



Correlation of Serum IL-17 with Level of Vitamin D and IgE in Asthmatic Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FN, NA and SJ designed the study, authors FN, FS, MK and KJ wrote the protocol, and wrote the first draft of the manuscript. Authors FN, NA, FS, SJ, WL and MK managed the literature searches, analyses of the study performed the ELISA analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Allergic asthma is a chronic inflammatory disorder of the airways caused by hypersensitivity and characterized by Th2 cytokine i.e. IL-4, that contribute to enhanced proliferation and differentiation of Th2 cells and switch of B cells from IgM to IgE production. Vitamin D also has a role in proliferation, differentiation, apoptosis of cells of the immune system, production of cytokines, and immunoglobulins. Elevated levels of IL-17 have been documented in bronchial submucosa and relationship of IL-17 and severe hyper responsiveness of airways have been established. Therefore, a study was conducted to determine serum level of vitamin D, IgE and IL-17 in patients of bronchial asthma.

Methodology: It was a comparative study, which included 82 subjects i.e. Group-I (41 subjects without asthma), and group-II (41 asthmatic patients). Serum levels of IL-17, IgE and vitamin D

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were determined by ELISA technique and the results were analyzed by using SPSS-20.0. Mann-Whitney-U, Chi-square and Pearson's correlation tests were used for data analysis.

Results: Mean \pm SD of total serum IgE level of bronchial asthma patients was high (861.6 \pm 559.2 IU/ml) compared to controls (204.7 \pm 237 IU/ml) and on comparison there was statistically significant difference (p value 0.000). Mean \pm SD of serum vitamin D level in asthmatics was 28.04 \pm 17.67 ng/ml while in non-asthmatics it was 27.27 \pm 17.01 ng/ml and on comparison the difference was not statistically significant ($p=0.915$). Mean \pm SD of serum level of IL-17 was high in asthmatics (920.08 \pm 732 pg/ml) as compared to non-asthmatics (767.71 \pm 811 pg/ml) and on comparison the difference was not statistically significant ($p=0.108$). In asthmatics, there was positive correlation of serum level of vitamin D and IL-17 ($r=0.101$) and there was an association ($p=0.001$) of family history with asthma.

Conclusion: Asthmatics had raised serum level of IgE, vitamin D and IL-17 as compared to controls and on comparison only the difference of IgE was statistically significant. Further, an association of family history of asthma was observed in asthmatic patients.

Keywords: Asthma; IL-17; Vitamin D; IgE.

1. INTRODUCTION

Allergic asthma is characterized as chronic inflammatory disorder of airways caused by hypersensitivity reactions that is characterized by wheezing, breathlessness, chest tightness, cough and bronchial hyper-responsiveness [1]. Around the world asthma prevails at variable frequency and it explains the influence of environmental factors on genetic predisposition of individuals for this disease [2,3]. It was documented that about 235 million people were suffering from asthma (WHO fact sheet N*306 May 2011), and this number is expected to be 400 million by 2025 [4].

In Pakistan, more than six million people were suffering from asthma, and Karachi, the largest city of Pakistan, had almost 8-10% of population suffering from chronic asthma and one out of 250 deaths in the city was due to exacerbations of asthma [5].

In allergic asthma, IL-4 enhances proliferation and differentiation of Th2 cells and switches B cells from IgM to IgE production [6]. On immunohistochemical analysis, bronchial biopsy of airways of asthmatic patients revealed infiltration of inflammatory cells such as mast cells, basophils, eosinophils, monocytes, and Th2 lymphocytes [7].

In asthma, elevated levels of IL-17 have been documented in bronchial submucosa and sputum [8]. A direct relationship of IL-17 production and severe airways hyper responsiveness has been established. Researchers suggest that aberrant interleukin (IL)-17 production is a key determinant of severe forms of asthma. The IL-17

and the factors regulating IL-17 production during the course of allergic inflammation remain unknown [9].

It is estimated that about one billion people throughout the world have vitamin D deficiency or insufficiency. This figure includes subjects of all age groups, races, and gender throughout Asia, America, Europe and even population of equator region [10].

Vitamin D has profound effects on human immune system, e.g. it acts as an immunomodulator, prevents excessive production of inflammatory cytokines and enhances 'oxidative burst' in tissue macrophages [11]. Vitamin D also has a role in proliferation, differentiation, and apoptosis of cells of immune system, in addition to production of cytokines, and immunoglobulin [12]. An inverse relationship has been observed between serum level of vitamin D with total IgE, eosinophil count, and airways hyper-responsiveness [13].

Some studies have reported a greater risk of asthma and atopy in adults who had received supplements of vitamin D in childhood. They suspected that increase risk of asthma could be due to food fortified with vitamin D [14].

By reviewing all this literature regarding the conflicting role of IL-17 and serum vitamin D level in asthmatic patients [15], the current study was carried out to compare the serum vitamin D, IL-17 and serum IgE levels in asthmatic patients and healthy individuals.

2. MATERIALS AND METHODS

The study was carried out in the Department of Immunology, University of Health Sciences (UHS)

Lahore after the approval of Ethical Review Committee and Advance Studies & Research Board of UHS. The samples were collected from Asthma Clinic, Gulab Devi Hospital Lahore and Chest Disease Unit, Sheikh Zayed Hospital Lahore (Pakistan). After an informed consent, a total of 82-subjects were recruited for this study and they were made into two groups of 41 individuals in each group. Group-I included non-asthmatic healthy individuals as control, whereas group-II had clinically diagnosed asthmatic patients with the history of allergy and they were selected with the help of a modified validated questionnaire of European Community Respiratory Health Survey (ECRHS).

2.1 Determination of serum IL-17A and Vitamin D [25(OH)D]

Serum level of IL-17A and Vitamin D were determined using ELISA kits from Komabiotech (Korea) and Euroimmun (USA). The plates were read at 450 nm using micro-plate reader (BioRad, USA). Calibrators' curve was made by reducing the data by using ELISA reader's computer software (Micro-plate Manager Version 2.2).

2.2 Determination of Serum IgE

Serum IgE was determined by ELISA technique by using kits from Komabiotech (Korea). According to Bischoff-Ferrari et al. [16], serum vitamin D level was classified into three categories i.e. sufficient level (30ng/ml), insufficient level (20-29 ng/ml), and deficient level (<20 ng/ml).

2.3 Statistical Analysis

The data was analysed using IBM SPSS (Statistical Package for Social Sciences) 20.0. Mann-Whitney-U test was applied for comparison of serum vitamin D and IL-17 levels whereas for their correlation Pearson's correlation test was used. Chi-square test was applied to determine association of family history of asthma. A p-value of ≤ 0.05 was considered as statistically significant.

3. RESULTS

3.1 Vitamin D

Mean±SD of serum level of vitamin D in asthmatic patients was 28.04±17.67ng/ml while in healthy controls it was 27.27±17.01ng/ml, and on comparison, the difference was not statistically significant (p=0.915). According to Bischoff-Ferrari et al. [16] serum vitamin D level of the study subjects was classified into three categories i.e. deficient, insufficient and sufficient. The level of Vitamin D in asthmatic and healthy control, in all the three categories was almost same (p=0.949) (Table 3).

3.2 IL-17

Mean±SD of IL-17 was high in asthmatic patients (920.08±732 pg/ml) as compared to healthy controls (767.71±811 pg/ml), and on comparison, the difference was not significant (p=0.108).

By applying Pearson's correlation test, positive correlation was observed between serum level of vitamin D and IL-17 in healthy subjects with correlation coefficient, r=0.194 (Fig. 1) and in asthmatic patients with correlation co-efficient of r=0.101 (Fig. 2).

3.3 IgE

Mean ± SD of total serum IgE concentration was high in bronchial asthma patients (861.6±559.2 IU/ml) compared to controls (204.7±237 IU/ml). On comparison the difference was statistically significant (p<0.001) (Table 2).

Among the asthmatic patients, high number of subjects 18 (43%) had family history of asthma, while only 5 (12%) of healthy controls had family history of asthma. On comparison, the difference between the two groups was statistical significant (p= 0.001). By applying Chi-square test, a positive association was observed in asthmatic patients with the family history of asthma. Number and comparison of subjects with family history of asthma in two groups is presented in Table 4.

Table 1. Mean±SD of age, number and percentage of gender and family history of asthma in two groups

Group	Age (Mean±SD)	Gender		Family history of asthma	
		F [n (%)]	Yes [n (%)]	Yes [n (%)]	No [n (%)]
I	26±3.99	30 (73)	11 (27)	5 (12)	36 (88)
II	27±8.43	16 (39)	25 (61)	18 (43)	23 (57)

n= number of subjects, M= male subjects, F= female subjects

Table 2. Mean±SD and comparison of Vitamin D, IL-17 and IgE level between two groups

Parameters	Group-I (Mean±SD)	Group-II (Mean±SD)	p-value
Vitamin D (ng/ml)	27.27±17.01	28.04±17.67	0.915
IL-17 (pg/ml)	767.71±811	920.08±732	0.108
IgE (IU/ml)	204.7±237	861.6 ±559	<0.001

*p value ≤0.05 = statistically significant

Table 3. Categorization and comparison of serum vitamin D level in two groups

Group (n)	Deficient (<20ng/ml)	Insufficient (20-29ng/ml)	Sufficient (≥30ng/ml)	p-value
I (41)	18	6	17	0.949
II (41)	17	7	17	
Total (82)	35	13	34	

n=number, *p value 0.05=statistically significant

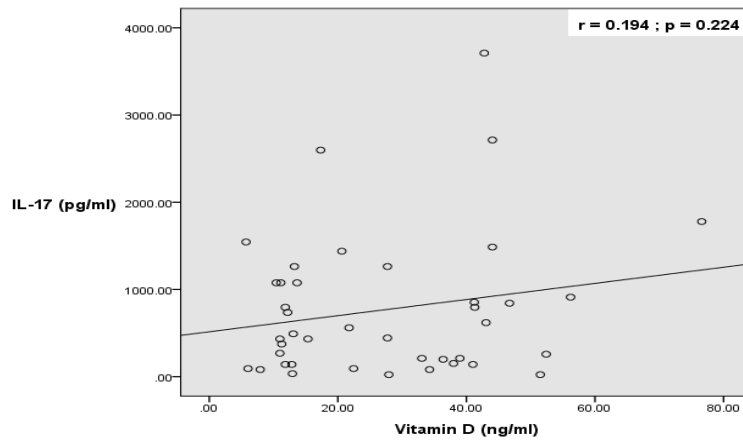


Fig. 1. Correlation of serum levels of vitamin D and IL-17 in healthy controls X-axis: Level of vitamin D, Y-axis: level of IL-17

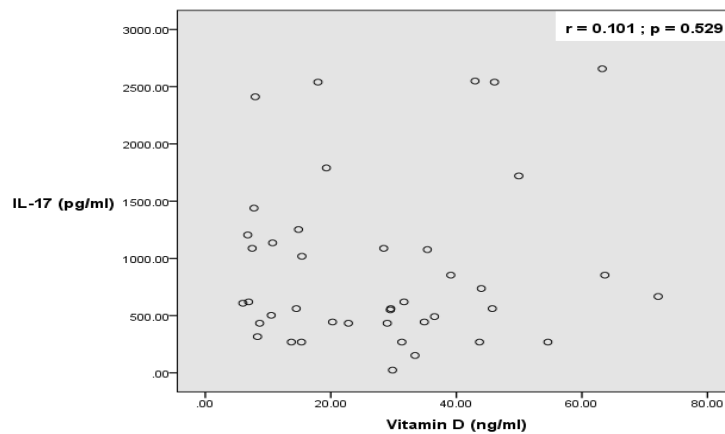


Fig. 2. Correlation of serum levels of vitamin D and IL-17 in asthmatic patients X-axis: Level of vitamin D , Y-axis: level of IL-17

Table 4. Number and comparison of subjects with family history of asthma in two groups

Groups (n)	Subjects with history of asthma	Subjects with no history of asthma	p-value
I (41)	05	36	0.001*
II (41)	18	23	
Total (82)	23	59	

*p value 0.05=statistically significant

4. DISCUSSION

According to Bischoff-Ferrari et al. [16], serum vitamin D level of the study subjects was classified into three categories i.e. sufficient level (≥ 30 ng/ml), insufficient level (20-29 ng/ml), and deficient level (< 20 ng/ml). Among the asthmatic patients serum level of vitamin D was sufficient in 42%, insufficient in 15%, and deficient in 43% of the subjects.

In the current study sufficient level of vitamin D in asthmatic patients was detected that could be due to the fact that asthmatic patients who visit Gulab Devi Hospital, Lahore are normally advised by the physicians to remain in the direct sun light for 1-2 hours daily.

These findings are in concordance with the study of Menon et al. [17] who performed the study in asthmatic patients of 2-19 years of age and could not determine significant difference between the serum level of vitamin D of asthmatic patients (28.64 ± 10.09) and healthy controls (28.42 ± 11.47) ($p=1.0$). Similar results have also been reported by American Academy of Allergy Asthma and Immunology (2012) that documented almost same level of vitamin D in asthmatics and general population. They, further reported that 50% of healthy controls and asthmatics had deficient serum level of vitamin D (< 20 ng/ml) and higher prevalence of vitamin D deficiency in adult population than children [18]. The findings of Maalmi and his co researchers are also in accordance with the present study because they also could not observe significant difference in the serum level of vitamin D of healthy subjects and asthmatic patients [19].

The current study is not in agreement with Berhm et al. [20] who demonstrated an association among low levels of vitamin D and serum IgE levels, eosinophil counts, hospitalizations in the last year and the use of inhaled corticosteroid in a group of 1024 children of 7.5-10.5 years of age. The current study is not in agreement with Berhm

et al. [13], who performed study on 1041 asthmatic subjects and suggested association of vitamin D insufficiency (< 30 ng/ml) with increased risks of severe asthma leading to visits of emergency room or hospitalizations. Further, current study is also not in agreement with Berhm et al. [21] who documented vitamin D deficiency in children of severe asthma as compared to healthy controls ($p=0.001$). Differences in geographic distribution of study population, ethnicity, disease duration, treatment modalities etc. may be the reason for these conflicting results.

Considering the serum level of IL-17, it was high (920.08 ± 732 pg/ml) in asthmatic patients as compared to healthy controls (767.71 ± 811 pg/ml) but it was not statistically significant ($p=0.108$) (Table 2). Although, at the time of sample collection, patients on anti-inflammatory drugs were excluded but since patient's level of education was low as they were recruited from a public hospital therefore asthmatic patients might had inflammation.

Wong et al. [22] documented high serum level of IL-17 in asthmatics as compared to controls with no statistically significant difference. Our findings are also in agreement with Frascchi et al. [23] who detected high serum level of IL-17 in asthmatics (78.8 ± 58.7 pg/dl) as compared to controls (17.7 ± 2.4 pg/dl). Similarly, Agachi et al. [24] and Alyasin et al. [25] detected increased serum level of IL-17 in severe asthma as they included asthmatic patients of different severity. They proposed > 20 pg/ml of serum level of IL-17 as a risk factor for severe asthma.

The current study is in agreement with Bullen et al. [26] who detected raised sputum level of IL-17 and its mRNA in 39 asthmatics as compared to 15 healthy subjects and a statistically significant difference between the two groups ($p=0.01$) on comparison. The probable reason for the significant difference could be use of sputum samples as there could be infiltration of Th-17 in air ways of asthmatics.

Sudha et al. [27] observed raised IL-17 levels in the cell cultures of peripheral blood mononuclear cells (PBMC) of 12 asthmatics (488.50±61.84 pg/dl) as compared to 12 healthy controls (187.96±19.6 pg/dl), and on comparison the difference was statistically significant between the two groups. The probable reason for their significant difference could be the use of cell culture of PBMC.

A study carried out in Pakistan by Afzal et al. [28] who documented significantly decreased serum levels of IL-17 in diabetic patients (718.05 pg/ml) as compared to healthy subjects (375 pg/ml) (p=0.0026), but their study was carried out in diabetic patient of retinopathy.

We found, a positive correlation between the serum level of vitamin D and IL-17 in asthmatic patients (r=0.101) but this correlation was statistically insignificant (p=0.529). Nanzer et al. [19] and Sun et al. [29], also documented a positive correlation between vitamin D and IL-17 in asthmatic patients ($\beta = 0.26$, $p = 0.025$).

A quite contradictory finding was reported by Maalmi et al. [15] who documented raised serum IL-17, deficient vitamin D levels and negative correlation of serum vitamin D and IL-17 levels in asthmatic patients (r=0.617).

Increased IgE levels have been documented in a number of conditions e.g. atopic diseases [30], parasitic and non-parasitic infections [31], inflammatory diseases, hematologic malignancies, primary immunodeficiency, etc [32]. Total serum IgE concentration is also affected by genetic makeup, race and immune status of the person [31]. Demirjian et al. [33] concluded increased total serum IgE level in atopic asthma patients. Masoud et al. [34] documented raised mean total IgE levels in positive skin prick test pediatric asthma patients. Similarly, Mishra et al. [35] reported significantly increased total serum IgE in pediatric bronchial asthma. The current study confirms the findings of the above mentioned studies.

5. CONCLUSION

Raised serum level of IL-17 was observed in asthmatic patients as compared to healthy controls but serum level of vitamin D was almost same in both the groups, therefore, targeting IL17 rather vitamin D, might be more useful in the clinical management of bronchial asthma.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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