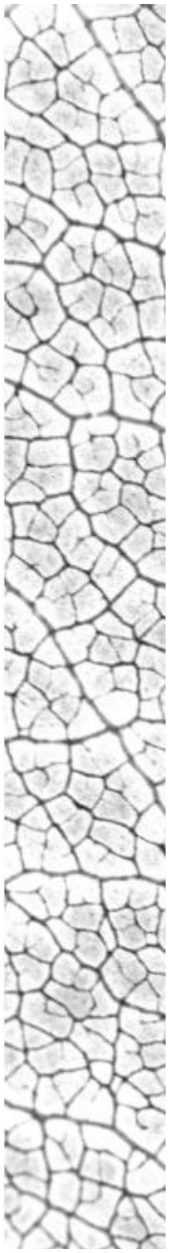

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Role of interleukin 25 and interleukin 33 as immunological markers in pediatric asthma

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Abstract

Background: Asthma is a chronic airways disease recognized by variable obstruction of the airflow, airway inflammation and hypperesponsiveness of the airway. The epithelial cytokines IL-33 and IL-25 have been implicated in asthma pathogenesis because they promote Th2-type cytokine synthesis.
Objectives: This study amid to show the role of both interleukins 25 and 33 in the airway inflammation.
Methods: The case- control study included 74 asthmatic children as patients group, 75 non asthmatic children as control group. Serum levels of IL-25 and IL-33 was measured by sandwich ELISA using ELISA kit (CUSABIO-china).
Results: Asthma was reported in a high frequency among males 56 (75.7%) than females 18 (24.3%). There was a high frequency of family history of asthma 50 (67.6%) and rhinitis 58(78.4%) in asthmatic patients. Also, there were a high frequency of aggravating by flu 50 (67.6 %) and dust 40 (54.1%) in asthmatic patients. According to the treatment, there was a high frequency of montelukaste 30 (40.5%) in asthmatic patients. In addition, there was a high frequency of mild severity in asthmatic patients. The serum level of IL-25 have a highly significant (*P= 0.000*) association with susceptibility to asthma, while IL-33 showing a non-significant (*P= 0.473*) association with the disease. Also, there were a non- significant correlation (*P= 0.688*) between IL-25 and IL-33 with the disease. Regarding correlation of IL-25 and IL-33 with Immunoglobulin E and eosinophil's count showing a non-significant association in asthmatic patients.
Conclusion: There was a highly significant association between the IL-25 serum level and susceptibility to asthma. Also, this study was reported a non-significant relationship between IL-33 and asthmatic disease.

Keywords: pediatric asthma, serum levels of IL-25 and IL-33, IgE level, eosinophils count

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the airway which can associate with functional and structural changes in the airway such as airway hyperresponsiveness and remodeling (Holgate, 2012). Asthma is a highly various disturbance with different trigger factors like allergens or environmental irritants and some patients with asthma participated common physiological disability and symptoms like shortness of breath, obstruction of the airway, cough and repeated wheeze attack (Bhakta and Woodruff, 2011; Coban and Ediger , 2018; Hassanpour et al., 2019).

Some Th2 cells cytokines and chemokine's perform a considerable role in the pathogenesis of asthma (Barnes, 2001). The Th2 cells immune response have ability to destroy the extracellular pathogens such as parasites and bacterial organisms by one types of the Th2 cell. Several types of Th2 cytokines like IL-4, IL-5 and IL-13 are associated with the pathology of asthma (Murdoch and Lloyd, 2010).

Interleukin 25 (IL-25) represents a member of IL-17 family and excreted locally in the airways, also, associated with inflammation of the asthmatic patient's airway. This cytokine is excreted by bronchial epithelium (Kouzaki et al, 2013). Eosinophils basophils and mast cells can produce IL-25 (Terrier et al, 2010).Allergic Th2 inflammation can be stimulated and augmented by IL-25 through release of IL-4. IL-5 and IL-13 (Tamachi et al. 2006). This will lead to increase concentration of the serum IgE, eosinophilia and hyperresponsiveness of the airway (Sharkhuu et al. 2006). The expansion of group 2 innate lymphoid cells can be activated by IL-25 and IL-33 and this will lead to the early irritation of type 2 immune response (Saenz et al. 2010).

The eosinophils can be activates directly by interleukin 25 through up-regulating the ICAM-1

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expression, promoting the production of pro-inflammatory chemokines like IL-8, IL-6, macrophage inflammatory protein-1 and delaying apoptosis. (Wong et al. 2005; Cheung et al. 2006).

A pro-inflammatory IL-33 is related with the IL-1 cytokines family and released in response to insults like smoke, allergens or viruses by damaged cells of barrier tissues (Leuthi et al. 2009; Cayrol and Girard, 2014; Molofsky et al. 2015; Kearley et al. 2015; Martin and Martin, 2016; Cayrol et al. 2018). In addition, IL-33 associated with severity of bronchial asthma and expression of this cytokine increased in the bronchoalveolar lavage fluid and epithelial cells of patients with this disease. (Préfontaine et al. 2009; Préfontaine et al. 2010)

MATERIALS AND METHODS

Patients

Seventy four patients were clinically diagnosed, 56 males and 18 female, at age ranged between (1-15) years old, attending to the asthmatic clinic in Kerbala Teaching Hospital for children in kerbala province. In addition, seventy five non asthmatic children attending the outpatient clinic were recruited as control subjects. All participants underwent a complete screening panel, including medical history and clinical examination. Five ml of venous blood were collected from each patients and control groups, collected in gel tubes, slow withdrawal of the blood sample via the needle of syringe to prevent hemolysis. The sample dropped into clean disposable gel tube, serum was separated after 20 minutes at room temperature. The samples were then centrifuged at 3500 rpm for 5 minute and then stored at freeze condition (-20C) until analyzed. Commercial ELISA kit was used to asses serum level of IgE (Bio Tek-USA).

Human interleukin-25 ELISA test

Serum levels of IL-25 were determined by quantitative sandwich enzyme immunoassay technique using (CUSABIO-china). Catalog Number. CSB-E11715h.

Human interleukin-33 ELISA test

IL-33 serum levels were determined by quantitative sandwich enzyme immunoassay technique using (CUSABIO-china). Catalog Number. CSB-E13000h.

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 eosinophil's and immunoglobulin in patients group.

Table 1. Socio-demographic and clinical features of 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	Positive	8	10.8
	Negative	66	89.2
Allergic rhinitis	Positive	40	54.1
	Negative	34	45.9
Allergic conjunctivitis	Positive	20	27.0
	Negative	54	73.0
Family history of eczema	Positive	16	21.6
	Negative	58	78.4
Family history of asthma	Positive	50	67.6
	Negative	24	32.4
Family history of allergic rhinitis	Positive	58	78.4
	Negative	16	21.6
Family history of smoking	Positive	34	45.9
	Negative	40	54.1
Aggravating by flu	Positive	50	67.6
	Negative	24	32.4
Aggravating by dust	Positive	40	54.1
	Negative	34	45.9
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

RESULTS

The socio-demographic characters for 74 patients with asthma disease were 56 (75.7%) males and 18 (24.3%) females with higher frequency at mean age (8.01). follow by the lower frequency 8 (10.8%) about history of eczema. About all rhinitis and all conjunctivitis the frequency were 40 (54.1%) and 20 (27.0%) respectively in asthmatic patients. Also, the frequency was high in patients regarding family history of asthma 50 (67.6%) and rhinitis 58 (78.4%) while lower frequency about family history of eczema 16 (21.6%) and smoking 34 (45.9%). In addition, aggravating by flu and dust were found in a high frequency 50 (67.6%) and 40 (54.1%). Treatment by montelukaste was found in a high frequency 30 (40.5%) in asthmatic patients. About severity of disease the high frequency was found in mild form 62 (83.8%) of asthmatic severity, as found in **Table 1**.

The mean rank of IL-25 in asthmatic children was significantly higher (*P= 0.000*) than in controls, (107.39) and (43.04), respectively.IL-33 showing a non-significant (*P= 0.473*) association with patients group, giving a mean rank of (72.45) and a mean rank of (77.52) among control group, as shown in **Table 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_ 25 pg/mL	107.39	43.04	378.000	0.000**
IL_ 33 pg/mL	72.45	77.52	2586.000	0.473

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

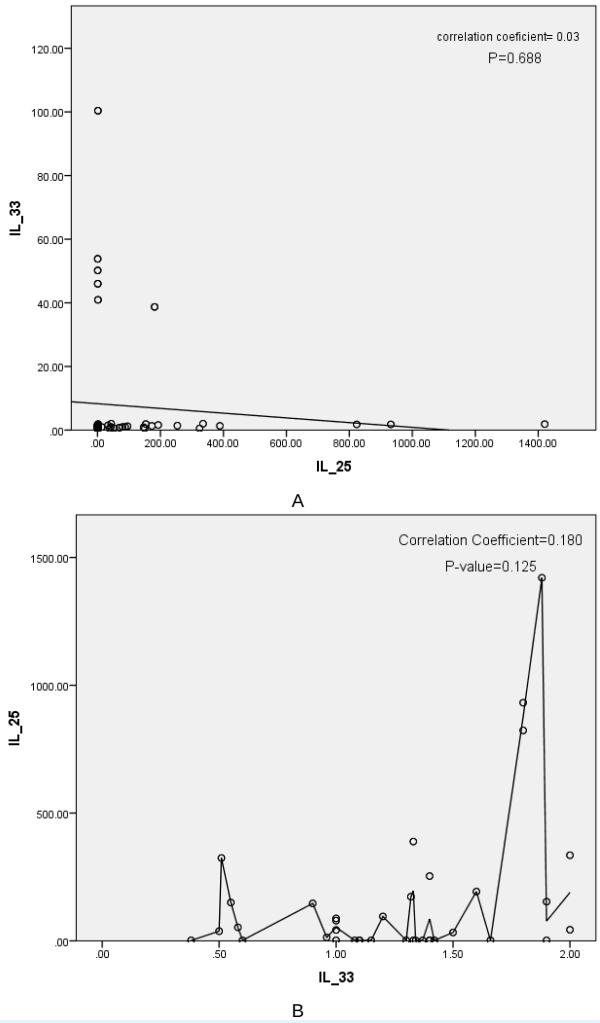

Fig. 1. A- Correlation between IL-25 and IL-33 in asthmatic cases and non -asthmatic control; B-Correlation between IL-25 and IL-33 in Asthmatic patients

Table 3. Correlation of interleukin-25 with Immunoglobulin E and eosinophils count in asthmatic patients (N=74)

Parameter	Correlation Coefficient	*P-value*
Eosinophils	0.108	0.358
Immunoglobulin E	-0.153	0.192

Fig. 1A showing a non-significant (*P= 0.688*) correlation between IL-25 and IL-33 in asthmatic children and non-asthmatic control with correlation coefficient (0.03), while in **Fig. 1B** found a non-significant (*P= 0.125*) correlation between these interleukins in asthmatic cases only.

Table 4. Correlation of interleukin-33 with Immunoglobulin E and eosinophils count in asthmatic patients (N=74)

Parameter	Correlation Coefficient	*P-value*
Eosinophils	0.147	0.212
Immunoglobulin E	0.029	0.808

Regarding IL-25, there was a non -significant correlation with Immunoglobulin E and eosinophils count in asthmatic patients, as shown in **Table 3**.

Table 4 showing a non- significant correlation of IL-33 with Immunoglobulin E and eosinophils count in asthmatic patients, as found in **Table 4**.

DISCUSSION

Asthma disease was found in males more than females at a percentage (75.7%) and (24.3%) respectively. This result was associated with a study accomplished by (Hubaida and Dawn, 2017) who revealed about asthmatic children, the boys are more prevalence of asthma than girls at a percentage (11.9%) versus (7.5%) respectively. Also, (Kynyk et al, 2011) reported about hospitalized asthma exacerbation that boys are twice as likely as girls. Another study of (Dold et al, 1992) found that boys with asthma have a higher prevalence rate than girls, but both sexes are comparable about risk factors of parental asthma.

Asthma was found high in children of age mean at (8.01). The result of present study were related to a study of (Hansel et al, 2013) who found that children more than five years old are susceptible to wheezing caused by viral organisms and these organisms with in the first three years is the most important risk factor for infected with asthma at age six years old.

Some disorders such as rhinitis, eczema and asthma are more prevalent with increasing age. These disorders are more related with IgE antibodies but in non -sensitized human, also can be occur. In atopic and non-atopic individuals, seasonal rhinitis is the prevalent symptom of these allergic disturbance with a prevalence about 10% to 30%. In addition, atopic eczema is found in 7% of allergic disorders and this result in the same line with result of current study (Steiner et al, 2018).

About family history of asthma, the predisposing factor for asthma in the offspring is single allergic disease of one parent with only asthma without allergic rhinitis. Several studies reported that asthma of one of the parents either mother or father can be elevated the risk of rhinitis. The results of these studies were corresponding with the results of present study (Dold et al, 1992).

Regarding smoking family history, our study was compatible with a study accomplished by (Polosa and Thomson, 2013) who found that smoking individual are significantly elevated risk of asthma disease. Also reported that female smokers have a prevalence rate of asthma at 70% higher than non -smokers female.

Moreover, a study of (Elliott et al, 2007) found that house dust may responsible for cause or exacerbate respiratory disease such as asthma. Also about the association between asthma and flu, a study conducted by (Trinh *et al,* 2018) found a significant association between influenza and asthma hospitalizations.

IL-25 is responsible for stimulation the immune response of Th2. Our study compatible with a study conducted by (Paplińska –Goryca et al, 2018) who reported the association between high concentration of IL-25 and the atopic status of asthmatic patients. Also, a study of (Wang et al, 2016) found the association between the IL-25 expression and asthmatic disease in human and this study were in the same line with present study. The IL-25 plays an essential role in the allergic disorders such as bronchial asthma because the release of this interleukin was increased in the damaged airway epithelium. The arrangement of IL-25 production is considered a new orientation for the treatment of bronchial asthma because this interleukin play important role in the bronchial asthma pathogenesis (Liu et al, 2018).

Current study found a non-significant association between IL-33 serum level and asthmatic disease and this result inconsistence with the result of (Momen et al, 2017) who confirms a highly significant between IL-33 serum level and disease. Interleukin-33 performs important role in the inflammatory diseases such as cardiovascular disease, rheumatoid arthritis and asthma. Also, it is responsible for induces the release of several cytokines such as IL-4 and IL-13 and stimulates many types of cells like mast cells, basophils and T helper cells (Fulgheri and Malinowski, 2011).

Our study reported a non- significant correlation between the interleukin 25 and interleukin 33 in the cases with asthma and also between asthmatic patients and control. This result were corresponding with the result of (Barlow et al, 2013) who found that IL-33 can stimulated ILC2s which considered a major cell target in the lung and it is a strong stimulator of the allergic response than interleukin 25. These cytokines may play different roles in the lung. In addition, IL-33 is responsible for induction of type 2 cytokines that stimulated lung airway hyperresponsiveness because IL-33 production proceeds the response of interleukin 25 through the allergic asthma.

Regarding the relationship between IL-25 and eosinophils, these cells represent the typical markers of airway inflammation in the bronchial asthma. Also, these cells can be activated by IL-25 in allergic condition of inflammation, while cytokine levels like IL-4, IL5, chemokines of eosinophil and immunoglobulin IgE increased (Liu et al, 2018). This study disagreement with our study that found a non- significant association of IL-25 serum level with eosinophil count and IgE level in asthmatic children.

Furthermore, present study revealed a non-significant correlation between IL-33 and IgE serum levels and this result disagreement with the results of several studies that reported the IL-33 may have a role in IgE mediated allergic disease because expansion of B cell and synthesis of IgE can be stimulated by IL-33 and this will lead to secretions of IL-4 by innate cells. The IL-4 stimulating the interaction of CD40 ligand on T cells and CD40 on B cells then trigger the IgE production (Momen et al, 2017). In addition, (Tworek et al, 2018) confirm a positive relationship between IL-33 serum level and eosinophil count in asthmatic disease. This result inconsistence with current study who revealed a negative correlation between IL-33 serum level and eosinophils count.

CONCLUSION

There was a highly significant association between the IL-25 serum level and susceptibility to asthma. Also, this study was reported a non-significant relationship between IL-33 and asthmatic disease.

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