

Serum vitamin D level as a predictor of bronchial asthma in Egyptian children

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Background/Aim

This study was designed to assess the relationship between the serum level of 25-hydroxyvitamin D and the clinical, functional severity and the level of asthma control among Egyptian asthmatic children.

Patients and methods

This case–control cross-sectional study was conducted on 50 asthmatic patients from those regularly attending the Pediatric Chest Clinic, Children's Hospital, Ain Shams University. Twenty healthy children of matched age and sex were recruited as the control group.

Results

Pulmonary function tests were significantly decreased in the asthmatic cases compared with the control ($P < 0.01$). The serum vitamin D level was found to be significantly decreased in asthmatic children compared with the control group ($P < 0.001$). Out of the 50 asthmatic children, 20 had a serum vitamin D level of less than 25 ng/ml, and were considered to be 'vitamin D deficient', whereas the remaining 30 children had a level ranging between >25 ng/ml and ≤ 30 ng/ml and were considered to be 'vitamin D insufficient'. The asthmatic children had a highly significant increase in the total leukocytic count, the eosinophilic count, serum immunoglobulin E, and serum alkaline phosphatase ($P < 0.001$ in all). There was also a significant increase in the serum phosphorus level in the asthmatic children group compared with the control group ($P < 0.01$). The serum vitamin D level was $24.1 \text{ ng/ml} \pm 2.9$ in the asthmatic patients on inhaled corticosteroid therapy and $28 \text{ ng/ml} \pm 1.4$ in those not on inhaled corticosteroid therapy. Significant positive correlations were found between the serum vitamin D level and the predicted percentage of forced expiratory volume in 1 s ($r = 0.871$, $P < 0.001$). A highly significant negative correlation was found between the serum vitamin D level and serum immunoglobulin E (marker of allergy; $r = -0.589$, $P < 0.001$). Moreover, serum vitamin D showed an inverse correlation with the clinical severity of bronchial asthma ($r = 0.903$, $P < 0.001$) and the level of control of asthma ($r = 0.923$, $P < 0.001$), classified according to the Global Initiative for Asthma (GINA) classification. The accuracy of vitamin D as a predictor of asthma was found to be 88% by automatic linear modeling.

Conclusion

Our results revealed an important support for the association between bronchial asthma in children and vitamin D deficiency, with a direct relationship between its serum level and pulmonary function test measures and the increased asthma severity. Vitamin D deficiency can be considered as a strong predictor of asthma. Improving the vitamin D status can help in the primary prevention of asthma and in decreasing exacerbations of attacks. Clinical trials of vitamin D supplementation to prevent asthma exacerbation are recommended.

Keywords:

bronchial asthma, children, pulmonary function tests, vitamin D

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Introduction

Bronchial asthma is one of the most prevalent diseases affecting people in both developed and developing countries. In some western countries, asthma and allergies affect more than one-third of the children from the general population [1,2]. Recently, it has been reported to reach similar levels in many Arabian countries such as Saudi Arabia (26.5%), Kuwait (16.8%), and in the United Arab Emirates (13.6%) where its prevalence is almost similar to that in industrialized or western countries [3–6]. In Egypt, it is one of the most common causes of emergency

and hospital admission as the prevalence of asthma among Egyptian children aged between 3 and 15 years was estimated to be 15–16%, and one in four children with asthma fails to attend school regularly because of poor asthma control [7]. However, it is well known that a positive atopic status, exposure and sensitization to environmental allergens, and/or familial history of allergic disease are significant risk factors associated with the development of asthma [8,9].

Many recent studies suggest that besides the already known risk factors, vitamin D deficiency is associated

with and may even predispose one to the development of the allergic phenotype of bronchial asthma in young children [10,11]. Vitamin D is a potent modulator of the immune system [12] and is involved in regulating cell proliferation and differentiation [13]. Prolonged breastfeeding without vitamin D supplementation, maternal vitamin D deficiency, poor diet and limited sunshine exposure (due to hot weather), dark skin [14] in Africa and Middle East have been suggested as major contributors to vitamin D deficiency [15,16].

The aim of this study was to assess the relationship between the serum level of 25-hydroxyvitamin D as a major circulating form of vitamin D and the clinical, functional severity, and level of asthma control among Egyptian asthmatic children.

Patients and methods

Patients

This cross-sectional case-control study was conducted in the Pediatric Chest Clinic, Children's Hospital, Ain Shams University during the period from April 2011 to October 2011. It included 50 asthmatic patients from those regularly attending the Pediatric Chest Clinic. Twenty healthy children of matched age and sex were recruited as the control group. Written informed consent was obtained from the study participants' parents. The study was conducted in accordance to the ethical procedures and policies approved by the Ethical Committee of Ain Shams Pediatric Hospital, Ain Shams University, Cairo, Egypt.

Materials and methods

Patients were diagnosed according to the clinical manifestations of asthma (cough, wheezing, shortness of breath, and exercise intolerance) according to GINA [17], and confirmed by spirometry. Dynamic spirometry (Master screen Pneumo, Erich Jaeger GmbH, Germany) was performed, with measurement of the forced expiratory volume in 1 s (FEV_1) (% of predicted) and the forced vital capacity (FVC). The ratio FEV_1/FVC is a measure of airflow obstruction. These measurements were performed according to the standards of the European Respiratory Society and the American Thoracic Society [18]. The highest values of FEV_1 of three forced expiratory maneuvers were used. The best FEV_1 , FVC, and FEV_1/FVC values were selected for the analysis. Information on age, sex, parental consanguinity, family history of allergic disease, duration of illness, and treatment modalities were collected through a questionnaire. The exclusion criteria were as follows: (a) any conditions known to affect bone metabolism (e.g. renal disease and hyperparathyroidism)

and other severe disease (e.g. cancer, gastrointestinal disorders, liver insufficiency, and diabetes mellitus) or grave physical disabilities, (b) intake of drugs that likely affect bone metabolism (e.g. corticosteroids), and (c) intake of dietary supplements containing calcium or vitamin D. The asthmatic children group consisted of 14 female and 36 male patients with a female to male ratio of 1 : 2.6. Their age ranged between 4 and 15 years, with a mean age of 8.5 ± 2.5 years.

Further subdivision of asthmatic children

According to asthma control during the past 3 months, the asthmatic children were subdivided into controlled (20 cases, 40%), partially controlled (12 cases, 24%), and uncontrolled (18 cases, 36%) cases on the basis of GINA [17].

According to asthma severity, they were subdivided into mild persistent (20 cases, 40%), moderate persistent (16 cases, 32%), and severe persistent (14 cases, 28%) cases on the basis of GINA [17].

According to the intake of inhaled corticosteroid therapy (ICS), they were subdivided into the ICS group (38 cases, 76%) and the non-ICS group (12 cases, 24%). Out of the 38 patients on ICS, 20 cases were on inhaled fluticasone and 18 were on inhaled ciclesonide.

After an overnight fast, venous blood samples were collected from all participants and the separated serum was stored at -20°C . Samples for alkaline phosphatase (ALP) was kept at room temperature and assayed within 4 h for biochemical assessment. Another portion of blood sample was collected on EDTA for an eosinophilic count using the Coulter counter technique. Quantitative determination of the 25-hydroxyvitamin D in serum samples was performed using enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostic EIA; Bensheim and Biomedica, Wien, Austria), according to the manufacturer's guidelines [19]. All wash steps were performed using an ELISA washer (Robonik ELISA plate washer, Mahape, Navi Mumbai, India Biotek EL $\times$ 800 Mumbai, India), whereas the absorbance of all samples were read using the ELISA reader (Biotek EL $\times$ 800) at 450 nm. A standard curve of the absorbance versus concentration was plotted using the calibrators. The concentration of vitamin D in the samples was determined directly from the curve. Serum vitamin D levels were categorized as deficient if the vitamin D level was less than 25 ng/ml, and insufficient if it was in the range of 25–30 ng/ml. The serum vitamin D level was considered to be normal or optimal if it was above 30 ng/ml.

Quantitative determination of serum total immunoglobulin E (IgE) was performed using ELISA,

[Rida screen total IgE (A0141); R-Biopharm AG, Darmstadt, Germany], according to the manufacturer's guidelines. All wash steps were performed using an ELISA washer (Robonik ELISA plate washer), whereas absorbance of all samples were read using the ELISA reader (Biotek EL × 800) at 405 nm. A standard curve of the absorbance unit versus concentration was generated using the results obtained from the standards provided with the kit. Total IgE values for samples were determined directly from the curve. The total and the differential leukocytic counts were determined using a cell counter by the examination of a Leishman-stained peripheral blood film [20].

The serum calcium level was analyzed according to the manufacturer's guidelines (Analyticon Biotechnologies AG, MesseDusseldorf GmbH, Germany) by a colorimetric assay with endpoint determination and a sample blank. The detection limit was 0.05 mmol/l (0.2 mg/dl) using the *o*-cresolphthalein complex one method, where the color intensity was measured photometrically at 580 nm. Serum phosphorus was assayed by measurement of the colorless phosphomolybdate complex at 340 nm by a spectrophotometer with respect to special sample precautions [21].

ALP was measured by the popular 4-nitrophenyl phosphate method according to the manufacturer's guidelines (Centronic GmbH, Germany). The enzyme activity was monitored by a spectrophotometer as the increase in the absorbance per minute at 405 nm for 3 min [22].

Statistical analysis

IBM-SPSS version 20 (Fayetteville, Arkansas, USA) was used for statistical analysis of the data on a personal computer. Normally distributed data were presented as the mean ± SD. The correlation between variables was determined using the Pearson correlation coefficient (*r*). A *P*-value below 0.01 was considered to be significant and a *P*-value below 0.001 was considered to be highly significant. Linear regression analysis and automatic linear modeling were carried out.

Results

A total of 50 asthmatic children aged between 4 and 15 years (mean 8.5 ± 2.5 years) were studied. There were 36 boys (72%) and 14 girls (28%). Passive smoking was found in 36 cases (72%), positive consanguinity in 34 cases (68%) and a positive family history in 30 cases (60%) out of the total sample (Table 1).

Pulmonary function tests were significantly decreased in the asthmatic cases compared with the control (*P* < 0.01). In the asthmatic cases, the FEV₁ ranged

between 50 and 85% of the predicted value (mean 69.2 ± 9.1) and the FVC ranged between 87 and 98% of the predicted value (mean 94.4 ± 2.3). The peak expiratory flow rate (PEFR) ranged between 46 and 79% of the predicted value (mean 62.5 ± 7.8). The FEV₁/FVC ratio ranged between 72 and 87% of the predicted value (mean 72.4 ± 8.3; Tables 1 and 2).

The serum vitamin D level was found to be significantly decreased in asthmatic children (25.1 ng/ml ± 3.2) as compared with the control group (41.4 ng/ml ± 4.2) (*P* < 0.001; Table 2). Out of the 50 asthmatic children, 20 had a serum vitamin D level of less than 25 ng/ml and therefore, they were considered to be 'vitamin D deficient', whereas the remaining 30 patients had a level ranging between >25 ng/ml and ≤30 ng/ml and were therefore considered to be 'vitamin D insufficient'. The asthmatic children had a highly significant increase in the total leukocytic count (*P* < 0.001), the eosinophilic count (*P* < 0.001), serum IgE (*P* < 0.001), and serum ALP (*P* < 0.001). There was also a significant increase in the serum phosphorus level in the asthmatic children compared with the control group (*P* < 0.01; Table 2).

The serum vitamin D level was 24.1 ng/ml ± 2.9 in the asthmatic group on ICS and 28 ng/ml ± 1.4 in those not on ICS.

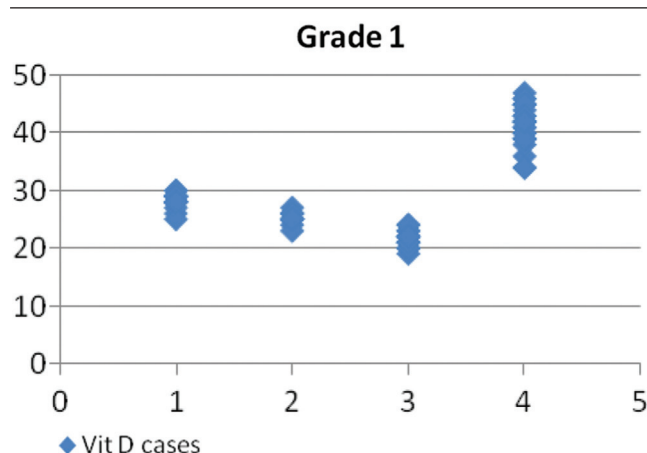
Table 1 Descriptive data of the asthmatic cases

Asthmatic group	Range	Mean ± SD
Age (years)	4–15	8.5 ± 2.5
Duration of illness (years)	2–12	5.5 ± 2.3
Diagnostic pulmonary functions		
FVC (% of predicted)	87–98	94.4 ± 2.3
FEV ₁ /FVC ratio	72–87	72.4 ± 8.3
Sex [N (%)]		
Males	36 (72)	
Females	14 (28)	
Residence [N (%)]		
Rural	32 (64)	
Urban	18 (36)	
Positive consanguinity [N (%)]	34 (68)	
Positive family history [N (%)]	30 (60)	
Passive smoking [N (%)]	36 (72)	
Treatment modality [N (%)]		
Inhaled corticosteroids	38 (76)	
No Inhaled corticosteroids	12 (24)	
Classification according to asthma control [N (%)]		
Controlled	20 (40)	
Partially controlled	12 (24)	
Uncontrolled	18 (36)	
Classification according to asthma severity [N (%)]		
Mild persistent	20 (40)	
Moderate persistent	16 (32)	
Severe persistent	14 (28)	

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

Regarding the correlation between serum vitamin D and pulmonary function measures, significant positive correlations were found between the serum vitamin D level and the predicted FEV₁% ($r = 0.871$, $P < 0.001$; Fig. 1). A highly significant negative correlation was found between the serum vitamin D level and IgE (marker of allergy) ($r = -0.589$, $P < 0.001$; Table 3 and Fig. 2). Moreover, the serum vitamin D level showed an inverse correlation with the clinical severity of bronchial asthma ($r = 0.903$, $P < 0.001$) and the level of control of asthma ($r = 0.923$, $P < 0.001$), classified according to the GINA classification (Table 3 and Figs 3 and 4).

Figure 1



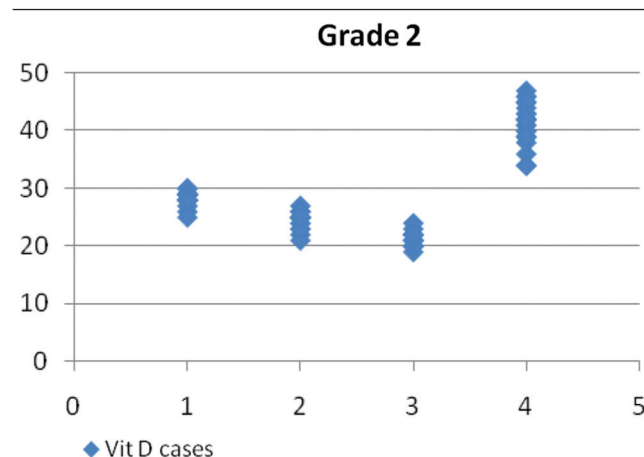
The relationship between the serum vitamin D level and the predicted percentage of forced expiratory volume in 1 s (FEV₁%) in asthmatic children.

The accuracy of vitamin D as a predictor of asthma was found to be 88% by automatic linear modeling (Fig. 5).

Discussion

The present study revealed that the serum vitamin D level was significantly decreased in all asthmatic children as compared with the healthy control group. Although our study involved only 50 asthmatic children, surprisingly, all the cases were vitamin D defective. Actually, 20 cases were vitamin D deficient

Figure 2



The relationship between the serum vitamin D level and the immunoglobulin E (IgE) level in asthmatic children.

Table 2 A statistical comparison between the asthmatic and the control group with regard to laboratory data and pulmonary function tests

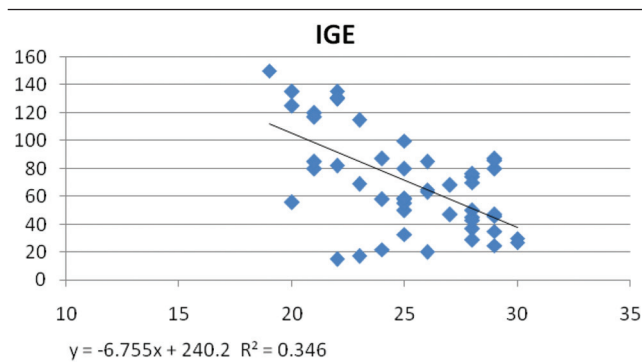
Variables	Asthmatic children		Control	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Serum IgE (U/ml)	15–150	70.6** $\pm$ 36.2	6–42	17.4 $\pm$ 9.3
Total leucocytic count $\times$ 1000/mcl	4.3–10	7.4** $\pm$ 2.4	2.7–7.2	4.8 $\pm$ 1.9
Eosinophilic count/mm ³	200–700	409.1** $\pm$ 137.3	100–210	161 $\pm$ 52.5
Alkaline phosphatase (U/l)	135–318	207.2** $\pm$ 51.6	59–140	98.7 $\pm$ 25.4
Serum calcium (mg/dl)	7.3–9.1	8.3 $\pm$ 0.5	8.4–10	9.3 $\pm$ 0.6
Serum phosphorous (mg/ml)	6.6–3.8	5.3* $\pm$ 0.7	3.1–4.5	3.9 $\pm$ 0.3
Serum vitamin D (ng/ml)	19–30	25.1** $\pm$ 3.2	34–47	41.4 $\pm$ 4.2
Prognostic pulmonary functions				
FEV ₁ % of predicted	50–85	69.2* $\pm$ 9.1	88–102	85.6 $\pm$ 5.1
PEFR% of predicted	46–79	62.5** $\pm$ 7.8	89–105	84.9 $\pm$ 6.9

FEV₁, forced expiratory volume in 1 s; IgE, immunoglobulin E; PEFR, peak expiratory flow rate; *significant, $P < 0.01$; **Highly significant, $P < 0.001$.

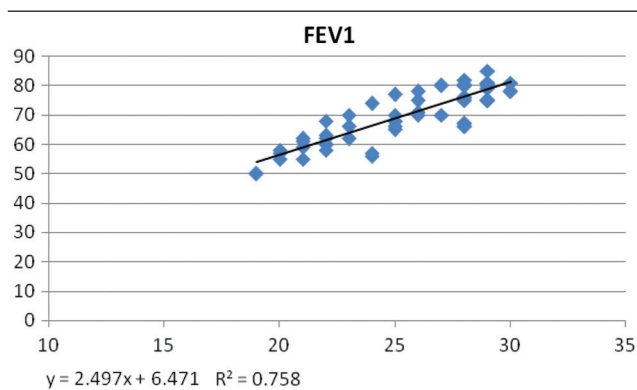
Table 3 Correlations with serum vitamin D

Variables	Serum IgE	Eosinophil	Alkaline phosphatase	FVC	FEV ₁	FEV ₁ /FVC	PEFR	Grade 2	Grade 1
Serum vitamin D									
Pearson correlation	-0.589**	-0.345*	-0.311*	0.442**	0.871**	0.844**	0.306*	-0.903**	-0.923**
Significance (two-tailed)	0.000	0.014	0.028	0.001	0.000	0.000	0.031	0.000	0.000

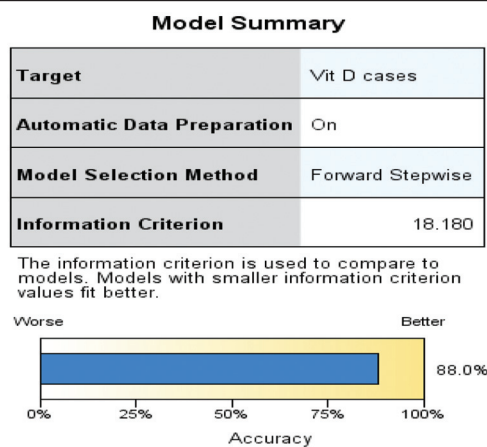
FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E; PEFR, peak expiratory flow rate; **Correlation is significant at the 0.01 level (two-tailed); *Correlation is significant at the 0.05 level (two-tailed).

Figure 3

The serum vitamin D level in asthmatic children with regard to the level of asthma control compared with the control group. 1, controlled cases; 2, partially controlled cases; 3, uncontrolled; and 4, control cases.

Figure 4

The serum vitamin D level in asthmatic children with regard to the level of asthma severity compared with the control group. 1, mild cases; 2, moderate cases; 3, severe cases; and 4, control cases.

Figure 5

Automatic linear modeling and the accuracy of vitamin D as a predictor of asthma.

(<25 ng/ml), whereas the other 30 were vitamin D insufficient (>25 ng/ml to ≤30 ng/ml). This finding could be considered as a respectful support for the hypothesis that bronchial asthma risk and vitamin D

deficiency (or even insufficiency) are inter-related. It is important to mention that the most important source of vitamin D is its natural cutaneous production due to the sun's ultraviolet radiation. In addition, food fortification with vitamin D besides eating egg yolk and fatty fish is crucial. Some behavioral factors result in vitamin D deficiency in healthy people, such as excessive clothing coverage, sunscreen use, increased time spent indoors and intrinsic factors such as the skin melanin content and increased cutaneous destruction of vitamin D₃.

Several mechanisms have been postulated to explain as to how vitamin D modulates the pathogenesis of asthma. Vitamin D may protect one from developing respiratory infections that serve as a trigger for the deterioration of asthma. It modulates the function of many immune cells including monocytes, macrophages, lymphocytes, and epithelial cells [23].

Poon *et al.* [24] reported that polymorphisms in the gene encoding the vitamin D receptor were associated with asthma phenotypes.

Vitamin D supplementation was reported to reduce the risk of disease exacerbations in children with asthma. The antiviral properties of vitamin D are further supported by a recent observational study of Finnish infants hospitalized with a wheezing illness, in whom the vitamin D level was inversely associated with coinfection with respiratory syncytial virus or rhinovirus [25].

Our study revealed a significant decrease in pulmonary function tests (FEV₁, FVC, FEV₁/FVC ratio, and PEFR) in the asthmatic cases. Significant positive correlations were found between the serum vitamin D level and the predicted pulmonary function tests in asthmatics (FVC, FEV₁, FEV₁/FVC ratio, and PEFR). These findings suggest the involvement of vitamin D in lung function and the development of airflow limitation. Vitamin D inhibits the formation of matrix metalloproteinase and fibroblast proliferation and influences collagen synthesis; these actions mean that 1, 25-dihydroxyvitamin D may influence tissue remodeling and probably lung function.

Chinellato *et al.* [26] have proved that children with mild to moderate persistent bronchial asthma and who were vitamin D deficient showed less improvement in FEV₁, after 1 year of treatment with inhaled corticosteroids compared with children with sufficient vitamin D. Moreover, vitamin D intake may influence the lung function, whereby several studies have

proved that children with higher vitamin D levels had better lung function measures (FEV₁ and FVC) with decreased airway resistance.

Moreover, Black and Scragg [27] reported that serum vitamin D was positively correlated with FEV₁ and glucocorticoid response as vitamin D insufficiency or deficiency (<30 ng/ml) was associated with airway hyper-responsiveness.

Some studies suggest a possible association between maternal intake of vitamin D during pregnancy and risk of childhood wheezing/asthma in the offspring, and so vitamin D intake during pregnancy may have a positive effect on lung growth and development in neonates [28].

Our study showed that the asthmatic children had a highly significant increase in asthma markers such as the total leucocytic count, the eosinophilic count, and serum IgE as compared with healthy control children. Significant negative correlations were found between the serum vitamin D level and eosinophil counts ($r = -0.345$, $P = 0.14$) and the serum IgE level ($r = -0.589$, $P = 0.000$) in asthmatic children. This is in agreement with the findings of Brehm *et al.* [29] in Costa Rica who reported an inverse relationship between circulating levels of vitamin D and several markers of allergy and asthma severity such as eosinophil count, IgE levels, asthma exacerbation, airway responsiveness, and skin test reactivity.

Moreover, Searing *et al.* [30] reported that the serum vitamin D was positively correlated with lung function and enhanced glucocorticoid action in peripheral blood mononuclear cells. Moreover, vitamin D was inversely correlated with total IgE, the degree of atopy and the use of inhaled or oral steroids.

Litonjua *et al.* [13] found that children with insufficient levels of 25-hydroxyvitamin D were more likely to have severe exacerbations, but they did not find any association between vitamin D and the bronchodilator response or airway hyper-responsiveness. This was in agreement with Alyasin *et al.* [31], who reported no association between the vitamin D level and the eosinophilic count. They relate this to the small number of cases studied.

Our results showed an 88% degree of 'accuracy' that vitamin D deficiency or even insufficiency can be an indicator of bronchial asthma and its severity. This was proved in our study by the highly significant positive correlation between the vitamin D level and the efficiency of intake of inhaled corticosteroids

($P < 0.001$). Another proof was the highly significant negative correlation between the vitamin D level and the grade of severity ($r = -0.903$, $P < 0.000$) and the degree of control of bronchial asthma ($r = -0.923$, $P < 0.000$). This means that the higher the level of vitamin D, the lesser the degree of bronchial asthma severity and the better the control. This highly beneficial finding is in accordance with the work conducted by Litonjua *et al.* [13], who proved that low vitamin D levels were associated with worse bronchial asthma symptoms, more use of medications and poorer lung function measures.

Ginde *et al.* [10] stated that vitamin D is potentially capable of overcoming the poor glucocorticoid responsiveness in severe asthmatics through the upregulation of interleukin-10 production (a potent anti-inflammatory cytokine) from CD4⁺ T cells. That is to say that vitamin D restores the capability of regulatory T cells from steroid-resistant patients with bronchial asthma to secrete interleukin-10 in response to steroids.

This is also in agreement with the work conducted by Bener *et al.* [32], who confirmed that vitamin D levels were predictive of asthma and allergy outcomes.

Conclusion

The present study provides an important support for the association between bronchial asthma in children and vitamin D deficiency or even insufficiency, with a direct relationship between its serum level and pulmonary function test measures. Vitamin D deficiency can be considered as a strong predictor of asthma. A strong association between the serum vitamin D level and increased asthma severity was found. Thus, improving the vitamin D status will help in the primary prevention of asthma and in decreasing exacerbations of attacks. Clinical trials of vitamin D supplementation to prevent asthma exacerbation are recommended.

Acknowledgements

Conflicts of interest

None

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