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Research Article

The Assessment of 25-Hydroxy Vitamin D Serum Level in Asthmatic Patients: A Case-Control Study

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Abstract

Background: The prevalence of vitamin D deficiency is increasing due to changes in lifestyle and dietary habits. The aim of this study was to compare the serum levels of 25-hydroxy vitamin D between patients with bronchial asthma and the healthy control group.

Patients and Methods: In this case-control study, 45 patients with asthma and 45 healthy subjects were enrolled and the level of serum 25 (OH) vitamin D was measured in both groups. In addition, a well-trained observer assessed airway reversibility, peak flowmetry and spirometry in the participants. The data were statistically analyzed using t test, one-way analysis of variance (ANOVA), and chi-square test with Stata software (version 11).

Results: The mean age (±SD) of participants were 49.06 ±16.43 and 46.13 ±16.10 years in case and control groups, respectively ($P=.394$). The prevalence of vitamin D deficiency was high in both groups (69% in case and 65.5% in control groups). The mean (±SD) serum 25 (OH) vitamin D was 16.24 (±14.98) ng/ml in case group and 17.70 (±16.07) ng/mL in control group ($P=.657$). We found a positive correlation between the levels of vitamin D and the amount of FEV1 ($r=0.2$).

Conclusions: According to the present study, the mean serum levels of vitamin D differences were not statistically significant between asthmatic patients and control group. However, the results of this study showed a positive relationship between forced expiratory volumes in first second (FEV1) and vitamin D levels.

Keywords: Asthma, Bronchial asthma, Obstructive lung disease, Vitamin D deficiency

Background

Asthma is one of the most common chronic diseases worldwide (1). It is a syndrome characterized by airflow obstruction which is usually resolved spontaneously or by using the medications. Asthmatic host has a special type of inflammation in the airway mucosa that make them more responsive to a wide variety of triggers compared with non-asthmatic individuals, leading to excessive narrowing with the consequent reduced airflow, dyspnea and wheezing (1). In the developing countries, its prevalence has increased due to the increased urbanization rate. Simultaneously, the prevalence of atopy and the other allergic diseases is increasing worldwide, indicating that the increased incidence of the disease can be due to its systemic feature (2). Asthma is a heterogeneous disease that is influenced by genetic and environmental factors. The diet and nutrition are suspected to be effective in asthma evolution. Some evidence confirmed the effect of zinc and other vitamins like E and D on the incidence of asthma (3). Vitamin D is one of the essential and fat-soluble vitamins which can be accumulated in the human body unlike water-soluble vitamins. The natural vitamin D can be synthesized via the skin by ultraviolet radiation (UV-B) (4). Foods except fish contain limited amount of vitamin D. The most specific screening test to measure the level of vitamin D deficiency is the serum levels of 25 (OH) vitamin D (5). Today, vitamin D deficiency is increasing worldwide depending on the nutrition, lifestyle and behavior changes. Aging, lack of sun exposure, dark skin, fat malabsorption and obesity are considered as risk factors for vitamin D deficiency (6). Vitamin D is vital in the normal function of skeletal and non-skeletal tissues such as muscles, immune cells in immune processes, mineral and calcium metabolisms and cerebral

processes. Studies have shown that vitamin D can help in the treatment of tuberculosis, psoriasis, multiple sclerosis and cancer prevention and vitamin D deficiency are associated with the susceptibility to infection ,especially the respiratory infections, cancer and autoimmune disorders (7). The association between vitamin D and asthma has not been clearly defined. Several studies have shown that vitamin D deficiency leads to increased airway reactivity and can increase the need for corticosteroids (8).

It is believed that vitamin D supplementation before or after birth can have a protective effect, while the others have suggested that vitamin D supplementation may increase the risk of allergies. Since asthma is considered as a common disease, it can affect patients' quality of life (9). Therefore, identification of the risk factors is required to prevent and improve the symptoms and the appropriate treatment with a lower cost is essential. Except genetic and environmental factors for prevalence and severity of asthma, other factors have been considered effective in asthma attacks, including reduction of antioxidants like vitamins A and C, magnesium and selenium, omega 3 and fish oil, the increased sodium, and omega-6 fat. Given the ambiguity of the role of vitamin D in the development and exacerbation of asthma, this study aimed to assess vitamin D deficiency in patients with asthma and its severity.

Patients and Methods

Methods

This case-control study was conducted in Pulmonology Clinic of Shahid Beheshti hospital, Hamadan, Iran, from January 2013 to December 2015. This study was approved by the Ethical Committee of the Hamadan University of Medical Sciences. Written informed consent was obtained from all subjects.

Patient Recruitment

The samples were selected from the patients with suspected bronchial asthma who referred to the Pulmonary Outpatient Clinic in Shahid Beheshti hospital in Hamadan, a province in western part of Iran. In addition, previously diagnosed asthmatic patients who did not receive any drug therapy in the past 3 months were also enrolled. First, All patients were interviewed and then examined by one executor. Only patients with bronchial asthma entered into the study. The patients underwent spirometry according to the standards to confirm the diagnosis and determine the severity of the disease, and the criteria for reversibility of over-responsiveness of the airways were also determined (10). In order to identify the bronchial asthma, a well-trained technician assessed airway reversibility, peak flowmetry and spirometry in the asthmatic patients. At least 3 acceptable maneuvers from American college of chest physicians standards were required with the minimum of the two reproducible forced expiratory volumes in first second (FEV1) and forced vital capacity (FVC) maneuvers within 5% of the best measurement required for each test (10). Airway reversibility was evaluated by the executor according to a standardized protocol using spirometry before and 15 minutes after inhalation of 2 puffs of a short-acting beta2-agonist spray (200 µg albuterol per dose) (10). Equal or more than 12% and at least 200 mL increase in FEV1 was diagnostic for bronchial asthma (11). Meanwhile, the severity of asthma was divided into 4 stages based on the asthma prevention and treatment guideline 2007 (12).

The control group was selected based on clinical examination performed by a physician to confirm no history or clinical sign of asthma or chronic obstructive pulmonary disease (COPD). The inclusion criteria for the patients group included having moderate to severe asthma, being over 18 years of age, having no history of smoking, and for the control group they were not having pulmonary or other respiratory or cardiovascular diseases, being over 18 years of age and having no history of smoking. In addition, the pregnant patients, patients who needed drugs effective in the metabolism and absorption of vitamin D, patients with other diseases affecting spirometry like COPD, chest disorders, those who used vitamin D supplementations in the past 3 months, patients with renal or hepatic diseases, malabsorption, and systemic diseases such as diabetes mellitus, and so on were excluded from the study. We considered seasonal variation in vitamin D level and for each person in the case group chose one control in the same season.

The sample size was considered 45 people for each group and the total sample size was 90 patients (at 90% CI and 80% statistical power).

A questionnaire was completed after sample selection and obtaining the consent and was completed via interview and questions. The questionnaire included demographic information, clinical findings, spirometry parameters and the test results for vitamin D was completed for patients, as well.

Lung Function Measurements

Spirometry was done before and after bronchodilator by 1 technician at Shahid Beheshti hospital. Baseline FVC and FEV1 measurements were obtained in the absence of bronchodilator use. Basal and post-bronchodilator FEV1 and FVC were measured. We used a ZAN100 spirometer (ZAN Messgeraete GmbH, Oberthulba, Germany) for measuring pulmonary variables and indexes (FEV1, FVC and FEV1/ FVC).

Laboratory Procedures

Venous blood (5 mL) was drawn by a technician in sterile syringe from case and control groups and serum was separated by centrifugation. The samples taken were put in the refrigerator at -20°C. Vitamin D levels were measured in serum samples by ELISA using Euroimmun kit (13). The sensitivity of this technique for 25-OH vitamins D2 and D3 was about 1.6 ng/mL, and no cross-reaction with vitamin D2 (ergocalciferol), vitamin D3 (cholecalciferol) and 24,25(OH)2 vitamin D3 was observed. All tests performed by 1 technician at the end of sampling. The technician was blinded to the patient and control groups and study protocols. The vitamin D3 level less than 20 ng/ml was considered deficiency, the level of 20-30 ng/mL was considered inadequate, and the level more than 30 ng/mL was considered as sufficient.

Statistical analysis

Statistical analysis was conducted using Stata software version 11.0 (Stata Corp., TX, USA). All continuous variables were tested for normal distribution before further statistical analysis with Shapiro-Wilk test. The results were expressed as the mean ± standard deviation (SD). Differences in categorical variables were analyzed by chi-square test (linear by linear association) and the Student's *t* test for continuous variables. A correlation between vitamin D and lung function parameters was evaluated with Pearson correlation in the case of normally distributed variables. The one-way analysis of variance (ANOVA) was used to determine the differences between the serum levels of vitamin D in different age groups. *P* value less than 0.05 was considered significant.

Results

Fifty-eight samples (64.4%) were males and 32 samples (35.6%) were females. The mean age was 47.6 ± 16.26 years with the age range of 18-86 years. The mean age of the patients group (49.06 ± 16.43 years) was higher than control group (46.13 ± 16.10 years), but it was not significantly different (*P* = .394). In this study, all patients had FEV1/FVC less than 0.7 and FEV1/FVC in the control group was above 0.7. FEV1 was classified in the patient group. The results showed that there was a positive dose-response relationship between FEV1 and vitamin D level. By increasing vitamin D levels, FEV1 also increased. The Pearson correlation coefficient was obtained 2.0, i.e. by increasing vitamin D level, FEV1 will be increased, though, it is not high according to Pearson correlation coefficient.

The mean level of vitamin D3 in all subjects (overall) was 16.97 ng/mL (ranged from 0.21 to 66). The mean level of vitamin D3 in the control group (non-asthmatic individuals) (17.70 ± 16.07) was more than patient group (16.24 ± 14.98), but there was no statistically significant difference (*P* = .657). The results showed that although vitamin D3 deficiency was more prevalent in the patient group than the control group, this difference was not significant (Table 1).

The analysis of variance showed that the mean level of vitamin D3 in age groups was not different, although it was observed that the mean level of vitamin D3 in the higher age groups was increasing (may be due to supplement consumption) (Table 2).

According to the results, none of the samples had mild asthma. Twenty- eight patients out of 45 in the patient group had moderate asthma and 17 patients had severe asthma. The mean vitamin D level in patients with severe asthma was lower compared with people with moderate asthma, but it was not significant (Table 2). Twenty-eight patients (62.2%) had moderate to severe asthma. Twenty-one patients (46.6%) suffered from respiratory problems every day. Twenty patients (44.4%) were awakened more than 2 times a week due to asthma.

Discussion

The aim of this study was to compare serum levels of 25 (OH) vitamin D in patients with bronchial asthma with non-asthmatic individuals. It has been shown that 25 (OH) vitamin D deficiency is associated with chronic diseases such as cancer, autoimmune, infectious, allergic and cardiovascular diseases (14-17). The results showed that the mean of 25 (OH) vitamin D was lower in people with asthma compared to non-asthmatic people (16.24 vs.17.70 ng/mL, respectively) but it was not statistically significant. It may be due to small sample size, high

Table 1. Vitamin D3 Levels Classified in 3 Groups Within Case and Control Groups

Vitamin D3 Level	Group [a]	
	Cases, No. (%)	Control, No. (%)
Deficiency (<20 ng/mL)	31 (69.0)	29 (65.5)
Inadequate (20-30 ng/mL)	8 (17.8)	9 (20.0)
Sufficient (>30 ng/mL)	6 (13.2)	7 (15.5)

[a] *P* = .904

Table 2. Mean Levels of Vitamin D3 in Different Age Groups Based on Asthma Severity

Variables	Vitamin D3 Level (Mean ± SD, ng/mL)	*P* Value
Age group (y)		0.054
≤30	13.10 ± 9.59	
31-40	13.80 ± 13.23	
41-50	14.20 ± 14.92	
51-60	17.80 ± 15.98	
>60	26.60 ± 18.78	
Asthma severity		0.239
Severe	14.17 ± 13.97	
Moderate	19.64 ± 11.22	

prevalence of vitamin D deficiency in both groups but these differences may be important in clinical practice. The result of this study, like previous studies, showed a high prevalence of 25-OHD vitamin deficiency, despite the supplements. It can be due to changes in lifestyles, incorrect dietary habits, and the high prevalence of obesity (18,19).

The results showed that by increasing levels of 25-OH vitamin D, pulmonary function also improved, so there is a positive correlation between 25-OH vitamin D levels with 0.28 Pearson correlation coefficients and FEV1. The results are consistent with Monadi et al reporting a dose-response relationship between 25-OHD and FEV1, i.e., vitamin D deficiency is more common in more severe forms of asthma (19). Although the desired level of 25-OHD vitamin is controversial among experts, in this study, the level less than 20 ng/mL was considered as vitamin deficiency acceptable to most of the experts (5). In this study, nearly 70% of people with asthma and 65% of the control group had 25-OHD vitamin level less than 20 ng/mL. The sufficient level of 25 (OH) vitamin D was found in 13.2% and 15.5% of the patient and control groups, respectively. Korn et al showed that by increasing the severity of asthma from mild to severe, the level of 25 (OH) vitamin D was downward. By decreasing the level of 25 (OH) vitamin D, FEV1 decreased. This study suggested 25 (OH) vitamin D consumption to control asthma and improve lung function (4).

The role of 25 (OH) vitamin D in the pathogenesis of asthma has not been established. 25 (OH) vitamin D may protect the lung infection at the onset of asthma. It is not still well clear whether 25 (OH) vitamin D deficiencies in patients with asthma is due to changes in lifestyle i.e., decreased exposure to sunlight or drugs, or it is not related to the disease (13). A clinical trial by Sutherland et al in 2010 showed that high levels of 25 (OH) vitamin D were associated with increased lung function, reduced airway excitability and increased response to the steroid which is consistent with the results of this study (13). Although it was reported that allergic diseases, asthma and decline in lung function are associated with 25 (OH) vitamin D deficiency, the association between 25 (OH) vitamin D with these diseases is still a controversial issue. For example, in a prospective study conducted by Thuesen et al, no significant relationship was found among atopy, asthma and 25 (OH) vitamin D, however, the 25 (OH) vitamin D levels had a positive correlation with FEV1, which is consistent with our results (16).

In this study, we had some limitations. None of epidemiological studies are without error. Our study had some limitations for example small sample size, single center based study and the presence of confounders like obesity.

The strengths of our study were having the exact criteria for asthma disease diagnosis and grading the severity of disease, excluding important confounders like chronic diseases and using drugs. All of interviews and examinations were performed by 1 person and all samples were analyzed at one time by 1 person. Also, we considered the seasonal variation in vitamin D levels, and for each person in the group of patients, we chose 1 person from the control group in the same season, but samples have been collected at different seasons and this may affect the outcome of the investigation.

Moreover, we recommend conducting the future studies as randomized controlled trials or cohort studies with large sample size with longitudinal follow up and more details.

Conclusion

According to the results, there was no statistically difference between mean serum levels of 25 (OH) vitamin D in patients with asthma and control group. However, there was a positive dose-response relationship between FEV1 and vitamin D levels, so by increasing the levels of vitamin D, FEV1 increased as well. In addition, in the more severe forms of asthma, vitamin D deficiency was more common.

Authors' Contribution

MRS interpreted the output data, wrote the manuscript and supervised the study designing. EN supervised all stages of the process. MV Developed the concept and designed the study, interpreted the data and helped in clinical aspects of study and revised final version of the manuscript. MJ gave technical support and conducted laboratory studies. JP helped in epidemiological aspect of the study and revised the final report.

Conflict of Interest Disclosures

None of the contributing authors had any conflict of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

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