

ORIGINAL ARTICLE

Serum levels of IL-10, IL-17F and IL-33 in patients with asthma: a case–control study

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Abstract

Objectives: The development of inflammation in asthma involves an intricate network of cytokines that recruit and activate numerous immune cells. This study was aimed to compare serum levels of IL-10, IL-17F, and IL-33 in asthmatic patients and non-asthmatic controls and correlate cytokine levels to asthma severity and various clinical, spirometric, and laboratory variables. **Methods:** Using ELISA, serum levels of IL-10, IL-17F, and IL-33 were evaluated in 44 asthmatics (14 mild persistent, 15 moderate persistent, and 15 severe persistent) and 44 controls. **Results:** This is one of the first reports showing a significant difference in serum levels of asthma-associated cytokines, anti-inflammatory IL-10, and pro-inflammatory IL-17F and IL-33, in the same subset of asthmatic patients. Our results showed diminished level of IL-10 and elevated levels of IL-17F and IL-33 in asthmatics than in controls ($p < 0.001$). Assessment of cytokine levels between subjects of different gender, age group, and BMI showed non-significant differences. Correlation analysis of cytokine levels to clinical variables showed that IL-17F is associated negatively to FVC % predicted (forced vital capacity) and FEV1% predicted (forced expiratory volume in one second) and positively to number of allergens sensitized and FEV1 reversibility. A strong negative correlation was found between IL-10 and IL-33 levels ($p = 0.001$). **Conclusions:** Negative correlation between IL-10 and IL-33 levels may reflect a converse relationship between anti-inflammatory and pro-inflammatory cytokines in an individually balanced pattern. The association between IL-17F level and asthmatic phenotypes such as reduced FVC and FEV1, higher degree of sensitization, and post-bronchodilator reversibility needs further assessments.

Keywords

Biomarkers, immunopathogenesis, phenotype

History

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Introduction

Asthma is a chronic inflammatory disorder of the airways characterized by bronchial hyper-responsiveness, reversible airflow obstruction, airway remodeling, allergen-specific IgE (Immunoglobulin E) production, as well as, eosinophils and T cells infiltration into the airways [1,2]. Due to increasing urbanizations, the number of asthmatics is likely to increase from the existing 300 million cases to 400 million by 2025 [3]. Although several therapeutic approaches have been taken so far, outcomes have been disappointing especially in case of severe asthma. One of the main reasons for such poor results is that the persistent airway inflammation in asthma is caused by a large variety of cytokines that recruit, activate,

and perpetuate the inflammatory cells in the asthmatic airways. Furthermore, because of redundancy in cytokine effects, several attempts to inhibit inflammatory cytokines in asthma with blocking antibodies have failed so far [4,5].

IL-10 is a potent anti-inflammatory cytokine that down-regulates the synthesis of Th1 (T helper 1) and Th2 (T helper 2)-associated cytokines, chemokines, and inflammatory enzymes [6,7]. It contributes to mitigation of allergic responses by induction of allergen tolerance [8,9] and inhibition of eosinophil survival [10], IgE synthesis [11], and monocyte/macrophage activation [12]. IL-33, also called IL-1F11 or nuclear factor from high endothelial venules (NF-HEV), is an inducer of Th2 immune responses that trigger the expression of pro-inflammatory cytokines, chemokines, and lipid mediators through a complex including IL-1 receptor ST2 protein [13–16]. Studies have shown that the expression of IL-33 is involved in allergen-induced airway inflammation, airway hyper responsiveness [17], mucus overproduction, serum IgE elevation, and eosinophils and

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plasma cells infiltration [13]. The Th17 – associated cytokine, IL-17F – is a pro-inflammatory cytokine that can induce the expression of various inflammatory cytokines, chemokines, adhesion molecules, and growth factors [18–21]. Expression of these molecules is responsible for leukocyte activation, neutrophils infiltration, pulmonary mucus hypersecretion, and airway remodeling, commonly observed in patients with asthma [22,23]. To the best of our knowledge, no study has made a direct comparison of IL-10, IL-17F, and IL-33 to evaluate their association with asthma and its severity; therefore, this study was conducted to evaluate serum cytokines IL-10, IL-17F, and IL-33 in asthmatic patients compared with non-asthmatic controls and investigate whether their levels were associated with asthma severity classified according to GINA guidelines.

Materials and methods

Study participants

This study was conducted on 44 asthmatic patients and 44 non-asthmatic healthy controls. Subjects with bronchial asthma were recruited from “Allergy, Asthma and Chest Center” (Mysore, India) while age and gender-matched controls were collected randomly from the general population. None of the control subjects had medical history of asthma or other respiratory diseases and all of them showed normal lung function in pre- and post-bronchodilator pulmonary function test (PFT). All the asthmatic cases were treatment naive (not on regular inhaled corticosteroids) at the time of serum sampling. Spirometry to confirm asthma was carried out according to American Thoracic Society (ATS) standards [24]. Diagnosis of asthma and its severity was performed according to the current Global Initiative for Asthma (GINA) guidelines. Asthmatic subjects were classified into three groups of mild persistent, moderate persistent, and severe persistent [25]. To avoid conditions that can affect cytokine serum levels, patients with other related inflammatory or immune diseases, liver diseases, cardiovascular diseases, type 2 diabetes, malignancy, hypertension, and organ transplantation were excluded from the study. This study was approved by the university ethical committee, with Institutional Human Ethical Committee (IHEC) numbered IHEC-UOM No. 80 PhD/2012-13, and accordingly an informed written consent was obtained from all the participants. Two milliliter of venous blood was collected from patients and controls. Samples were allowed to clot, and serum was separated by centrifuging at 2000g for 10 min. Finally, isolated serum samples were transferred to sterile eppendorf tubes and stored at -80°C for the future analysis.

Determination of the levels of IL-10, IL-17F, and IL-33 in serum samples:

Commercial ELISA kits from Ray Biotech Inc. (Norcross, GA) were used to measure the serum levels of IL-10, IL-17F, and IL-33 [Catalog nos.: ELH-IL10-001, ELH-IL17F-001, and ELH-IL33-001]. The test procedure was performed according to the manufacture's guidelines. In brief, 100 μl of each standard and samples were added into the pre-coated wells and allowed to incubate overnight at 4°C . The wells

were washed, 100 μl of biotinylated antibody was added and allowed to incubate for 1 h at room temperature. After washing (3 times), 100 μl HRP-conjugated streptavidin solution is pipetted to the wells, and the plate was incubated at room temperature for 45 min. The wells were washed, 100 μl of TMB (3,3',5,5'-tetramethylbenzidine) substrate solution was added. The plate was incubated for 30 min at room temperature and the color development stopped by adding 50 μl stop solution. The intensity of color was measured at 450 nm in a plate reader (BIO-RAD, Berkeley, CA). The minimum detectable level of IL-10, IL-17F, and IL-33 is less than 1 pg/ml, 20 pg/ml, and 2 pg/ml, respectively.

Association of cytokine serum levels with asthma duration, allergen sensitization, and severity of the sensitization

In skin prick test (SPT), sensitization to a total of 35 allergens (HollisterStier Allergy, Spokane, WA) was assessed in which the wheal size greater than 3 mm of saline control was considered as positive [26]. The severity of sensitization was characterized by comparing the largest wheal diameter measured for an allergen with that of histamine. Histamine was used as a positive control. The severity of sensitization on SPT was assessed according to Aas and Belin [27] in which allergen wheal size of greater than histamine was graded as 4+. For correlation studies, subjects with different cytokine levels were classified according to the duration of asthma, the number of allergens sensitized, and the severity of sensitization.

Association of serum cytokine levels with absolute eosinophils count (AEC), total serum IgE, and E (eosinophil) count

AEC and E count was carried out in a complete blood count (CBC) analyzer (Mindray, BC3000 plus, Guangdong, China). Total serum IgE measurement was performed using ELFA (Enzyme-Linked Fluorescent Assay) (Minividas, Biomerieux Vitek Inc, Marcy, France). For correlation studies, subjects with different cytokine levels were classified into two groups, namely (a) participants with normal values of AEC, total serum IgE, and E count; (b) patients with values of AEC, IgE, and E count more than the normal controls. The AEC value of 440 cells/mm^3 (cells per cubic millimeter), total serum IgE value of 120 kUA/l (kilo units per liter, where A represents the amount of allergen-specific IgE antibody in the blood), and E% value of 6% were considered as upper limits of normal.

Association of serum cytokine levels with FEV1 improvement following inhaled corticosteroids (ICS) and long acting β_2 agonist (LABA) treatment

FEV1 (mL) in asthmatic patients was compared before and after 2 months of treatment with ICS and LABA and the improvement in FEV1 (mL) was correlated to serum cytokine levels in asthmatic subjects undergoing treatments.

Statistical analysis:

Data are presented as mean $\pm$ standard error of mean. The sample results were calculated by interpolation from a

standard curve that was carried out in the same assay as the sample. Final results were obtained by multiplication of the results with the dilution factors. Statistical analysis was carried out using the Statistical Package for the Social Sciences version 19 (SPSS, Inc, Chicago, IL). As with non-parametric data, Mann–Whitney *U*-test and Kruskal–Wallis test were used to assess differences between asthmatic and control groups and within asthmatic subgroups, respectively [28]. A *p* value < 0.05 was considered as significant. To establish the upper and lower limits of cytokines normal ranges, 95% confidence interval of the mean was calculated for all three cytokines. Correlations were evaluated using Spearman rank (*rs*) for non-parametric data and Pearson correlation coefficient (*r*) for parametric data [29].

Results

Demographic characteristics of the study population are presented in Table 1. Asthmatic patients were classified into three subgroups of mild persistent (*N* = 14), moderate persistent (*N* = 15), and severe persistent (*N* = 15) according to the severity of disease. The average age of the patients was 38.4 ± 13.77 years. The mean concentration of anti-inflammatory cytokine IL-10 has decreased from 14.95 ± 1.65 pg/ml in controls to 3.16 ± 0.42 pg/ml in cases (nearly 5-fold decrease); while the mean concentration of pro-inflammatory cytokines IL-17F and IL-33 was increased from 224.29 ± 23.84 pg/ml in controls to 3526.41 ± 573.81 pg/ml in cases (nearly 16-fold increase) and from 0.51 ± 0.26 pg/ml in controls to 79.10 ± 20.62 pg/ml in cases (about 155-fold increase), respectively (Table 1). On subgroup analysis, a significant difference in the levels of all three cytokines was observed between each of the severity groups and the controls

(*p* < 0.001, Figure 1). While no significant difference in the levels of IL-10 and IL-33 was observed within asthmatic subgroups, a significant difference in the level of IL-17F was noticed across asthma severity groups (Figure 2). Comparison of IL-10, IL-17F, and IL-33 serum levels between subjects of different gender (male and female), age group (≤ 40 years, > 40 years) and BMI (normal and overweight) in both cases and controls revealed that while neither asthmatics nor controls show significant difference within their own group, a significant difference is observed between asthmatic and control groups (Figure 3).

Further evaluation of cytokines levels between subjects with either normal or above normal values of total serum IgE, E count, and AEC showed no difference in the cytokines levels in subjects with either above normal or normal values of total serum IgE and AEC, however, a significant difference in all three cytokine levels was demonstrated in patients with different E% groups (Table 2). Assessment of IL-10, IL-17F, and IL-33 levels between asthmatic patients with different numbers of allergens sensitized (≤ 5 allergens, > 5 allergens), asthma duration (≤ 5 years, > 5 years), and sensitization level ($\leq$ histamine, $>$ histamine) showed a significant difference only with respect to the serum level of IL-17F in patients with higher number of allergens sensitized (*p* = 0.041, Table 2).

Correlation analysis of cytokine levels to various clinical variables including age, gender, BMI, asthma duration, number of positive allergens, sensitization level, total serum IgE level, E count, AEC, FEV1% predicted (forced expiratory volume in 1 s), FVC1 % predicted (forced vital capacity), FEV1/FVC (% predicted), PEF% predicted (peak expiratory flow), and degree of FEV1 reversibility showed that serum level of IL-17F is associated negatively to FVC % predicted

Table 1. Demographic characteristics of the study population.

	Controls	Cases	<i>p</i> Value
Age			
≤ 40 years	25	24	
> 40 years	19	20	
Sex			
Male	22	22	
Female	22	22	
Smoking status			
Smoker	0	1	
Non-smoker	44	43	
Pulmonary function test	Pre-bronchodilator	Pre-bronchodilator	
Mean $\pm$ SE of FVC [L]	3.05 ± 0.10	2.58 ± 0.17	0.020
Mean $\pm$ SE of FEV1 [L]	2.52 ± 0.08	2.02 ± 0.14	0.004
Mean $\pm$ SE of FEV1/FVC%	83.38 ± 0.83	73.34 ± 2.99	0.009
Mean $\pm$ SE of PEF [L/s]	7.09 ± 0.29	37.56 ± 16.93	0.009
Mean $\pm$ SE of MEF25-75 [L/s]	2.91 ± 0.17	2.00 ± 0.20	0.001
Severity			
Mild persistent	–	14	
Moderate persistent	–	15	
Severe persistent	–	15	
Mean concentration $\pm$ SE of IL-10 (pg/ml)	14.95 ± 1.65	3.16 ± 0.42	<i>p</i> < 0.001
95% CI lower and upper limits of IL-10 (pg/ml)	12.10–18.71	2.31–3.95	
Mean concentration $\pm$ SE of IL-17F (pg/ml)	224.29 ± 23.84	3526.41 ± 573.81	<i>p</i> < 0.001
95% CI lower and upper limits of IL-17F (pg/ml)	180.11–269.17	2365.89–4627.08	
Mean concentration $\pm$ SE of IL-33 (pg/ml)	0.51 ± 0.26	79.10 ± 20.62	<i>p</i> < 0.001
95% CI lower and upper limits of IL-33 (pg/ml)	0.07–1.10	44.55–121.68	

CI, confidence interval, SEM, standard error of mean. Values are presented as mean concentration in pg/ml $\pm$ SE. *p* Value < 0.05 is considered as significant.

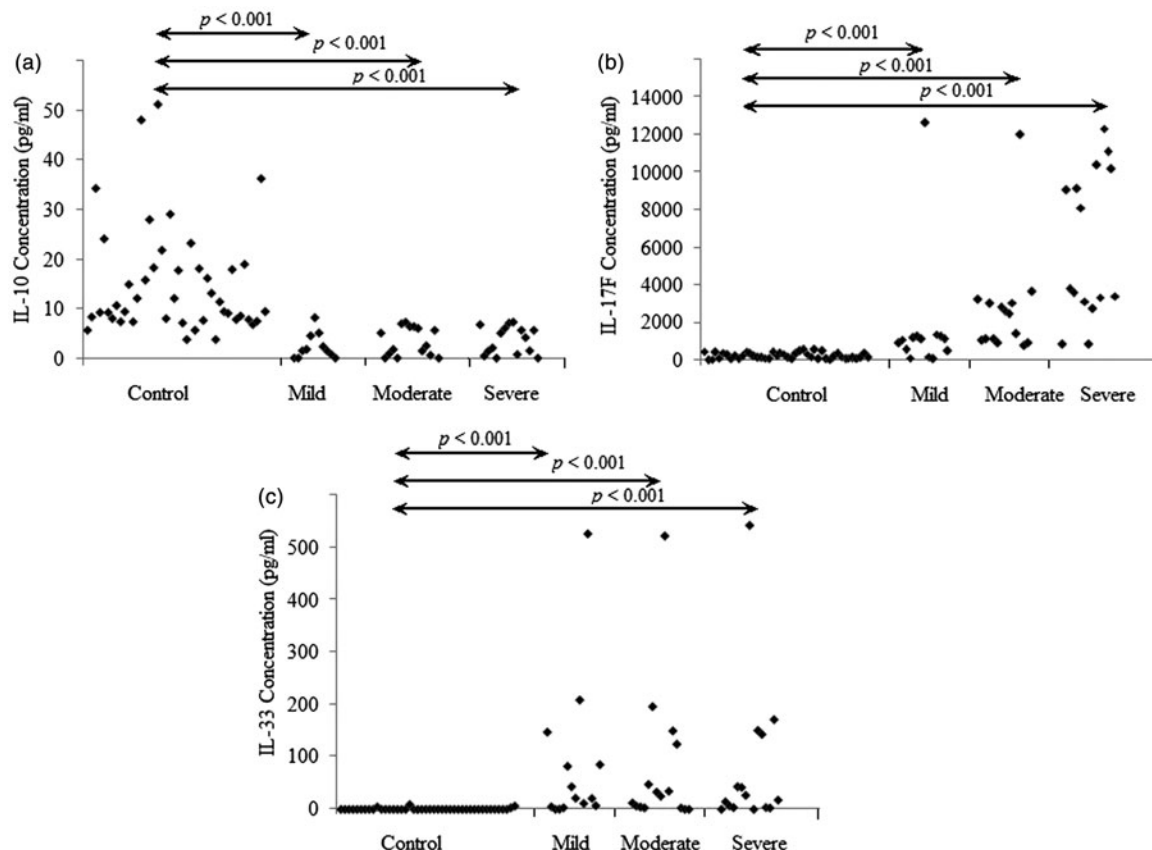


Figure 1. Comparisons of cytokine serum levels between asthmatic patients and healthy controls. Using the ELISA method, serum levels of (a) IL-10, (b) IL-17F, and (c) IL-33 were evaluated in 44 asthmatic patients (14 mild persistent, 15 moderate, and 15 severe persistent) and 44 non-asthmatic controls. Our results showed a significant difference in serum levels of all three cytokines between each of the severity groups and controls ($p < 0.001$). p Value < 0.05 is considered as significant.

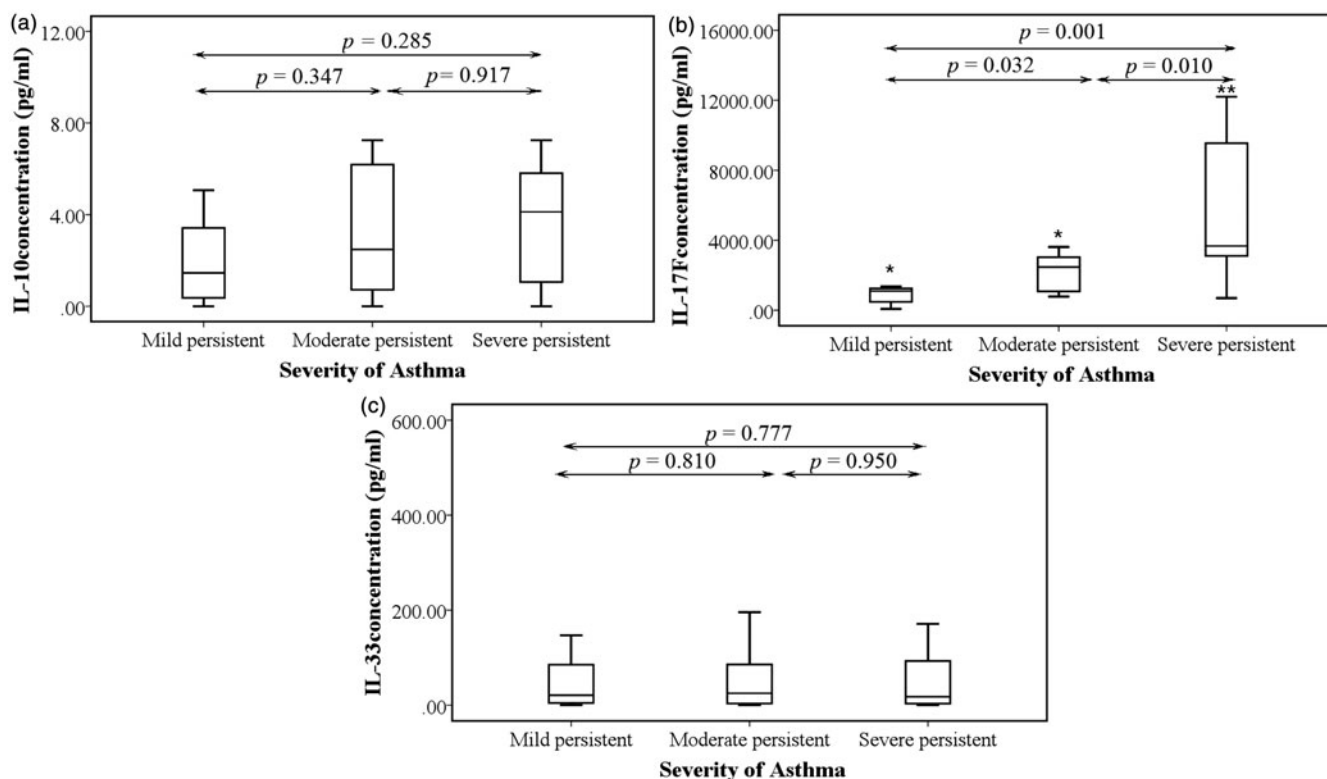


Figure 2. Comparisons of cytokine serum levels among asthmatic patients classified according to increasing severity (GINA classification). Serum levels of (a) IL-10, (b) IL-17F, and (c) IL-33 were measured and compared among 14 mild persistent, 15 moderate persistent, and 15 severe persistent asthmatic patients. Of all three cytokines analyzed, only serum level of IL-17F was significantly different between subjects of different disease severity. * p Value < 0.05 , ** p value < 0.01 is considered as significant.

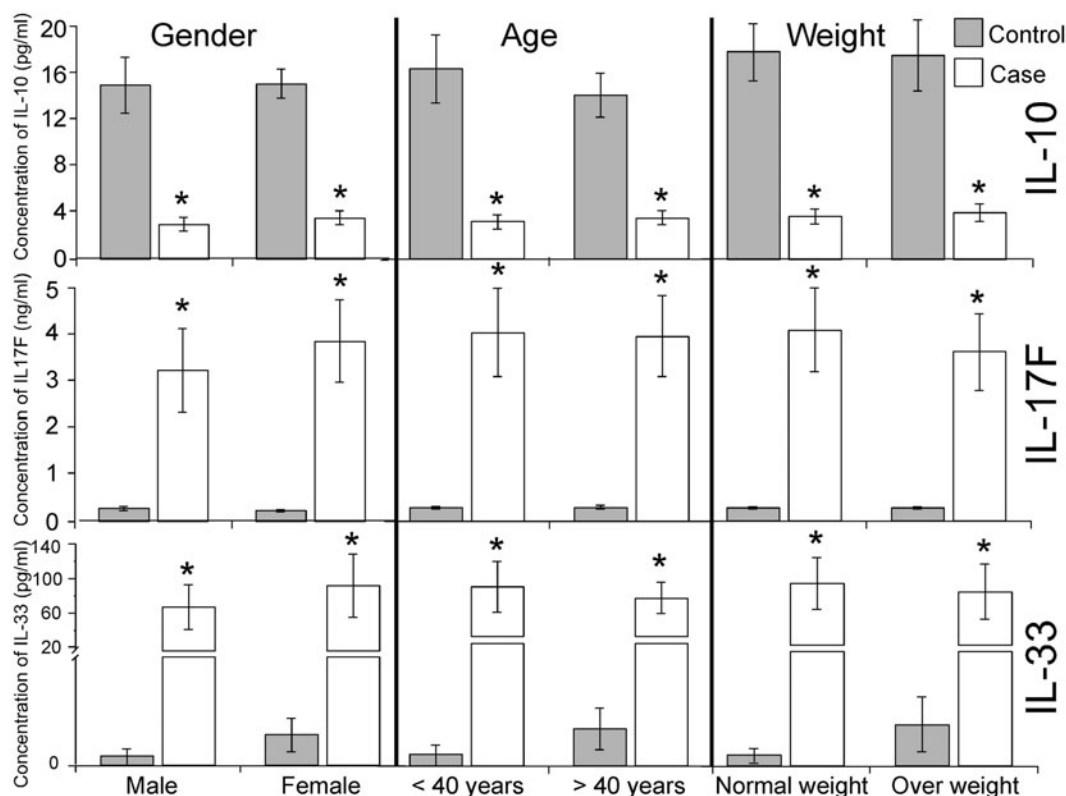


Figure 3. Subgroup analysis showing the effect of gender, age, and BMI on levels of IL-10, IL-17F, and IL-33 among cases and controls. Serum levels of IL-10, IL-17F, and IL-33 were measured and compared among subjects of different genders (male and female), age group (≤ 40 years, > 40 years), and BMI (normal, overweight). Our results showed that while neither asthmatics nor controls show significant difference within their own group, a significant difference is observed between asthmatic and control groups. * p Value < 0.05 is considered as significant.

Table 2. Serum concentrations of IL-10, IL-17F, and IL-33 in different subgroups of asthmatic patients.

Clinical variables	IL-10	IL-17F	IL-33
Asthma duration			
≤ 5 years ($N = 31$)	2.96 ± 0.52	3573.35 ± 765.69	95.83 ± 28.38
> 5 years ($N = 13$)	3.59 ± 0.74	3414.50 ± 704.80	39.21 ± 12.93
p Value	0.298	0.242	0.125
No. of positive allergens			
≤ 5 ($N = 31$)	2.57 ± 0.67	2329.00 ± 735.62	103.20 ± 35.29
> 5 ($N = 13$)	3.36 ± 0.58	4629.21 ± 821.29	40.01 ± 11.78
p Value	0.683	0.041	0.487
Sensitization			
$\leq$ Histamine ($N = 22$)	3.48 ± 0.68	3566.59 ± 793.09	97.28 ± 35.73
$\geq$ Histamine ($N = 22$)	2.66 ± 0.53	3486.23 ± 710.60	60.93 ± 27.03
p Value	0.917	0.589	0.860
Total serum IgE [kUA/l]			
≤ 120 kUA/l ($N = 2$)	3.70 ± 1.36	1241.37 ± 130.37	2.53 ± 2.19
> 120 kUA/l ($N = 42$)	3.13 ± 0.44	3635.23 ± 599.76	82.75 ± 21.20
p Value	0.670	0.652	0.159
Eosinophil%			
$\leq 6\%$ ($N = 12$)	4.80 ± 0.71	1853.55 ± 803.93	38.53 ± 18.46
$> 6\%$ ($N = 32$)	2.56 ± 0.48	4153.74 ± 703.88	94.32 ± 27.17
p Value	0.019	0.022	0.034
AEC (cells/mm³)			
≤ 440 cells/mm ³ ($N = 0$)	–	–	–
> 440 cells/mm ³ ($N = 44$)	2.16 ± 0.48	3388.14 ± 749.81	79.43 ± 25.14
p Value	–	–	–

Values are presented as mean concentration in pg/ml $\pm$ SE. p Value < 0.05 is considered as significant.

($r = -0.387$, $p = 0.010$) and FEV1 % predicted ($r = -0.460$, $p = 0.002$), and positively to the number of allergens sensitized ($r_s = 0.312$, $p = 0.039$), and FEV1 reversibility ($r = 0.427$, $p = 0.004$) (Table 3, Figure 4). Of all three cytokine analyzed, a strong negative correlation was observed between serum levels of IL-10 and IL-33 ($r = -0.517$, $p = 0.001$). On spirometric follow-up after ICS and LABA treatment introduction, we observed that patients with higher levels of IL-10 show a greater improvement in FEV1 following 2 months of treatment with ICS and LABA (Figure 5a) while patients with higher levels of IL-17F and IL-33 show poorer response to the same treatment (Figure 5b and c).

Discussion

Based on the existing guidelines, diagnosis of asthma and choice of treatments mainly rely on clinical symptoms and pulmonary function test [25]. However, spirometry parameters are actually indicative of airway geometry and not endobronchial inflammations [30]. As a result, there is an interest on serum cytokines as biomarkers to diagnose asthma, predict attacks, estimate severity, and select treatments [31]. This is the first study to evaluate three important cytokines associated with asthma, pro-inflammatory IL-17F and IL-33, and anti-inflammatory IL-10 in the same subset of patients. Our results demonstrate a highly significant difference in cytokine levels between diseased and healthy individuals ($p < 0.001$). We observed a decrease in serum level of IL-10 and an increase in serum levels of IL-17F and

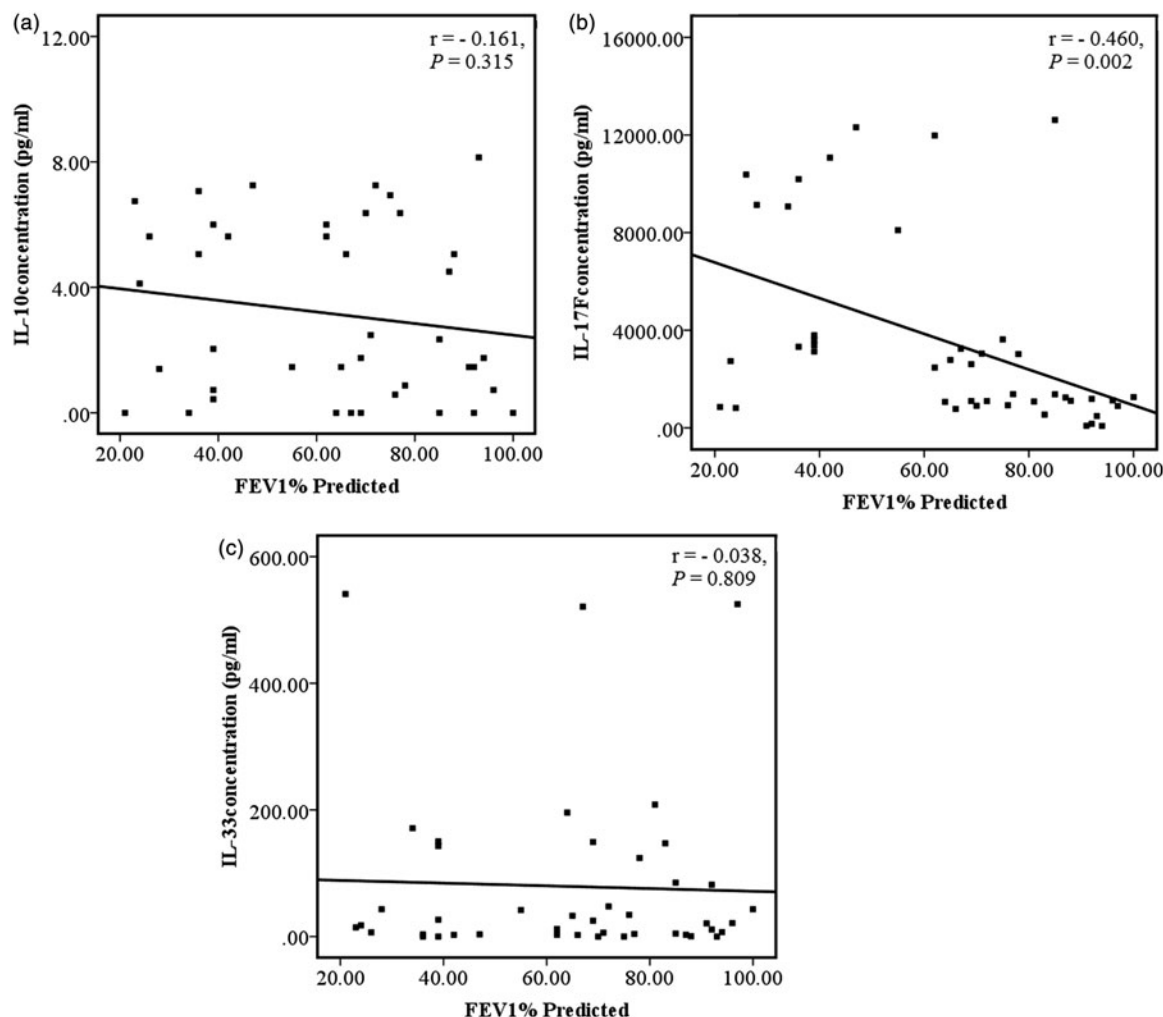


Figure 4. Correlation analysis of cytokine serum levels to pre-bronchodilator FEV1 (% predicted) in patients with asthma. Serum levels of (a) IL-10, (b) IL-17F, and (c) IL-33 were evaluated in asthmatic patients and correlated with pre-bronchodilator FEV1 (% predicted). Of all three cytokine analyzed, only serum level of IL-17F was associated negatively to FEV1 (% predicted). p Value <0.05 is considered as significant.

IL-33 in asthmatics than in healthy controls. On subgroup analysis, we report a significant difference in serum levels of the assessed cytokines between each of the asthma severity groups and controls ($p < 0.001$).

IL-10 is an anti-inflammatory cytokine with a major protective role in asthma. It inhibits the expression of a wide variety of pro-inflammatory cytokines and chemokines including IL-2, IFN- γ , IL-4, IL-5, IL-6, IL-8, GM-CSF, IL-1 β , and TNF- α [32]. The immunosuppressive function of IL-10 is further established by its ability to induce allergic tolerance, inhibit IgE synthesis, debar eosinophil survival, and down-regulate the inflammatory mediators [6–11]. It is incessantly expressed in the airways of healthy individuals but its expression is decreased in patients with asthma [23]. Several studies have reported diminished levels of IL-10 in BAL fluid, peripheral blood mononuclear cells, alveolar macrophages, sputum, as well as sera of the asthmatic subjects compared to healthy controls [33–38]. We observed a decrease in serum level of IL-10 in asthmatic patients compared with non-asthmatic controls ($p < 0.001$). Our data on IL-10 serum analysis is conflicting with other studies showing either higher or same levels of IL-10 in asthmatics compared to healthy controls [39,40] (Table 4). The possible explanation for this paradoxical results could be that the

subjects of these studies were on treatment with regular inhaled steroids that are shown to inhibit the inflammatory response in asthma by increasing the level of anti-inflammatory cytokines such as IL-10 [41] whereas, all our study subjects were inhaled corticosteroid treatment naive. As the expression of IL-10 protein correlates with the appearance of other pro-inflammatory cytokines such as IL-4 and IL-2 [42], the difference in IL-10 levels among different populations may be the result of differences in pro-inflammatory cytokine panels within these populations. Discrepancies in IL-10 levels among different population can also be explained by the involvement of genetic variations as a polymorphism in IL-10 transcription initiation site has been associated with decreased IL-10 expression [33]. Overall, asthma as an intricate and heterogeneous disorder with different features and various phenotypes in different ethnicities may contribute to the differences observed across studies [31].

Several studies support a role for IL-17F in asthma. Allergen challenge-mediated elevation of ML-1 (shorter isoform of IL-17F) in basophils, mast cells, and allergen-specific T cells of patients with asthma supports the role of this cytokine in allergic responses of asthma [43]. Further supports come from studies on mouse model of asthma in which challenge with ovalbumin induced the expression of

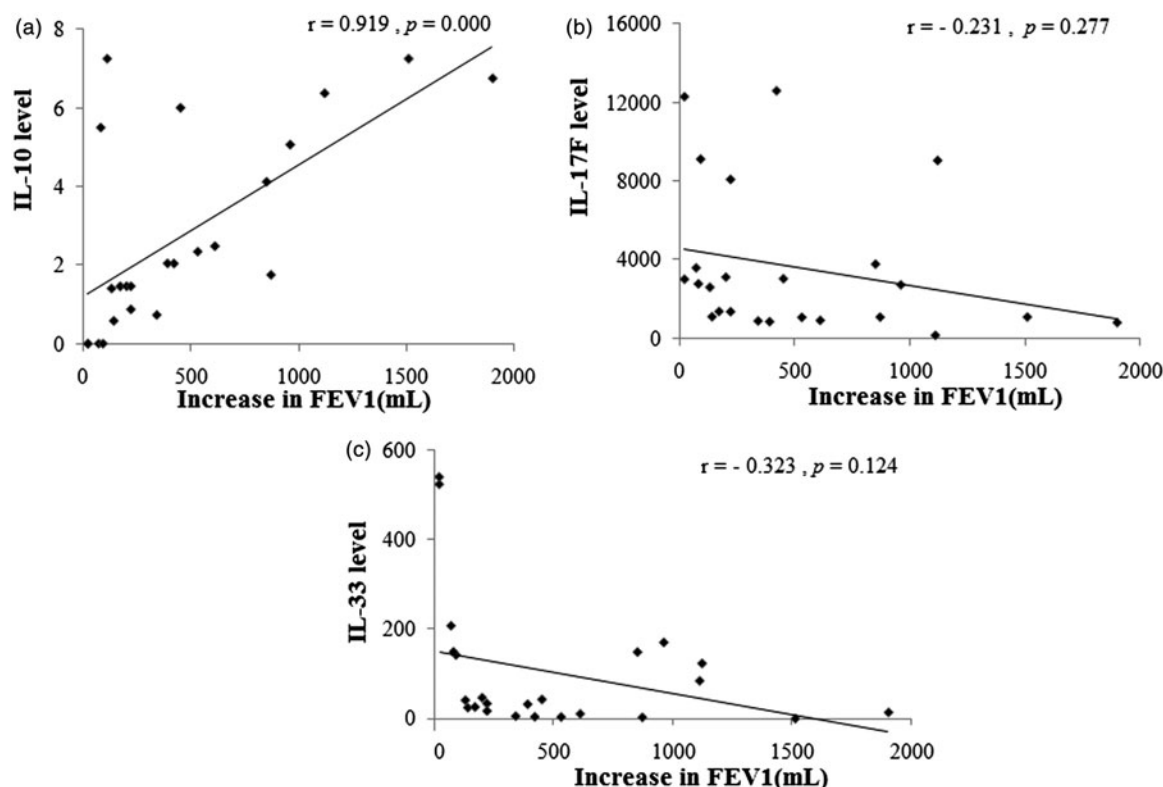


Figure 5. Improvement in FEV1 levels after treatment with ICS/LABA and relation to baseline (pre-treatment) levels of IL-10, IL-17F, and IL-33. FEV1 was compared before and after treatment and the degree of improvement in FEV1 was correlated to serum cytokine levels. Our results showed that patients with higher levels of IL-10 show a greater improvement in FEV1 following 2 months of treatment (a) while patients with higher levels of IL-17F and IL-33 show a trend towards the same treatment (b and c). Correlation analysis was carried out using Pearson correlation (r).

Table 3. Correlation analysis of cytokine serum levels to various clinical variables.

Clinical variables	IL-10	IL-17F	IL-33
Total serum IgE	$r = -0.093, p = 0.561$	$r = 0.177, p = 0.250$	$r = 0.175, p = 0.256$
AEC	$r = -0.227, p = 0.154$	$r = 0.225, p = 0.143$	$r = 0.020, p = 0.896$
E%	$r = 0.190, p = 0.233$	$r = 0.102, p = 0.510$	$r = -0.092, p = 0.553$
Asthma duration	$rs = 0.165, p = 0.304$	$rs = 0.178, p = 0.246$	$rs = -0.002, p = 0.990$
Number of positive allergens	$rs = 0.065, p = 0.689$	$rs = 0.312, p = 0.039$	$rs = -0.106, p = 0.493$
Sensitization	$rs = -0.017, p = 0.918$	$rs = 0.082, p = 0.595$	$rs = 0.027, p = 0.863$
Age	$r = 0.044, p = 0.787$	$r = 0.136, p = 0.378$	$r = -0.254, p = 0.097$
Gender	$rs = 0.048, p = 0.767$	$rs = 0.132, p = 0.391$	$rs = -0.050, p = 0.746$
BMI rate	$r = -0.143, p = 0.372$	$r = -0.166, p = 0.280$	$r = -0.056, p = 0.719$
FVC (% predicted)	$r = -0.160, p = 0.317$	$r = -0.387, p = 0.010$	$r = -0.077, p = 0.618$
FEV1-FVC (% predicted)	$r = -0.051, p = 0.750$	$r = -0.245, p = 0.109$	$r = -0.017, p = 0.911$
PEF (% predicted)	$r = -0.119, p = 0.457$	$r = -0.273, p = 0.073$	$r = -0.063, p = 0.682$
FEV1 reversibility	$r = 0.051, p = 0.753$	$r = 0.427, p = 0.004$	$r = -0.017, p = 0.913$

r : Pearson correlation coefficient; rs : Spearman correlation coefficient. p Value < 0.05 is considered as significant.

IL-17F in bronchial epithelial as well as infiltrating inflammatory cells [44]. Studies on IL-17F-deficient mice showed defective airway neutrophilia on challenge with specific allergens [45]. The results of our study showed a different serum pattern between healthy and asthmatic individuals with respect to IL-17F level ($p < 0.001$). Furthermore, on subgroup analysis, a significant difference in the level of IL-17F was demonstrated across the severity groups. Our results concur with the findings of others who have reported the overexpression of IL-17F in bronchial submucosa, airway epithelium, inflammatory infiltrates, and sera of the patients with mild to severe asthma [46–48] (Table 4). It has been shown that IL-33 can induce IL-17F gene and protein

expression through ST2-ERK1/MSK1 signaling pathway in bronchial epithelial cells [49]. Furthermore, IL-10 can inhibit the monocyte-dependent proliferation as well as cytokine synthesis of CD4⁺ T cells and CD8⁺ T cells as cellular sources of IL-17F [50–52]. With diminished level of inhibitory IL-10 in our study subjects, an increase in CD4⁺ T cells and CD8⁺ T cells proliferation could elevate serum levels of IL-17F in the asthmatic patients.

IL-33 is the most effective inducer of Th2-type immune responses in mucosal tissues [53]. It induces the expression of several inflammatory cytokines such as IL-1 β , IL-6, IL-13, and TNF- α in mast cells. Enhanced amount of IL-13 and IL-5 is produced by *in vitro*-polarized Th2 cells in the presence of

Table 4. Serum concentrations of IL-10, IL-17F, and IL-33 in different ethnic populations.

Study	Year	Country	Numbers (case/control)	ICS treatment	Atopic/ non-atopic status	Asthmatics	Controls
IL-10							
This study	2014	India	44/44	No	Atopic	3.16 ± 0.42 ^a	14.95 ± 1.65 ^a
Wong et al. [39]	2001	Hong Kong	41/30	Yes	Atopic	^c 2.51 (0.0–6.92) ^c	^c 0.05(0.0–4.62) ^c
Ceyhan et al. [40]	2004	Turkey	17/5	Yes	Atopic	4.1 ± 3.8 ^b	2.3 ± 2.5 ^b
Pukelshiem et al. [31]	2010	Germany/Sweden	103/321	Yes	Both	2.4 (1–6) ^c	1.6 (1–4) ^c
Deo et al. [54]	2010	India	24/20	No	Atopic	54.20 ± 12.52 ^c	43.54 ± 9.22 ^c
Shi et al. [55]	2011	China	39/20	No	Atopic	^c 50.5 ± 27 ^a	^c 61 ± 36 ^a
IL-17F							
This study	2014	India	44/44	No	Atopic	3526.41 ± 573.81 ^a	224.29 ± 23.84 ^a
Bazzi et al. [48]	2011	Saudi Arabia	100/102	Not defined	Not defined	700.237 ± 2010.77 ^a	236.009 ± 382.82 ^a
IL-33							
This study	2014	India	44/44	No	Atopic	79.10 ± 20.62 ^a	0.51 ± 0.26 ^a
Yang et al. [45]	2012	China	30/23	Yes	Not defined	208 ± 95 ^b	143 ± 32 ^b
Rudha et al. [56]	2013	Iraq	30/30	Not defined	Not defined	676.5 ± 224.3 ^b	132.4 ± 50.12 ^b
Azazi et al. [57]	2013	Egypt	30/30	Yes	Atopic	846 ± 202.150 ^d	174 ± 41.200 ^d

^aMean concentration in pg/ml ± SEM, ^bmean concentration in pg/ml ± SD, ^cmedian concentration in pg/ml, and range in parenthesis,

^dmean concentration in ng/ml ± SEM, ^eplasma is used as the study sample.

IL-33 [13]. It is associated with allergen-induced airway inflammation, airway hyper responsiveness, and eosinophil infiltration [13,17] and is observed to be reduced after systemic glucocorticoid treatment [58]. Our study on serum level of IL-33 as an inflammatory biomarker showed higher level of IL-33 in asthmatic patients than in controls. Consistent with our findings, previous studies have shown the increased level of IL-33 in bronchial epithelial cells, smooth muscle cells, sputum, and sera of the asthmatics compared with healthy controls [56–62] (Table 4). Taken together, the increased levels of IL-17F and IL-33 as pro-inflammatory cytokines and decreased level of IL-10 as anti-inflammatory cytokine may act as a collective effect to mediate the expression of other pro-inflammatory cytokines and chemokines such as IL-6, IL-8, and GM-CSF that are known to be associated with asthma in human [63,64] and murine model of asthma [65]. The expression of these inflammatory mediators can amplify the allergic response through the recruitment of secondary cells like eosinophils that themselves may release a variety of cytokines [66].

Correlation analysis of serum cytokine levels to distinct clinical parameters of asthma such as lung function, E count, total serum IgE, and duration of disease showed unclear patterns. Of all three cytokines analyzed, only serum concentration of IL-17F was associated negatively to FVC (%predicted) and FEV1 (%predicted) and positively to the number of allergens sensitized and FEV1 reversibility. Reduced FVC (%predicted) and FEV1 (%predicted) is reported in asthmatic patients compared to healthy controls [38,67,68]. The positive correlation between the number of allergens sensitized and IL-17F levels suggests the role of this cytokine in atopic sensitization. IL-17F was initially located in BALF from atopic asthmatic patients on stimulation with ragweed allergen [43]. It plays a crucial role in interceding innate immune responses to allergens through an IL-17RA-dependent manner [69]. Earlier studies have shown an effect of IL-17F on fibrocytes-enhancing collagen I expression [70]. As elevated levels of IL-17F is known to be associated with airways neutrophilia and remodeling [46], our conflicting results on positive correlation of IL-17F levels and degree of

reversibility needs further study to explore the mechanisms for association in our study population. The strong negative correlation between serum levels of IL-10 and IL-33 may reflect the probability of a converse relationship between anti-inflammatory and pro-inflammatory cytokines in an individually balanced cytokine pattern. Finally lack of correlation between serum levels of IL-10 and IL-33 and clinical parameters may indicate that serum cytokine level and clinical tests demonstrate discrete aspects of asthma [31,71,72]. Correlation analysis of serum cytokine levels to ICS/LABA-mediated FEV1 improvement showed a trend in FEV1 among our study population. Although this can be used as a reliable marker for clinical investigations, further studies with larger sample size are needed to confirm the trend observed in our study population.

The fact that analyzing the cytokine pattern in serum samples do not give us information on the source of the assessed cytokine may reflect the significance of local environment like lung tissues in analyzing the cytokine levels, but meanwhile, the feasibility of serum sampling as a non-invasive method to analyze cytokine levels has directed the interests toward non-invasive methods rather than invasive ones [31]. As our study subjects were treatment naive at the time of sampling, we were able to determine the actual levels of cytokines without the modifying effects of treatment. Therefore, the difference in the cytokine levels between the severity groups is more likely to reflect the actual differences in disease severity and unmodified by the dose of anti-inflammatory medications used in treatment.

In conclusion, the present study sheds lights on the potential of cytokines serum analysis as a biomarker tool in asthma. Our results showed a highly significant difference in serum levels of the assessed cytokines in asthmatic patients compared to healthy controls. Further studies on cytokine biology may help us to understand the asthma pathogenesis and attain more effective therapeutic strategies.

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Declaration of interest

The authors have no conflicts of interest to declare.

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