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Elevated serum levels of interleukin -22 and interleukin-23 in pediatric asthma

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Abstract---Background: There are few studies had addressed the association between serum levels of Interleukin -22 and Interleukin-23 with severity of asthma, this study aims to evaluate whether elevated IL-22 and IL-23 serum levels is related to the severity of asthma. Methods: This study is a case-control study that included 60 asthmatic children (35 male and 25 female) as the patients group age ranged between 4 months to 14 years old, and 60 non-asthmatic (32 male and 28 female) age-matched patients group attending the outpatient clinic were recruited as control group. Serum levels of IL-22 and IL-23 was measured by sandwich ELISA using ELISA kits. Results: The results of this study have shown an elevated serum level of IL-23 in patients group compared with control group with significant ($p= 0.008$) and there was no significant difference between patients and control regarding IL-22 serum level ($p = 0.721$) in asthmatic children. Conclusion: According to the current study the serum level of IL-23 showing a significant association with the asthma severity in children.

Keywords---Asthmatic children, IL-22, IL-23, ELISA test

Introduction

Asthma is considered as an inflammatory disease in the airway, leading to airway hyper-responsiveness, obstruction, mucus hyper-production and airway wall remodeling (Kudo, Ishigatsubo, and Aoki 2013). In the development of this complicated illness, the interplay of hereditary and environmental variables is crucial (Mukherjee and Zhang 2011). Chronic inflammation is associated with airway hyperresponsiveness (an exaggerated airway-narrowing response to specific triggers such as viruses, allergens and exercise) that leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity (Vogelmeier et al. 2017). There are many cytokines implicated in the development of the chronic inflammatory processes that are often observed in asthma. Ultimately, these cytokines cause the release of mediators such as histamine and leukotrienes (LT), which in turn promote airway remodeling, bronchial hyperresponsiveness and bronchoconstriction. The CD4+ T-lymphocytes from the airways of asthmatics express a panel of cytokines that represent the Th2 cells (Ayakannu et al. 2019). Eosinophils, which tend to accumulate at sites of allergic inflammation, contribute to the development of bronchial asthma (Weller 1997). The allergic asthmatic response to allergen exposure is associated with immunoglobulin E (IgE) mediated mast cell activation, resulting in the accumulation of leukocytes such as eosinophils and Th2 lymphocytes in the airway (Umetsu and DeKruyff 1997; Rajakulasingam et al. 1997). IL-22 is produced by Th17 cells at the site of allergic airway inflammation and attenuates eosinophilic inflammation and airway hyperresponsiveness (AHR), presumably by inhibiting cytokine and chemokine production from lung epithelial cells. In asthma patients, however, excessive production of IL-22 may lead to the progression of airway remodeling by enhancing the proliferation and migration of airway smooth muscle cells (ASMCs) (Hirose, Takahashi, and Nakajima 2013). A study was performed by (Ciprandi et al. 2012) demonstrated that serum IL-23 was up-regulated in asthmatic children and it change well related with lung function improvement. Thus, it is presumable that IL-23 could be a suitable marker of allergic inflammation in asthma.

Materials and Method

One hundred and twenty blood samples were taken and divided into sixty clinically diagnosed asthmatic children and sixty non asthmatic children. The asthma samples were taken from both sexes (25) males and (35) females at age ranged between 4 months to 14 years old attending to asthma clinic of Imam AL-Hussein Medical City, Karbala /Iraq during the period extended from November (2020) to March (2021). Case information sheets involving age, gender and others were carried out for each patient. Exclusion criteria include patients with autoimmune disease (rheumatoid arthritis, inflammatory bowel disease and others), tumour and COPE. One ml of venous blood was collected from each participants in EDTA tube was used for absolute eosinophils count determination Using Sysmex XN-350 five differential automated hematology analyzers used, while four ml of venous blood were collected from patients and control 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 stored at -20 C° for subsequent analysis. Serum levels of IL-22 and IL-23 were determined by classic sandwich-ELISA using ELISA kits (Bioassay Technology, China).

Statistical analyses

All statistical tests were calculated using SPSS. *P* value less than 0.05 was regarded statistically significant.

Result

Asthmatic patients age ranged between 4 months to 14 years old, there were no significant differences ($P = 0.413$) in age distribution between asthmatic patients and control, the means of ages are (7.89 ± 4.29) and (8.50 ± 3.88) for asthmatic children and control respectively, as shown in table (1). The study result revealed that a higher distribution of asthma in male compared with female with a percentage 35(58.3%) and 25(41.7%) respectively. There was no significant difference ($P = 0.317$) in gender distribution between the patients and control. In the present study found that 2(3.3%) and 1 (1.7%) of patients and control respectively had a positive result for history of eczema, while 58(96.7%) and 59(98.3%) of those have a negative result. There was no significant difference ($P = 0.679$) in history of eczema distribution between the patients and control. Regarding the family history of eczema, found 7(11.7%) and 0 (0.0%) of patients and control respectively had a positive family history of eczema while 53 (88.3%) and 60 (100%) of those have a negative result. There was a significant difference ($P = 0.013$) in family history of eczema distribution between the patients and control. In the current study found that 11(18.3%) and 12 (20%) of patients and control respectively had a positive allergic rhinitis, while 49 (81.7%) and 48(80%) of those have a negative result. There was no significant differences ($P = 0.817$) in allergic rhinitis distribution between the patients and control. Concerning family history of allergic rhinitis, found 30(50%) and 8(13.3%) of patients and control respectively had a positive result, while, 30(50%) and 52 (86.7%) of those have a negative result. There was a significant difference ($P = 0.005$) in family history of allergic rhinitis distribution between the patients and control. Also, this study found that 17(28.3%) and 8 (13.3%) of patients and control respectively had a positive allergic conjunctivitis, while 43(71.7%) and 52(86.7%) of those have a negative result. There was no significant difference ($P = 0.071$) in allergic conjunctivitis distribution between the patients and control. In addition, current study found that 30 (50%) and 12 (20%) of patients and control respectively had a positive result for exposure to cigarette smoke while, 30 (50%) and 48(80%) of those have a negative result. There were a highly significant differences ($P = 0.001$) in exposure to cigarette smoke distribution between the patients and control.

Table 1: Distribution of demographic characteristics for patients and control

Variables	Group				<i>P</i> value
	Patients N=60		Control N=60		
	Mean	SD	Mean	SD	
Age	7.89	4.29	8.50	3.88	0.413

		Count	%	Count	%	
Gender	Male	35	58.3%	32	53.3%	0.317
	Female	25	41.7%	28	46.7%	
History of Eczema	+ve	2	3.3%	1	1.7%	0.679
	-ve	58	96.7%	59	98.3%	
Family History of eczema	+ve	7	11.7%	0	0.0%	0.013*
	-ve	53	88.3%	60	100.0%	
Allergic rhinitis	+ve	11	18.3%	12	20.0%	0.817
	-ve	49	81.7%	48	80.0%	
Family History of allergic rhinitis	+ve	30	50.0%	8	13.3%	0.005*
	-ve	30	50.0%	52	86.7%	
Allergic conjunctivitis	+ve	17	28.3%	8	13.3%	0.071
	-ve	43	71.7%	52	86.7%	
Exposure to cigarette smoke	+ve	30	50.0%	12	20.0%	0.001**
	-ve	30	50.0%	48	80.0%	

* *p* value is significant ($P < 0.05$), ** *p* value is highly significant, Student's t-test, Chi-square test

The current study showed that the (mean \pm SE) of eosinophils count were (0.40 ± 0.06) and (2.14 ± 0.24) in patients and control respectively there was a significant difference ($p = 0.005$) between patients and control in eosinophil counts. Also, this study found that the IgE serum level were (126.21 ± 14.57) and (37.65 ± 5.14) in patients and control respectively there was a significant differences ($p = 0.005$) between patients and control in IgE serum level. In addition, serum level of IL-22 were (41.54 ± 10.05) and (36.57 ± 9.55) in patients and control respectively, there was no significant differences ($p = 0.721$) between patients and control regarding IL-22 serum level. In current study found that serum level of IL-23 were (54.12 ± 17.81) and (5.12 ± 2.50) in patients and control respectively there was a significant differences ($p = 0.008$) between patients and control regarding IL-23 serum level, as clarified in table (2).

Table 2: Mean differences of IL-15, IL-22 and IL-23 serum level among the patients and control

Variables	Patients		Control		<i>P</i> value
	Mean	SE	Mean	SE	
EOS	0.40	0.06	2.14	0.24	0.005*
IgE	126.21	14.57	37.65	5.14	0.005*
IL 22	41.54	10.05	36.57	9.55	0.721
IL 23	54.12	17.81	5.12	2.50	0.008*

* *p* value is significant ($P < 0.05$), Student's t-test

Correlation between Eosinophils count and the duration of asthma

Figure (1) showed the correlation between EOS count and duration of disease in months about asthmatic patients. No significant negative correlation were found between EOS count and duration of disease, $r = -0.61$ ($p = 0.646$).

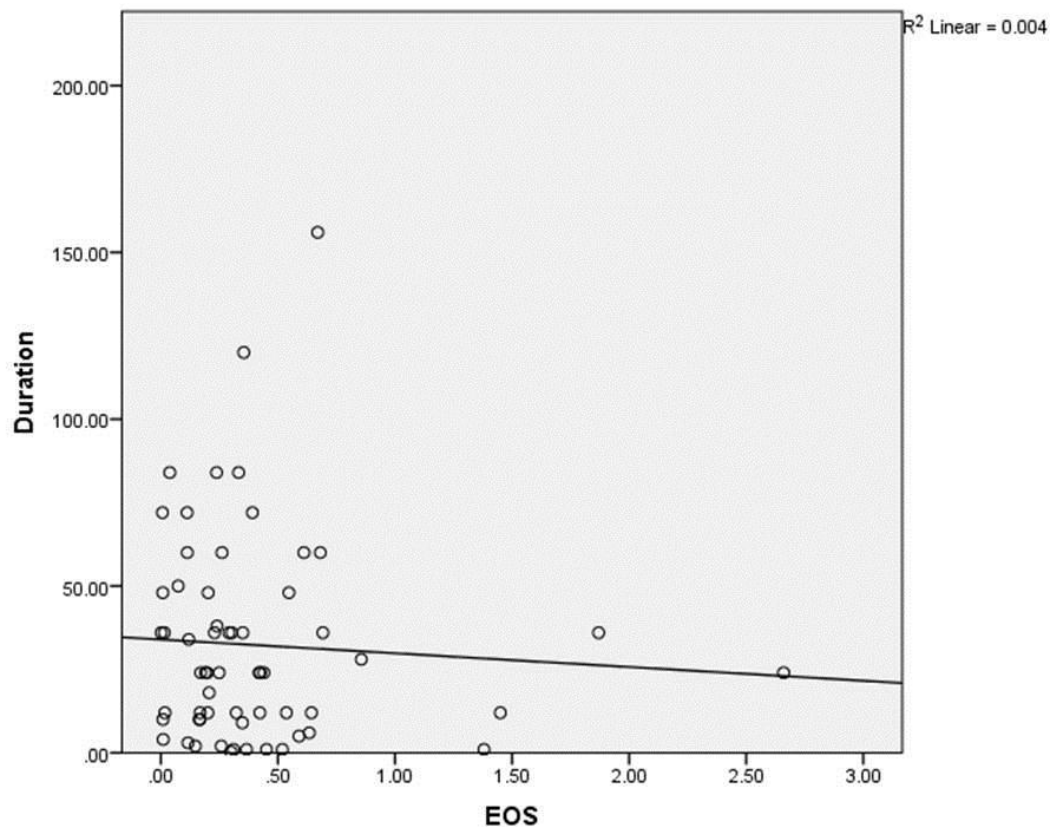


Figure (1): Correlation of eosinophil count with the duration of asthma in months.
 Pearson correlation coefficient: $r = -0.61$ ($p = 0.646$)

Correlation between IgE serum level and duration of asthma

Figure (2) showed the correlation between IgE and duration of disease in months of asthmatic patients. No significant negative correlation were found between IgE serum level and duration of disease, $r = -0.017$ ($p = 0.898$).

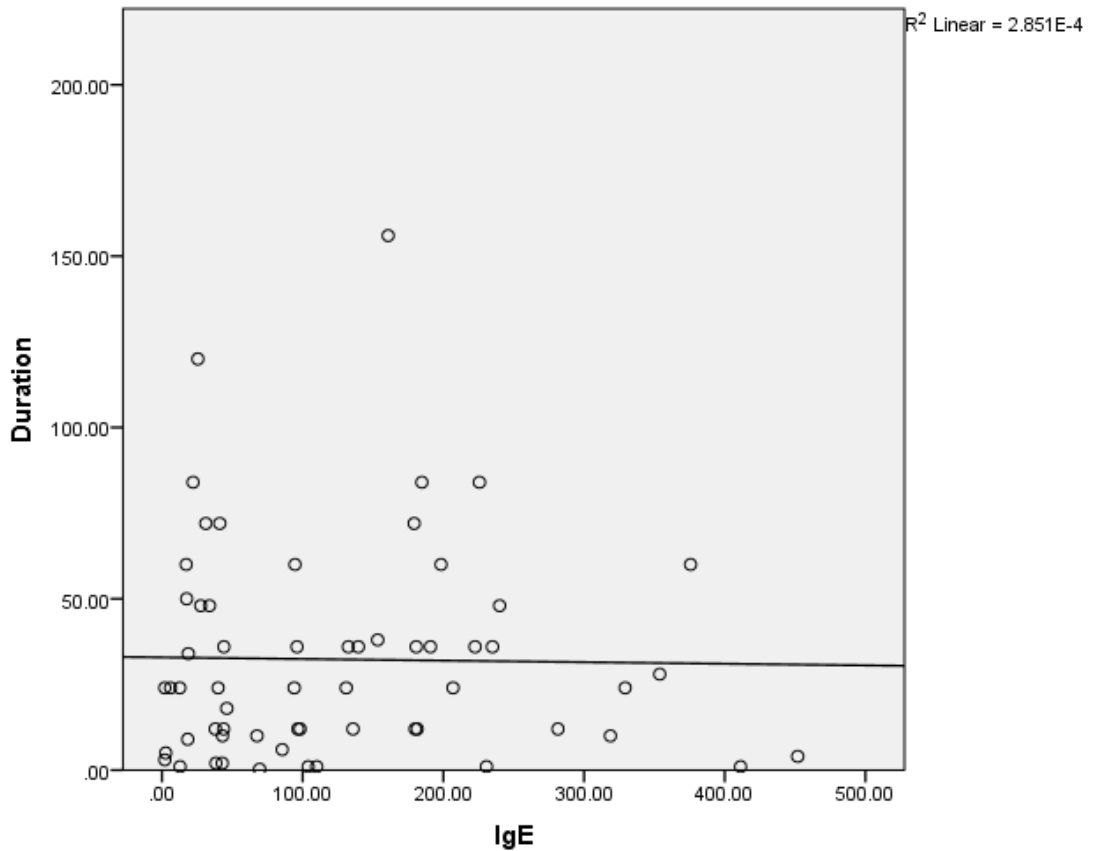


Figure (2): Correlation of IgE serum level with the duration of asthma in months.
 Pearson correlation coefficient: $r = -0.017$ ($p = 0.898$).

Correlation between interleukin 23 (IL-23) serum level and duration of asthma

Figure (3) showed the correlation between IL-23 and duration of disease in months of asthmatic patients. There were a significant negative correlation between IL-23 level and duration of asthma $r = -0.280$, ($p = 0.030$).

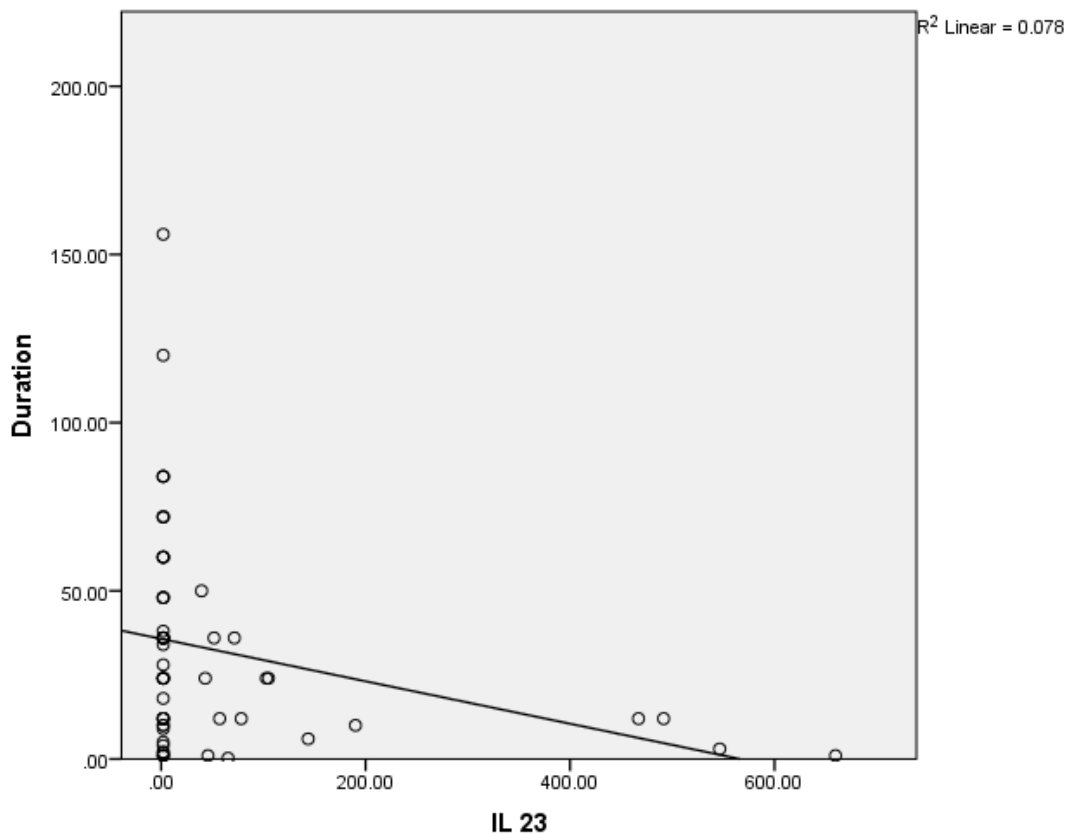


Figure (3): Correlation of IL-23 serum level with the duration of asthma in months. Pearson correlation coefficient: $r = -0.280$ ($p = 0.30$)

The Correlation between IL-23 serum level and eosinophil count in asthmatic children

Figure (3-14) showed the correlation between IL-23 serum level and eosinophil count in asthmatic children. No significant positive correlation was found between IL-23 serum level and eosinophil count, $r = +0.064$ ($p\text{-value} = 0.625$).

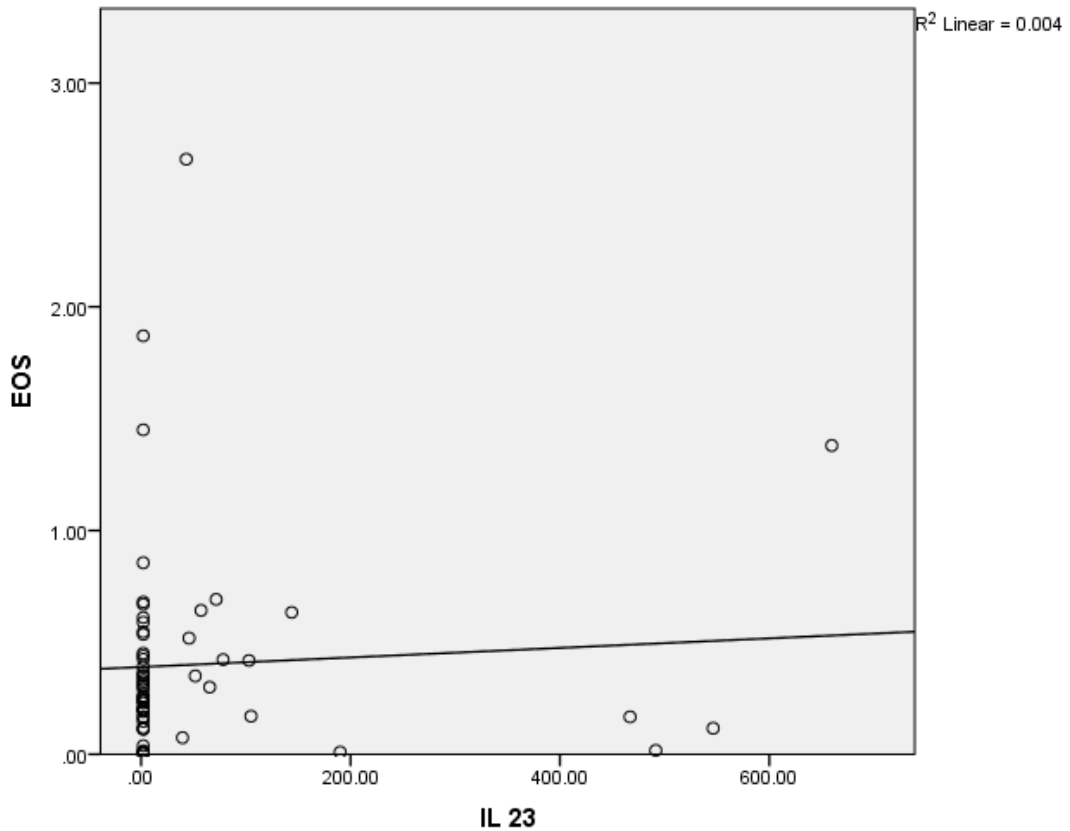


Figure (4): Correlation of IL-23 pg/ml serum level with the eosinophil count.
 Pearson correlation coefficient: $r = +0.064$ ($p\text{-value} = 0.625$).

The Correlation between IL-23 and IgE serum levels in asthmatic children

Figure (3-15) showed the correlation between IL-23 and IgE serum levels. No significant negative correlation were found between IL-23 and IgE serum levels, $r = -0.170$ ($p\text{-value} = 0.194$).

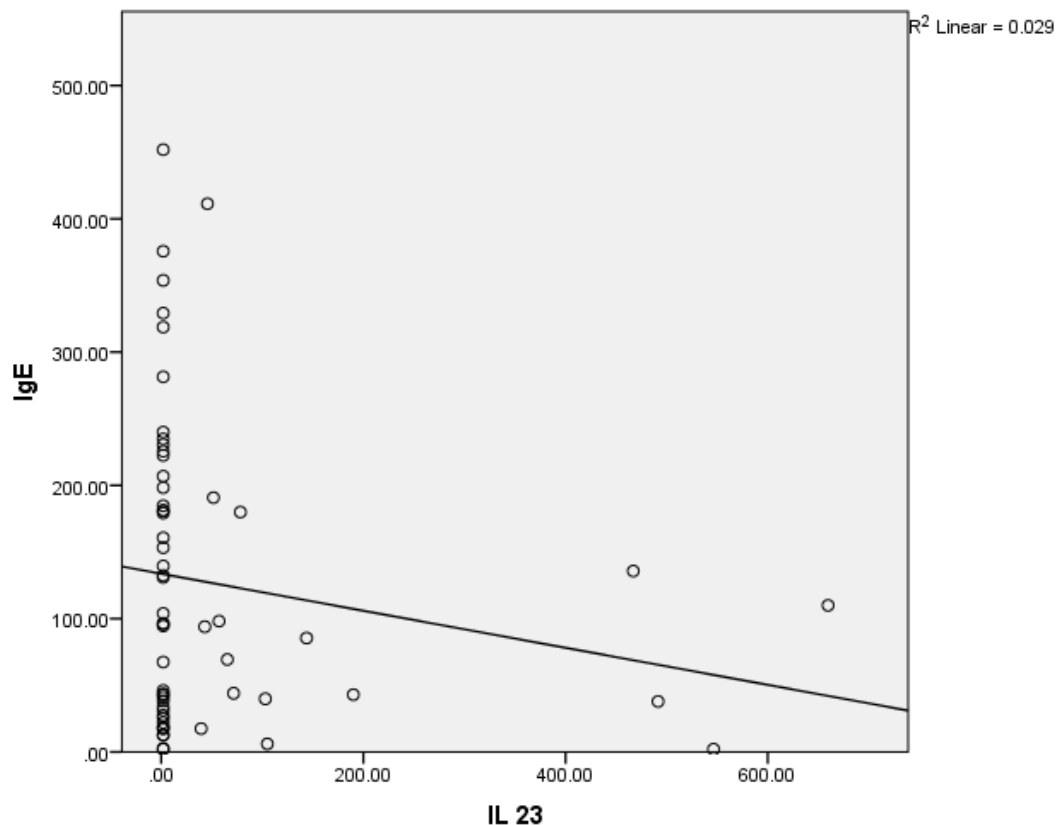


Figure (5): Correlation of IL-23 pg/ml serum level with the serum IgE. Pearson correlation coefficient: $r = -0.170$ ($p\text{-value} = 0.194$)

Discussion

The current study showed that there is no significant difference ($P = 0.413$) in age between the patients and control groups. This result was agreed with a study achieved by Chen et al. (2020) indicated no significant differences in age among asthmatic children and control, as shown in table-1. The present study showed that males suffered from asthma more than females. The majority of patient's group were males 35 (58.3%) compared with 25 (41.7%) of females. This result was associated with a study conducted by (Hameed et al. 2019) who revealed that the asthma were higher in males which was (69.4 %) compared with females (30.6%). Also, a study conducted by (Wright et al. 2006) found that showed that boys were significantly more likely than girls to have asthma (53.8% vs. 43.3%). Also (Zein and Erzurum 2015) revealed to that onset of asthma more in boys than girls in childhood. The mechanism of the changing gender ratio appears to be the late incidence of asthma among girls because the latter constitute a considerable part of adult asthma cases. (Nicolai et al. 2003).

Regarding the history of eczema, the present study found 2(3.3%) of asthmatic patients had a positive result compared to 1(1.7%) of control. In the same line (von Kobyletzki et al. 2012) revealed that children with eczema at baseline had more

than a 2-fold increase in the odds of developing asthma. However, in this study the association is not highly significant the differences of these results may be due to the ethnicity in different populations.

Regarding the family history of eczema, the present study found that 7(11.7%) of asthmatic patients had a positive family history of eczema result compared with 0 (0%) of control. This study detected the family history of eczema is effective in asthmatic patients, the association was significant between family history of eczema and pediatric asthma ($P=0.013$). In the same line (Spergel 2010) showed that eczema was demonstrated to be a major risk factor for the development of asthma and hay fever. This might be due to genetic predisposition. (Weidinger et al. 2008) demonstrated that Filaggrin (FLG) must be considered to be a major gene in the development of eczema, atopic sensitization, and allergic rhinitis, with a significant effect in asthma that is restricted to patients with a history of eczema. About family history of rhinitis, the current study found that 30 (50%) of asthmatic patients had a positive result compared with 8 (13.3%) of control. The result showed a high association between family history of rhinitis and asthma. (Bergeron and Hamid 2005) showed that Allergic rhinitis is an important risk factor for developing asthma and is also an important cause of nonoptimal control of asthma. Some other studies compatible with result of this study, showed the family history of rhinitis risk factor for asthma (Dold et al. 1992; Gelardi et al. 2014).

Regarding the Allergic conjunctivitis, the present study found that 17 (28.3%) of asthmatic patients had a positive result compared with 8(13.3%) of control. Agreed with (Michailopoulos et al. 2017) who showed that Allergic conjunctivitis was found to be very common among those with symptoms of allergies. In the same line (Gradman and Wolthers 2006) whose found (33%) of asthmatic patients have allergic conjunctivitis and suggested adding allergic conjunctivitis as an important co-morbidity in future guidelines on asthma.

There was a highly significant association ($P=0.001$) between exposure to cigarette smoke and pediatric asthma appeared 30(50%) of patients had positive exposure to cigarette smoke compared to 12(20%) of control. This result was compatible with (Jing, Wang, and Liu 2019), who found that passive smoking was closely associated with the severity of childhood asthma by affecting the balance of Treg/Th17 cell.

In the present study there was a significance association ($P= 0.005$) between eosinophils count in control compared with asthmatic patient as shown in table - 2. This result was compatible with other studies indicated a non-significant eosinophil count in asthmatic patients such as a study achieved by (Ullmann et al. 2013), who showed that (86%) of asthmatic patient had normal eosinophils count.

In this study there was a significant association ($P=0.005$) between serum IgE level in asthmatic patients compared with control. This result related with previous studies such as a study achieved by (Strømgaard et al. 2011), who found a strong positive relationship between total serum IgE level and asthma in

children. Also, (AN Abood, RI Ghazal, and M Al-Musawi 2013) mentioned that (48.7%) of asthmatic patients showed positive IgE screening.

About the IL-22 serum level, this study found that there is no significant differences between asthmatic patients and control ($P = 0.721$). Several studies showed a contrary results such as a study achieved by (Besnard et al. 2011). who showed that IL-22 play a crucial role in antigen sensitization in a murine asthma model. And a study achieved by (Zhu et al. 2011)who demonstrated that IL-22 level was increased in the sera of asthma patients and are positively correlated with disease severity. The differences of these results may be due to the ethnicity in different populations.

In addition, the present study showed a significant association ($P=0.008$) between the IL-23 serum level and asthmatic patients compared with control. This result was agreed with several studies, such as a study established by(Ciprandi et al. 2012), who revealed that IL-23 levels were higher in asthmatic patients than in control ($p < 0.001$) and who also suggested that the serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children. This study showed no significant correlation were found between EOS count and duration of disease ($p = 0.646$) as in figure -1. This result disagreed with a study achieved by(Hancox, Pavord, and Sears 2018) whose assured that increased blood eosinophils are associated with airflow obstruction and enhanced decline in lung function.

This result showed no significant association regarding the IgE serum level and duration of asthma ($P = 0.898$) as in figure -2. This result in the same line with a study conducted by (Mediaty and Neuber 2005) whose found that total serum IgE was significantly decreased as a function of age in patients with asthma. Also similar to a study conducted by (Ahmad Al Obaidi et al. 2008), who demonstrate that specific immunotherapy reduced total IgE serum level in 36% of patients with asthma.

The present study also showed a significant negative correlation between the duration of asthma and the IL-23 serum level ($p=0.030$) as in figure -3. This result agreed with a study conducted by (Ciprandi et al. 2012) who showed that serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children.

This study showed that there is no significant association between IL-23 level and EOS count ($P= 0.625$) with a non-significant positive correlation ($r=+ 0.064$). On the contrary, a study achieved by (Guerra et al. 2002) who confirmed that IL-23 heterodimer was indeed produced in acutely infected lungs, and that their levels as measured by ELISA were significantly diminished in the absence of eosinophils.

Also, this study reported that there is no significant association between IL-23 and IgE serum levels($P= 0.194$) with a non-significant negative correlation ($r=-0.170$). IL-23 signaling may regulate allergic asthma through modulation of Th2 cell differentiation (Peng et al. 2010). The Th2 cell differentiation plays a triggering role in the activation and/or recruitment of IgE antibody (Maggi 1998).

Conclusion

The results of this study have shown an elevated interleukin -23 serum level is associated with severity of asthmatic children; therefore, this marker may have an important role in diagnosis of progression of severe asthma.

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