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Elevated serum levels of Interleukin-13 and Interleukin-17A in Pediatric Asthma

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ABSTRACT

Background: Pediatric asthma is a chronic serious disease, which is difficult to diagnose with heterogeneous etiology. Till now it is not clear how T helper (Th)2 & Th17 pathways interact in pathophysiology of asthma, the dominant pathway can lead to different phenotypes.Th2-low (non Th2) which is mediated mainly by Th17, Th17-high and Th2-high type mediated by Th2 cell subset of lymphocytes are two different phenotypes of asthma.
Objectives: The present study focused on interleukin-17A (IL-17A), a major Th17 cytokine and interleukin-13 (IL-13) a cytokine secreted by Th2 cells trying to elucidate their combined role in airway inflammation.
Method: This is a case-controlled study include 149 children with a mean age of 1-15 years, 74 children with asthma and 75 control healthy subjects performed at asthma clinic outpatient during October 2019 through December 2019. Blood samples from all participants subjected to analysis by ELISA for measurement of IL-17A & IL-13 serum levels. Statistical Package for the Social Science, SPSS, (version 20, IBM, Chicago, Illinois) program was used for data entry and analysis.
Result: High serum IL-13 and IL-17A concentrations (P-value = 0.000), both cytokines are positively correlated, although non-significant statistically (P-value=0.133). IL-13, IL-17A &IgE are more associated with moderate asthma than mild form.
Conclusion: Elevated IL-13 & IL-17A serum levels among asthmatic children compared to control subjects, study suggest Th2-high / Th17-high phenotype in pediatric asthma pathogenesis.

Keywords: IL-13, IL-17A, Th2, Th17, Pediatric asthma

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INTRODUCTION

Asthma is a clinical disorder characterized by chronic airway hyper responsiveness with airway obstruction, eosinophilic and neutrophilic inflammation which manifested by cough, wheeze and shortness of breath. (1)
The role of Th2 CD4 cells with their corresponding secreted cytokines as interleukin (IL)-4, IL-5 and IL-13 is well defined in pathophysiology of adult asthma (2). IL-13 has both proinflammatory and anti-inflammatory features, so it is important IgE synthesis regulator and can mediate allergic inflammatory response in asthma. (3)
However, previous studies showed that different pathophysiological phenotypes present in asthma (4) and revealed a controversial role for Th2 activity (5) that can affect the targeting pharmacotherapies .(5,6)
Like Th2 pathway, Th17 cytokines has been implicated in pathophysiology of severe asthma with great concern (7). Th17 CD4 cells detected within lung tissue of sever asthmatic patients (8) and secret IL-17A, IL-17F, IL-21 & IL-10 (8), from which IL-17A is of major importance in mild to severe asthma. (9,10) IL-17A has also a proinflammatory action and involved in different disease processes. (11,12) Recruitment of neutrophil is the main action of IL17A in asthma. (13,14)
Previous researchers explained that IL-13 secretion can downregulate IL-17A secretion, others reported expression of IL-13 receptors on Th17 cells (15,16,17). The counteract activity of Th2 & Th17 is a matter of conflict in asthma pathophysiology. Th2 cytokines can downregulate Th17 cytokines production in vivo and in vitro (16). Other studies declared that Th2 can enhance Th17 activity and so increase air way inflammation. (18)
Finally, the exact role of Th2 & Th17 in asthma pathophysiology is still a complex and unclear process, which could interfere with therapy strategies pointed against Th2 or Th17 cytokines that can lead to worsening and more severe form of air way inflammation. Therefore, the present study tried to identify this correlation between these two immunological pathways and their relationship with pediatric asthma severity. Finally, the exact role of Th2 & Th17 in asthma pathophysiology is still a complex and unclear process, which could interfere with therapy strategies pointed against Th2 or Th17 cytokines that can lead to worsening and more severe form of air way inflammation. Therefore, the present study tried to identify this correlation between these two immunological pathways and their relation with pediatric asthma severity.

MATERIAL AND METHODS

This is a case control performed at Karbala Teaching Hospital for Children in (asthma clinic outpatient) during October 2019 through December 2019. All the included patient children diagnosed based on history and clinical examination and met the criteria of the American Thoracic Society for asthma.(19) Total 149 for patient and control groups distributed as 74 patients, 56 males and 18 females with age group range between one to fifteen years old (patients with inflammatory conditions other than asthma were excluded from the study). The control groups consist of75 children (50 male and 25females, with the same age distribution of the patient's group), with no history of allergies, asthma or inflammatory diseases.
Ethical approval for study was obtained from Karbala Health Directorate Committee, verbal and written approval also obtained from the patients' parents before taking the samples. The demographic and clinical information were recorded through an interview questionnaire.

The degree of asthma severity was identified based on the international standards diagnosed per the in the NAEPP/EPR 3 Guidelines (20) by the specialist paediatrician. About 5 cc of venous blood were drawn from each participant, serum was separated and stored in deep freeze (-20°C) till the time of immunoassay. Commercial ELISA kits were used to assess serum levels of IL-13, IL-17A (CUSABIO)and IgE (BioTek, USA), withdetection range equal to 62.5 pg/ml-4000 pg/ml for IL-13 and6.25 pg/ml-400 pg/m for IL-17A. The recommended protocols for the manufacturers were followed.

Statistical analysis: Statistical Package for the Social Science, SPSS, (version 20,IBM, Chicago, Illinois) program was used for data entry and analysis. Data were summarized into tables and graphs. Frequency, and percentage were used to describe the demographic and clinical data of the patients. As a Shapiro-Wilk's test ($p<0.05$), and visual appearance of their histogram, normal Q-Q plots and box plots showed that data was approximately non normally distributed for different variables of cases and controls, Mann Whitney U test was used for comparison of mean rank of interleukins between patients and control groups, Sperman rho was used to measure correlation between interleukins, and between interleukins and eosinophils and immunoglobulin in patients group.

RESULT

Sociodemographic and clinical characteristics of asthmatic patients were shown in table (1). 54.1% have allergic rhinitis, 27% with allergic conjunctivitis and 10.8% with allergic eczema. Table (2) reveal high statistical significance of IL-13 AND IL-17A in asthmatic patients compared to control group(p-value=0.000). Negative correlation between IL-13 and eosinophil count and IgE , it is significant for eosinophil (p=-0.297). No significant correlation for IL-17A with these parameters as shown in table (3). When we compared the study parameters with severity of asthma, the only significant association is for IgE, although serum levels of IL-13 and IL-17A were more in moderate severity cases compared to mild one as clarified in table (4). Significant correlation between serum concentration of IL-13 and IL-17A for all studied groups(p-value=0.000), although no statistical significance correlation between them among asthmatic group still there is positive correlation. Figure 1 & 2

DISCUSSION

The present study reports a significant high level for both IL-13 and IL-17A in asthmatic patients compared to control group. Within the asthmatic groups there was no significant association between severity and levels of both cytokines, although the mean level was more in moderate severity subgroup compared to mild subgroup. The role of IL-13 (one of TH2 cytokines) is well identified in pathophysiology of asthma. (2) It mediates allergic hyperactivity response of airways by regulation IgE secretion because it has proinflammatory and anti-inflammatory characters. (3) Past studies have reported increased IL-13 levels in pediatric asthmas. (21,22) Asthmatic patient's lung tissue analysis revealed the presence of Th17 CD4 cells with secretion of IL-17A (8) , which had a relevant role in mild to severe asthma (9, 10) The current finding similar to previous results that declared increment in plasma IL-17A in asthmatic patients compared to control subjects.(23)

Previous studies confirmed current findings by linking IL-17A levels with airway inflammation especially moderate to severe form asthma (7, 9, 23, 24)

In present study no significant correlation between IL-13 & IL-17A detected, but still it is a positive correlation which is against previous researchers' findings which concluded a downregulation of IL-17A by IL-13 secretion (15,16). others reported that the down regulation could be explained by IL-13 receptors expression on Th17 CD4 cells. (15,16,17) Other studies revealed that air way inflammation induced due to activity of Th17 could be enhanced by Th2 (19) Also previous research identified different types pf Th2 & Th17 in asthma (Th2-high, Th17-high & Th2-17 low). (25) The same study concluded that a positive direct correlation between Th2 & Th17(Th2-high, Th17-high) existed depending on a murine study, the study also suggests targeting both IL-13 and IL-17A in pharmacotherapy. (25) Th2-low or non-type 2-driven asthma represents several specific endotypes produced by different mechanisms rather than a single entity. (26)

A dual-positive Th2/Th17 cells correlation in bronchoalveolar lavage fluid of asthmatic patients is proved in another study. (27) They concluded that airway inflammation and obstruction is more in Th2/Th17 (predominant) group compared to Th2 (predominant) and Th2/Th17 (low) groups of asthma. (27) Cosmi *et al.*, reported that isolated human CD4+CD161+ T cells subset could develop into Th2 cells and Th17 with their corresponding cytokines as IL-17A in vitro study. (28)

Branchett *et al.*, explained the dual response of both IL-13 & IL17A on the basis of repeated house dust mite inhalation that lead to accumulation of CD41 lymphocytes in the lung tissue secreting IL-10. (29) Present finding reveals a significant association between IgE& severity, similar results for IgE association with severity confirmed by Momen *et al*. (30) Regarding the relationship between IL-13 and IgE which is important as IL-13 was defined as an active regulator for IgE synthesis in allergic patients (31), addition of IL-13 to culture significantly enhance IgE synthesis as demonstrated by Doleck *et al*. (31) which is against current findings that shows negative correlation between IL-13 with eosinophil count and IgE However, other study declared that IL-13 lead to development of asthma in a way which is independent on IgE and eosinophils. (32) Afshari *et al*. demonstrate no correlation between IL-13 mRNA expression and IgE levels. (33)

For conclusion, present data reported high serum levels of IL-13 & IL-17A in pediatric asthma that are positively correlated and associated with moderate form of the disease. These findings could be explained on bases of heterogenicity of asthma with different causal underling pathophysiology. Consequently, it is difficult to depend on IL-13 & IL-17A concentrations alone for determining role of Th2 & Th17 pathway in asthma. In addition, peripheral cytokines level does not give a relevant insight for its source compared to local environment analysis and cellular level, keeping in mind serum analysis for this study has advantages of being more feasible and non-invasive procedure. The severity of asthma was not the main focus of study because of limitation of participants inpatient for severe form asthma with more detailed severity, control and management lines data.

This study highlights the need for more local, cellular and molecular investigations and extended studies for determine role of Th2, Th17 cells subset in pediatric asthma in Iraq which could change the strategies of therapy and diagnosis in pediatric asthma.

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Tables and Figures

Table 1: sociodemographic and clinical features of asthmatic cases (N=74)

Variables		Frequency	Percentage
Gender	Male	56	75.7
	Female	18	24.3
Age(year)(mean±SD)		8.01±4.13	
History of eczema		8	10.8
allergic rhinitis		40	54.1
allergic conjunctivitis		20	27.0
Family history of eczema		16	21.6
Family history of asthma		50	67.6
Family history of allergic rhinitis		58	78.4
Family history of smoking		34	45.9
Aggravating by flu		50	67.6
Aggravating by dust		40	54.1
Treatment	Montelukaste	30	40.5
	Inhaled corticosteroid	4	5.4
	Mixed	2	2.7
	Nil	38	51.4
Severity	Mild	62	83.8
	Moderate	12	16.2

Table 2: Differences in the concentration of interleukin between the studied patients and control groups.

Parameter (Mean Rank)	Cases N=74	Controls N=75	Mann-Whitney U	P-value
IL-13 pg/Ml	89.76	60.44	1683.000	0.000**
IL-17A pg/Ml	107.72	42.72	354.000	0.000**

**** P value is of high statistically significant. IL =interleukin, pg/ml=picogram/milliliter.**

Table3: Correlation of interleukin-13 and interleukin-17A with Immunoglobulin E and eosinophils count in asthmatic patients (N=74).

Parameter	IL-13		IL-17A	
	Correlation Coefficient	P-value	Correlation Coefficient	P-value
Eosinophil count	-0.297*	0.010	0.789	0.740
IgE	-0.222	0.058	-0.075	0.527

*** Correlation is significant at the 0.05 level (2-tailed).**

Table 4: Association of interleukin-13, interleukin-17A, IgE and Eosinophil count with severity of asthma (N=74)

Parameter	Mild		Moderate		P-value
	Mean	S.E.	Mean	S.E.	
IL-13	0.73(0.4-1.09)	0.31	1.017(0.44-1.9)	0.39	0.084
IL-17A	20.49(1.8-35.3)	6.24	25.36(0.47-192.67)	4.79	0.766
IgE	609.03(421.8-768.3)	63.87	750.37(24.17-855.9)	34.68	0.036*
Eosinophil count	252.27(2.8-1000)	461.50	93.73(0.3-1000)	284.04	0.168

One-way ANOVA* P value is significant ≤ 0.05.

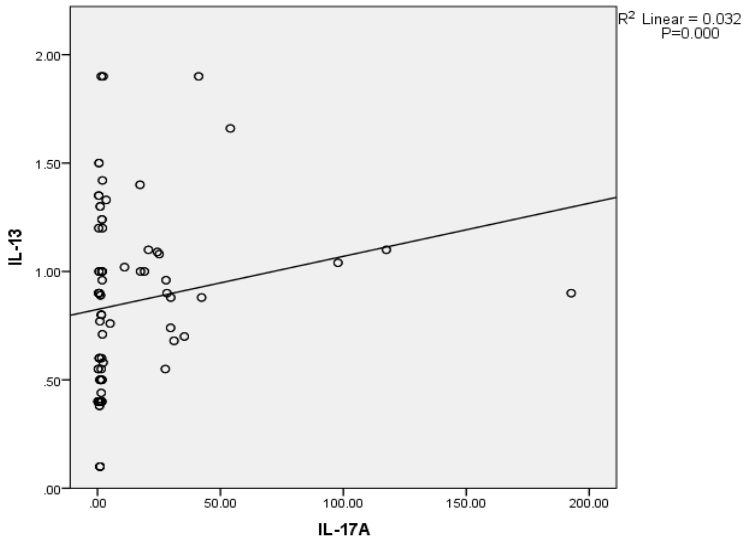

Figure 1: Correlation between IL-13 and IL-17A in studied group (N=149).

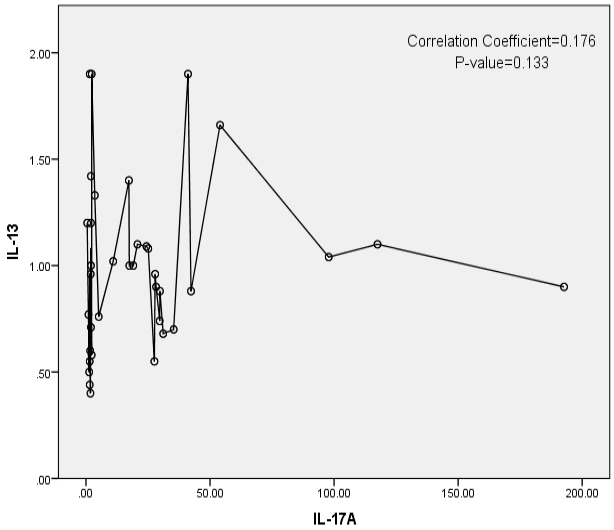

Figure 2: Correlation between IL-13 and IL-17A in Asthmatic patients (N=74).