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Association between Hemoglobin, Serum Calcium, IgE, and Vitamin D Status among Children

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Abstract

Introduction: *Documentation of the association of Hypovitaminosis D with anaemia, serum calcium level and IgE in children was limited, especially in South-Asian population. This study was conducted to observe the association of vitamin D status with hemoglobin, serum calcium, and IgE in the children of Bangladesh.*

Aim of the study: *The study aimed to explore the association of hemoglobin, serum calcium, and IgE with vitamin D status in infants and children in South- East region of Bangladesh.*

Methods: *This was a cross-sectional observational study conducted at a pediatrics clinic in Chittagong Metropolitan City from July 2012 to December 2017. In this study, a total of 524 participants between the age of 0-18 years were included.*

Results: *In this study, more than half (269, 51.3%) of the study subjects were in the 1- 5 years preschool age group, followed by 5-10 years aged school children (171, 32.6%). Out of 524 study subjects, hypovitaminosis D (<20ng/dl) was noticed in more than half of the cases 265(50.57%) based on measurement of serum 25 (OH) D. Only 259 (49.42%) cases were found to be sufficient (20- 100ng/ml). A significant negative association was observed between hemoglobin and vitamin D for serum calcium. A significant positive association was observed between hemoglobin and serum calcium findings with respect to serum IgE levels, and there was a significant negative association (-0.048) between Vitamin D and serum IgE levels.*

Conclusion: *Hypovitaminosis D (<20ng/dl) prevails significantly among Infants and children of South- East region of Bangladesh. Hemoglobin and Vitamin D levels had a significant negative association with serum calcium. A significant negative association was also observed between Vitamin D levels and IgE levels, but serum IgE had a positive association with both hemoglobin and serum calcium.*

Keywords: *Hypovitaminosis D, Hemoglobin, IgE, Serum Calcium*

1. Introduction

Hypovitaminosis D is a risk factor for many diseases, including the endocrine system, immune system, CVS, neuropsychological functioning, and many more.[1,2] It is surprising to see that hypovitaminosis D persists even in regions with sufficient sunlight.[3,4] Numerous factors, such as the amount of skin exposure, the time and length of sun exposure, air pollution, skin pigmentation, usage of sunblock, and genetic and nutritional factors help to explain this paradox of hypovitaminosis D despite an abundance of sunshine.[5,6,7] Cutaneous vitamin D production is at its peak between 10 AM and 3 PM when the majority of kids are either at school or inside. Ultraviolet B, having a shorter wavelength, tends to scatter earlier to later in the day. There are noticeable changes in vitamin D synthesis between exposure to the face, hands, and arms solely owing to clothes and exposure from the entire body.[8] The quantity of ultraviolet B that reaches the earth's surface can be decreased by cloud cover, rising water vapor, and industrial pollution.[9] An Asian would require three times the sun exposure than a light-skinned person to produce equivalent amounts of vitamin D.[10] Dietary factors like very low calcium intake and high fiber diet may deplete vitamin D stores.[11]

A lack of vitamin D in the diet can lead to Osteomalacia, a condition that softens the bones. In children, this is known as rickets. In the developed world, this is a rare disease.[12,13] A circulating level of 25(OH)D of >75nmol/L or 30ng/ml is required to maximize vitamin D's beneficial effect on health. In the absence of adequate sun exposure, 800-1000 IU vitamin D3/day may be needed to achieve this in children.[13] Low blood calcidiol (25- hydroxy D) can result from avoiding the sun.[14] Deficiency results in impaired bone mineralization and leads to bone-softening diseases.[15] Low dietary calcium intakes, which are typical of cereal-based diets with limited availability of dairy products, have been linked to the condition in sunny nations like Nigeria, South Africa, and Bangladesh, where it affects older toddlers and children.[16] In the past, rickets were a significant public health issue in the US. A sharp reduction in rickets cases was accompanied by an increase in the consumption of milk supplemented with relatively small amounts of vitamin D as well as an increase in the proportion of animal protein. [16-19] in the American diet of the 20th century.[4,5] It is suggested that the major effect of calcium supplementation is on reducing the bone remodeling space rather than structurally increasing bone size or volumetric bone density.[19]

According to new research led by Johns Hopkins Children's Center investigators, low levels of the "sunshine" vitamin D appear to increase a child's risk of anemia. The study also demonstrates a complex interaction between low vitamin D levels and hemoglobin, the oxygen-binding protein in red blood cells.[18] The researchers believe that several mechanisms, including vitamin D's effects on red blood cell production in the bone marrow and its ability to regulate immune inflammation, a known cause of anemia, could account for the link.[20]

Vitamin D aids calcium absorption in the intestines. Overcrowding, air pollution, and a lack of vitamin D-fortified foods are major contributors to vitamin D deficiency.[21] Low calcium intakes are commonly observed as a result of a diet low in dairy products and high in phytates and oxalates. Calcium supplementation may have a greater effect on bone remodeling space than on structurally increasing bone size or volumetric bone density.[22] New evidence indicates that vitamin D deficiency is widespread in many countries, and some studies suggest a possible link between low vitamin D status and atopy-related phenotypes in children.

In the Prediction of Allergies in Taiwanese Children (PATCH) study, serum 25(OH)D concentrations were measured using a new automated electrochemiluminescence-based assay, Elecsys Vitamin D total assay (Roche Diagnostics, Mannheim, Germany), in a population-based sample of 1315 Asian children aged 5-18 years. ImmunoCAP and ImmunoCAP Phadiatop Infant were used to measure total and allergen-specific IgE levels in the blood. The mean serum 25(OH)D concentration was 20.4 ng/ml (standard deviation: 7.1 ng/ml). 90.3 percent of children had vitamin D deficiency (defined as serum 25(OH) D 30 ng/ml). 51.0 percent of children had vitamin D deficiency (defined as serum 25(OH)D 20 ng/ml).

The serum 25(OH)D concentration was not found to be significantly related to total IgE ($P > 0.05$) or allergic sensitization, as defined by a positive Phadiatop Infant test result ($P > 0.05$). This population-based study finds no link between vitamin D status and total IgE levels or allergic sensitization in Asian children.[23] Another study found a negative relationship between age and Vitamin D concentration in both healthy children and children with allergic respiratory diseases. Vit D levels were found to be negatively associated with total and nonspecific IgE levels, but positively associated with cow's milk-specific IgE levels. Serum vitamin D levels may be linked to an increased risk of allergic disease development. [24]

Although there are a lot of studies regarding vitamin D status in patients of all ages, very few studies have focused on its effects on children. Further studies showing an association between atrophy and anemia are scarce.

Therefore, the present study was conducted to observe this association of vitamin D status with hemoglobin, serum calcium, and IgE among the children of Bangladesh region.

2. Materials & Methods

This cross-sectional study was conducted in a private pediatrics clinic in Chittagong Metropolitan City in the south-eastern region of Bangladesh over a period of sixty-six months from July 2012 to December 2017. In this study, a total of 524 participants between the age of 0-18 years were primarily selected, following the inclusion and exclusion criteria of the study and children with Vitamin-D malabsorption, anticonvulsants, nephrotic syndrome, severe hepatocellular disease, renal diseases or chronic diseases were excluded.

These are the following criteria to be eligible for enrollment as our study participants:

Inclusion Criteria

a) Children aged 0 to 18 years old;

b) Children of both genders;

c) Children whose parents/guardians were willing to let them participate in the study.

Exclusion Criteria

a) Children with Vitamin-D malabsorption;

b) Children with anticonvulsants, nephrotic syndrome,

c) Children with severe hepatocellular disease, renal diseases, or any other chronic diseases

Vitamin D Status Classification

Vitamin D status was classified depending upon the measured serum 25(OH) D (ng/ml) as follows: Insufficiency <20, Sufficiency 20-100, following Misra et al.'s study.[5] A maximum of 5 ml venous blood sample was taken for each case by disposable syringe and was collected in a red-capped tube (no anticoagulant) which was sent to a centrifuge machine (Rotofix 32 A, 3000 RPM) after 1 hr. of collection in a standard lab in the Metropolitan city under the supervision of Medical Biochemistry consultant. With 10 minutes' centrifugation the separated serum of the sample cup (300 microliter) was put in the Auto machine (Cobas: Elecsys 2010) for measuring 25(OH)D by Eletro-Chemiluminescence in ADVIA centaur XP/ Elecsys 2010/ Immulite 2000 xPi and, the result was provided 24 minutes later.

Calcium was measured using the Photometric method in Siemens Dimension EXL 200 Integrated Chemistry System. Hemoglobin was measured following the Cyan-methaemoglobin method in the Sysmex XN-1000 Hematology Analyzer. IgE was measured by the CLIA method (Chemiluminescence immune assay) in the ADVIA centaur XP immune assay system.

Data Collection Procedure

Informed consent was obtained from the legal guardians of the study participants, and ethical approval was obtained from the ethical review committee of the Department of Statistics, Faculty of Science of Chittagong University.

Statistical Analysis

All data were recorded systematically in preformed data collection form. Quantitative data was expressed as mean and standard deviation and qualitative data was expressed as frequency distribution and percentage. Data were analyzed by chi-square tests, Z score calculator for proportion, and MedCalc statistical software for Windows. A one-tailed P value of less than 0.05 was considered statistically significant. The data were analyzed by using SPSS 16 (Statistical Package for Social Sciences) for Windows version 10.

3. Results

Table 1. *Distribution of Age among the study subjects*

Age in Groups	Frequency	Percentage (%)
Infancy	72	13.7
1 – 5 Years	269	51.3
5 – 10 Years	171	32.6
10 Years	12	2.3
Total	**524**	**100.0**

In this study, more than half (269, 51.3%) of the study subjects were in the 1- 5 years Preschool age group, followed by 5-10 years aged school children (171, 32.6%). The average age is 51.87 months (SD 39.91 months), the median age is 43 months and the age range is between 1 and 191 months.

Table 2. *Association between age group and vitamin D status*

Age Groups	Vitamin-D Status			P Value
	Hypovitaminosis (N=265)	Sufficiency (N=259)	Total (N=524)	
Infancy	39 (14.7%)	33 (12.7%)	72 (13.7%)	**< 0.01**
1 – 5 Years	113 (42.6%)	156 (60.2%)	269 (51.3%)	
≥ 5 Years	113 (42.6%)	70 (27%)	183 (34.9%)	

Among the study subjects, hypovitaminosis D (<20ngm/dl) was noticed in more than half of the cases (265, 50.57%), Only 259(49.42%) cases were found to be sufficient (20-100ng/ml).

It was observed that there is a highly significant association between the age groups and vitamin D status proven by the chi-square test.

Table 3. *Association between hemoglobin, serum calcium, IgE level, and vitamin D status*

Biomarkers	Level	Vitamin-D status			P value
		Sufficiency (%)	Hypovitaminosis D (%)	Total (%)	
Hemoglobin [N=524]	Normal	190 (51)	182(49)	372 (100)	> 0.05
	Low	69 (45)	83 (55)	152 (100)	
Serum Calcium [N=486]	Normal	136(48)	150 (52)	286 (100)	> 0.05
	Low	106 (53)	93 (47)	199 (100)	
	High	1 (100)	0 (00)	1 (100)	
IgE [N=406]	Normal	29 (59)	20 (41)	49 (100)	> 0.05
	Low	10 (50)	10 (50)	20 (100)	
	High	167 (49)	170 (51)	337 (100)	

Among all 524 children, though hemoglobin level was possible to measure for all, serum calcium measurement was available in 486 children and IgE was recorded for 406 children. Among the 372 children with normal hemoglobin levels, 182 (49%) had hypovitaminosis D but among the 152 children with low hemoglobin levels, 55% (83) had hypovitaminosis D. Though hypovitaminosis D was higher among low hemoglobin children, no association between hemoglobin and vitamin D status (p=0.238) was found. Also, there was no association between serum calcium and vitamin D status (p=0.846). There was no association between vitamin D status and serum IgE level observed by the chi-square test (P value= 0.451).

Table 4. *Vitamin D levels according to hemoglobin, serum calcium, and IgE levels among the study subjects*

Biomarkers	Level	Frequency	Mean vitamin D (ng/dl)	SD	Median	Minimum	Maximum
Hemoglobin [N=524]	Normal	372	22.17	11.47	20.08	3.00	70.00
	Low	152	20.80	10.91	19.35	3.00	58.59
Serum Calcium [N=486]	Normal	286	21.24	10.88	19.29	3.00	53.84
	Low	199	22.70	12.18	21.50	3.00	70.00
	High	1	22.90	.	22.90	22.90	22.90
IgE [N=406]	Normal	49	23.46	11.61	23.12	3.00	53.84
	Low	20	21.65	14.39	19.93	3.74	50.40
	High	337	21.83	11.10	19.80	3.00	70.00

Table 4 shows that of all 524 children, 372 had normal hemoglobin level and their mean vitamin D concentration was 22 ng/dl (SD 11.4), which is higher than those 152 with low hemoglobin (20 ng/dl). Among the patients with available calcium data (n=486), the mean vitamin D concentration was 21.2 ng/dl (SD 10.8) in patients with normal calcium levels (n=286) and 22.7 ng/dl (SD 12.18) in patients with low calcium levels (n=199). Out of the patients with available IgE data (n=406), the mean vitamin D concentration was 23.46 ng/dl (SD 11.61) in patients with normal IgE level (n=49) and 21.65 ng/dl (SD 14.39) in patients with low IgE level (n=20). However, most of the patients had high IgE (n=337) with a mean vitamin D concentration of 21.83 ng/dl (SD 11.10). The differences in mean vitamin D concentration varied with the different levels of hemoglobin, serum calcium, and IgE concentrations in our study respondents. However, the differences were not statistically significant when examined by the F test (p=0.212, p=0.381, and p=0.638) respectively.

Table 5. *Pearson Correlations of Vitamin D level, hemoglobin, serum calcium, and IgE level*

Parameters	Hemoglobin	Vitamin D Level	Serum calcium	IgE
Hemoglobin	1	0.123	-0.025	0.023
Vitamin D		1	-0.018	-0.048
Serum calcium			1	0.004
IgE				1

**Significant at P value<0.05*

The Pearson correlation coefficient between vitamin D Level and hemoglobin level was 0.123, which was not significant (p>0.05 for a two-tailed test), based on 479 complete observations. A significant negative association was observed between hemoglobin and vitamin D with respect to serum calcium. A significant positive association was observed among hemoglobin and serum calcium findings with respect to serum IgE levels, but there was a significant negative association (-0.048) between Vitamin D and serum IgE levels.

4. Discussion

Vitamin D deficiency is a pandemic throughout the world irrespective of high and low altitude. Vitamin D deficiency has been reported in Europe, America, the Middle East, Africa, and Asia. Even it is more frequent in the sunny Mediterranean, south Asia, and neighboring countries like China, Japan, and Thailand. [14,15,16]

In this study, most of the subjects (269, 51.3%) were in the 1-5 years age group followed by 5-10 years aged school children (171, 32.6%). Infants were 72(13.8%) and more than 10 years age group were 12 in number (2.3%). The mean age was 51.87 ± 39.91 months with a range (1 – 191).

Among the study subjects, hypovitaminosis D (<20ng/dl) was noticed in more than half of the cases (265,50.57%), where serum 25(OH)D level of ≥ 20 ng/dl, was taken as cut off value for sufficiency, only 259(49.42%) cases were found to be sufficient(20-100ng/ml). The mean age differences (Table 06) between sufficiency and hypovitaminosis D status (44.72 ± 35.86 SD Vs 58.86 ± 42.41 SD) were statistically significant (P < 0.001).

In developing countries, the prevalence of hypovitaminosis D varies widely by and within regions, prevalence ranges between 30- 90% according to the cut-off value used within specific regions and is independent of latitude. A high prevalence of this disorder exists in China and Mongolia, especially in children of whom up to 50% are reported to have serum 25(OH)D <12.5nmol/L. Despite ample sunshine throughout the year, 1/3 to 1/2 of individuals living in sub-Saharan Africa and the Middle East have serum 25 (OH) D levels <25 nmol/L, according to studies published in the past decade. Hypovitaminosis D is also prevalent in children living in Latin America.[17]

In this study, although there was no statistically significant association between vitamin D status and patients' hemoglobin concentration, serum calcium and IgE level (p >.05), there was a correlation between the level of hemoglobin and level of vitamin D among the study patients.

Greater vitamin D was associated with greater hemoglobin concentration. The Pearson correlation coefficient between vitamin D Level and hemoglobin level was 0.123, which was not significant, but a significant negative association was observed between hemoglobin and vitamin D with serum calcium. Significant positive association was observed among hemoglobin and serum calcium findings with respect to serum IgE levels, but there was a significant negative association (-0.048) between Vitamin D and serum IgE levels.

Low levels of the "sunshine" vitamin D appear to increase a child's risk of anemia, according to new research led by investigators at the Johns Hopkins Children's Center. The study, published online Oct. 10, 2013 in the Journal of Pediatrics, is evidence of a complex interplay between low vitamin D levels and hemoglobin.18 Among 340 preschool children in urban Srilanka, weight for age was compiled where the prevalence of underweight & anemia was found 7.1% & 7.4% respectively. [19]

5. Limitations Of The Study

The study was conducted in a single hospital with purposive sampling. So, the results may not represent the whole community.

6. Conclusion

In this study, out of 524 study subjects, based on serum 25(OH) D level hypovitaminosis D (<20ngm/dl), is noticed in more than half (265, 50.57%) of the cases. Only 49.42% of cases have sufficient (20-100ng/ml) vitamin D. The differences in hypovitaminosis D status are statistically insignificant with serum calcium and IgE levels. However, hemoglobin and Vitamin D levels had a significant negative association with respect to serum calcium. A significant negative association was also observed between Vitamin D levels and IgE levels, but serum IgE had a positive association with both hemoglobin and serum calcium.

7. Recommendation

As hypovitaminosis D prevails significantly among Infants and children of South East region of Bangladesh, further multicenter large sample-sized study should be done in making immediate plan for awareness generation in the community. At the same time management of widespread low vitamin D level is very much urgently needed for proper health benefits of our future citizens.

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Conflict of Interest

None declared

Ethical Approval

The Institutional Ethics Committee approved the study

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