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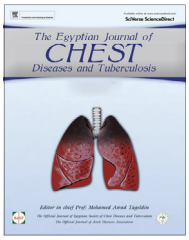

ORIGINAL ARTICLE

Vitamin D and phenotypes of bronchial asthma

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KEYWORDS

Asthma;
Vitamin D;
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Abstract *Background:* Many studies have suggested the role of vitamin D deficiency in both T-helper1 and T-helper2 diseases. The existence of associations of vitamin D with asthma and allergy remains uncertain. While some suggest that vitamin D may be protective, others suggest that vitamin D supplementation may increase the risk of allergy.

Aim of the work: The aim of the study was to evaluate the state of vitamin D in asthmatic patients and its potential relationship with asthma phenotypes.

Patients and methods: This study was conducted on 66 nonsmoker asthmatic patients and 30 healthy controls. Serum 25-hydroxy vitamin D3 levels were determined and compared between the two groups. The relationship between serum vitamin D levels and asthma phenotypes were examined.

Results: Vitamin D level was significantly lower in asthmatic patients than in control group, in asthmatic patients, vitamin D levels had a significant positive correlation with FEV1% predicted and a significant negative correlation with body mass index, the number of atopic patients was significantly higher in bronchial asthma patients with vitamin D insufficiency than those with sufficient vitamin D.

Conclusion: Vitamin D deficiency was highly prevalent in asthmatic patients and it was associated with atopy and asthma severity.

Introduction

Asthma has been recognized as a typical complex disease with a high number of factors modulating the expression of asthma-related phenotypes and/or outcomes [1]. The innate and adaptive immune systems play an important role in the pathogenesis of asthma. Many genes involved in inflammation and immunoregulation pathways have been associated with asthma. The immune system is complex in nature with multiple redundant and interfering pathways. Recently, the vitamin D pathway has emerged as a new pathway contributing to the outcome of immune responses [2].

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Vitamin D is a nutrient and a hormone that can be obtained from a few natural foods and fortified foods and it can be generated endogenously from sunlight exposure via a photosynthetic mechanism in the skin. Vitamin D deficiency has been reported in many populations, even in those living in areas with abundant sun exposure. 25-Hydroxyvitamin D [25(OH)D] is the major circulating form of vitamin D, and its concentration in serum has been thought to reflect the status of vitamin D [3]. Although there is no consensus regarding optimal 25(OH) Dserum levels, based on epidemiologic studies, a desirable level of serum vitamin D, i.e., 25(OH)D, for general health is at least 30–40 ng/mL (75–100 nmol/L) [4]. Recently the effects of vitamin D as a hormone have gained attention. Vitamin D appears to have regulatory effects on every part of the immune system, with vitamin D deficiency being linked to an array of immunologically based diseases. Because Vitamin D promotes steroid sensitivity in the body and can down regulate an inflammatory state via gene expression and cytokine production, its action in this case 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 an inability to switch off the inflammatory state, following an acute inhalational insult, with up regulation of prostaglandin, leukotrienes, macrophages, and T cell activity and recruitment [2,5].

There have been numerous studies looking at vitamin D status in association with various lung diseases focusing on asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and respiratory infections. These studies have demonstrated a high prevalence of vitamin D deficiency in their participants [6]. Vitamin D deficiency has been associated with lower lung function in COPD and CF patients [7]. Other studies have shown an association between vitamin D deficiency and infections such as *Mycobacterium tuberculosis* [8,9] and upper respiratory tract infections [10–12]. The existence of associations of vitamin D with asthma and allergy remains uncertain. While some suggest that higher maternal intake of vitamin D during pregnancy was associated with a decreased risk of recurrence of wheezing in young children, others suggest that vitamin D supplementation may increase the risk of allergy [13] therefore, the aim of this study was to evaluate the state of vitamin D in asthmatic patients and its potential relationship with asthma phenotypes.

Patients and methods

Patients

This study included 66 newly diagnosed bronchial asthma patients (34 females and 32 males) with a mean age of (37.8 ± 10) who attended outpatient clinic between July 2011and July 2012 after giving written informed consent and 30 healthy control without history of any allergic disorders (16 females and 14 males). Asthma was diagnosed by history which included episodic respiratory symptoms and reversible airflow obstruction (documentation of reversibility of FEV1 and/or FVC by 12% or an increase of FEV1 by 200 cc either spontaneously or after inhalation of 400 μg of salbutamol) [14].

Exclusion criteria

Smokers, and patients who had a co-morbid disease in addition to bronchial asthma that could affect vitamin D serum levels were excluded. Such diseases included rheumatoid arthritis, cystic fibrosis, multiple sclerosis, ulcerative colitis, crohn's disease, celiac disease, osteomalacia, sarcoidosis, thyroid dysfunctions, and individuals who had received medications including systemic corticosteroids, barbiturates, bisphosphonates, sulfasalazine, omega3 and vitamin D components such as calcium-D.

Methods

The following was done for all patients:

- Complete medical history.
- General and local chest examination.
- Plain postero-anterior chest X-ray.
- Laboratory investigations (total and differential blood cells count, ESR, renal, hepatic function tests and fasting blood sugar).
- Spirometry performed according to American Thoracic Guidelines (before and after inhalation of 400 μg of salbutamol) [15] by using Chestgraph HI-701 spirometer.
- Body mass index (BMI) was calculated from measured height and weight.
- *Eosinophils in blood and sputum:* eosinophil's count in blood was measured using sysmex kx-21, while in sputum was assessed as follows:
- Sputum induction: Participants were asked to expectorate a spontaneous sputum sample or, if unable to do so, they underwent sputum induction with the inhalation of hypertonic saline (4.5%) through an ultrasonic nebulizer (Suchatzki, Rennerod, Germany). The nebulization was continued for at least 10 min and stopped after 15 min or earlier if a $\geqslant$2 mL sputum sample of good quality was obtained [16].
- Sputum processing and cell counts: The sputum was selected from saliva and dispersed using dithiothreitol as described by Pavord et al. (1997) [16]. The suspension was filtered, and a total cell count of leukocytes and viability was performed. Cytospins were stained (May-Grunwald Geimsa) and a differential cell count was obtained from 400 non-squamous cells [16].
- Allergy skin testing**:** allergy skin test was performed on the volar side of the forearm as was previously described [17] with standardized extracts of common aeroallergen (house dust, dog hair, cat hair, grass and tree pollen, cotton dust and mixture of molds). Skin response was measured after 15 min, considering skin wheal diameter. Skin test reaction was considered positive if the diameter of the skin wheal was $>$3 mm, while a wheal diameter $<$3 mm was defined as negative response. A $>$3 mm wheal is a net wheal compared to the negative control. Atopy was defined when a patient had suggestive history with a significant positive skin test reaction to at least one of the applied aeroallergen [17].

- Vitamin D level: Two milliliters of venous blood was obtained centrifuged; serum was separated and stored at −20 °C until assayed. Serum level of 25(OH) D was measured using the immunodiagnostic enzyme immunoassay (EIA) developed by immunodiagnostic system kits Ltd. In this study vitamin D levels were categorized as insufficient (< 30 ng/mL), or sufficient (⩾30 ng/mL) according to Holick (2007) [3].

Statistical analysis

Statistical analysis was performed with SPSS version19software package (SPSS, Inc. Chicago). The results of this study were analyzed 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 testing.

Results

A total of 66 asthmatic patients (34 females and 32 males) and 30 controls were enrolled in this study, the mean age of participants with asthma was 37.8 ± 10, with mean FEV1% (73.3 ± 7.6) and BMI (28.8 ± 4.9 kg/m^2) (Table 1).

In this study 44% of asthmatic patients suffered from vitamin D insufficiency (25(OH) D < 30 ng/mL) while in control group vitamin D insufficiency was present in 20% of them (Table 1).

The studied bronchial asthma patients with vitamin D insufficiency had significantly higher sputum eosinophils % and a significantly lower FEV1% than bronchial asthma patients with sufficient vitamin D (Table 2), moreover, there was a significant increase in the number of severe bronchial asthma patients with vitamin D insufficiency (41.4%) compared with those with sufficient vitamin D (13.6%) (Table 2).

In this study the presence of atopy was significantly higher in asthmatic patients with vitamin D insufficiency (58.6%) than those without vitamin D insufficiency (41.4%) (Table 3).

Table 1 Physiological and demographic characteristics of participants.

Characteristics	Asthma patients No. = 66	Control group No. = 30	*P*
Age (years)	37.8 ± 10	38.2 ± 13	NS
Female	34 51.5%	16 53.3%	NS
Male	32 48.5%	14 46.7%	NS
BMI (kg/m^2)	28.8 ± 4.9	29.2 ± 6.1	NS
FEV1%	73.3 ± 7.6	93.6 ± 8.7	< 0.05
FEV1/FVC %	64.8 ± 8.4	87.3 ± 9.2	< 0.05
25(OH)D, ng/mL (mean ± SD)	43.6 ± 16.3	65.8 ± 11.6	< 0.05
25(OH)D < 30 ng/mL (*n*%)	29(44%)	6(20%)	< 0.05

Table 2 Characteristics of the studied patients according to vitamin D levels.

	Asthmatics with insufficient vitamin D (*N* = 29)	Asthmatics with sufficient vitamin D (*N* = 37)	*P* value
BMI (mean ± SD)	31.3 ± 6.3	27 ± 2.1	< 0.001
FEV1%	67.8 ± 4	77.6 ± 7	< 0.001
Sputum eosinophils %	13.6 ± 5.1	4.7 ± 2.1	< 0.001
Asthma severity n (%)			
Intermittent	5(17.2%)	11(29.7%)	
Mild	6(20.7%)	13(35.1%)	< 0.05
Moderate	5(17.2%)	8(21.6%)	
Severe	13 (44.8%)	5(13.6%)	

Table 3 Association of atopy with vitamin D insufficiency.

	Asthmatics with insufficient vitamin D (*N* = 29)	Asthmatics with sufficient vitamin D (*N* = 37)	*P*
Atopy			
Present	17(58.6%)	10(26.4%)	< 0.05
Absent	12(41.4%)	27(73.6%)	

A significant correlation between serum vitamin D with the FEV1% ($P < 0.05$) was present in this study ($P = 0.000$, $r = 0.706$) Fig. 1.

In this study a significant negative correlation between serum 25(OH) D and BMI (kg/m^2) was observed in the studied asthmatic patients ($P = 0.000$, $r = 0.458$) (Fig. 2).

Discussion

The existence of associations of vitamin D with asthma and allergy remains uncertain, therefore, the aim of this study was to evaluate the state of vitamin D in asthmatic patients and its potential relationship with asthma phenotypes.

In this study 44% of asthmatic subjects had serum vitamin D levels < 30 ng/mL compared with 20% in the control group ($P < 0.05$) which was statistically significant. This finding agrees with Brehm et al. [18] who found that 25% of asthmatic patients had serum vitamin D levels < 30 ng/mL, and 3.4% had levels < 20 ng/mL and found a strong inverse association between serum vitamin D levels and asthmatic state. A number of confounding factors may influence these relationships; one is that the subjects with asthma spend more time indoors, so they may be exposed to less sunlight [18].

The studied bronchial asthma patients with vitamin D insufficiency had significantly higher sputum eosinophil % and a significantly lower FEV1% than bronchial asthma patients with sufficient vitamin D (Table 2), moreover, there was a significant increase in the number of severe bronchial asthma patients with vitamin D insufficiency (41.4%) compared with those with sufficient vitamin D (13.6%) (Table 2). Vitamin D deficiency could be involved in asthma pathogenesis through several mechanisms. Vitamin D appears to have regulatory effects on every part of the immune system,

with vitamin D deficiency being linked to an array of immunologically based diseases. Because Vitamin D promotes steroid sensitivity in the body and can down regulate an inflammatory state via gene expression and cytokine production, its action in this case 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 an inability to switch off the inflammatory state, following an acute inhalational insult, with up regulation of prostaglandin, leukotrienes, macrophages, and T cell activity and recruitment [19].

A positive correlation was found between 25(OH) D levels and FEV1% in the studied asthmatic patients in the present study (Fig. 1) this finding agrees with that of Damera et al. (2009) [20] who reported that in patients with asthma, higher serum 25(OH) D concentrations were associated with higher FEV1% and explained this finding by that vitamin D inhibits the formation of matrix metalloproteinase as well as fibroblast proliferation and influences collagen synthesis; these actions mean that 1,25-dihydroxy vitamin D may influence tissue remodeling and probably lung function [20]. It has been shown that, in addition to affecting immune cells, vitamin D affects smooth muscle function and proliferation, which has a direct relevance for lung function in asthma and in airway remodeling [21]. Airway remodeling is an important feature of asthma and is correlated with airflow limitation [22]. Vitamin D influences airway remodeling by affecting smooth muscle cell movement, growth, and contractility and by inhibiting transforming growth factor-β and matrix metalloproteinase as well as fibroblast proliferation [23]. Furthermore, in animal models, vitamin D has been shown to affect lung development and maturation in utero and in the immediate postpartum period [21].

Brehm et al. [24] found an inverse relationship between circulating levels of vitamin D and markers of allergy in asthmatic patients such as eosinophil count, IgE levels and skin-test reactivity. These findings are in line with the results of this study as the number of atopic asthmatics was significantly higher in asthmatics with insufficient levels of 25(OH) D than those with sufficient 25(OH) D (Table 3).

In this study there is an inverse relationship between BMI and vitamin D levels (Fig. 2), a finding previously reported

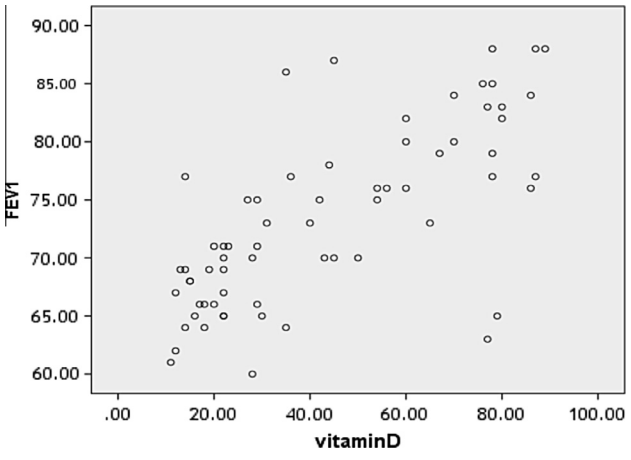

Figure 1 Correlation between 25(OH) D and FEV1%.

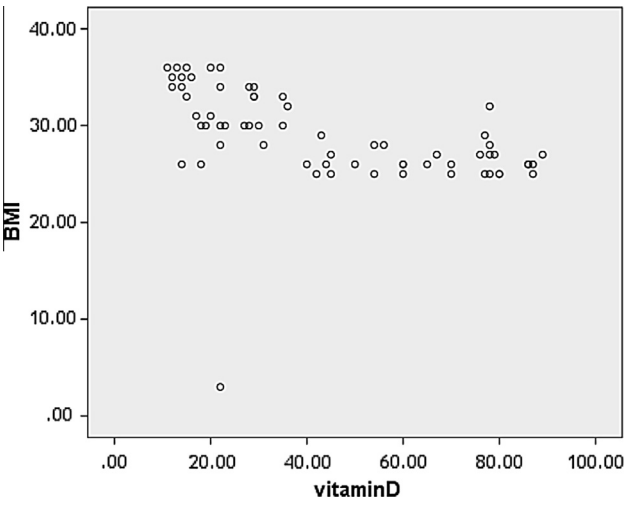

Figure 2 Correlation between 25(OH) D and BMI.

[25]. One of the most significant effects of obesity on asthma relates to phenotype, particularly its association with reduced response to steroids, both clinically and in vitro [5]. A cross-sectional study on 616 asthmatic Costa Rican children found that higher serum 25(OH) D concentrations were associated with a reduction in the need for anti-inflammatory medications and hospitalization [18]. Others have demonstrated an inverse correlation between serum 25(OH) D concentrations and the amount of steroid medication prescribed to asthmatic patients. This suggests that vitamin D may potentiate the effects of steroids in asthmatic patients and suggests that vitamin D, the levels of which are reduced in overweight and obese subjects with asthma, may be one pathway by which obesity and reduced steroid response are related [26].

Conclusion

These results showed that vitamin D deficiency was highly prevalent in asthmatic patients, and there was a direct and a significant relationship between vitamin D levels and pulmonary function test outcomes in asthmatics patients, therefore measuring serum levels of vitamin D could be considered in the routine assessment of patients with bronchial asthma.

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