ORIGINAL ARTICLE

LEVELS OF VITAMIN D IN PATIENTS OF CHILDHOOD ASTHMA

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Abstract

Background: Lack of vitamin D has been linked to increased incidence of asthma in children. In this study we tried to find out the relation of vitamin D status in childhood asthma.

Methods: Hospital based analytical case control study. Vitamin D level was assessed in both cases and controls and its association with asthma was assessed in the age group 5- 12 years.

Results: Median serum 25 (OH) D was very low among cases 15.0 (IQR: 10.0, 19.5) as compared to controls 20.5 (IQR: 15.5, 28.0) and this difference was highly significant ($p<0.001$). However, Vitamin D deficiency was also found to be prevalent in our control population, with 47.6% having low serum Vitamin D levels. With multivariate analysis no relation was found between Vitamin D and the allergic markers [IgE & eosinophil count]. There was a significant relation between the low level of Vitamin D and the severity of asthma ($p<0.001$). The mean value of calcium was low and alkaline phosphatase and phosphate were higher in cases than the control group.

Conclusion: Though the causality of childhood asthma due to Vitamin D deficiency is not established, low Vitamin D is an aggravating factor for childhood asthma. Therefore, Vitamin D supplementation may be considered as an adjuvant therapy in asthmatic children.

Keywords: childhood asthma, serum vitamin D level, serum calcium, eosinophil count, serum IgE level

Introduction

Bronchial asthma remains the most common chronic disease of childhood (1-3) and is one of the leading causes of morbidity in children worldwide. (4) In India, the estimated burden of asthma is more than 30 million. In children, reported incidence by 6-7 years and 13-14 years are 2.3% & 3.3% respectively. (5-6). Epidemiological evidence suggests that there is a worldwide epidemic of vitamin D deficiency (7-8) and lack of vitamin D has been linked to increased incidence (9-12) and severity (13) of asthma in children. Given the emerging association between low vitamin D levels and asthma, in this study we tried to find out the correlation of vitamin D status in childhood asthma in the age group 5- 12 yrs. This age group was selected as children in this age group are exposed to the sunlight adequately because of the outdoor activities. The smaller children have their activities inside. Secondarily, we studied the association between Vitamin D deficiency and allergy markers of childhood asthma (IgE, eosinophil counts) and tried to correlate Vitamin D deficiency with severity of childhood asthma.

Methods & Materials

This hospital based analytical case control study was conducted in department of Pediatrics, VMMC and Safdarjang hospital, a tertiary care hospital in New Delhi, India from June 2012 to May 2013.

Using alpha equal to 0.05 and power equal to 80%, minimum sample size taken under each group was 60. All asthma patients of 5 to 12 years age group coming to Pediatric out-patient department (OPD) or emergency were classified as per Global Initiative for Asthma (GINA) guidelines 2013 and were taken as cases. Children of age group between 5-12 years attending pediatrics OPD for minor illness with no features of asthma were taken for control purpose. Children having clinical rickets, protein energy malnutrition (PEM), taking drugs which interferes with vitamin-D metabolism like anti epileptic drugs, anti-metabolites, who has received vitamin D either oral / injection in last 6 months and children having any chronic liver, kidney or lung diseases were excluded. A written informed consent was obtained from the parents for enrolling their child for the study and was approved by the Ethical Committee of the Hospital. Clinical features, frequency of asthma attack and history of allergy and wheeze were noted in each case. Blood was sent for serum calcium, serum phosphorus and serum alkaline phosphatase (ALP) level. Separate samples were sent for Vitamin D, IgE and eosinophil count. Kit used for estimation of vitamin D was DLD Diagnostika GMBH 25(OH) D ELISA from Germany. This test is free for the patients in our hospital. Vitamin D (25-hydroxy cholecalciferol) deficiency was defined as 25-hydroxy vitamin D levels < 50nmol/L or 20ng/ml (cut off level). Vitamin D deficiency was further classified as: mild: 25-OH vitamin D level between 26-50nmol/L or 10-20 ng/ml, moderate: Between 12.5-25 nmol/L or 5-10 ng/ml and severe: Level <12.5nmol/L or <5ng/ml. IgE was measured by ELISA method. Reference Range for IgE was taken as: 4-7 years :< 50 IU/ml and 7-14 years :< 100IU/ml.

Data Analysis

Chi-square test was used for the difference of their distribution between two groups. T test was used to test the difference of mean between cases and controls when it was normally distributed and for other clinical variables Mann-Whitney U non parametric test was used. Association of sex and vitamin D was assessed through Mann-Whitney U, test and Association of age and vitamin D was assessed through Kruskal-Wallis test. Coefficients for medians along with 95% confidence interval (CI) were provided for the association of calcium, phosphate, alkaline phosphatase, IgE and eosinophil with vitamin D using quantile regression models as bivariate analyses. Again, quantile regression model with covariates was used to find out the difference of vitamin D between cases and controls after adjusting the variables significantly associated in

bivariate analyses. Associations between vitamin D, IgE and eosinophil were tested using quantile regression model, again. Fisher exact test was used to associate vitamin D deficiency (<20ng/ml) and severity of asthma. The associations were considered statistically significant if the p value ≤ 0.05. All the analyses were done using statistical software SPSS 17.0.

Results

A total of 63 cases and same number of controls were included in the study. Baseline characteristics of cases and controls are depicted in Table 1. Levels of various biochemical parameters in the cases and controls are depicted in Table 2. In cases, calcium and ALP are significantly correlated with vitamin D and bivariate analysis shows that median value of vitamin D, 15.0ng/ml (IQR= 12.96-17.04) is significantly less from that of (20.5ng/ml) controls (IQR= 16.69-24.31). After adjusting calcium and ALP which are significantly associated with vitamin D, it still remains significantly different between cases and controls. (Table 3) The association of asthma marker IgE with demographic and other investigated parameters in bivariate analysis of vitamin D and calcium were significantly associated with IgE as 1 ng/ml increase in vitamin D level decreased 6.019 IU/ml IgE (p=0.003) and 1 mg/dl increase in calcium level made 55.8 IU/ml decrease in IgE (p=0.039). The association of vitamin D and Eosinophil was significantly different between cases and controls (p<0.001). Vitamin D was significantly associated with eosinophil in bivariate analysis as 1ng/ml increase in vitamin D level decreased 7.264 cell/mm3 eosinophil (p<0.001). Calcium was also significantly associated with eosinophil as one mg/dl increase in calcium level made -78.519 IU/ml decrease in IgE (p=0.005). Again, phosphorus was significantly associated with eosinophil (p=0.05) (Table 3). The result of the multivariate analysis, the median vitamin D value after adjusting calcium and ALP was 16.1 ng/ml in cases and 22.2ng/ml in controls (p=0.01). Multivariate association shows that when study group calcium and phosphorus were adjusted the significance of the association between IgE and vitamin D lapsed, (p=0.321). Multivariate association shows, that when study group, calcium and phosphorus were adjusted, the significance of the association between eosinophil and vitamin D lapsed. (p=0.914). Severity of Asthma was significantly associated with vitamin D deficiency, (p<0.001) as shown in Table 4.

Table 1. Baseline characteristics of cases and controls

Parameter	Cases (Mean ± SD)	Controls (Mean ± SD)	P Value
Sample Size	63	63	-
Age (years)	7.6±2.2	8.1±2.5	0.607
Male	37	38	0.856
Female	26	25	

Table 2. Difference in the distribution of investigated parameters of patients between two study groups

Clinical characteristics	Mean ± SD		p value	Median (IQR)		p value
	Case (n=63)	Control (n=63)		Case (n=63)	Control (n=63)	
Vitamin D (ng/ml)	15.8 ± 7.8	23.6 ± 11.8		15 (10.0-19.5)	20.5 (15.5-28.0)	<0.001
Calcium (mg/dl)	9.2 ± 1.1	9.7 ± 0.9	0.01	9.1 (8.5-9.8)	8.6 (8.9-10.2)	
Phosphorus (mg/dl)	4.2 ± 0.6	3.9 ± 0.9		4.2 (3.8-4.7)	3.7 (3.2-4.5)	0.014
Alkaline phosphatase (IU/l)	171.4 ± 110.6	163.3 ± 75.9		152.5 (105.0-196.2)	143.7 (107.7-199.5)	0.915
IgE (IU/ml)	424.1 ± 285.1	121.1 ± 75.9		343.5 (212.0-615.9)	115.0 (73.8-164.0)	<0.001
Eosinophil (cell/mm3)	521.9 ± 215.8	147.6 ± 82.9		462.0 (360.0-630.0)	136.0 (76.0-210.0)	<0.001

Table 3. Bivariate Association between Vitamin D, IgE and Eosinophil with Demographic and Other Investigated Parameters of Study Population

Vitamin D	**Median**	**95% CI**	**p value**
Case	15.0	12.96-17.04	0.004
Control	20.5	16.69-24.31	
Male	18.0	15.6-20.4	0.999
Female	18.0	16.0 -20.0	
	Coefficient	**Standard error**	
Calcium (mg/dl)	7.692	0.768	<0.001
Phosphorus (mg/dl)	0.455	1.021	0.657
Alkaline phosphate (IU/l)	-0.035	0.008	<0.001
IgE			
Case	343.5	275.3-411.7	<0.001
Control	115.0	91.1-138.9	
Male	187	105.3-267.7	0.577
Female	160	121.6-198.4	
	Coefficient	**Standard error**	
Vitamin D (ng/ml)	-6.019	1.966	0.003
Calcium (mg/dl)	-55.8	26.754	0.039
Phosphorus (mg/dl)	30.818	24.588	0.212
Alkaline phosphate (IU/l)	0.147	0.195	0.452
Eosinophil			
Case	462.0	388.3-535.7	<0.001
Control	136.0	94.4-177.6	
Male	310	203.0-417.0	0.791
Female	288	197.8-378.2	
	Coefficient	**Standard error**	
Vitamin D (ng/ml)	-7.264	1.624	<0.001
Calcium (mg/dl)	-78.519	27.186	0.005
Phosphorus (mg/dl)	88.235	45.073	0.05
Alkaline phosphate (IU/l)	0.198	0.305	0.516

Table 4: Association between Severity of Asthma and Severity of Vitamin D Deficiency (Only For Cases)

Severity of Asthma	**Vitamin D severity**				**Total**	
	No deficiency (>=20ng/ml)	**Mild (10-20ng ml)**	**Moderate (5-10ng/ml)**	**Severe (<5ng/ml)**		
Intermittent	7 (53.85%)	5 (38.46%)	1 (7.69%)	0	13	**p value <0.001**
Mild	7 (26.92%)	18 (69.23%)	1 (3.85%)	0	26	
Moderate	0	11 (55.0%)	7 (35.0%)	2 (10.0%)	20	
Severe	0	0	2 (50.0%)	2 (50.0%)	4	

Discussion

Being a tropical country, Vitamin D deficiency is supposed to be uncommon in India. (14) However from the data available in published literature Vitamin D deficiency is very common in India in all the age groups and both the sexes across the country. (15-17) Prolonged breastfeeding without vitamin D supplementation, maternal vitamin D deficiency, poor diet and limited exposure to sunshine have been suggested as major contributors to vitamin D deficiency. (18,19) Several epidemiological studies have suggested that vitamin D deficiency is associated with an increased incidence of asthma and other allergy symptoms.(6) Some have reported Vitamin D deficiency was the strongest predictor of asthma stronger than familial history of asthma or serum IgE levels and familial history of vitamin D deficiency also being a predictor of asthma. (20)

In this present study, it was found that median serum 25 (OH) D was significantly lower among cases as compared to controls. This means that asthma in children of 5 to 12 years is likely to be associated with low serum vitamin D levels. Thus vitamin D deficiency appears to be a major risk factor for childhood asthma. Brehm et al conducted a cross sectional study on 616 asthmatic children between the ages of 6 and 14 years in Costa Rica to examine the relation between 25 OH Vitamin-D level and markers of allergy and asthma severity. (8) Linear, logistic and negative binomial regression was used for the univariate and multivariate analysis. Of the 616 children with asthma, only 175(28%) had insufficient levels of 25-hydroxyvitamin D (<30ng/ml).In multivariate linear regression models, vitamin D levels were significantly and inversely associated with total IgE and eosinophil count. In multivariate logistic regression a unit increase in vitamin D levels was associated with reduced odds of hospitalization, any use of anti-inflammatory medication and increased airway responsiveness. These findings were confirmed by the same group of researchers in a subsequent study based on the childhood asthma management program (CAMP) cohort of 1024 children, apart from the association between vitamin D levels and allergy markers. (21)

Another cross sectional study of Italian children 5 to 11 years by Chinellato et al had similar result like us that Vitamin D levels was deficient in 53.3% and 37.3% children had insufficient levels [20 to 30ng/ml]. Only 9.4% children with asthma had sufficient vitamin D. (22) As there were no control in these studies, the vitamin D level of general population are not known. Freishtat et al did a cross sectional case control study of children 6 to 20 years old with and without asthma in USA where 86% of cases had insufficient out of which 54% had deficient vitamin D levels. Median vitamin D level of those with asthma 18.5 (IQR: 11.3, 25.1) was significantly lower than that of non asthmatic controls 40.4 (IQR: 34.6, 49.5) and p=0.002. (23) It indicates that low level of Vitamin D is associated with childhood asthma in both developed and the developing countries.

However, Vitamin D deficiency was also found to be prevalent in our control population, with 47.6% having low serum Vitamin D levels. Harinarayan and Marwaha had already shown in population studies that Vitamin D deficiency is very common in India in all the age groups and both the sexes across the country. (17,18, 24) In the present study 6.4% cases were found to be severely deficient, moderate deficiency was seen in 17.5% while 53.9% of cases were found to be mildly deficient. Among controls no one was severely deficient but only had mild deficiency in 47.6%.

The severity of Vitamin D deficiency was found to be associated with severity of childhood asthma. Our finding are in agreement with studies showing that insufficient vitamin D status is associated with an increase in the risk of asthma exacerbations as shown in patients of CAMP cohort and with augmented airway responsiveness and increased risk of asthma hospitalization in children with asthma as shown by Brehm et al. (20,25) Litonjua and colleagues recently found association between serum 25(OH)Vitamin-D levels and risk of an asthma related emergency department visit or hospitalization (26) and Vitamin D deficiency was found to be the strongest predictor of asthma, stronger than familial history of asthma or serum IgE levels. (20)

Analyzing the allergy markers, IgE was significantly different between cases and controls. In bivariate analysis vitamin D was significantly associated with IgE as 1ng/ml increase in vitamin D level decreased 6.019 IU/ml IgE. But multivariate analysis, when study group and calcium were adjusted, showed that the significance of the association between IgE and vitamin D lapsed. Similar result was seen in relation between Vitamin D and eosinophil count. Eosinophil count was significantly different between cases and controls and Vitamin D was significantly associated with eosinophil count in bivariate analysis as one ng/ml increase in vitamin D level decreased 7.264 cell/mm3 eosinophil count ($p<0.001$) but in multivariate analysis, when calcium and phosphorus were adjusted, the significance of the association between eosinophil and vitamin D lapsed. So this does not established the causality between these two with Vitamin D deficiency. In contrast, Brehm et al had shown that in multivariate linear regression models, vitamin D levels were significantly and inversely associated with IgE and eosinophil count. (8) The reason may be that they only included asthmatic children without control. Another explanation may be the narrow range of vitamin D concentration in our study population. The CAMP cohort study done by the same authors showed no relationship between vitamin D and both IgE and eosinophil count. Same findings were also seen in the cross sectional case control study by Chinellato et al. (22)

Few studies have shown that children with insufficient vitamin D levels were found to have a slightly lower mean FEV1 and association between lower vitamin D levels and higher requirement of inhaled and oral corticosteroids. (25,27)

In our study, serum calcium was higher and phosphorus and ALP were lower in controls than the cases ($p<0.001$). But none of the children have any feature of clinical rickets. Calcium was significantly

associated with IgE as one mg/dl increase in calcium level made -55.8 IU/ml decrease in IgE. Calcium level was also significantly associated with eosinophil as one mg/dl increase in calcium level made -78.519 IU/ml decrease in IgE. Again, phosphorus was significantly associated with eosinophil.

Camargo and his team looked at the levels of vitamin D in newborn cord samples collected from a group of 922 children in New Zealand and correlated it with filled out periodic questionnaires about their children health, until the child turned five. (28) They found that lower the amount of vitamin D in cord blood, the higher the risk of wheezing. Keet et al assessed the relationship between serum vitamin D levels and self reported wheeze and asthma in a large nationally representative survey. (29) Among 6857 subjects, they found that lower serum vitamin D levels were associated with higher risk of both wheeze and asthma.

Though it seems there is an increase in incidence of asthma exacerbation with low vitamin D level, larger studies particularly in tropical areas are necessary. This study also does not prove that a normal level of vitamin D will prevent the acute exacerbations of asthma.

Conclusion

All these above mentioned studies indicate improving Vitamin D status holds promise in the primary prevention of asthma and in decreasing exacerbations and better control of the disease. Our study has shown that there is definite relation between decrease in vitamin D level and increase in severity of asthma. Though the causality of childhood asthma due to Vitamin D deficiency is not established, from different studies discussed above, Vitamin D deficiency is well correlated with the incidence, frequency, severity and negative response to corticosteroid in childhood asthma. Therefore, Vitamin-D supplementation can be considered as an adjuvant therapy in asthmatic children.

Funding: None

Conflict of Interest : None

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DOI No. 10.7199/ped.oncall.2014.71