'sufficient' [29]. For the cognitive outcomes, we calculated a combined WISC-IV^{INDIA} and CVS z-score based on converted z-scores for the three index scores in the WISC-IV^{INDIA} and the total CVS score. We also calculated a combined NEPSY-II z-score based on converted z-scores in the seven subtests. For the BRIEF 2, we used the overall Global Executive Composite score in the analyses. Children's height-for-age z-score (HAZ) at follow up was calculated based on WHO growth standards [30]. The wealth of an individual was determined by a wealth index created using principal component analysis based on assets owned by the household [31]. Using the score from the principal component analysis, the population was divided into five equal wealth quintiles i.e. poorest, very poor, poor, less poor and least poor.

We used multivariable linear regression to investigate the association between vitamin D status at baseline and the cognitive outcomes and HAZ score at follow up. We used generalized linear model (GLM) with the gaussian distribution family, and identity link function to calculate beta-coefficients for the cognitive outcomes and the HAZ scores. We used GLM with the poisson distribution family, and log link to calculate the relative risk (RR) for stunting [32].

Table 1 Demographic information and nutritional status of the 716 North Indian children at baseline (6 to 30 months) and follow up (6 to 9 years)

	Vitamin D sufficient ($n = 154$)	Vitamin D inadequate ($n = 234$)	Vitamin D deficient ($n = 328$)
Child characteristics at baseline (6 to 30 months)			
Boys, n (%)	85 (55.2)	125 (53.4)	161 (49.1)
Ever breastfed, n (%)	154 (100)	232 (99.1)	323 (98.5)
Growth Z scores, mean (sd)			
Weight-for-height (WHZ)	−0.9 (0.9)	− 0.9 (0.9)	− 0.8 (0.9)
Height-for-age (HAZ)	−1.7 (1.1)	− 1.6 (1.1)	− 1.6 (1.2)
Weight-for-age (WAZ)	− 1.6 (0.9)	−1.5 (1)	− 1.5 (1)
Wasted (<−2 WHZ)	18 (11.7)	28 (12)	34 (10.4)
Stunted (<−2 HAZ)	59 (38.3)	86 (36.7)	128 (39)
Underweight (<−2 WAZ)	47 (30.5)	77 (32.9)	105 (32)
Biomarkers:			
Cobalamin, mean (sd) pmol/L	312.7 (186.7)	332.6 (215.3)	305.9 (175.2)
Cobalamin < 200 pmol/L, n (%)	47 (30.5)	72 (30.8)	106 (32.3)
Folate, mean (sd) nmol/L	16.7 (14.6)	16.1 (13.7)	14.9 (14.4)
Folate < 7.5 nmol/L, n (%)	48 (31.2)	69 (29.5)	115 (35.1)
Homocysteine, mean (sd) μmol/L	14.4 (8.8)	13.3 (6.7)	13.7 (7.3)
Homocysteine > 10 μmol/L, n (%)	104 (68.4)	144 (62.1)	204 (62.4)
Soluble transferrin receptor, mean (sd) nmol/L	4.3 (2.8)	4.4 (3.3)	4.7 (3.1)
Soluble transferrin receptor> 4.7 nmol/L, n (%)	40 (26)	76 (32.5)	111 (33.8)
Child characteristics at follow up (6 to 9 years)			
Age follow up (yrs) mean (SD)	7.9 (0.6)	7.8 (0.6)	7.9 (0.6)
Schooling			
No School, n (%)	3 (1.9)	6 (2.6)	4 (1.2)
Hindi medium, n (%)	61 (39.6)	88 (37.6)	129 (39.3)
English medium, n (%)	90 (58.4)	140 (59.8)	195 (59.4)
Family characteristics at follow up			
Mothers years of schooling			
No schooling, n (%)	54 (35.5)	65 (28)	76 (23.3)
Primary (1–5 years), n (%)	23 (15.1)	18 (7.8)	49 (15)
Middle (6–12 years), n (%)	65 (42.8)	123 (53)	153 (46.9)
Higher (> 12 years), n (%)	10 (6.6)	26 (11.2)	48 (14.7)
Fathers occupation			
Government job or private services, n (%)	88 (57.1)	135 (57.9)	165 (50.8)
Self-employed, n (%)	31 (20.1)	56 (24)	95 (29.2)
Daily wager/farming, n (%)	29 (18.8)	31 (13.3)	52 (16)
No job/other, n (%)	6 (3.9)	11 (4.7)	13 (4)
Wealth Quintile			
Poorest, n (%)	39 (25.3)	47 (20.1)	60 (18.3)
Very Poor, n (%)	36 (23.4)	38 (16.2)	65 (19.8)
Poor, n (%)	31 (20.1)	57 (24.4)	60 (18.3)
Less Poor, n (%)	32 (20.8)	43 (18.4)	69 (21)
Least Poor, n (%)	16 (10.4)	49 (20.9)	74 (22.6)

Table 2 The association between baseline Vitamin D status and cognitive scores at follow up in North Indian children 6 to 9 years

	WISC-IV^{INDIA} and CVS z-score		NEPSY z-score		Global BRIEF score	
	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)[a]	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)[a]	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)[a]
Vitamin D sufficient	**Reference**		**Reference**		**Reference**	
Vitamin D inadequate	0.03 (−0.17 to 0.23)	−0.12 (−0.30 to 0.05)	−0.01 (−0.21 to 0.19)	−0.15 (−0.33 to 0.04)	0.21 (−2.10 to 2.48)	0.87 (−1.40 to 3.14)
Vitamin D deficient	0.02 (− 0.16 to 0.22)	− 0.13 (− 0.29 to 0.04)	−0.02 (− 0.21 to 0.17)	−0.16 (− 0.34 to 0.02)	−1.10 (−3.21 to 1.05)	−0.38 (− 2.53 to 1.77)

[a]adjusted for log folate, log soluble transferrin receptor and log homocysteine level at baseline, and the wealth index, paternal occupational status and maternal education at follow-up and intervention group

We used a method of purposeful selection of covariates to identify variables for the multivariable models [33, 34]. We included in the multivariable models the variables that changed the beta coefficient or relative risk of the outcome variables by 20% from the univariable models. We present the adjusted models, including variables that were identified in the process. The candidate variables for these models were age and sex of the child, maternal and paternal years of schooling, paternal occupation, wealth quintiles at follow up and baseline log (base e) cobalamin, folate, and total homocysteine concentration and the intervention groups.

Statistical analyses were performed using STATA version 15 (Stata Corporation, College Station, TX). We used generalized additive models in the statistical software R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) to explore nonlinear associations between the plasma vitamin D concentration at baseline and the combined WISC-IV^{INDIA} and CVS z-score, the combined NEPSY-II z-score and the Global BRIEF-2 score at follow up after adjustment for potential confounders [35].

Results

Of the 1000 children in the main study, 791 children consented to participate in the follow-up study. Fig. 1 shows the flow of the participants. The demographic information and nutritional status of the children at baseline and follow up are presented in Table 1. Baseline vitamin D status was available for 716 children who consented. Of these, 328 (45.8%) were deficient, 234 (32.7%) were inadequate, and 154 (21.5%) sufficient [29]. The mean (SD) and median (IQR) of vitamin D concentration at baseline were 14.6 (8.6) ng/ml and 12.8 (8.3–18.7) ng/ml, respectively.

The estimates from both univariable and multivariable analyses comparing the combined WISC-IV^{INDIA} and CVS z-score, the combined NEPSY-II z-score and the Global BRIEF-2 score between vitamin D inadequate, deficient, and vitamin D sufficient children are shown in Table 2. There were no significant differences between the vitamin D sufficient children, inadequate and deficient children on any of the cognitive outcomes.

Table 3 shows the association between baseline vitamin D status and linear growth at follow up. Of the children, 15.8, 12.4 and 17.5% were stunted in the vitamin D deficient, inadequate and sufficient group, respectively. Vitamin D status was not associated with the HAZ score or the proportion of children stunted at follow up.

The association between vitamin D concentration at baseline and the cognitive outcomes at follow up are depicted in Fig. 2. The GAMs did not reveal any nonlinear associations between vitamin D level at baseline and the combined WISC-IV^{INDIA} and CVS z-score and the Global BRIEF-2 score at follow up.

Table 3 The association between baseline Vitamin D status and linear growth at follow up in North Indian children 6 to 9 years

	HAZ scores at follow up		Stunted at follow up	
	Unadjusted β coefficient (95% CI)	Adjusted β coefficient (95% CI)*	Unadjusted RR (95% CI)	Adjusted RR (95% CI)[a]
Vitamin D sufficient	**Reference**		**Reference**	
Vitamin D inadequate	0.06 (−0.13 to 0.26)	−0.07 (− 0.26 to 0.12)	0.70 (0.42 to 1.20)	0.87 (0.50 to 1.50)
Vitamin D deficient	0.08 (−0.10 to 0.27)	−0.06 (− 0.24 to 0.11)	0.90 (0.57 to 1.44)	1.14 (0.70 to 1.86)

[a]adjusted for log folate, log soluble transferrin receptor and log homocysteine level, at baseline and the wealth index, paternal occupational status and maternal education at follow-up and intervention group

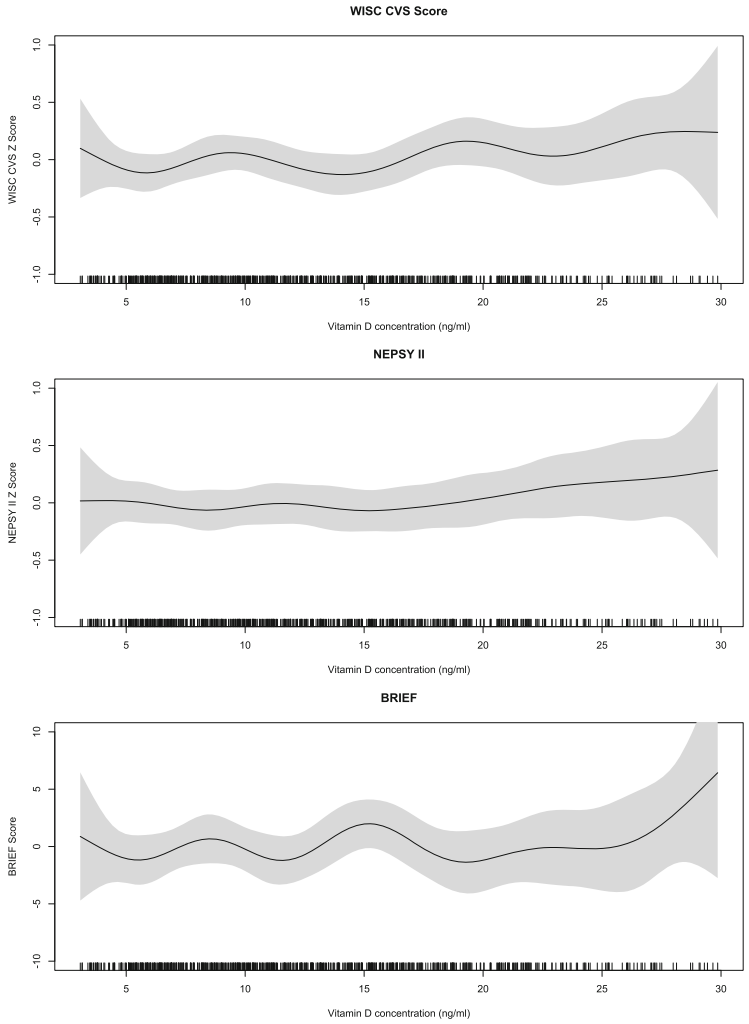

Fig. 2 Association between baseline vitamin D level and the combined WISC IV^{INDIA} and Crichton Vocabulary Scale (CVS) z-score, the combined NEPSY II z scores and the BRIEF P Global Executive composite score at follow up in North Indian children 6 to 9 years. The graph was constructed using generalized additive models in R, the solid line depicts the association of vitamin D level at baseline and Global BRIEF score at follow up. The shaded area spans the 95% confidence interval of this association

Discussion

We did not find any evidence for a link between early vitamin D status and long-term cognition and linear growth.

To our knowledge, this is the first study that has examined the relationship between vitamin D status in early childhood and cognitive development at school age. The findings from this study are in accordance with findings from the same cohort when neurodevelopment was measured in early childhood [13]. The findings are also similar to those from a cohort study in India, where vitamin D status in early childhood was not associated with the gross motor functioning among school aged children [36]. Studies that have examined the association between cord blood vitamin D concentrations and neurodevelopment measured in early and middle childhood have shown mixed results [37, 38]. Furthermore, studies that have examined the associations between vitamin D

deficiency during pregnancy and neurodevelopment during early and middle childhood have also shown inconsistent results [7–12, 39]. Three studies found an association between pregnancy vitamin D status and neurodevelopment outcomes in children before 4 years of age [8, 11, 38], while one study found marginal associations with language scores at 10 years of age [10]. The inconsistencies are likely due to differences in the populations, the timing of vitamin D assessment during pregnancy, the use of different cut-offs for vitamin D deficiency, age of the child at developmental assessments, and the way potential confounders were handled. The large sample size, the broad range of cognitive assessments, many with Indian norms, and the timing of the assessments at school age provide strengths to our findings that early vitamin D status most likely is not associated with cognitive function on long term.

We found no association between vitamin D status at baseline and linear growth at follow up. There are similar findings described in pre-school children in Nepal and in uninfected HIV exposed infants in Africa [40, 41]. In contrast, low-birth-weight (1.8 to 2.5 kg) infants in India, who received 1 RDA of vitamin D supplementation showed significantly higher length and weight at 6 months of age compared to those who received placebo [42]. Vitamin D helps the growth plate cells to be more sensitive to growth hormone action which plays an important role in linear growth at school age [43]. Vitamin D also maintains bone health to ensure normal calcium and phosphate levels in the blood [44]. Thus, our findings of no association between vitamin D deficiency and linear growth may be unexpected. A probable explanation could be that the children have to be severely deficient in vitamin D before it has consequences for their bone growth. There may be deficiencies of other growth-limiting macro and micronutrients such as calcium, zinc, vitamin B12 that account for the variance of growth between these study children. Furthermore, lower proportion of animal source protein in food, may also contribute to poor growth in this population [45]. The role of vitamin D might thus be negligible in the light of other growth limiting factors in this population.

Dietary sources of vitamin D are limited mainly to oily fish, eggs, and fortified foods [46]. As most people in the northern part of India are vegetarians, the predominant dietary source of vitamin D among the study children would be milk. Milk is rarely fortified with vitamin D in India and the vitamin D content of unfortified milk is very low (2 IU/100 mL). Prevalence of lactose intolerance also contributes to vitamin D deficiency in this setting [47]. Currently, there is no national program of vitamin D supplementation for infants and children, but the Indian Academy of Pediatrics guidelines recommend daily vitamin D supplementation in doses of 400 IU up to 1 year of age and 600 IU from 1 to 18 years of age [48].

The main strength of the study is that we measured vitamin D status in a large sample of children during what is considered a critical window for the brain development, and measured the cognitive outcomes during a period where valid and stable estimates can be obtained. The study includes high quality and comprehensive assessment of cognitive development, with the use of validated tests with Indian norms. We were able to include 80% of the children from the primary cohort after more than 5 years with no significant differences between the children who were included in the follow-up and not. An immunoassay method was used to assess vitamin D concentration. The immunoassay can underestimate serum 25(OH) D2 concentration compared to liquid chromatography-tandem mass spectrometry (LC-MS/MS) [49].

Conclusion

The results from the current study do not support that vitamin D status in early childhood is of importance for long-term growth and cognition.

Abbreviations

BRIEF: Behavior rating inventory of executive function; CI: Confidence interval; CVS: Crichton Verbal Scale; NEPSY: A developmental neuro psychological assessment; RCT: Randomized controlled trial; RDA: Recommended daily allowance; SD: Standard deviation; tHcy: Total Homocysteine; WISC-IV ^{INDIA}: Wechsler intelligence scale for children, 4th edition ^{INDIA}

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Authors' contributions

RC, TS, ST, IK designed the research with contributions from all authors. RC and TS did the statistical analysis with support from ST, IK, MH, and NB. RC wrote the first draft. All authors contributed to the critical interpretation and writing of the paper and saw and approved the final version.

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Availability of data and materials

Request for data pertaining to the current analysis may be sent to Dr. Sunita Taneja (Email id: sunita.taneja@sas.org.in).

Ethics approval and consent to participate

The follow-up study was approved from the ethics committee of Society for Applied Studies (India) 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 participating children's blood specimen for use in future research. All parents consented for the same.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Holick MF. Vitamin D: extraskeletal health. Rheum Dis Clin N Am. 2012;38:141–60. https://doi.org/10.1016/j.rdc.2012.03.013.
2. Kamboj P, Dwivedi S, Toteja GS. Prevalence of hypovitaminosis D in India & way forward. IJMR. 2018;48(5):548–56.
3. Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). Endocrinol Metab Clin N Am. 2010;39(2):255–69. https://doi.org/10.1016/j.ecl.2010.02.007.
4. Kesby JP, Eyles DW, Burne TH, McGrath JJ. The effects of vitamin D on brain development and adult brain function. Mol Cell Endocrinol. 2011;347(1–2):121–7. https://doi.org/10.1016/j.yfrne.2012.07.001.
5. O'Loan J, Eyles DW, Kesby J, Ko P, McGrath JJ, Burne TH. Vitamin-D status during various stages of pregnancy in the rat; its impact on development and behaviour in adult offspring. Psychoneuroendocrinology. 2007;32:227–34. https://doi.org/10.1016/j.psyneuen.2006.12.006.
6. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin-D3 and brain development. Neuroscience. 2003;118:641–53. https://doi.org/10.1016/s0306-4522(03)00040-x.
7. Strom M, Halldorsson TI, Hansen S, Granström C, Maslova E, Petersen SB, Cohen AS, Olsen SF. Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. Ann Nutr Metab. 2014;64:254–61. https://doi.org/10.1159/000365030.
8. Morales E, Guxens M, Llop S, Rodríguez-Bernal CL, Tardón A, Riaño I, Ibarluzea J, Lertxundi N, Espada M, Rodriguez A. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics. 2012;130:e913–20. https://doi.org/10.1542/peds.2011-3289.
9. Keim SA, Bodnar LM, Klebanoff MA. Maternal and cord blood 25(OH)-vitamin D concentrations in relation to child development and behaviour. Paediatr Perinat Epidemiol. 2014;28:434–44. https://doi.org/10.1111/ppe.12135.
10. Whitehouse AJ, Holt BJ, Serralha M. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics. 2012;129:485–93. https://doi.org/10.1542/peds.2011-2644.
11. Hanieh S, Ha TT, Simpson JA, Thuy TT, Khuong NC, Thoang DD, Tran TD, Tuan T, Fisher J, Biggs BA. Maternal vitamin D status and infant outcomes in rural Vietnam: a prospective cohort study. PLoS One. 2014;9:e99005. https://doi.org/10.1371/journal.pone.0099005.
12. Brouwer-Brolsma EM, Vrijkotte TG, Feskens EJ. Maternal vitamin D concentrations are associated with faster childhood reaction time and response speed, but not with motor fluency and flexibility, at the age of 5–6 years: the Amsterdam born children and their development (ABCD) study. Br J Nutr. 2018;120(3):345–52. https://doi.org/10.1017/S0007114518001319.
13. Chowdhury R, Taneja S, Bhandari N, Kvestad I, Strand TA. Bhan MK. Vitamin-D status and neurodevelopment and growth in young north Indian children: a secondary data analysis. Nutr J, 2017; 16: 59 doi: https://doi.org/10.1186/s12937-017-0285-y.
14. Månsson J, Stjernqvist K, Serenius F, Ådén U, Källén K. Agreement between Bayley-III measurements and WISC-IV measurements in typically developing children. J Psychoeduc Assess. 2018. https://doi.org/10.1177/0734282918781431.
15. Schneider W, Niklas F, Schmiedeler S. Intellectual development from early childhood to early adulthood: The impact of early IQ differences on stability and change over time. Learn Individ Differ 2014;32:156–162. doi: https://doi.org/https://doi.org/10.1016/j.lindif.2014.02.001.
16. Winje BA, Kvestad I, Krishnamachari S, Manji K, Taneja S, Bellinger DC, et al. Does early vitamin B₁₂ supplementation improve neurodevelopment and cognitive function in childhood and into school age: a study protocol for extended follow-ups from randomised controlled trials in India and Tanzania. BMJ Open. 2018;8(2):e018962.
17. Chowdhury R, Taneja S, Bhandari N, Sinha B, Upadhyay RP, Bhan M, Strand TA. Vitamin-D deficiency predicts infections in young north Indian children: a secondary data analysis. PLoS One. 2017;12(3):e0170509. https://doi.org/10.1371/journal.pone.0170509.
18. Taneja S, Strand TA, Kumar T, Mahesh M, Mohan S, Manger MS. Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: a randomized placebo-controlled trial. Am J Clin Nutr. 2013;98(3):731–7. https://doi.org/10.3945/ajcn.113.059592.
19. Wechsler D. WISC-IV India. Wechsler intelligence scale for children - fourth (India edition). New Delhi: Pearson; 2016.
20. Raven J, Rust J, Squire A. Manual for Coroured progressive matrices and Crichton vocabulary scale. NCS Pearson Inc: UK; 2008.
21. Raven J, Rust J, Squire A. Raven's Coloured Progressive Matrices and Crichton Vocabulary Scales (Hindi Edition). Chennai, India: Pearson Clinical & Talent Assessment; 2015.
22. Brooks BL, Sherman S, Strauss E. NEPSY-II: A developmental neuropsychological assessment, second edition. 2010 Child Neuropsychol 16(1):80–101. https://doi.org/10.1080/09297040903146966.
23. Gioia GA, Isquith PK, Roth RM. Behavior Rating Inventory for Executive Function. In: Kreutzer JS, DeLuca J, Caplan B, eds. Encyclopedia of Clinical Neuropsychology. Cham: Springer International Publishing; 2018:532–8. https://doi.org/10.1007/978-3-319-57111-9_1881.
24. Cobas E411 Vitamin-D Total Reagent Insert (06268668001V1). Roche Diagnostics Web site. Available online: http://www.cobas.com/home/product/clinical-and-immunochemistry-testing/elecsys-vitamin-d-total-assay.html Accessed 17 April 2019.
25. Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst (e) ine with the Abbott IMx analyzer. Clin Chem. 1995;41(7):991e4.
26. O'broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol. 1992;45(4):344e7.
27. Kelleher BP, Walshe KG, Scott JM, O'Broin SD. Microbiological assay for vitamin B12 with use of a colistin-sulfate-resistant organism. Clin Chem. 1987;33(1):52e4.
28. Cotton F, Thiry P, Boeynaems J. Measurement of soluble transferrin receptor by immunoturbidimetry and immunonephelometry. Clin Biochem. 2000;33(4):263e7.
29. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull 2014;39(4):322–350 doi:https://doi.org/10.1111/nbu.12108.
30. Onis M. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr 2006; 95(S450): 76–85 doi: https://doi.org/https://doi.org/10.1111/j.1651-2227.2006.tb02378.x.
31. International Institute for Population Sciences (IIPS) and Macro International. National Family Health Survey (NFHS-4), 2015-16. Mumbai: International Institute for Population Sciences (IIPS) and Macro International; 2015.
32. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004;159(7):702–6. https://doi.org/10.1093/aje/kwh090.
33. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol and Med. 2008;3:17. https://doi.org/10.1186/1751-0473-3-17.
34. Hosmer DW, Lemeshow S, Sturdivant RX. Applied logistic regression. New York: Wiley; 2013.
35. Wood SN. Modelling and smoothing parameter estimation with multiple quadratic penalties. J Royal Stat Soc. 2000;62:413–428 doi: https://doi.org/https://doi.org/10.1111/1467-9868.00240.
36. Filteau S, Rehman AM, Yousafzi A, Chugh R, Kaur M, Sachdev HPS, Trilok-Kumar G. Association of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term low birth weight. BMJ Open. 2016;6:e009268. https://doi.org/10.1136/bmjopen-2015-009268.
37. Zhu P, Tong SL, Hao JH, Tao RX, Huang K, Hu WB, Zhou QF, Jiang XM, Tao FB. Cord blood vitamin D and neurocognitive development are

nonlinearly related in toddlers. J Nutr. 2015;145(6):1232–8. https://doi.org/10.3945/jn.114.208801.
38. Gould JF, Anderson AJ, Yelland LN, Smithers LG, Skeaff CM, Zhou SJ, Gibson RA, Makrides M. Association of cord blood vitamin-D with early childhood growth and neurodevelopment. J Paediatr Child Health. 2017;53(1):75–83. https://doi.org/10.1111/jpc.13308.
39. Darling AL, Rayman MP, Steer CD, Golding J, Lanham-New SA, Bath SC. Association between maternal vitamin D status in pregnancy and neurodevelopmental outcomesin childhood: results from the Avon longitudinal study of parents and children (ALSPAC). Br J Nutr. 2017;117(12):1682–92. https://doi.org/10.1017/S0007114517001398.
40. Avagyan D, Neupane SP, Gundersen TE, Madar AA. Vitamin D status in preschool children in rural Nepal. Public Health Nutr. 2016;19(3):470–6. https://doi.org/10.1017/S136898001500083X.
41. Sudfeld CR, Duggan C, Abound S, Kupka R, Manji KP, Kisenge R, Fawzi WW. Vitamin D status is associated with mortality, morbidity, and growth failure among a prospective cohort of HIV-infected and HIV-exposed Tanzanian infants. J Nutr. 2015;145(1):121–7. https://doi.org/10.3945/jn.114.201566.
42. Kumar GT, Sachdev HS, Chellani H, Rehman AM, Singh V, Arora H, Filteau S. Effect of weekly vitamin-D ements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. BMJ. 2011;342:d2975. https://doi.org/10.1136/bmj.d2975.
43. Esposito S, Leonardi A, Lanciotti L, Cofini M, Muzi G, Penta L. Vitamin D and growth hormone in children: a review of the current scientific knowledge. J Transl Med. 2019;17(1):87.
44. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014;21(3):319–29.
45. Eaton JC, Rothpletz-Puglia P, Dreker MR, Iannotti L, Lutter C, Kaganda J, Rayco-Solon P. Effectiveness of provision of animal-source foods for supporting optimal growth and development in children 6 to 59 months of age. Cochrane Database of Systematic Reviews. 2019(2):CD012818. https://doi.org/10.1002/14651858.CD012818.pub2.
46. Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Mawer EB, Puliyel JM. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi. India. 2002;87(2):111–3.
47. Ritu G, Gupta A. Vitamin-D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014;6:729–75. https://doi.org/10.3390/nu6020729.
48. Khadilkar A, Khadilkar V, Chinnappa J, Rathi N, Khadgawat R, Balasubramanian S, et al. Prevention and treatment of vitamin D and calcium deficiency in children and adolescents: Indian academy of pediatrics (IAP) guidelines. Indian Pediatr. 2017;54:567–73.
49. Li L, Zeng Q, Yuan J, Xie Z. Performance evaluation of two immunoassays for 25-hydroxyvitamin D. J Clin Biochem Nutr. 2016;58(3):186–92.

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