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To cite this article: Amira Ibrahim Mansour, Eman Rateb Abd Almonaem, Ola Galal Behairy & Tahany Mahmoud Gouda (2017): Predictive value of IL-35 and IL-17 in diagnosis of childhood asthma, Scandinavian Journal of Clinical and Laboratory Investigation, DOI: [10.1080/00365513.2017.1328739](https://doi.org/10.1080/00365513.2017.1328739)

To link to this article: <http://dx.doi.org/10.1080/00365513.2017.1328739>



Published online: 30 May 2017.



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ORIGINAL ARTICLE



Predictive value of IL-35 and IL-17 in diagnosis of childhood asthma

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ABSTRACT

This study aimed to evaluate the correlation between serum levels of IL-17 and IL-35 and the presence and severity of childhood asthma. The study was performed on 60 diagnosed asthmatic children, who were further classified into four groups according to the Global Initiative for Asthma Guidelines for Asthma Severity and Control (GINA) 2016, plus 30 age- and sex-matched apparently healthy children. All participants were subjected to full medical history, clinical examination, pulmonary function tests and laboratory evaluation in the form of complete blood count (CBC), serum total IgE, IL-17 and IL-35 by ELISA. Our results revealed that eosinophils count, IgE and IL-17 were significantly higher in the asthmatic group than the control group ($p < .001$), while IL-35 levels were significantly lower in asthmatics than control ($p < .001$). A strong negative correlation was found between serum IL-17 and serum IL-35; a positive correlation was found between serum IL-17 and both of serum total IgE and eosinophils counts in atopic asthmatic patients, and serum IL-35 showed significant negative correlations with both. ROC analysis of the data showed that the cut-off value of IL-35 level was <189.5 pg/mL and for IL-17 level, it was >13.1 pg/mL; this value could predict childhood asthma with sensitivity of 81.7% and 83.3%, and specificity of 76.7% and 70%, respectively. A combination of both cytokines yielded an increase in sensitivity to 95%. In conclusion, in the current study, IL-17 is upregulated while IL-35 is downregulated in childhood asthma with a significant negative correlation between both. These results suggest that both may play an important role in the pathogenesis of childhood asthma.

ARTICLE HISTORY

Received 31 January 2017
Revised 27 April 2017
Accepted 7 May 2017

KEYWORDS

Asthma; cytokines; ELISA; eosinophils; IgE

Introduction

Childhood asthma is a chronic inflammatory disorder of the airways in children [1], characterized by interaction between many cells of the innate and adaptive immune systems that interact with epithelial cells to cause bronchial hyper-reactivity [2]. It manifests as recurrent attacks of wheezing, breathlessness, chest tightness, and coughing, particularly at night and in the early morning. These attacks are usually associated with variable airflow obstruction that is often reversible either spontaneously or with treatment. Childhood asthma prevalence worldwide is rising dramatically making it regarded as a major healthcare problem in children [3].

Different immunological mechanisms contribute to asthma in children, mainly CD4 + Th2 cells play a central role in the inflammatory process via its released cytokines, IL-4 and IL-13 [1], which lead to recruitment of B cells, eosinophils and mast-cell leading to airway inflammatory reaction [4]. Other T cell populations and their cytokines also play an important role in the activation or inhibition of Th2-dependent airway inflammation including Th1, Th17 and CD4 + CD25 + Foxp3 + regulatory T (Treg) cells, and the understanding of such interaction between these cytokines and their immunoregulatory role in asthma can contribute to new therapeutic strategies [5].

Endothelial cells also play a role in pathogenesis of asthma through upregulation of the transendothelial

migration (TEM), increasing the expression of endothelial adhesion molecules (such as ICAM-1) and increasing the production of chemokines to attract and activate circulating eosinophils such as pulmonary endothelial tissue transglutaminase 2 (TG2) [6].

IL-35 is a member of the IL-12 cytokine family, which contains IL-12, IL-23, IL-27 and IL-35, and all of the family members consist of α chain (P19, P28 or P35) and β chain (P40 or Epstein-Barr virus induced 3 [EBI3]) [7]. IL-35 is secreted only by regulatory T cell populations [8]. In contrast to other IL-12 relatives, IL-35 carries important inhibitory properties [9]. It has been implicated in the pathogenesis of many autoimmune and inflammatory disorders through inhibition of CD4 + effector T cells [10].

IL-35 plays an important role in the suppression of the development and the differentiation of T helper 17 in autoimmune diseases [11] by inducing cell cycle arrest in G1 without inducing apoptosis [12]. Also, it has been observed to be critical to the development of Th2 cells and inhibition of allergen-specific Th2 responses and so decreasing production of Th2 cytokines of allergic airways disease [13].

IL-17 (IL-17A) is a proinflammatory cytokine secreted by T helper 17 cells upon induction by IL-23 [14]. IL-17 family consists of five members (IL-17B, IL-17C, IL-17D, IL-17E and IL-17F), which are characterized by the four highly conserved cysteine residues in their 3-dimensional shape

with no similarity to other known cytokines [15]. Increased IL-17 A expression was observed to be related with numerous chronic inflammatory disorders as rheumatoid arthritis, asthma, systemic lupus erythematosus and allograft rejection [16]. It has been suggested that IL-17 is involved in the neutrophilic inflammation and severe asthma [17].

The current study was undertaken to assess the correlation between serum levels of IL-17 and IL-35 and the presence and severity of childhood asthma.

Subjects and methods

This case control study was conducted at the Pediatric Department of Benha University Hospital, Egypt, from January 2015 until June 2016. The study was approved by the Ethical Scientific Committee of Benha University and was carried out according to the guidelines of the Helsinki Declaration [18]. An informed consent was obtained from one of the parents before enrollment of their children in the study. Eighty children were included in the study and they were divided into two groups:

I – Patients group. This group included 60 asthmatic children from attendants to outpatient chest clinic and/or admitted to the Pediatric Department of Benha University Hospital. Their diagnosis was verified according to the Global Initiative for Asthma Guidelines for Asthma Severity and Control (GINA 2016) [19], after consideration of the exclusion criteria which included, age below 5 years, receiving systemic corticosteroid treatment during the last 2 months; chronic illness involving congenital heart or lung disease, liver and kidney diseases. Then they were further classified into four groups according to type and severity of asthma: (1) intermittent bronchial asthma; (2) mild persistent bronchial asthma; (3) moderate persistent bronchial asthma; and (4) severe persistent bronchial asthma.

II – Control group. This included 30 apparently healthy non-atopic, age- and sex-matched children, without personal or family history of asthma or other allergic conditions.

All individuals enrolled in the study were subjected to the following: full history taking, with special attention to history of atopy for the child and family, and complete clinical examination. Pulmonary function tests were performed by using spirometry (performed by Erich Jaeger 95 GmbH 1992–1997 for measurement of pulmonary functions) before and after bronchodilator (administration of four separate puffs at 30-sec intervals of the short acting B2-agonist salbutamol (total of 400 mcg) using a spacer device) [20]. The following indices were also measured: forced vital capacity (FVC), forced expiratory volume in 1st second (FEV1) and FEV1/FVC and post bronchodilator change in FEV1 which are displayed automatically by the apparatus. Chest X-rays posteroanterior and lateral views were carried out. Laboratory investigations were carried out for all enrolled subjects including complete blood count by automated hematology system (Sysmex XE 5000) [21], ESR (by Westergren method) [22]. Liver and renal function tests were also carried out: Serum IgE: using enzyme immunoassay for the quantitative determination of immunoglobulin

E (IgE) concentration in human serum (Immunospec Corporation, Ref: E29-006); Serum IL-17: using Human IL-17 Immunoassay (Quantikine® ELISA, Catalog No. D1700); and Serum IL-35: using Human IL-35ELISA Kit (Biosource, Catalog No: MBS2511987).

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SPSS Inc., Chicago, IL, USA). Categorical data were presented as number and percentage using Chi square (χ^2) test to analyze them. Continuous variables were expressed as mean $\pm$ standard deviation. Data were tested for normality using Shapiro Wilks test, Student's *t*-tests for normally distributed variables, or Kruskal-Wallis test, Mann-Whitney U (MWU) test and Spearman's correlation coefficient (ρ) for non-parametric variables. Significant Kruskal test was followed by post hoc multiple comparisons to detect the significant pairs using the Bonferroni adjusted Mann-Whitney U test at adjusted *p* values of .005. ROC curve was used to determine the cut-off value of IL-17 and IL-35 with optimum sensitivity and specificity in prediction of patients with asthma and differentiating severe persistent type. The accepted level of significance in this work was stated at 0.05 ($p < .05$ was considered significant).

Results

The study was conducted on 60 asthmatic patients: 29 males (48.3%) and 31 females (51.7%), and their mean age was 9.1 ± 3.29 SD, range 5–16 years old. In addition, there were 30 healthy control subjects of matched age and sex, 15 males and 15 females with their mean age was 8.6 ± 3 SD, range 5–13 years old. Regarding their history of atopy, 13.3% (two) of the intermittent asthmatic group, 20.0% (three) of the mild persistent group, 80.0% (12) of the moderate persistent group, and 86.7% (13) of the severe persistent group were atopic.

Our data revealed that, eosinophils count, IgE and IL-17 were significantly higher in the asthmatic group than the control group ($p < .001$), while IL-35 was significantly lower in asthmatics than control ($p < .001$); also, FEV1, FVC and FEV1/FVC were significantly higher in moderate and severe persistent asthmatic patients than mild persistent and intermittent asthmatic patients (Table 1).

In this study, peripheral blood eosinophilic count, serum IL-17 and serum total IgE levels were significantly higher in atopic asthmatic patients than non-atopic asthmatic patients ($p < .001$), while serum level of IL-35 were significantly higher in non-atopic asthmatic patients than atopic asthmatic patients ($p < .001$) (Table 2).

As demonstrated in Table 3, serum IL-17 levels were negatively correlated with serum IL-35, FEV1, FVC and FEV1/FVC, and positively correlated both of IgE and eosinophils. IL-35 also showed positive correlations with FEV1 and FVC but showed negative correlations with IgE and eosinophils.

As indicated in Table 4, serum IL-17 levels were positively correlated with both serum totals of IgE and

Table 1. Comparison of the studied variables between the studied groups.

Variables	Control group (n = 30)		Intermittent asthma (n = 15)		Mild persistent (n = 15)		Moderate persistent (n = 15)		Severe persistent (n = 15)		Kruskal-Wallis test	p
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD		
Eosinophils cells $\times 10^9/L$	$0.213 \times 10^9/L$	0.66	$0.217 \times 10^9/L$	0.069	$0.241 \times 10^9/L$	0.074	$0.408 \times 10^9/L$	0.028	$0.539 \times 10^9/L$	0.040	61.3	<.001 (HS)
IgE IU/mL	165.3	34.3	474.0	123.1	493.8	118.2	633.8	157.5	1015.5	129.0	75.8	<.001 (HS)
IL-17 pg/mL	10.6	3.7	14.0	2.1	14.3	1.8	17.1	1.7	24.4	3.2	59.7	<.001 (HS)
IL-35 pg/mL	240.2	75.4	188.7	39.7	171.8	46.6	95.8	28.8	73.6	32.6	60.3	<.001 (HS)
FEV1/FVC	84.5	3.6	81.4	5.01	73.7	3.05	73.4	2.79	44.3	7.65	76.1	<.001 (HS)
FEV1%	93.4	2.66	84.5	4.77	82.4	1.76	72.2	5.14	39.0	6.61	79.4	<.001 (HS)
FEV1 liter	3.03	0.32	3.3	0.35	2.7	0.52	2.7	0.68	1.6	0.28	45.07	<.001 (HS)
FVC%	86.4	6.1	83.1	6.72	81.0	4.55	75.3	6.00	59.5	8.10	52.9	<.001 (HS)
FVC liter	3.27	0.41	4.2	0.32	3.5	0.69	3.8	0.88	3.2	0.72	31.8	<.001 (HS)

HS: high significance.

Table 2. Comparison of the studied variables between atopic and non-atopic patients.

Variable	Atopic (n = 30)		Non-atopic (n = 30)		MWU test	p
	Mean	± SD	Mean	± SD		
Eosinophils cells $\times 10^9/L$	$0.442 \times 10^9/L$	0.116	$0.260 \times 10^9/L$	0.104	4.97	<.001 (HS)
IgE IU/mL	776.3	268.1	532.2	170.8	3.48	<.001 (HS)
IL-17 pg/mL	20.3	4.79	14.7	2.67	4.87	<.001 (HS)
IL-35 pg/mL	99.9	51.2	165.1	53.1	4.18	<.001 (HS)

MWU test: Mann-Whitney U test; HS: high significance.

Table 3. Correlations between IL-17, IL-35 and the studied variables among patients group.

With	IL-17		IL-35	
	rho	p	rho	p
Age	-0.411	.001 (HS)	0.219	.092 (NS)
Eosinophils cells $\times 10^9/L$	0.792	<.001 (HS)	-0.716	<.001 (HS)
IgE IU/mL	0.648	<.001 (HS)	-0.526	<.001 (HS)
IL-17 pg/mL	—	—	-0.635	<.001 (HS)
IL-35 pg/mL	-0.635	<.001 (HS)	—	—
FEV1/FVC	-0.723	<.001 (HS)	0.692	<.001 (HS)
FEV1%	-0.792	<.001 (HS)	0.783	<.001 (HS)
FEV1 liter	-0.594	<.001 (HS)	0.581	<.001 (HS)
FVC%	-0.642	<.001 (HS)	0.611	<.001 (HS)
FVC liter	-0.425	.001 (HS)	0.335	.009 (S)

Rho: Spearman's correlation coefficient; NS: non-significant; HS: high significant.

eosinophils count in atopic asthmatic patients, and positively correlated with eosinophils in non-atopic asthmatic patients, while serum IL-35 levels showed significant negative correlations with IgE and eosinophils in atopic asthmatic patients, and these correlations were non-significant in non-atopic asthmatic patients.

ROC analysis of the data showed that the best cut-off value of IL-35 level was <189.5 pg/mL and for IL-17 level, it was >13.1 pg/mL. This value could predict childhood asthma with sensitivity of 81.7% and 83.3%, specificity of 76.7% and 70%, positive predictive value of 87.5% and 84.7%, negative predictive value of 67.6% and 67.7%, accuracy of 80% and 78.9% and area under curve (AUC) 0.86 and 0.87, respectively. A combination of both cytokines yielded an increase in sensitivity to 95% (Table 5, Figure 1).

Discussion

In this study, we tried to evaluate serum levels of two important cytokines in childhood asthma, IL-35 and IL-17, to explore their role in asthma among various patient subgroups.

Eosinophils play important role in pathogenesis of asthma and allergic diseases, activation of eosinophils correlates significantly with asthma severity [23]. Bousquet et al. (1990) concluded that eosinophils count increases in peripheral blood, bronchoalveolar lavage fluid and lung biopsy specimens of chronic asthmatic patients and its count correlated with the severity of asthma [24].

In our study, a significant increase in eosinophils count was found in asthmatic patients and atopic asthmatics compared with non-atopic and healthy controls; its count significantly increases with an increase of severity of the asthma.

This finding agrees with previous studies of peripheral blood eosinophil count which mentioned a correlation between eosinophilic count and asthma [24–27].

Comparing the eosinophil count between atopic and non-atopic asthmatics in previous studies have been focused on their count in patients' mucosal epithelium [28–30]. Some of these studies are consistent with our findings, and conclude that eosinophils count by immunohistochemistry were increased in atopic than non-atopic asthmatics [29], but others reported that bronchial submucosa of non-atopic contains more eosinophils than in atopic asthmatics [28,30].

In the current study, serum IgE levels were significantly elevated in severe persistent asthmatic patients than other asthmatic grades and normal control and in atopic than non-atopic patients. These results are consistent with the previous study by Ying et al. (2001) who found that all atopic subjects included in their study had elevated serum total IgE concentrations compared to non-atopic subjects [30].

Kovač et al. (2007) also, reported higher total IgE concentrations in severe persistent asthmatics than other grades of asthma [31].

In addition, Chandran and his colleagues (2015) demonstrated that serum IgE levels were elevated in severe persistent asthmatics followed by moderate persistent, mild

Table 4. Correlations between IL-17 and both IgE and eosinophils among atopic and non-atopic asthmatic patients.

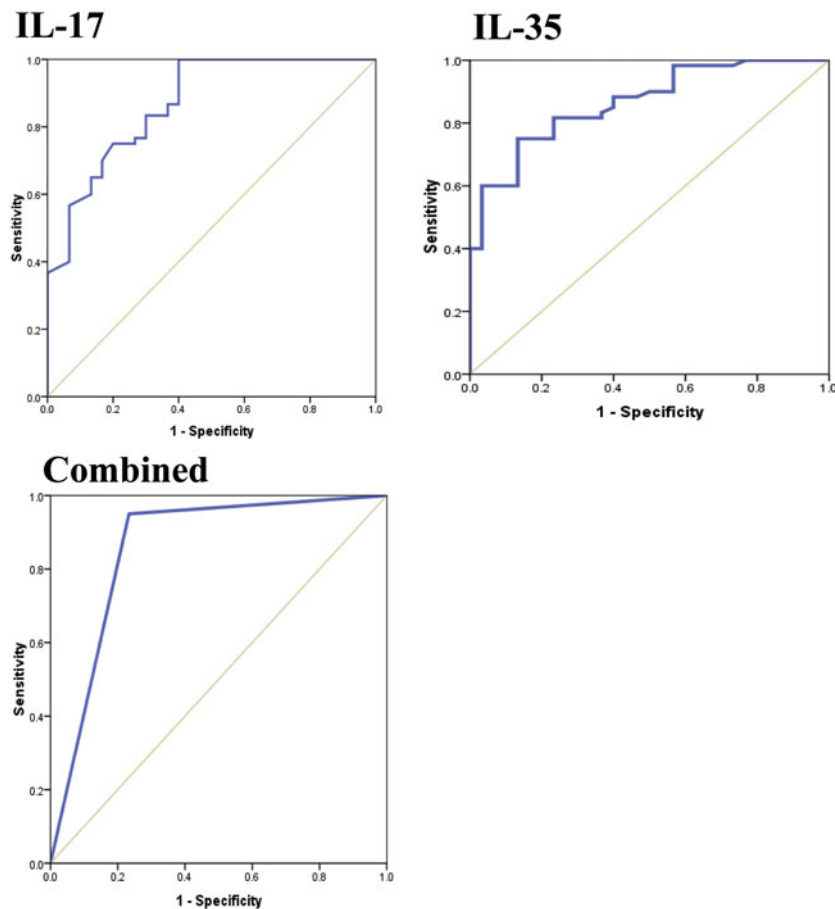
With	IL-17 pg/mL				IL-35pg/mL			
	Atopic		Non-atopic		Atopic		Non-atopic	
	ρ	p	ρ	p	ρ	p	ρ	p
Eosinophils cells $\times 10^9/L$	0.854	<.001 (HS)	0.385	.036 (S)	−0.664	<.001 (HS)	−0.242	.19 (NS)
IgE IU/mL	0.777	<.001 (HS)	0.045	.81 (NS)	−0.507	.004 (S)	−0.042	.82 (NS)

ρ : Spearman's correlation coefficient; NS: non-significant; HS: high significant.

Table 5. Performance of IL-17 and IL-35 in prediction of patients with asthma.

Cut-off	Sens%	Spec%	PPV%	NPV%	Accuracy%	AUC	95% CI	p
IL-17 > 13.1 pg/mL	83.3%	70%	84.7%	67.7%	78.9%	0.87	0.79–0.95	<0.001 (HS)
IL-35 < 189.5 pg/mL	81.7%	76.7%	87.5%	67.6%	80%	0.864	0.79–0.94	<0.001 (HS)
Combined markers	95%	76.7%	89%	88.5%	88.9%	0.868	0.76–0.96	<0.001 (HS)

PPV: positive predictive values; NPV: negative predictive values; NS: non-significant; HS: high significant.

**Figure 1.** ROC curves for the performance of IL-17 and IL-35 in the prediction of patients with asthma.

persistent asthma, and they concluded that values of IgE levels were a good diagnostic and prognostic marker of allergic diseases like asthma [32].

The role of IgE levels in allergic diseases is well established, as B cells are responsible for the synthesis of allergen-specific IgE; the binding of antigen-specific IgE to Fc ϵ RI sensitizes mast cells and other effector cells to release mediators in response encountered allergen [33], blood eosinophilia and raised serum total IgE level are now considered strong predictors for allergy in asthmatic children [34].

IL-17 is a proinflammatory cytokine, secreted mainly by a distinct CD4+ T helper cell subset (Th17), and proposed to

be included in the neutrophilic inflammation and airway remodeling processes in severe asthma [35].

Imbalance between Th1 and Th2 plays a significant role in the pathogenesis of asthma. In our study, serum levels of IL-17 were significantly increased in asthmatic patients compared to healthy controls and in severe persistent asthmatic patients compared to moderate, mild persistent and intermittent asthmatic groups. Also, serum IL-17 levels were significantly higher in atopic asthmatic patients than non-atopic asthmatic patients ($p < .001$).

These results agree with previous findings of Bullens et al. (2006), who found a significant increase in IL-17

mRNA in sputum of patients with severe asthma under inhaled steroids and found it correlated with IL-18 mRNA and sputum neutrophilia [17].

In 2009, Al-Ramli et al. demonstrated a significant increase in the number of IL-17A+ and IL-17F+ cells in the lung biopsies of patients with severe and moderate asthma compared with mild asthma and controls [36]. Also, Irvin and co-workers (2014) analyzed Th2/Th17 cells in bronchoalveolar lavage (BAL) of patients with severe asthma and concluded that TH2/TH17-predominant pattern of cytokine expression were increased in BAL of asthmatic patients than control and in severe asthmatic patients than other grades of asthma [37].

In addition, Agache et al. (2010) reported increased serum IL-17 in severe asthma patients compared to mild/moderate asthma patients. They also concluded that serum IL-17 above 20 pg/mL could be considered as an independent risk factor for severe asthma [38].

Al-Kufaidy and co-workers (2016) studied the expression of IL-17A and IL-17F in the bronchial tissues of asthmatic patients and normal controls by RT-PCR and found a significant elevation in IL-17A and IL-17F expression in the bronchial tissues of patients with mild/moderate and severe asthma relative to control and in tissues of severe asthmatic patients than tissues of mild/moderate asthmatics [39].

Also, Wong et al. (2001) found higher plasma IL-17 and IL-6 levels in allergic asthmatic patients compared to normal controls, but these differences were statistically insignificant [40].

Our study revealed that serum IL-35 levels were significantly lower in severe persistent asthma patients followed by moderate persistent, mild persistent asthma, than intermittent asthma and control, and, its levels were lower in atopic patients than non-atopic patients.

Interleukin (IL)-35 is a potent immunoregulatory cytokine, but how IL-35 participates in the immunopathogenesis of allergic asthma patients is still unknown [41].

These results are consistent with the study by Ma et al. (2014), who concluded that serum IL-35 levels in asthmatic patients were significantly lower than those in healthy controls ($p < .05$) [1].

Our results also agree with that of Wang et al. (2015), who stated that serum IL-35 levels and its mRNA expression were decreased in allergic asthmatics. They concluded that IL-35 may be a new target in the immunotherapy for asthma patients [41].

Chen and co-workers (2014) also showed that the values of plasma IL-35 concentration in asthmatic ($p = .001$) and a chronic obstructive pulmonary disease (COPD) ($p = .000$) group were lower than those of control subject. They concluded that IL-35 may be involved in the Th2- and Th17-mediated inflammation process of asthma and COPD [4].

The study by Yao-Yuan and Kong (2012) also agreed with our results as they investigated the serum IL-35 levels in asthmatic patients during controlled, partly controlled and uncontrolled periods and found that serum IL-35 concentrations of the asthma group during uncontrolled and partly controlled periods were lower than the control group ($p < .001$) and no significant differences were found between

serum IL-35 concentrations in the asthma group and control group during the controlled period ($p = .36$) [42].

In the present study, atopic asthmatic patients showed significant positive correlations between serum IL-17 and serum total IgE and eosinophils counts ($r = 0.777$ and 0.854 , respectively), and significant negative correlations between serum IL-35 and both ($r = -0.507$ and -0.664 , respectively). These correlations were non-significant in the non-atopic group except for a positive correlation between IL-17 and eosinophils ($r = 0.385$). The study also revealed a strong negative correlation between serum IL-17 and serum IL-35 ($r = -0.635$); strong negative correlations were also found between serum IL-17 and FEV1, FVC and FEV1/FVC. IL-35 also showed positive correlations with FEV1 and FVC.

These results agree with the results of Chen et al. (2014), as they found that plasma IL-35 was positively correlated with increased FEV1% ($p = .004$), FEV1/FVC ($p = .020$) and FVC% ($p = .043$) in asthmatic patients [4].

However, our results are inconsistent with Ma et al. (2014), who found non-significant negative correlations of serum IL-35 with IgE ($r = -0.2909$, $p = .0807$) and eosinophil count ($r = -0.1206$, $p = .4586$) [1].

In our study, the best cut-off value of IL-35 level was <189.5 pg/mL and for IL-17 level, it was >13.1 pg/mL; this value could predict childhood asthma with sensitivity of 81.7% and 83.3, and specificity of 76.7% and 70%, respectively. A combination of both cytokines yielded increase in sensitivity to 95%.

Conclusion

Our study demonstrated that IL-17 is upregulated while IL-35 is downregulated in the serums of asthmatic children with significant negative correlations between both. These results suggest that both play an important role in the pathogenesis of childhood asthma, and both can be used in the prediction of childhood asthma, which gives a promising hope in the treatment of this disease via modulation of these pathways.

Further studies are needed at cellular level on a large number of patients for better understanding of factors that regulate both cytokines and their involvement in asthma.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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