Original Article

Iran J Pediatr
Dec 2014; Vol 24 (No 6), Pp: 710-714

Relationship between Vitamin D and Childhood Asthma: A Case–Control Study

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Received: *Nov 24, 2013*; Accepted: *Sep 03, 2014;* First Online Available: *Nov 07, 2014*

Abstract

Objective: Studies determining the relationship between serum vitamin D status and childhood asthma have yielded controversial results. Findings indicated that vitamin D deficiency is associated with asthma and airway hyper responsiveness. The aim of this study was to assess the relationship between serum vitamin D status and childhood asthma.

Methods: Data were obtained from 200 asthmatic children (age 3-12 years) and 200 healthy controls. Serum levels of 25(OH) vitamin D, total IgE, calcium, phosphorus, parathormone (PTH) and eosinophil count were measured in both asthmatic children and healthy controls. Also, the mean values of 25(OH) vitamin D were compared with asthma symptom severities.

Findings: There was a significant decrease in the concentration of serum 25(OH) vitamin D in the asthmatic patients as compared with the controls (20.34±2.8 vs 25.39±4.1 ng/mL, 95%CI: 1.46-3.86, *P*=0.01). Out of total asthmatic subjects, 40 (20%) were vitamin D sufficient, 48 (24%) were insufficient, and 112 (56%) were deficient. Total IgE concentration was also significantly higher in asthmatic patients having vitamin D deficiency (132.4±20.1 IU/ml, 95%CI: 1.38-3.75, *P*=0.03). Comparing asthmatic patients with healthy controls, odds of having vitamin D level less than 20ng/mL was 2.47.

Conclusion: Our findings suggest that vitamin D deficiency or insufficiency may be positively related to the prevalence of asthma in children.

Iranian Journal of Pediatrics, Volume 24 (Number 6), December 2014, Pages: 710-714

Key Words: Vitamin D status; Asthma; Children; Total IgE

Introduction

Asthma is a common chronic inflammatory disease affecting an estimated number of 300 million individuals throughout the world. It has a significant social and financial burden on public health[1]. The prevalence of asthma is increasing in the developed and developing countries[2]. The increased prevalence of asthma, as a heterogeneous disease, is not only due to genetics, but also may be influenced by a number of environmental factors associated with urban lifestyles or dietary habits. Among the numerous dietary proposed hypotheses associated with asthma, the decrease in serum concentration of vitamin D due to increased time spent indoors, decreased exposure to sunlight, less exercise, obesity, and inadequate calcium intake are of particular interest[1,3].

Recent epidemiologic studies have shown an

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association between vitamin D deficiency and asthma[3-6]. Besides the central role of vitamin D in calcium and bone physiology, the protective effects of vitamin D in asthma could be attributed to its immunomodulatory properties[7]. In addition, vitamin D has effects on epithelial cell, T and B lymphocytes, and antigen presenting cell functions. Moreover, by induction of regulatory T (T reg) cells to produce interleukin (IL)-10, vitamin D modulates inflammatory processes and could help to control asthma severity[8].

The aim of the present study was to find out the relation between serum vitamin D deficiency and asthma severity in children living in province of Bushehr, southwest Iran, based on the Global Initiative for Asthma (GINA) classification[9].

Subjects and Methods

By considering the confidence level as 0.05 with the power of 80%, 44% as the proportion of persons with serum vitamin D levels lower than 20 ng/ml in asthmatic patients and 30% with the same levels of serum vitamin D in healthy controls, the sample size was estimated to be 186 cases in each group. At last, 200 patients in each group entered the study. Finally two hundred children with asthma (aged 3-12 years, 127 females and 73 males), who were diagnosed according to the clinical criteria and classified with GINA guideline[9] and 200 healthy controls were enrolled for the study. The investigation was performed in Abolfazl Pediatric Clinic, affiliated to Bushehr University of Medical Sciences. All patients were on regular treatment with oral or inhaled corticosteroids based on GINA classification. Subjects presenting the other chronic respiratory diseases and a history of consumption of any supplements of vitamin D or drugs that modulate serum vitamin D levels were excluded. Participants with no history of allergic diseases and negative atopic family history were enrolled as the healthy controls. The study was approved by the ethics committee of the University and written informed consent was obtained from the parents.

Blood samples were collected from the subjects by venipuncture. Cellular assay (Peripheral blood eosinophil count) was performed and the serum samples collected were stored at -70°C until the analysis. The values for Vitamin D levels ≥30 ng/ml were considered as sufficient, between 20 and 30 ng/ml as insufficient and lower than 20 ng/ml as deficient[8]. Serum concentration of 25 (OH) vitamin D was assayed with an electrochemi-luminscence (ECL) method [Cobas E-411(Elecsys), Rosche, Germany].

Total IgE was measured using ELISA kit purchased from Radim, (Pomezia, Italy) and total IgE levels >100 IU/mL were defined as high. Biochemical assays were done using diagnostic kits made by Bioactiva Diagnostica (Hamburg, Germany). The levels of PTH were also measured by the Electrochemiluminescence Immunoassay (ECLIA) method using Roche Elecsys kits.

The data were analyzed using student's t-test, one way ANOVA, Kruskal-Wallis test and chi-square (linear by linear correlation), as applicable (with a preset probability of $P<0.05$). Quantitative data are presented as mean±SD. Statistical tests were conducted using the SPSS software package, version 13 (SPSS Inc., Chicago, IL, USA). Independent t test was used to compare different quantitative variables (such as age, body mass index, serum level of biochemical markers) between asthmatic patients and healthy controls. Chi-square test was used to check the categorical variables (such as sex, residence) between the two groups. Additionally, using simple, multiple and logistic regression analysis, the simultaneous effects of confounding variables such as age, sex, body mass index (BMI) and residence on the asthmatic state were measured. Normality assumptions of distributions were applicable based on the Kolmogorov–Smirnov test. Due to the lack of normal distribution of vitamin D levels in both groups, only logarithmic transformation of vitamin D was compared in the two groups.

Findings

Demographic data of asthmatic children and healthy controls are shown in Table 1. The severity and treatment of asthma was presented according to GINA guidelines. The intermittent asthma was assayed in 148 (74%) subjects, mild

Table 1: Characteristics of patients in asthmatic and non-asthmatic groups

Parameter	Asthmatic Children (n=200)	Healthy Controls (n=200)	*P*. value
Age (Year)	5.77 (2.2)	5.93 (2.1)	0.5
Sex (Female/ Male)	127/73	124/76	0.8
Vitamin D	20.34 (2.8)	25.39 (4.1)	0.01
Calcium	9.24 (0.59)	8.96 (0.43)	0.001
Phosphorus	4.78 (0.77)	4.31 (0.82)	0.01
Parathormone	49.28 (3.5)	47.85 (2.8)	0.6
Total IgE	121.4 (22.1)	37.9 (2.1)	0.001
Residence (Urban/Rural)	143/57	119/81	0.01

persistent 38 (19%) and moderate persistent 14 (7%). There was no difference in the BMI between asthmatic children (16.2±4 kg/m^2) and the healthy controls (15.1±3 kg/m^2) (*P*>0.05) (Table 2).

There was no difference in sex and age between the healthy controls and asthmatic children. A significant difference was observed between the concentration of 25 (OH) vitamin D in the serum of healthy controls (25.39±4.1 ng/ml) and the asthmatic patients (20.34±2.8 ng/ml; 95%CI: 1.46-3.86, *P*=0.01) (Table 1). Vitamin D deficiency was shown in 112 (56%) patients with asthma and 80 (40%) in the healthy controls (*P*=0.002). Also, 48 (24%) of asthmatic children and 42 (21%) from the control group had insufficient amount of Vitamin D. Sufficient levels of vitamin D was observed in 40 (20%) patients and 68 (34%) of healthy controls. In comparison with the control group, chance of having vitamin D level less than 20 ng/ml in asthmatic patients was 2.38 (*P*=0.0001; 95%CI: 1.46-3.86). However, after adjustment for residence, the chance changed to 2.47 (*P*=0.0001; 95%CI: 1.51-4.04). There was no difference between the concentration of vitamin D in the serum and the severity of asthma in both groups (*P*=0.6) (Table 3).

Asthmatic patients showed significantly higher concentration of total IgE in the serum (121.4±22.1 IU/ml) compared to the healthy controls (37.9±2.1 IU/ml, 95%CI: 1.38-3.75, *P*= 0.001). Also total IgE concentration was significantly higher in asthmatic patients who had vitamin D deficiency (132.4±20.1 IU/ml, 95% CI: 1.38- 3.75, *P*=0.03). Moreover, serum calcium level (9.24±0.59 mg/dl) was found to be decreased significantly in asthmatic patients compared to the healthy subjects (8.96±0.43 mg/dl, 95%CI: 1.48-4.77, *P*=0.001). Serum phosphorus concentration in patients (4.78±0.77 mg/dl) was significantly higher than in the controls (4.31±0.82 mg/dl, 95% CI: 1.33-3.42, *P*=0.01). There was no considerable difference between the serum level of PTH in asthmatic children (49.28±3.51 pg/dl) and the controls (47.85±2.8 pg/dl, *P*=0.6). Also, no change was noticed in the eosinophil count of asthmatic patients as compared with the healthy controls (*P*= 0.2).

Discussion

To the best of our knowledge, the effect of vitamin D on the development of asthma is not well investigated. Recent studies have shown the relation between the low serum levels of vitamin D and the prevalence of asthma with especial reference to immunomodulatory effects of vitamin D on the lungs[8,10-12].

In the present study, we have shown that

Table 2: Comparison of serum vitamin D levels in asthmatic patients and healthy controls

Vitamin D Levels	Asthmatic Children	Healthy Controls	*P*. value
Deficient (<20 ng/ml)	112 (56%)	80 (40%)	
Insufficient (20-30 ng/ml)	48 (24%)	42 (21%)	0.002
Sufficient (>30 ng/ml)	40 (20%)	78 (39%)	

Table 3: Relationship between serum level of vitamin D and asthma severity based on GINA classification

Vitamin D	Asthma Severity (GINA)*		
	Intermittent	Mild Persistent	Moderate Persistent
Deficient (<20 ng/ml)	86 (58.1%)	20 (52.6%)	6 (42.9%)
Insufficient (20-30 ng/ml)	32 (21.6%)	12 (31.6%)	4 (28.6%)
Sufficient (>30 ng/ml)	30 (20.3%)	6 (15.8%)	4 (28.6%)

GINA: Global Initiative for Asthma; *P*-value=0.6

serum levels of vitamin D in asthmatic children were lower than in the healthy controls, this is in agreement with the report from Freishtat et al[13]. Also a higher prevalence of vitamin D deficiency was observed in asthmatic patients in our investigation. The reason may be that asthmatic children spend less time outdoors, and thus they are less exposed to sunshine. Interestingly, the result of our investigation showed that the prevalence of asthma in the population living in the city was higher than in those living in rural areas. Therefore, we suggest that vitamin D may be involved in the immunopathology of asthma. Asthma is a chronic respiratory disease characterized by airway inflammation with enhanced activity of Th2 cells, which induce IgE production, and promote eosinophilic airway inflammation and hyper-responsiveness[14,15]. Vitamin D has also some immunomodulatory effects on different cells of the immune system. So that it reduces Th2 cells activity to produce IL-4 with direct down regulation of vitamin D receptor (VDR), which results in the attenuation of allergic airway inflammation[1]. Also, Lange et al reported that vitamin D impairs recruitment of eosinophils and reduces levels of IL-5 in a marine model of eosinophilic inflammation[16]. In vivo and in vitro studies have shown that vitamin D enhances the production of IL-10 as an anti-inflammatory cytokine by human T and B cells. In addition, some studies reported that vitamin D promotes mouse and human regulatory T cells (T reg) directly and after affecting antigen presenting cells (APCs) and so enhances anti-inflammatory effect[17].

During our study we did not find any relation between vitamin D deficiency or insufficiency and the severity of asthma, which is not in agreement with other studies[18,19]. It was suggested that vitamin D deficiency or insufficiency due to down regulation of glucocorticoid pathways leads to the need for increased steroid doses and this may itself increase asthma severity[19]. Moreover, some studies have shown that respiratory infections such as rhinoviruses enhance allergic airway inflammation, reduce pulmonary function and increase asthma exacerbation severity, especially in asthmatic patients having vitamin D deficiency or insufficiency[20,21].

We also found that mean serum level of total IgE in asthmatic patients with vitamin D deficiency or insufficiency was higher than in healthy controls, while eosinophilic count was not changed. In a recent cohort study from United States, it was reported that cord blood vitamin D levels below 20 ng/ml were associated with increased IgE levels and aeroallergen sensitization[22]. This is in line with our findings that vitamin D may have a role in the induction of allergic inflammation.

In addition to the critical role that vitamin D plays in calcium and phosphorus homeostasis, it regulates signaling pathways in many cells and tissues, indicating that vitamin D may limit prohypertrophic signaling associated with excessive airway hyper-responsiveness[23]. It seems that the decreased level of serum calcium, increase in the concentration of the serum phosphorus and no change in PTH level of our asthmatic patients may be related to the main role of vitamin D. However, additional studies are needed to determine the effects of vitamin D and calcium in biology of airway smooth muscle and epithelial cell biology in asthma.

Conclusion

Finally, we conclude that vitamin D deficiency is associated with incidence of asthma in children exposed to more sunshine, and this could be the reason for higher incidence of vitamin D deficiency among children living in southern Iran. Therefore,

we suggest that treatment with vitamin D supplement in patients with asthma might reduce airway inflammation, probably by reducing the production of IgE or eosinophil count and thus could be therapeutically effective in preventing and/or reversing allergic airway inflammation and airway hyperresponsiveness.

Acknowledgment

This study was funded by Bushehr University of Medical Sciences (BPUMS). We are especially thankful to Sadegh Golbini for his assistance.

Authors' Contribution

All authors have equally contributed for the concept / design, collection/analysis of data, and preparation/critical revision of the manuscript.

Conflict of Interest: None

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