Original Article

Comparison of Serum Vitamin D Levels Between Asthmatics and Healthy Individuals: A Cross-Sectional Study

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Abstract

Background: Serum vitamin D3 plays a pivotal function not solely in managing mineral homeostasis but also in modulating allergic reactions, cancer and autoimmune diseases. It manages over 200 genes, thus governing cell replication, apoptosis and differentiation. Deficiency of Vitamin D3 in asthmatics is significantly correlated with increased airway sensitivity, impaired pulmonary functions, reduced management of asthma and insensitivity to corticosteroids.

Objective: This research aimed to determine and compare serum levels of vitamin D3, counts of inflammatory cell, and lung function indices between asthmatics and healthy subjects in Lahore.

Methods: This was an observational and cross-sectional study executed at the University of Health Sciences, Lahore, over a 1-year span, involving 60 asthmatic patients as sample and 30 healthy controls. Following a desired clinical assessment, pulmonary function evaluation was conducted using a spirometer, and blood samples were collected for the analysis of inflammatory cell counts and serum levels of vitamin D3. All data were inputted and examined utilizing SPSS version 22. The Kruskal-Wallis test was employed to compare differences in group medians. For Pairwise comparison of groups Mann Whitney U test was applied. A p-value of < 0.05 was considered statistically significant.

Results: The serum vitamin D3 levels were significantly lower among asthmatics compared to controls. Additionally, all participants, including normal controls, exhibited severe vitamin D3 deficiency. Moreover, asthmatic subjects showed a significant decrease in lung function parameters.

Conclusion: The vitamin D3 levels were significantly lower in asthmatics compared to the controls.

Key words: Vitamin D3, Asthma, Lung function test.

Introduction

Bronchial asthma presents as an airway disorder marked by inflammation and hyperresponsiveness of the airway mucosa, resulting in intermittent exacerbations and periods of remission accompanied by symptoms such as cough, dyspnea, and chest tightness.[1,2] As per the 2004 Global Initiative for Asthma (GINA) report, the prevalence of asthma in Pakistan stands at 4.3%.[3]

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Prominent triggers include upper respiratory tract infections, food allergies, exposure to dust, a familial predisposition to allergic conditions, residing in urban areas, and premature cessation of breastfeeding.[4,5]

Inflammation mediated through eosinophils and helper T (Th) cells is pivotal for causing swelling in the mucosal, submucosal, and adventitial layers during airway inflammation, as well as for remodeling the epithelial and subepithelial regions.[6,7] Eosinophil levels in the blood act as an indicator of the severity and marker of lung inflammation.[8] T helper lymphocytes (CD4 cells) can differentiate into Th1 cells, which produce INF-γ, or Th2 cells, responsible for generating Interleukin 4 and 5.

The cytokines from Th2 cells attract more eosinophils and promote increased production of IgE, contributing to delayed allergic responses. Conversely, INF-γ from Th1 cells stimulates higher production of IgG, helping to alleviate delayed allergic reactions.[9]

The initiation and maintenance of immune responses are facilitated by antigen-presenting cells (APCs), which can be directly inhibited by vitamin D3.[10] This vitamin acts as an immune modifier by interacting with its receptor (VDR) on various cells, including T cells and activated B cells.[11] The immune modulating effect may shift the cytokine equilibrium generated by the Th1 and Th2 lymphocytes towards predominance of Th2.[12] However, studies on T cells from human cord evidenced that both Th1 and Th2 lymphocytic cells can be inhibited by vitamin D3.[13] Therefore, these immune-modulating effects of the vitamin D3 on these lymphocytes of Th origin in asthma might depend upon the duration of exposure period to this vitamin.

Furthermore, vitamin D3 plays a critical role in mitigating chronic infections (as tuberculosis) and upper airway infections (UAIs) may be influenza or the common cold, which frequently trigger asthma, particularly in regions like Pakistan.[14] Additionally, vitamin D3 impedes the airway smooth muscle growth, thereby the process of remodeling. Although bronchial smooth muscles in asthmatic as well as in healthy airways tend to proliferate in response to Platelet-Derived Growth Factor (PDGF), the active form of vitamin D3 (calcitriol), counteracts this phenomenon.[15] This remodeling contributes to the bronchial wall thickening, a significant contributor to asthma exacerbations.[16]

The purpose of this study was to measure and compare the serum levels of the vitamin D3 and blood inflammatory cell count in asthmatic individuals and in control group.

Methods

It was a comparative study executed at the Department of Physiology and Cell Biology in the University of Health Sciences Lahore (UHS) and at Gulab Devi Hospital from February 2011 till January 2012. The study approval was by the Ethics review committee of UHS Lahore.

A total number of 60 asthmatics from Gulab Devi Chest Hospital Lahore were included and were segregated into the three groups as: 20 mild asthmatics, 20 moderate asthmatics and 20 severe asthmatics in accordance with the National Heart Lung and Blood Institute (NHLBI) recommendations. A total of 30 age matched healthy individuals were enrolled for the purpose of comparison.[17] Purposive sampling was done.

Desired history was undertaken and clinical assessment was performed after written informed consent. Patients having chronic obstructive airway bronchitis, acute or long term tuberculosis, emphysematous lung problems or any other cardiovascular, endocrinological or some other systemic illness; patients with the vitamin D3 deficiency features such as unexplained muscular pains, bone aches or obvious deformities; parasitic infestation evidence in the last 1 year and those on long term medicines including corticosteroids and supplementation with calcium and vitamin D3 were excluded from the study. Spirometry was performed by using the digital spirometer, Spiro lab II. Forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), ratio between FEV1/FVC and maximum voluntary ventilation were performed. Blood samples were obtained by aseptic techniques and stored in separate aliquots. Serum vitamin D3 levels were assessed by using ELISA and white blood cell count was performed by using the 5-part differential Hematology analyzer.

For statistical conclusion, the software SPSS version 22 was used. Data was presented as median with IQR and Kruskal Wallis statistics was used to analyse group means. For Pairwise comparison of groups Mann Whitney U test was applied. A p-value of < 0.05 was considered statistically significant.

Results

The median (IQR) age, anthropometric indices including height in cm, weight in Kg and BMI in Kg/m^2 of the

Table I: *Baseline anthropometric parameters of the study subjects.*

	N S	M A	Mod A	S A
Parameter	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Age (in years)	22 (18.25-22.75)	19.5 (16.2-22)	20.5 (18.2-22)	18.5 (16.2-22)
Height (cm)	169.5 (157-175)	155 (150-165)	155 (150-167)	156 (152-160)
Weight (kg)	57 (50-71.7)	49.5 (43-55)	47.5 (43.2-58.7)	45.5 (42.5-49.7)
BMI (kg/m^2)	21.95 (18.7-23.7)	20.2 (17.4-23.6)	20.56 (18.4-22.7)	18.7 (17.3-20.1)

A (asthma), M (mild), Mod (moderate), N (normal), S (severe) study subjects is given in table I. The comparison of spirometry values between the normal subjects and the three groups of asthmatic subjects is presented in table II. It was shown that FEV1, FVC, ratio of FEV1/FVC and MVV were all lower in asthmatics as compared to healthy subjects. A comparison of blood parameters such as serum vitamin D3 levels and white blood cells counts is presented in table IV which shows that serum vitamin D3 levels were significantly less in the asthmatics than in the healthy ones. Conversely, the white blood cells were significantly higher in the asthmatics than in the normal ones.

Table II: *Comparison of lung function tests among the study groups.*

Indices	N A	M A	Mod A	S A	*p*-value
FEV_1 (in liters)	3.35 (2.78-3.62)	2.32 (2.0-2.6)	1.75 (1.4-2.06)	1.15 (0.96-1.17)	0.001*
FVC (liters)	3.66 (3.04-4.18)	2.72 (2.4-3.4)	2.46 (2.07-2.76)	1.71 (1.35-2.2)	0.001*
FEV_1 to FVC (%)	88.48 (85.28-93.9)	81.03 (76.8-90.3)	74.25 (67.6-82.1)	71.73 (56.5-75.1)	0.001*
MVV (liters/min)	126.2 (103.8-144)	77.45 (71.9-94.4)	68.7 (56.7-82.7)	41.9 (35.3-51.4)	0.001*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Kruskal Wallis stats was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

Table III: *Comparison of lung function tests pairwise*

Variables	NS. vs SA.	*p*-value	N S. vs Mod A.	*p*-value	SA. vs Mod A.	*p*-value
FEV_1 (liters)	3.35 vs 1.15	0.002*	3.35 vs 1.75	0.002*	1.15 vs 1.75	0.02*
FVC (liters)	3.66 vs 1.71	0.001*	3.66 vs 2.46	0.003*	1.71 vs 2.46	0.04*
FEV_1/ FVC (%)	88.48 vs 71.73	0.001*	88.48 vs 74.25	0.001*	71.73 vs 74.25	0.02*
MVV (liters/min)	126.2 vs 41.9	0.001*	126.2 vs 68.7	0.002*	41.9 vs 68.7	0.002 *

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Mann Whitney Test was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

Discussion

The current study investigated serum levels of vitamin D3 and asthma severity markers in both normal individuals and those with asthma. It also assessed pulmonary function through tests, inflammatory cell counts, and anthropometric measurements. The findings revealed notable disparities in vitamin D3 levels across the study cohorts. Specifically, among normal subjects, the median vitamin D3 level was 13.48 ng/ml. Mehmood and companions (2009) described deficiency of vitamin D3 as

Table IV: *Comparison of blood parameters among the study groups*

Variables	N S	M A	Mod A	S A	*p*-value
Total leukocytic count ($x10^3/mm^3$)	8.24(7.2-8.7)	8.86(7.6-10.6)	9.04(7.9-10.5)	12(8.9-12.09)	0.002*
Eosinophilic count ($x10^3/mm^3$)	0.30(0.08-0.56	0.56(0.45-0.68)	1.35(0.42-1.4)	1.2(0.5-1.9)	0.001*
Lymphocytic count ($x10^3/mm^3$)	2.42(1.6-2.6)	2.28(2.1-3.05)	3.20(2.24-3.3)	2.90(2.2-3.1)	0.003*
Levels of Vitamin D_3 in serum (ng/ml)	13.48(8.8-21.9)	7.21(4.5-9.6)	9.51(6.2-12.5)	6.65(4.51-8.59)	0.001*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Kruskal Wallis stats was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

Table V: *Comparison of blood parameters pairwise*

Variables expressed in median	NS. vs SA.	*p*-value	N S. vs Mod A.	*p*-value	SA. vs Mod A.	*p*-value
Total leukocytic count ($x10^3/mm^3$)	8.24 vs 12	0.001*	8.24 vs 9.04	0.032*	12 vs 9.04	0.001*
Eosinophilic count ($x10^3/mm^3$)	0.3 vs 1.2	0.001*	0.3 vs 1.35	0.001*	1.2 vs 1.35	0.04*
Lymphocytic count ($x10^3/mm^3$)	2.42 vs 2.9	0.021*	2.42 vs 3.20	0.001*	2.9 vs 3.20	0.25
Levels of vitamin D_3 in serum (ng/ml)	13.48 vs 6.65	0.001*	13.48 vs 9.51	0.002*	6.65 vs 9.51	0.002*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Mann Whitney Test was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

levels below 20 ng/ml in serum, indicating that the study healthy population group exhibited this deficiency.[18] Individuals with mild asthma showed a median vitamin D3 level of 7.21 ng/ml, those with moderate asthma had median of 9.51 and those with severe asthma had a level of 6.65 + 2.84 ng/ml. The measures differed significantly from each other.

According to Karim et al.'s classification from 2011, levels of vitamin D3 less than 10 ng/ml are considered severely deficient, which can lead to various health complications.[19] This cut off is supported by several studies conducted in Lahore. For instance, Haque et al. found that 66% of healthy population had vitamin D3 measure less than 20 ng/ml.[20] In this study, there is a significant difference in vitamin D3 levels among asthmatics, and levels were as low as 6.65 ng/ml in severely asthmatic group. Restricted data exists regarding vitamin D3 deficiency in the healthy Pakistani population, and no study has specifically evaluated serum vitamin D3 levels in asthmatics until now.[19] To our knowledge, this study is the first of its kind among asthmatics aged 12 to 25 years.

The pulmonary function parameters analyzed in our study exhibited significant variations among the different groups examined, encompassing FEV1, FVC, the FEV1/FVC ratio, and MVV. These outcomes are consistent with earlier findings by Ayub and companions in 1997, suggesting that lung volumes even in healthy adults are comparatively lower than those in similar European population.[21] The median values for FEV1, FVC, and the FEV1/FVC ratio in our study align closely with those reported by Memon et al. in 2007, although their study encompassed individuals aged 15 to 65 years.[22] However, our results diverge from those presented by Khan and Saadia in 2006, who observed higher mean values for FEV1 (2.38 liters), FVC (3.35 liters), and FEV1/FVC ratio (70%) in subjects aged 19.5-30 years.[23] In our study, these parameters were notably lower in the asthmatic group. Additionally, our findings contrast with the values documented by Williams et al. in 1978, who noted lower FEV1 (2.28 liters) and FVC (2.76 liters) values in adult nonsmoker females from Lahore aged 21.8 years in classical literature.[24]

Conclusion

A marked insufficiency of serum vitamin D3 is evident in both asthmatic patients and in the control group of healthy individuals. Asthmatic participants demonstrate a decline in lung function test outcomes, while there is a rise in the number of inflammatory leukocytes, which correlates with the severity of the disease.

Conflict of Interest: *None*

Funding Source: *None*

Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution

All the authors contributed equally in accordance with ICMJE guidelines and are accountable for the integrity of the study.

HSK: Conception of idea & design, acquisition, analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

HMW: Acquisition of data, drafting the article, critical review, final approval of the manuscript

AS: Analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

FA: interpretation of data, final approval of the manuscript

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