

## Research Article

# Cytokine Patterns Associated with Bronchial Asthma in Saudi Patients

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## Introduction

Asthma is the most common serious chronic lung disease developed due to a combination of strong genetic and environmental factors. It has a high prevalence in industrialized as well as in developing countries [1]. Asthma prevalence has been increasing to epidemic levels, especially in areas with high urbanization. Over 300 million people worldwide are affected by asthma, with a high negative impact on quality of life, productivity, and health care costs [2]. Allergic asthma (atopic asthma) is the most common, accounting for 90% of cases less than 30 years of age [3].

Cytokines play an integral role in the coordination and persistence of inflammatory processes in chronic inflammation of the airways of asthmatic patients [4]. Cytokines are small, extracellular signaling proteins usually less than 80 KD [4]. Based on the recent understanding of the pathogenesis of asthma, cy-

## Abstract

Asthma is a common respiratory disease worldwide; most patients exhibit mild to moderate states of clinical severity. The aim of this study was designed to measure different cytokines (IL-4 and IL-5) levels in Saudi asthmatic patients and healthy volunteers' serum, in addition to evaluating of the role of cytokines in clinical severity and diagnosis of bronchial asthma in Saudi patients. Through this study, 70 cases of Saudi bronchial asthma patients at different ages and case severity will be included in addition to 20 apparently healthy cases as a control. Serum cytokines (IL-4 and IL-5) levels will be measured by cytokines assay using Enzyme immunoassay. The results proved that the total levels of IL-4 and IL-5 were significantly higher in asthmatic patients than in control volunteers. Moreover, the concentrations of IL-4 in severe were significantly higher than those detected in moderate cases at different age groups for IL-4 but not in all age groups for IL-4. The Mean±SD for IL-4 in moderate was 13.55±1.25 pg/ml whereas in severe was 15.49±1.73 pg/ml while the Mean±SD of control was 7.55±1.47 pg/ml. The concentrations of IL-5 were non-significant in severe when compared with moderate cases. The Mean±SD for total IL-5 in moderate was 4.24±1.38 pg/ml whereas in severe was 4.46±1.40 pg/ml although the total Mean±SD in control was 2.29±0.37 pg/ml. Our results prove that there was a non-significant role of gender in bronchial asthma severity and there were no significant differences between serum cytokines levels in males and females in bronchial asthma. These results also expressed a significant role of IL-4 in the disease severity of asthmatic patients. Furthermore, detecting serum levels of IL-4 and IL-5 indicates the role of these cytokines in the development and pathogenesis of bronchial asthma.

**Keywords:** Asthma; Interlukein-4; Interlukein-5; EIA; cytokines

tokines are classified into (i) lymphokines, (ii) pro-inflammatory cytokines, (iii) anti-inflammatory cytokines, and (iv) chemokines [4]. Allergen-induced IgE synthesis can trigger eosinophils, basophils, and mast cells to release cytokines for the differentiation of progenitor Th cells into Th2 cells to secrete IL-4 and IL-5. Moreover, basophils, mast cells, and eosinophils act as effectors of allergic inflammation through the release of pro-inflammatory, vasoactive, and fibrogenic factors (histamine, peptide leukotrienes, platelet-activating factor, tryptase, chymase, etc.) that are responsible for symptoms of bronchial asthma [5].

For assessing the presence of allergen-specific IgE antibodies, two different methods can be used, a skin pricks test or an allergy blood test. Both methods are recommended and have similar diagnostic values in terms of sensitivity and specificity [7,8].

This study was designed to determine the circulating patterns of cytokines in bronchial asthma of Saudi patients in relation to age and gender as well as disease severity, to record the serum levels of different cytokines (IL-4 and IL-5) in Saudi asthmatic patients, and to evaluate the role of cytokines in pathogenesis and diagnosis of bronchial asthma.

### Materials and Methods

A total of 90 blood samples were involved in this study, out of them 70 blood samples were collected from asthmatic patients at different ages and genders as well as case severity (moderate or severe). In addition, 20 blood samples were collected from control volunteers. These samples were collected from King Fahd Hospital in Medinah, kingdom of Saudi Arabia. The samples were classified according to age, disease severity, and gender. Asthmatic blood samples were 38 moderate and 32 severe cases, based on patients' gender 34 and 36 were male and female respectively. Regarding to age groups the asthmatic patients and controls were classified into 7 age groups starting from age groups (10-<20), (20-<30), (30-<40), (40-<50), (50-<60) and ( $\geq$  60) years as presented in Table (1).

Enzyme Immunoassay (EIA) was used for quantitative determination of human IL-4 and IL-5 concentration using commercially available kits. Blood samples were collected from asthmatic patients and control volunteers in plain tubes, left at room temperature for 30 min until coagulation, after that serum samples were separated by centrifugation at 1000 rpm for 15 min then liquated and stored at  $\leq -20$  °C.

**Table 1:** Collective table of serum samples collected from moderate, and severe asthmatic patients and control in relation to age group and gender.

Groups of Age/Year	Asthmatic Patients					Control Volunteers		
	Gender		Severity		Total	Gender		Total
	M	F	Moderate	Severe		M	F	
(0-<10)	7	5	5	7	12	2	2	4
(10-<20)	4	6	6	4	10	1	2	3
(20-<30)	5	5	6	4	10	2	1	3
(30-<40)	4	5	5	4	9	2	1	3
(40-<50)	4	4	4	4	8	1	1	2
(50-<60)	5	4	5	4	9	1	1	2
( $\geq$ 60)	7	5	7	5	12	1	2	3
Total	34	36	38	32	70	10	10	20

**Table 2:** Serum IL-4 concentration in moderate asthmatic patients and control in different age groups.

Groups of Age/ Year	Number	IL4 (pg/ml)					
		Moderate		Number	Control		P value
		M $\pm$ SD	Range		M $\pm$ SD	Range	
(0- <10)	5	14.26 $\pm$ 1.12	13.17- 15.91	4	9.08 $\pm$ 1.77	7.31-11.47	0.014
(10- <20)	6	14.28 $\pm$ 1.28	13-16.43	3	8.60 $\pm$ 1.08	7.78-9.83	0.02
(20- <30)	6	13.71 $\pm$ 0.94	12.48- 15.05	3	7.14 $\pm$ 1.25	6.12-8.54	0.02
(30- <40)	5	13.30 $\pm$ 0.57	12.65- 14.19	3	7.60 $\pm$ 1.82	5.74-9.38	0.024
(40- <50)	4	13.92 $\pm$ 1.65	12.14- 15.91	2	8.41 $\pm$ 2.64	6.54-10.28	0.064
(50- <60)	5	12.56 $\pm$ 2.65	8.10- 14.53	2	6.64 $\pm$ 0.99	5.94-7.34	0.053
( $\geq$ 60)	7	12.83 $\pm$ 0.57	11.97-13.51	3	5.35 $\pm$ 0.70	4.83-6.14	0.017
Total	38	13.55 $\pm$ 1.25	8.10-16.43	20	7.55 $\pm$ 1.47	4.83-11.47	P < 0.001

\* Significant P value:  $\leq$  0.05

**Table 3:** P Values between IL-4 concentrations in moderate asthmatic patients for different age groups.

Age groups	(0-<10)	(10-<20)	(20-<30)	(30-<40)	(40-<50)	(50-<60)	( $\geq$ 60)
(0- <10)							
(10- <20)	0.712						
(20- <30)	0.783	0.470					
(30- <40)	0.395	0.168	0.521				
(40- <50)	0.537	0.392	0.593	0.806			
(50- <60)	0.753	0.200	0.584	0.753	0.806		
$\geq$ 60	0.072	0.031	0.074	0.251	0.507	0.463	

\* Significant P value:  $\leq$  0.05

The assay was carried out according to the manufacturer's manual of the Kits. All reagents, working standards, and samples have been prepared as directed in the previous sections. Excess microplate strips have been removed from the plate frame, returned to the foil pouch containing the desiccant pack, and resealed. Add 100  $\mu$ l of assay diluent has been added to each well, and then add 50  $\mu$ l of standard, control, or sample has been added per well, plate covered with the adhesive strip provided and incubated for 2 hours at room temperature. Aspiration and washing of each well has been done by repeating the process twice for a total of three washes, by filling each well with wash buffer (400  $\mu$ l) using a squirt bottle, manifold dispenser, or autowasher. Add 200  $\mu$ l of conjugate has been added to each well and incubated for 2 hours at room temperature. The aspiration/wash as in step 5 has been repeated.

Add 200  $\mu$ l of substrate solution has been added to each well and incubated for 20 minutes at room temperature in the dark. then add 50  $\mu$ l of stop solution has been added to each well. Then the color of the wells changed from blue to yellow. The optical density of each well within 30 minutes has been determined using a microplate reader set to 450 nm with a wavelength correction set to 570 nm.

The Mann-Whitney rank sum test was used to assess the differences in the concentration of cytokines in asthmatic patients and control subjects. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) v.20 to have a Mean (M), Standard Deviation (SD), range, and P-value for all the raw data. A probability of P < 0.05 was considered significantly different [9].

### Results

#### Interleukin 4 Levels in Asthmatic Patients and Control Sera

Tables (2&3) have presented the mean and standard deviations (M $\pm$ SD) of serum IL-4 concentration in moderate and severe asthmatic patients as well as control sera. The results of IL-4 levels of asthmatic patients and control volunteers were tabulated based on the severity of the disease (moderate and severe), as well as age groups.

It was clear from these results, that there were significant differences between serum IL-4 levels in moderate and severe asthmatic patients when compared with results obtained from

**Table 4:** Serum IL-4 concentration in severe asthmatic patients and control in different age groups.

Groups of Age/ Year	IL4 (pg/ml)						P value
	Number	Severe		Number	Control		
		M±SD	Range		M±SD	Range	
(0- <10)	7	16.71±2.78	14.19– 21.48	4	9.08±1.77	7.31-11.47	0.008
(10- <20)	4	16.96±2.73	13.17-19.03	3	8.60±1.08	7.78-9.83	0.034
(20- <30)	4	15.70±1.61	14.02- 17.46	3	7.14±1.25	6.12-8.54	0.034
(30- <40)	4	15.48±1.60	13.85– 17.64	3	7.60±1.82	5.74-9.38	0.034
(40- <50)	4	14.99±1.39	13.51- 16.25	2	8.41±2.64	6.54-10.28	0.06
(50- <60)	4	14.97±1.54	13.51- 17.12	2	6.64±0.99	5.94-7.34	0.064
( ≥60 )	5	13.61±0.49	13.17 –14.36	3	5.35±0.70	4.83-6.14	0.024
Total	32	15.49±1.73	13.17-21.48	20	7.55±1.47	4.83-11.47	P < 0.001

**Table 5:** P Values Between IL-4 concentration in severe asthmatic patients for different age groups.

Age groups	(0-<10)	(10-<20)	(20-<30)	(30-<40)	(40-<50)	(50-<60)	(≥ 60)
(0- < 10)							
(10-< 20)	0.999						
(20-< 30)	0.636	0.386					
(30-< 40)	0.571	0.386	0.999				
(40-< 50)	0.449	0.245	0.561	0.772			
(50-< 60)	0.394	0.386	0.564	0.468	0.661		
≥ 60	0.009	0.174	0.027	0.036	0.048	0.064	

\* Significant P value: ≤ 0.05

controls in all different age groups except age group (40-<50) and (50-<60). The highest levels of IL-4 were found in the age group (10- <20) in both moderate and severe (14.28 and 16.96 respectively). Whereas age groups (50-<60) had the lower level in moderate which was 12.56 and age group (≥60) had the lower level in severe which was 13.61pg/ml. The total IL-4 concentration in both moderate and severe asthmatic patients were 13.55pg/ml and 15.49 pg/ml respectively which were significantly higher when compared with control which was 7.55 pg/ml.

**Comparison Between Serum IL-4 Concentration in Moderate and Severe Asthmatic Patients of Different Age Groups**

Table (4&5) shows comparisons that have been carried out to clarify the relationship between serum IL-4 levels in moderate and severe asthmatic patients in addition to recording their role in the development of disease in relation to age. The result indicated significant differences between serum IL-4 levels in moderate and severe asthmatic patients in age groups (0-<10), (30-<40), and ≥60. On the other hand, no significant differences were found in age groups (10-<20), (20-<30), (40-<50) and (50-<60). The highest concentrations of IL-4 in both moderate and severe were found in age group (10-<20) which were 14.28 pg/ml and 16.96 pg/ml respectively. Comparison between total IL-4 concentration in moderate and severe asthmatic patients indicated significant differences between moderate and severe asthmatic patients.

**Table 6:** Comparison between serum IL-4 levels in moderate and severe asthmatic patients in different age groups.

Groups of Age/ Year	IL4 (pg/ml)						P value
	Number	Moderate		Number	Severe		
		M±SD	Range		M±SD	Range	
(0- <10)	5	14.26±1.12	13.17– 15.91	7	16.71±2.78	14.19–21.48	0.028
(10- <20)	6	14.28±1.28	13-16.43	4	16.96±2.73	13.17-19.03	0.108
(20- <30)	6	13.71±0.94	12.48- 15.05	4	15.70±1.61	14.02- 17.46	0.069
(30- <40)	5	13.30±0.57	12.65– 14.19	4	15.48±1.60	13.85–17.64	0.027
(40- <50)	4	13.92±1.65	12.14- 15.91	4	14.99±1.39	13.51- 16.25	0.245
(50- <60)	5	12.56±2.65	8.10- 14.53	4	14.97±1.54	13.51- 17.12	0.138
( ≥60 )	7	12.83±0.57	11.97 –13.51	5	13.61±0.49	13.17 –14.36	0.049
Total	38	13.55±1.25	8.10-16.43	32	15.49±1.73	13.17-21.48	P <0.001

**Interleukin 5 Levels in Asthmatic Patients and Control Sera**

Tables (6&7) have presented the mean and standard deviations of serum IL-5 concentration in moderate and severe asthmatic patients as well as control sera. The results of IL-5 levels of asthmatic patients and control volunteers were tabulated based on the degree of the disease severity (moderate and severe) and age.

It was clear from these results, that there were significant differences between serum IL-5 levels in moderate and asthmatic patients when compared with results obtained from controls in age groups (10- <20), (20- <30), (30- <40) and (≥60). On the other hand, severe asthmatic patients showed significant differences in age groups (0- <10), (10- <20), (20- <30) and (30- <40) when compared with controls. The highest levels of IL-5 in the moderate were found in the age group (0- <10) which was 5.68 pg/ml whereas the highest levels in the severe were found in the age group (10- <20) which was 5.86 pg/ml. whereas the age group (≥60) had the lower level in both moderate and severe (3.46 and 3.80 respectively). The total concentration of IL-5 in both moderate and severe was 4.24 pg/ml and 4.46 pg/ml respectively which were significantly higher when compared with control which is 2.29 pg/ml.

**Comparison between Serum IL-5 Concentration in Moderate and Severe Asthmatic Patients of Different Age Groups**

Tables (8 & 9) show comparisons that have been carried out to clarify the relationship between serum IL-5 levels in moderate and severe asthmatic patients in addition to recording their role in the development of disease in relation to age. The result indicated no significant differences between serum IL-5 levels in moderate and severe asthmatic patients in all different age groups.

The highest concentrations of IL-5 in moderate and severe were found in young age groups (0-<10) and (10-<20) respectively. Comparison between total IL-5 concentrations the results indicated no significant differences between moderate and severe asthmatic patients.

**Table 7:** Comparative table of serum IL-4 levels in moderate and severe asthmatic patients compared to control in different age Ggroups.

Groups of Age/ Year	IL4 (pg/ml)								
	Number	Moderate		Number	Control		Number	Severe	
		M±SD	Range		M ± SD	Range		M± SD	Range
(0- <10)	5	14.26±1.12	13.17-15.91	4	9.08±1.77	7.31-11.47	7	16.71±2.78	14.19-21.48
(10- <20)	6	14.28±1.28	13-16.43	3	8.60±1.08	7.78-9.83	4	16.96±2.73	13.17-19.03
(20- <30)	6	13.71±0.49	12.48- 15.05	3	7.14±1.25	6.12-8.54	4	15.70±1.61	14.02-17.46
(30- <40)	5	13.30±0.57	12.65- 14.19	3	7.60±1.82	5.74-9.38	4	15.48±1.60	13.85-17.64
(40- <50)	4	13.92±1.65	12.14- 15.91	2	8.41±2.64	6.54-10.28	4	14.99±1.39	13.51- 16.25
(50- <60)	5	12.56±2.65	8.10- 14.53	2	6.64±0.99	5.94-7.34	4	14.97±1.54	13.51- 17.12
( ≥60 )	7	12.83±0.57	11.97 -13.51	3	5.35±0.70	4.83-6.14	5	13.61±0.49	13.17 -14.36
Total	38	13.55±1.25	8.10-16.43	20	7.55±1.47	4.83-11.47	32	15.49±1.73	13.17-21.48

**Table 8:** Serum IL-5 concentration in moderate asthmatic patients and control in different age groups.

Groups of Age/ Year	IL5 (pg/ml)						
	Number	Moderate		Number	Control		P value
		M±SD	Range		M ± SD	Range	
(0- <10)	5	5.68±1.91	3.04- 7.85	4	2.43±0.28	2.13-2.73	0.014
(10- <20)	6	5.11±1.45	3.11-7.31	3	2.19±0.28	2-2.51	0.02
(20- <30)	6	3.99±1.68	2.58- 7.23	3	2.31±0.37	2.05-2.73	0.039
(30- <40)	5	3.57±0.49	2.66- 5.09	3	2.48±0.44	1.98-2.73	0.099
(40- <50)	4	4.33±1.58	2.89- 6.24	2	1.90±0.32	1.67-2.13	0.064
(50- <60)	5	3.51±1.34	2.43- 5.86	2	2.24±0.48	1.90-2.58	0.118
( ≥60 )	7	3.46±0.73	2.89 - 4.86	3	2.46±0.42	1.98-2.73	0.016
Total	38	4.24±1.38	2.43-7.85	20	2.29±0.37	1.67-2.73	P < 0.001

\* Significant P value: ≤ 0.05

**Table 9:** Serum IL-5 concentration in severe asthmatic patients and control in different age groups.

Groups of Age/ Year	IL5 (pg/ml)						
	Number	Severe		Number	Control		P value
		M±SD	Range		M±SD	Range	
(0- <10)	7	5.50±1.74	3.11- 8.08	4	2.43±0.28	2.13-2.73	0.008
(10- <20)	4	5.86±2.42	4.41-9.47	3	2.19±0.28	2-2.51	0.034
(20- <30)	4	4.14±0.75	3.27- 5.09	3	2.31±0.37	2.05-2.73	0.032
(30- <40)	4	4.08±0.79	3.34- 5.17	3	2.48±0.44	1.98-2.73	0.032
(40- <50)	4	3.99±1.27	2.89- 5.78	2	1.90±0.32	1.67-2.13	0.064
(50- <60)	4	3.84±1.35	2.81- 5.70	2	2.24±0.48	1.90-2.58	0.064
( ≥60 )	5	3.80±1.52	2.36 -5.93	3	2.46±0.42	1.98-2.73	0.297
Total	32	4.46±1.40	2.36 -9.47	20	2.29±0.37	1.67-2.73	P < 0.001

\* Significant P value: ≤ 0.05

**Table 10:** Comparison between serum IL-5 levels in moderate and severe asthmatic patients in different age groups.

Groups of Age/ Year	IL5 (pg/ml)						
	Number	Moderate		Number	Severe		P value
		M±SD	Range		M±SD	Range	
(0- <10)	5	5.68±1.91	3.04- 7.85	7	5.50±1.74	3.11- 8.08	0.807
(10- <20)	6	5.11±1.45	3.11-7.31	4	5.86±2.42	4.41-9.47	0.67
(20- <30)	6	3.99±1.68	2.58- 7.23	4	4.14±0.75	3.27- 5.09	0.388
(30- <40)	5	3.57±0.49	2.66- 5.09	4	4.08±0.79	3.34- 5.17	0.217
(40- <50)	4	4.33±1.58	2.89- 6.24	4	3.99±1.27	2.89- 5.78	0.885
(50- <60)	5	3.51±1.34	2.43- 5.86	4	3.84±1.35	2.81- 5.70	0.999
( ≥60 )	7	3.46±0.73	2.89 - 4.86	5	3.80±1.52	2.36 -5.93	0.999
Total	38	4.24±1.38	2.43-7.85	32	4.46±1.40	2.36 -9.47	0.385

**Discussion**

The pathophysiological basis underlying reversible airway obstruction in bronchial asthma is inflammation [9]. Many studies have suggested that the severity of asthma is related to the degree of inflammation [10,11]. Animal models and clinical studies in humans have indicated an important role for Th2 cells producing IL-4 and IL-5 in the pathogenesis of allergic asthma [5], with the induction of a Th1 response that seems to aggravate an inflammatory process [6].

The results of our study proved that the levels of serum IL-4 and IL-5 in asthmatic patients agreed to some extent with the results published before, which demonstrated that both pro-inflammatory and Th2 cytokines play critical roles in the inflammatory characteristics of allergic asthma. Our results indicated that serum IL-4 was significantly higher in asthmatic patients than in healthy individuals. Comparison of the study results between the groups (moderate, severe asthma, and control) evidenced that serum concentrations of IL-4 in the group with moderate (p<0.001) and severe asthma (p<0.001) were statisti-

cally significantly higher in comparison to the group with control. These results might be indicative of different degrees of the disease (disease severity) with their underlying degrees of chronic inflammation also being different and IL-4 as possible markers. Moreover, other data proved that IL-4 is necessary for the differentiation of the naive CD-positive T-cells within the Th2 subpopulation secreting IL-4 and IL-5. Although IL-4 induces IgE synthesis and enables the immediate type of hypersensitivity reaction, there is certain evidence suggesting in vitro and in vivo anti-inflammatory effects of IL-4. IL-4 is critical in switching B lymphocytes to produce IgE, for expression of Vascular Cell Adhesion Molecules-1 (VCAM-1) on endothelial cells. IL-4 is of critical importance in the differentiation of Th2 cells and is therefore an 'upstream' cytokine that is an attractive therapeutic target in the treatment of atopic diseases. My results agree with those that proved that excessive IL-4 production by Th2 cells has been associated with elevated allergy [12]. Other results demonstrated that Th1/Th2 imbalance results in the clinical expression of allergy and/or asthma. Studies on mice and humans have shown Th2 cytokines [IL-4 and IL-5] as major contributors to allergy and asthma. In those studies, serum IL-4 was below detectable level in all the subjects, those results disagreed with our results. Nevertheless, IL-5, an IgE-stimulating cytokine was associated with bronchial asthma in children.

It has been reported that IL-5 has a critical role in eosinophilia which has been confirmed by the use of an anti-IL-5 antibody in asthmatic patients, which almost depletes circulating eosinophils and prevents eosinophil recruitment into the airway after allergen. IL-5 is a cytokine that is not encountered at high levels in healthy individuals. Control of IL-5 protein production takes place at the level of transcription [13]. IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including cytokines (TNF- $\alpha$ , granulocyte-macrophage colony-stimulating factor, IL-5, chemokines) and inflammatory enzymes (inducible nitric oxide synthesis) that are over-expressed in asthma [14].

Our finding showed elevation of serum IL-5 in asthmatic patients concurs with previous reports that there are significantly lower IL-5 serum concentrations in the group of healthy controls in comparison to the group with bronchial asthma. Comparison of the study results between the groups (moderate, severe asthma, and control) evidenced that serum concentrations of IL-5 in the group with moderate ( $p < 0.001$ ) and severe asthma ( $p < 0.001$ ) were statistically significantly higher in comparison to the group with control. However, no significant differences ( $p = 0.385$ ) have been detected between the groups of severe and moderate cases.

These findings are in agreement with many other results applied for measurements of IL-5. Motojima et al [15] determined IL-5 serum concentrations in 78 asthma patients (the control group comprised 30 healthy individuals). Results of the study revealed that IL-5 serum concentration in asthma patients was statistically significantly higher in comparison to IL-5 concentration values in the healthy controls.

A comparison of the study results between the groups (mild, moderate, and severe asthma) shows that IL-5 serum concentrations are significantly higher in the group with moderate and severe asthma in comparison to the group with mild asthma. Serum concentrations in patients with moderate and severe asthma were significantly higher in comparison to those obtained in the group with mild asthma. The study conducted by Hacken et al [16] also verified significantly higher IL-4 and IL-5

serum concentrations in 17 patients with asthma in comparison to concentrations of the cytokines in healthy controls, however, they have failed to evidence correlation between IL-4 and IL-5 concentrations and parameters of clinical manifestations of asthma.

The lack of significant difference in IL-4 and IL-5 serum concentrations between patients with moderate and severe asthma supports the attitudes of the majority of different authors considering that the groups with these forms of asthma may be practically treated during the studies as one common group of patients with prominent and advanced inflammation which is significantly different from inflammation found in mild asthma.

It may be concluded that the results of our study are consistent with other results reported so far, suggesting that cytokines IL-4 and IL-5 obviously represent good markers of chronic inflammation of the lower airways in bronchial asthma. Their application for the diagnostic as well as predictive purpose may be reasonably recommended, however only within the research projects for the time being. Additional studies on the role of IL-4 and IL-5 in chronic inflammation associated with asthma are needed.

Comparison between different age groups (10-<20), (20-<30), (30-<40), (40-<50), (50-<60), and ( $\geq 60$ ) years indicated higher serum cytokines levels in young age groups than elderly in many cases whereas no significant differences have been found in middle age groups. Serum IL-4 concentrations in severe asthmatic patients in the age group ( $\geq 60$ ) were statistically significantly lower in comparison to the age group (0-<10) ( $p = 0.009$ ), age group (20-<30) ( $p = 0.027$ ), age group (30-<40) ( $p = 0.036$ ) and age group (40-<50) ( $p = 0.048$ ). Serum IL-5 concentrations in moderate asthmatic patients in the age group ( $\geq 60$ ) were statistically significantly lower in comparison to the age group (0-<10) ( $p = 0.042$ ) and age group (10-<20) ( $p = 0.032$ ).

These observations are in agreement to some extent with the findings of other investigators as Drazen J [17] who shows that asthma approximately affects 5% of the adult population, most cases begin before the age of 25 years but it may develop at any time through life. Other studies conducted by Sudha S Deo et al [18], observed the pattern of cytokines in patients with asthma most of them were in the age group 25-45 years. Age differences in different groups were not seen. There was also no significant difference as far as the duration of the disease was concerned.

## Conclusion

This study showed that total serum levels of Th2 cytokines are elevated in asthmatic patients which may be responsible for the pathological features of asthma. The role of age in the development of disease symptoms may be uncertain with a tendency to occur in young ages more frequently than elderly which may be due to that most cases have onset before the age of 25 years, but it may develop at any time through life. Finally, additional studies may be recommended to demonstrate the use of anti-cytokine therapy which may help in treating inflammatory diseases for asthmatic patients.

## Author Statements

### Conflict of Interest

The authors declare that is no conflict interest.

### Authors contribution

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

### Data Availability

All datasets generated or analyzed during this study are included in the manuscript.

### Ethics Statement

This study was approved by the institutional review board (IRB 23-091) of the General Directorate of Health Affairs in Madinah.

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