Original Article

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Paediatrics Section

Vitamin D Levels In Children with Bronchial Asthma

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ABSTRACT

Introduction: Bronchial Asthma is a common chronic inflammatory disorder of the airways in childhood. Vitamin-D, required for bone mineralization, is also a potent immune system regulator having a potential role in various allergic diseases. This study was undertaken to determine the difference in serum levels of Vitamin-D in asthmatic children and to determine the association between vitamin-D and asthma in children.

Materials and Methods: This cross-sectional study included 88 (44 asthmatic children and 44 healthy controls) children aged between 5 and 13 y. Serum 25-hydroxy vitamin-D levels were determined and compared between the two groups. The association between vitamin-D levels and lung function was studied in the asthmatic children.

Results: Serum vitamin-D level was significantly lower in asthmatic children than in control group and in the asthmatic group, vitamin-D levels had a significant positive correlation with FEV1% and FEV1/FVC%.

Conclusion: Vitamin-D deficiency is highly prevalent in asthmatic children and is associated with airway limitation.

Keywords: Airway limitation, Asthma, Children, Lung function, Vitamin-D

INTRODUCTION

Bronchial Asthma is a common chronic inflammatory disorder of the airways in childhood [1]. It is related with increased airway inflammation, airway hyper responsiveness and airflow obstruction in response to specific triggers leading to repeated episodes of wheezing, dyspnea, a feeling of tightness in the chest and cough. These episodes are usually associated with bronchial obstruction that resolves, spontaneously or after treatment. The exact causes for asthma are not well understood. Varied aetiological factors and changing environmental factors such as atmospheric pollution, dietary changes, allergens and lifestyle changes may be responsible for the rising prevalence of asthma [1,2]. Impaired immunogenic tolerance and interplay between cells and inflammatory mediators may ultimately promote airway obstruction associated with the disorder [3].

Vitamin-D, increases calcium absorption from the gastrointestinal tract, determines bone health and regulates neuromuscular function. Some non classical actions of Vitamin-D include cellular differentiation, insulin secretion, and blood pressure. It is also believed to be a potent immune system regulator having a potential role in various allergic diseases [4]. A potential role of Vitamin-D in tuberculosis, pneumonia, inflenza and respiratory infections has been proposed [4]. The existence of associations of vitamin-D with asthma and allergy remains uncertain. While some studies found hypovitaminosis D more prevelant amongst asthmatics, others suggest that vitamin D supplementation may increase the risk of allergy [5-8]. Therefore, this study was undertaken to determine the difference in serum levels of Vitamin-D in asthmatic children and controls and to determine the association between vitamin-D and asthma in children.

MATERIALS AND METHODS

This cross-sectional study was conducted at our institutional hospitals, between March 2013 and August 2013. Bangalore is located at average latitude of 12.96° N which has a minute seasonal variation in the peak of sunlight. The Ethics committee of the institute approved of the study and a written informed consent was obtained from a parent of each participant of the study. The study subjects were 88 in number aged between five and thirteen years. Fourty four children who were diagnosed as asthmatic by clinical evaluation as per GINA (Global Initiative for Asthma Guidelines), 2012 were enrolled in the study as cases [1].

A study done by Bener A et al., [9] revealed that 68.1% of the asthma patients are likely to be deficient with regard to vitamin-D levels. Based on that study, with an absolute precision of 15% and a confidence level of 95%, 44 subjects needed to be included for the present study (44 asthmatic patients and 44 normal healthy controls matched for the age and sex). Fourty four normal healthy children aged between 5 to 13 y, without a history or family history of asthma, coming to the paediatric outpatient department for follow-up, were enrolled as controls. Childern who had any underlying liver, kidney, endocrine diseases, or were on drugs that might affect vitamin-D levels for the past one year were excluded from the study. Data was collected on demographic variables (age, gender, height, weight, place of residence) and details of daily sun exposure during effective period (10am to 3pm) and its average duration in hours per day during the last one month. Between 10 am to 3pm, enough UVB photons reach the earth's surface to produce vitamin-D irrespective of the season [10]. Our study subjects were aged between 5 to 13 yrs and went to school. History of duration of exposure was collected from the parents and/or from children themselves by asking for duration of exposure during travel to and from the school, during lunch and snack breaks and the games period during school hours. Three ml of a random sample of blood was collected into a plain vacutainer using all aseptic precautions from the study subjects. After separation, the sample was analysed for the following tests. Serum calcium, serum phosphorous and serum alkaline phosphatase were estimated on Roche Cobas 6000 in the Biochemistry Diagnostic laboratory of our hospital. Serum calcium was estimated by colorimetric assay using o-cresolphthalein complexone method [11]. Serum phosphorous was estimated by colorimetric assay using molybdate [12]. Serum alkaline phosphatase was measured by colorimetric assay using para nitro phenyl phosphate method [13].

After separation serum samples were stored at -20^{0}C. 25 hydroxy cholecalciferol levels were estimated using ELISA kit (DLD

MRI pattern	Gender		Total n (%)	Mean Age in years	SD
	Male n (%)	Female n (%)			
Controls	30	14	44	7.17	2.175
	(68.2%)	(31.8%)	(100.0%)		
Cases	31	13	44	7.50	2.338
	(70.5%)	(29.5%)	(100.0%)		
Total	61	27	88	-	-
	(69.3%)	(30.7%)	(100.0%)		

[Table/Fig-1]: Gender and age distribution of the study group

	Controls (n=44) Mean±SD	Cases (n=44) Mean±SD	p value
Weight (in kg)	23.9±6.80	24.19±6.04	0.18
Height (in cms)	120.95±16.21	117.36±16.71	0.04*
BMI(Kg/m^2)	16.93±2.60	17.36±3.93	0.53
Duration of daily sunlight exposure between 10am and 3pm (in minutes)	142.39±4.33	120.00±5.11	0.00*
FEV1%	92.21±7.8	75.3±8.5	0.02*
FEV1/FVC%	87.3±9.2	66.4±7.6	0.02*
Serum Calcium (mg/dL)	9.68±0.6	9.45±0.6	0.04*
Serum Phosphorus (mg/dL)	5.03±0.7	5.07±0.54	0.76
Serum Alkaline Phosphatase (U/L)	235.84±63.3	240.23±63.71	0.74
Serum 25 hydroxy cholecalciferol (ng/mL)	16.49±1.13	12.88±1.79	0.02*

[Table/Fig-2]: Comparison of baseline variables among study subjects
**Indicates statistical significance*

	CONTROLS n=44 (%)	CASES n=44 (%)	TOTAL N=88 (%)
Severe Deficiency (≤5 ng/mL)	0(0)	5 (11.36)	5 (5.68)
Deficiency (≤15 ng/mL)	7 (16)	25 (56.82)	32 (36.36)
Insufficiency(>15ng/mL and <20 ng/mL)	37 (84.09)	14 (31.82)	51(57.95)
Sufficiency(≥20 ng/mL to 100ng/mL)	0 (0)	0(0)	0(0)

[Table/Fig-3]: Grouping of study Subjects based on their vitamin D levels

Diagnostika GMBH) and values expressed in ng/ml. Study subjects were categorized based on their vitamin D levels as severely deficient (≤5 ng/mL), deficient (≤15 ng/mL), insufficient (>15ng/mL to <20 ng/mL) and sufficient (≥20 ng/mL to 100ng/mL) [14]. A pulmonary Spirometry performed according to American Thoracic Guidelines (before and after inhalation of salbutamol) by using TM diagnostic spirometer {(Model-2001) manufactured by ndd Medizintechnik AG, 8005 Zurich, Switzerland} [15].

STATISTICAL ANALYSIS

The data were collected in preformed questionnaires and then entered into Microsoft excel sheet. Statistical analysis was performed with SPSS version19 software package (SPSS, Inc. Chicago). The results of this study were analysed and presented as numbers, percentage or mean ± standard deviation (SD). Student t-test, analysis of variance (ANOVA) and Chi-square were used for comparison between groups. The correlations were analyzed by Pearson correlation coefficients. A p-value less than 0.05 was considered to be significant for statistical hypothesis.

RESULTS

Fourty four children with asthma and 44 children as controls were chosen to be a part of our study. The mean age in year's ±SD in the controls was 7.17 ± 2.17 and 7.50 ± 2.33 in cases. The age and gender distribution of the study group is shown in [Table/Fig-1]. [Table/Fig-2] shows a comparison of the baseline variables and biochemical parameters between the two groups. Statistically significant differences were found between the two groups in terms

	Asthmatics with Serum 25 hydroxy cholecalciferol levels ≤15 ng/mL (Vit D deficiency n=30)	Asthmatics with Serum 25 hydroxy cholecalciferol levels >15ng/mL and <20 ng/mL (Vit D insufficiency n=14)	p-value
BMI(Kg/m^2)	17.5±3.0	17.2±3.2	0.62
FEV1%	68.7±4.1	81.5± 3.7	<0.001
FEV1/FVC	55.9±2.1	75.8±3.5	<0.001

[Table/Fig-4]: Characteristics of cases according to Serum 25 hydroxy cholecalciferol levels

of duration of daily exposure to the sun, FEV1%, FEV1/FVC%, Serum calcium levels and Serum 25 hydroxy cholecalciferol levels as shown in [Table/Fig-2]. The asthmatic group had a mean ±SD FEV1% of 75.3±8.5, FEV1/FVC% of 66.4±7.6 and Serum 25 hydroxy cholecalciferol levels of 12.88±1.79 ng/mL.

The Study subjects were grouped as shown in [Table/Fig-3], based on their Serum 25 hydroxy cholecalciferol(vitamin D) levels as severely deficient (≤5 ng/mL), deficient (≤15 ng/mL), insufficient (>15ng/mL to <20 ng/mL) and sufficient (≥20 ng/mL to 100ng/mL) [12]. There were no children in our study either amongst the cases or controls with sufficient Serum 25 hydroxy cholecalciferol levels.

Based on the symptoms, clinical findings and spriometry results the children with asthma (44 cases) were grouped as follows- mild intermittent 18 (40.90%), mild persistent 14 (31.81%), moderate persistent 10 (22.72%) and severe persistent 2 (4.54%). Further, the children with asthma were divided into two groups based on serum 25 hydroxy cholecalciferol levels as shown in [Table/Fig-4].

A significant correlation between serum 25 hydroxy cholecalciferol levels with the FEV1% (p <0.05) was found in the cases(P=0.000, r=0.706) as also a significant correlation between serum 25 hydroxy cholecalciferol levels and FEV1/FVC was observed in the studied asthmatic patients (p = 0.000, r =0.458). There was no significant correlation between serum 25(OH) D and BMI (kg/m^2).

DISCUSSION

Bronchial Asthma is a common respiratory disorder in children worldwide. Vitamin-D is a fat-soluble nutrient and steroid hormone that has classical actions like increasing calcium absorption and maintenance of bone health. Recently, cardiovascular diseases, inflammation, infectious diseases, multiple sclerosis, impaired physical functioning, allergic diseases and all-cause mortality have been related inversely to serum Vitamin-D concentrations [4,16]. The existence of associations of vitamin-D with asthma remains uncertain. The aim of this study was to determine the levels of serum 25 hydroxy cholecalciferol in asthmatic children and to determine the role of vitamin-D in asthma.

The mean serum 25 hydroxy cholecalciferol (Vitamin-D) levels in asthmatic children was 12.88±1.79ng/mL, which was significantly lower than the mean vitamin-D levels in the healthy controls (16.49±1.13ng/mL). 68.18% of the asthmatic children had deficient serum vitamin-D levels (≤15ng/mL) and 31.28% of them had insufficient vitamin-D levels (>15ng/mL to <20ng/mL). These findings are consistent with a similar study done in Thailand, where 61.4% of the asthmatic children had vitamin-D deficiency (31.2% of uncontrolled asthmatics, 17.4% of partly controlled and 12.8% of controlled asthmatic children) [17].

None of the subjects in our study had sufficient vitamin-D levels. Several studies from across the world and India have shown a high prevalence of vitamin-D deficiency in children and adolescents [18-20]. Indian children are increasingly predisposed to vitamin-D deficiency as they are entirely breast fed or not exposed to sunlight for prolonged periods and the lack of food fortified with vitamin-D [20,21]. An Indian is also likely to require three times as much UV-B exposure as a light-skinned person to achieve equivalent vitamin-D concentrations [14].

In addition to all these factors that determine vitamin-D levels in a general population, asthmatic children tend to stay indoors, with their bodies covered with warm clothes and are less likely to be exposed to sunlight or involved in outdoor activities. The mean duration of daily sunlight exposure between 10am and 3pm was 120.00± 5.11 minutes in children with asthma, which was significantly lesser than the 142.39±4.33 minutes in the healthy controls. This has been seen previously in a study done by Uysalol M et al., in Turkey [22]. The children with bronchial asthma having vitamin D deficiency(≤15ng/mL) in our study had a significantly lower FEV1% and FEV1/FVC% than bronchial asthma patients with insufficient vitamin D levels(>15ng/mL to <20ng/mL).

Vitamin-D deficiency could be involved in asthma pathogenesis through a number of mechanisms. Vitamin-D deficiency has been linked to many disorders having an immunological basis like multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, type 2 diabetes mellitus and cardiovascular disease [15,17,22]. Vitamin-D has been proposed to have modulatory actions on the immune system. Vitamin-D has been proposed to promote steroid sensitivity and to down regulate an inflammatory state via gene expression and cytokine production. This action could be directly on the airway. Vitamin-D receptors are present in the airways and are thought to inhibit proinflammatory cytokines, with effects on CD4+ T cells, interleukin-2, interferon-gamma, and macrophages. A deficiency of Vitamin-D could be associated with a failure to turn off the inflammatory state, following an acute inhalational insult, with up regulation of prostaglandin, leukotrienes, macrophages, and T cell activity and recruitment [23,24]. A positive correlation was found between 25(OH) D levels and FEV1% in the asthmatic children in the present study. This finding agrees with that of other studies who reported that in patients with asthma, higher serum 25(OH) D concentrations were associated with higher FEV1% [25,26].

Irreversible loss of lung function can be caused by airway adaptation occurring early on in asthma. Airway adaptation or remodelling involves structural changes in the walls of the airways caused by continual injury and repair processes. It is shown by changes in tissue, cellular, and molecular composition affecting the airway smooth muscle, epithelium, blood vessels, extracellular matrix, and defective lung–blood barriers. Vitamin-D may modulate airway remodelling and lung function by affecting the growth and contractility of airway smooth muscle. Vitamin-D inhibits transforming growth factor- β (TGF-β), matrix metalloproteinases and fibroblast proliferation [27]. There are a few studies that could not find a correlation between serum vitamin D levels and pulmonary function and explained this to be due to a number of confounding factors that affect vitamin-D levels and asthma severity [17,28].

The present study has some limitations: Even though there is a strong relation of airway limitation and compromised lung function and 25(OH) D level, we could not establish a direct mechanistic link between vitamin D deficiency and asthma in children. The sample size was relatively small (n =88). A larger sample size would have increased our statistical power to detect associations. As with most other studies investigating the role of vitamin-D in asthma, our design was cross-sectional, thus limiting our ability to establish a causal link between vitamin-D and asthma morbidity. Furthermore, many confounding factors can affect vitamin-D levels or asthma severity. Long-term follow-up studies focusing on changes in vitamin-D status and asthma parameters will be needed to elucidate the effect of vitamin-D status on asthma. Future clinical trials are also necessary to determine if vitamin-D truly has effects upon asthma and to determine if there are beneficial effect of vitamin-D supplementation on airway adaptation. Although vitamin-D is definitely necessary for optimal lung function, the low levels observed in asthma could be due to the inflammatory process and simply considered as a marker of airway limitation. Recommendations cannot be made regarding vitamin-D in asthma until long term follow up studies and clinical trials are done.

CONCLUSION

To conclude, in our study, we found that vitamin-D levels were considerably lower in children with asthma than in healthy children. There was a direct and a significant relationship between vitamin-D levels and pulmonary function test outcomes in children with bronchial asthma. Hence, measuring serum levels of vitamin-D could be considered in the routine assessment of children with bronchial asthma. We propose to undertake a long term study in the near future in order to determine the effect of vitamin-D supplementation on pulmonary function in the same group of study subjects in order to establish whether a cause and effect relationship exists between Vitamin-D and pulmonary function or is it just a coincidental finding.

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