Research Paper

Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

Bacterial Colonization of Lower Airways in Asthmatic Patients and its Association With Interleukin17 Level

Medical Science

KEYWORDS : Airways, Asthmatic, Colonization, Interleukin17.

Ashwaq Fouad Abu Talb	Medical Microbiology & Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Shymaa Abdel-Azim Mansour	Medical Microbiology & Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Waleed Mansour Mohamed	Chest Department , Faculty of Medicine, Zagazig University, Zagazig, Egypt
Reem Mohamed Abdel-Rahman	Clinical Microbiology Department, Al Ahrar Hospital, Zagazig, Egypt

ABSTRACT

Background: The central role of the Th2 subset in the disease, inducing eosinophilic asthma while neutrophilic asthma may be due to an increased propensity for asthmatics to carry more bacterial pathogens in their lower airways. During the development of asthma, IL-17 and Th-17 cells play a role in induction of the disease through acting as a proinflammatory cytokine, also they negatively regulates established asthma. The aim of this study was to assess the association between bacterial colonization of lower airways and Interleukin 17 level in different asthmatic subtypes.

Methods: This study included 60 subjects (30 asthmatic patients and 30 healthy volunteers). All studied cases and control groups were subjected to history taking, clinical examination including both general and chest examination, chest X-ray, and high resolution CT chest. Diagnosis of bronchial asthma was done. Sputum investigations to detect asthma phenotype and bacterial colonization were done. Serum investigations include detection of serum total IgE level to detect atopy and the level of serum interleukin 17 level.

Results: There was bacterial colonization in 33.3% of cases. Serum IL-17 was higher in patients than controls (p value <0.003). Serum IL-17 level was higher in patients with bacterial colonization than in patients without bacterial colonization (p value <0.001). Serum IL-17 level was higher in mixed granulocytic asthma and neutrophilic asthma (with no significant difference between the 2 groups) than in other asthma phenotypes (eosinophilic and paucigranulocytic) with a statistically high significant difference.

Conclusion: Bacterial colonization and subsequent elevation of serum IL-17 levels are associated with neutrophilic asthma. Also, IL-17 can be used to predict asthma severity since it is increased in severe asthma compared to mild and moderate forms of the disease.

Introduction

Bronchial asthma is one of the most important challenges to public health because of its increasing prevalence, financial burden and uncommon remission [1].

Two hallmarks of asthma are chronic airway inflammation and airway dysfunction. Measurement of the airway inflammation might provide a better guide to the need for corticosteroid treatment than assessment of functional abnormality. In clinical practice, it is difficult to assess airway inflammation and the effects of medication on such inflammation [2].

Asthma is divided into eosinophilic and non-eosinophilic asthma. Eosinophilic asthma is either pure eosinophilic or mixed granulocytic [3]. Non-eosinophilic asthma is divided into neutrophilic and paucigranulocytic [4].

Eosinophilic asthma is as a result of allergen mediated activation of mast cells and T-helper2 (Th2) lymphocytes. In contrast, there are many triggers of neutrophilic asthma including endotoxin, viral, and bacterial infection, constituents of cigarette smoke, and many occupational agents [5].

Eosinophilic asthmatics makes up between 70-80% of asthmatics, while neutrophilic asthmatics make up between 20-30% of asthmatics [6]. Corticosteroids is unlikely to be effective in improvement of symptoms and airway responsiveness in non-eosinophilic asthma [3].

The lower airways have been considered a sterile environment, but in airway diseases such as bronchiectasis and chronic obstructive pulmonary disease (COPD), the isolation of bacteria in sputum is not an uncommon. These pathogens are often present during exacerbations and stable phase of disease indicating chronic colonization [7].

On the other hand, the detection of bacterial pathogens in chronic asthma remains under studies. In a study, 27% of asthmatic patients presenting with an exacerbation of asthma had bacteria in sputum with Streptococcus pneumoniae (S.pneumoniae), Streptococcus pyogenes (S.pyogens), Staphylococcus aureus (S.aureus), Moraxella catarrhalis (M.catarrhalis) and Haemophilus influenzae (H.influenzae). This spectrum of bacterial species was also isolated from induced sputum samples in 15% of patients during a stable period of asthma [8].

Interleukin -17 is a pro-inflammatory cytokine produced by "Th17", neutrophils, and macrophages. It was implicated in the pathogenesis of asthma where it is correlated with the severity of neutrophilic inflammation [9].

The aim of this study was to assess the association between bacterial colonization of lower airways and Interleukin 17 level in different asthmatic subtypes.

Methods

I- Technical design

A) Site of the study:

This study was carried out at the Medical Microbiology and Immunology Department, the Allergy and Immunology Unit of Microbiology and Immunology Department, and the Chest department, Faculty of Medicine, Zagazig University during the period from July 2012 to August 2013.

B) Sample size:

This study included 60 subjects that were divided into two

groups: group I (Patient group); it included 30 asthmatic patients and group II (Control group); it included 30 healthy non-smoker volunteers with no history of chest infection during the previous 6 weeks.

C) Subjects

All patients and controls fulfill the following criteria:

- **Inclusion criteria:**

Bronchial asthma patients were totally controlled on inhaled corticosteroids and/or long acting β2 agonist bronchodilators. The criteria of controlled bronchial asthma were according to levels of asthma control of Global Initiative for Asthma (GINA) 2012. Severe asthma here is asthma that requires high intensity treatment according to step 4 GINA guidelines [10].

Patients were middle age and male or female.

- **Exclusion criteria:**

Smokers, history of chest infection within the previous 6 weeks, antibiotic treatment within the previous 6 weeks, patients with previous diagnosis of chronic obstructive pulmonary disease (COPD), bronchiectasis, immuodeficiency diseases, receiving immunotherapy and receive systemic steroids in preceding 4 weeks [11].

II- Administrative design:

§ Approval for performing the study was obtained from IRB review, faculty of medicine, Zagazig university and Medical Microbiology and Immunology Department and Chest Department, faculty of medicine, Zagazig university.

§ Witten consent was obtained from all subjects.

III- Operational design

A) Type of study

It is a case-control study.

B) Steps of performance

All subjects were undergone the following: clinical evaluation included history taking and clinical examination included both general and chest examination. Investigations included chest X-ray and high resolution CT chest (HRCT chest): to exclude any associated radiological abnormality and other alternative diagnosis e.g. bronchiectasis, diagnosis of bronchial asthma were according to GINA guidelines 2012 [10], sputum investigations to detect asthma phenotype and bacterial colonization. Serum investigations include detection of serum total IgE level to detect atopy and serum interleukin 17 level.

1-Collection of sputum and blood samples:

Sputum samples: induced sputum samples were obtained from patients. For induction of sputum 5 to 7 ml hypertonic saline (3% NaCl) was used [12].

Blood samples: for separation of the serum

2. Sputum Investigations

A) Sputum homogenization:

Equal amount of sterile physiological saline was added to the sputum sample then ten sterile glass beads (size 3.5-4.5 mm) (Loba Chemie, India) were added and was vortexed for about 5 minutes then incubated at 37 ° C incubated for another 15 minutes [13].

B) Total leukocytic count (TLC):

Part of the homogenized sputum was filtered using sterile surgical gauze [14], the supernatant removed away while the filtrate was taken for TLC using white blood cell count solution (EDM Egypt). The result were reported as "number by 109/l)".

C) Differential leukocytic count (DLC) in sputum

Ten microliter of homogenized sputum was taken to be examined for differential cell count using leishman stain (EDM Egypt). Macrophage, lymphocyte, neutrophil, and eosinophil values were calculated as percentages of total inflammatory cells, excluding squamous epithelial cells. Sputum samples that contained more than 30% squamous epithelial cells were not analyzed [15]. Asthma is divided into eosinophilic and non-eosinophilic asthma. Eosinophilic asthma is either pure eosinophilic (eosinophil count alone ≥1%) or mixed granulocytic (eosinophil count ≥1% and neutrophil count ≥61%)[10].3 Non-eosinophilic asthma is divided into neutrophilic (neutrophil count alone ≥61%) and paucigranulocytic (neutrophil count ≤61% and eosinophil count ≤1%) [4].

D) Quantitative bacterial culture

Sputum samples were incubated for 24-48 hours at 5% CO2 for blood (Oxoid UK) and chocolate agar while in atmospheric air for MacConkey agar (Oxoid UK) [16]. Serial tenfold dilution of the homogenized sputum was done using a sterile saline (0.9% NaCl). 10-3, 10-4 and 10-5 dilutions were used to inoculate chocolate, blood and MacConkey agar plates [13]. They were spread over one-half of the plate and then streaked over the other half [17]. Plates yielding between 30 and 300 colonies were counted and the bacterial load was counted and expressed as colony forming units per milliliter (CFU/ml) of original sputum according to the equation: CFU/ml= Number of colonies/agar plate x Dilution factor x Inoculation factor [18]. Bacterial colonization (Significant bacterial load): was considered in subjects who had a bacterial load >106 CFU/ml for any individually potentially pathogenic bacteria [19].

E) For bacterial identification:

***Gram stain (EDM Egypt):** For microscopical examination of the isolated colonies from primary culture plate.

***Biochemical tests for bacterial identification:**

catalase test to differentiates streptococci (-ve) and staphylococci (+ve) [20], coagulase test for differentiation between coagulase positive and coagulase negative S.aureus [21], Optochin disks (Oxoid UK) to provide a rapid presumptive identification of S.pneumoniae [22], Triple sugar iron medium (Merck UK) used for differentiation of Gram negative bacilli [21], Indole test to demonstrate the ability of Gram negative bacilli for indole production[21] and Oxidase reagent (Oxoid, UK): for oxidase test to differentiate between P.aeroginosa (positive reactions) and other Gram negative organisms (enterobacteria, acinetobacter) (negative reactions) [23].

3- Serum investigations:

A. Serum Total IgE level:

In-vitro quantitative determination of serum total IgE Level was done by Immundiagnostik IgE, Enzyme Linked Immunosorbent Assay (ELISA) kit (Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim, Germany)

B. Serum interleukin-17:

In-vitro quantitative determination of serum IL-17 was done by R&D system, ELISA kit (614 mcKinley place NE, Minneapols,MN 55413, USA).

Statistical analysis

Data were checked, entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 19 for data processing and statistics. Data were expressed as numbers and percentages for qualitative variables and mean±standard deviation (SD) for quantitative one.

The comparison was done using: The student "t" test, analysis of variance (ANOVA) test, Kruskall-Wallis test and Chi- square test

Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

(X2). The threshold of significance was fixed at 5% level (P-value). P value of > 0.05 indicates non-significant results, P value of < 0.05 indicates significant results and P value of < 0.01 indicates highly significant results.

Results

The ages of patients (group I) ranged from 22-72 with mean±SD= 44.8±14.6 and they were divided into 20 (66.7%) males and 10 (33.3%) females. The table also shows that the ages of controls (group II) ranged from 20-69 with mean±SD= 40.2 ±15.1 and they were divided into 23 (76.6%) males and 7 (23.3%) females. There were statistically non-significant differences between ages and sex of cases and control groups (p value 0.235 and 0.39); respectively. According to severity of asthma, 5 (16.6%) of patients were classified as mild asthma, while 15 (50.0%) as moderate asthma and 10 (33.3%) as severe asthma.

Figure (1) shows the frequency of different asthma phenotypes among patients (group I) that was 12 cases (40%) eosinophilic, 10 cases (33.3%) neutrophilic, 5 cases (16.7%) pauci-granulocytic and 3 cases (10%) mixed granulocytic phenotypes.

Table (1) shows frequency of bacterial colonization in sputum samples among asthmatic patients (group I) and controls (group II). Bacterial colonization was detected in 10 cases (33.3%) and bacterial isolates were 70% (23.3% of total cases) k. pneumoniae, 20% (6.7% of total cases) S. pneumoniae and, 10% (3.3% of total cases) S. aureus while no bacterial colonization was detected in control group.

Table (2) shows that serum IL-17 was higher in patients (group I) than controls (group II) with statistically high significant difference (p value <0.001).

Table (3) shows that serum IL-17 level was higher in patients with bacterial colonization than in patients without bacterial colonization with statistically significant difference (p value <0.003).

Table (4) shows comparison between different asthma severity groups as regards asthma phenotypes, bacterial colonization and serum IL-17 levels. It shows that severe cases were associated with mixed granulocytic and neutrophilic asthma. Also, it shows that bacterial colonization was found in severe cases 10 (100%) while it was absent in mild and moderate cases with statistically high significant difference (p value 0.001).It also shows that serum IL-17 level is higher in severe cases than in mild and moderate cases with statistically high significant difference (p value <0.001).

Table (5) shows laboratory findings among groups of different asthma phenotypes. As regards total cell count, there were statistically non-significant differences (p value 0.076) between different asthma phenotypes groups.

The table also shows statistically high significant difference (p value <0.001) between asthma phenotype as regards the percentages of inflammatory cell types in each group.

The table also shows statistically no significant difference was found when rate of bacterial colonization and serum total IgE level in neutrophilic asthma was compared with that of mixed granulocytic asthma, while statistically highly significant difference was found when rate of bacterial colonization and serum total IgE level in neutrophilic and mixed granulocytic asthma as one group compared with eosinophilic and paucigranulocytic asthma as separate groups.

Table 6 shows statistically no significant difference was found when serum IL-17 level in neutrophilic asthma was compared with that of mixed granulocytic asthma, while statistically highly significant difference was found when its level in neutrophilic and mixed granulocytic asthma as one group compared with eosinophilic and paucigranulocytic asthma as separate groups.

Discussion

Prevalence increased markedly worldwide during the second half of the 20th century, but seems to have plateaued thereafter except in USA. The prevalence of asthma in Egypt is 8.4% [24].

Individually, the Th2 cytokines can explain many features of asthma, including airway eosinophilia (IL-5) and bronchial hyperreactivity (IL-13) acting on bronchial smooth muscle cells. However, some individuals with asthma display airway neutrophilia rather than eosinophilia [25].

In this study, patients were classified according to differential leukocytic count into 40% eosinophilic, 33.3% neutrophilic, 16.7% paucigranulocytic and 10% mixed granulocytic (Table 3). In a study done by Manise et al, (2013) they patients were 41% eosinophilic, 16% neutrophilic and 43% paucigranulocytic according to sputum cellular profile.26 The higher percentage of neutrophilic asthma in our study was due to higher percentage of severe asthma which is correlated with neutrophilic type [8].

Sputum homogenization was done using sterile glass beads according to Pye et al., (1995)13 instead of N-acetyl L-cysteine (NALC) as it has a bactericidal effect according to Wang et al., (2014) [27].

In our study, bacterial colonization with potentially pathogenic organisms was detected in 33.3% of asthmatic patients. Our study was in agreement with Zhang et al., (2012) who found that 51.7% of patients had bacterial colonization but the higher rate in their study as all patients were with chronic severe stable asthma [8].

In our study, the isolated bacteria was k. pneumonia in 23.3% of total cases and 70% of colonizers, S. pneumonia in 6.7% of total cases and 20% of colonizers and eventually S. aureus in 3.3% of cases and 10% of colonizers. Our study was in agreement with the study done by Jounio et al., (2009) who detected S. pneumoniae in 8.5% of patients [28]. On the other hand, Jounio et al., (2009) isolated other organisms that was 49.9% H. parainfluenzae, 35.7% group A beta-haemolytic streptococci,15.1% Group C streptococci and 6.1% Group G streptococci [28].

Also, Zhang et al., (2012) [8] and Wood et al., (2009) [19] reported that the most frequently isolated organism was H. influenza in 35% and 60% of cases; respectively.

As regards the mechanism of bacterial colonization in asthma, several studies have shown that asthma causes chronic inflammation and epithelial shedding in the airways, which may lead to chronic bacterial colonization and predispose the subject to local or even systemic infection [29].

No H.influenzae was isolated and it was explained by studies that found coculture of *S. pneumoniae* and *H. influenzae* led to a rapid decrease in viable counts of *H. Influenzae where it* was killed by concentrations of hydrogen peroxide similar to that produced by *S. pneumoniae* through the action of pyruvate oxidase (SpxB) under conditions of aerobic growth [30].

In addition, *our study included adult patients who may have immunity against H.influenzae* infection as although Hib vaccine is given to children, Hib infections have also decreased in adults. This decrease occurred because of herd immunity; children infected with Hib carry the bacteria in their nasal passages while clearing the infection. These Hib-carrying children would regu-

larly infect adults [31]. Also, this difference may be due to different environmental conditions and inadvertent use of antibiotics.

In our study it was detected that there was a significant increase in serum IL-17 levels in patient group more than that measured in control group. Our study also was in agreement with study by Lu et al., (2012) who showed that there were significant differences in serum IL-17 expression between asthmatic patients and normal controls [32]. In contrast, our study wasn't matched with results by Pukelsheim et al. (2010) who identified similar levels of IL-17 in both asthmatic and control groups [33].

As regards the relation between serum IL-17 levels and bacterial colonization, it was found that serum IL-17 level was significantly higher in patients with bacterial colonization than in patients without bacterial colonization. The elevated levels of serum IL-17 may be an altered systemic response to the presence of bacterial antigens in such patients [34].

Wood et al., (2009) reported that bacterial pathogens had been shown to induce an inflammatory response dominated by neutrophil recruitment to the infection site and increases in IL-8 expression [19].

In our study the relation between severity of asthma, on one side and asthma phenotype, bacterial colonization and serum IL-17 levels on the other side was detected. A significant relationship between the previous factors was detected.

It was found that severe asthma was associated with mixed granulocytic cases (100%) and neutrophilic cases (70%) with statistically significant difference. Our study was in agreement with Yong-chang et al., (2005). They confirmed that the presence of sputum neutrophilia in patients with severe asthma providing further evidence that neutrophilic inflammation may play an important role in pathogenesis of severe asthma. They thought that this may explain the mechanism underlying neutrophil influx into the airway as IL-17 released from activated T-cells may play an important role in neutrophil airway inflammation in severe asthma by stimulating the expression and production of IL-8 from a variety of cells including airway epithelial cells [35].

It also was found that serum IL-17 level was significantly higher in cases with severe asthma than those with mild and moderate asthma. Our study was in agreement with Alyasi et al., (2013) who detected increased serum IL-17 level as an independent risk factor for severe asthma which are in accordance with the well known biological function of IL-17. One possibility is that IL-17 could induce the release of the inflammation factor IL-6 to cause neutrophil recruitment and activation related to local inflammation [36].

In contrast, in study by Pukelsheim et al., (2010) serum cytokine levels and its correlations to blood cell count of asthma did not reveal any clear pattern [33].

In our study the laboratory findings among different asthma groups were detected. Bacterial colonization was (100%) in mixed granulocytic asthma and (70%) in neutrophilic asthma while, it was absent in other asthma phenotypes (p value <0.001). Our study was in agreement with Wood et al., (2009) who found that there was a relationship between higher neutrophil count in asthmatic patients and significant bacterial colonization with bacterial load >106 CFU/ml, while eosinophils showed higher levels in cases with non-significant bacterial load [19].

Also, our study was in agreement with Simpson et al., (2006) who determined that neutrophilic asthma group had an increased frequency of chronic bacterial colonization of the airway when compared with other asthma phenotypes.They found that neutrophilic asthma was characterised by increased expression of several key innate immune receptors: TLR2, TLR4 and CD14, as well as pro-inflammatory cytokines IL8 and IL1β. They also found high levels of airway endotoxin in subjects with neutrophilic asthma [37].

Our study was also in agreement with Wood et al., (2009) who found that not all cases of neutrophilic asthma were associated with significant bacterial load. This may indicate that bacteria were present didn't grow under the experimental conditions used in the study. Alternatively, other mechanisms may have led to airway neutrophilia independent of typical bacteria. Stimuli such as viruses and atypical bacteria lead to increased levels of IL-8, neutrophils and neutrophil degranulation. Similarly stimuli such as ozones, endotoxins and air pollution can lead to neutrophilic inflammation via mechanisms such as the activation of the Toll-like receptor pathway that may sense non infectious stimuli. Thus mechanisms other than the presence of typical bacteria may have led to the development of airway neutrophilia in these subjects [19].

In this study statistically highly significant difference was found when serum total IgE in neutrophilic and mixed granulocytic asthma as one group compared with eosinophilic and paucigranulocytic asthma as separate groups. This study was in agreement with study by Manise et al., (2013) who found that serum total IgE were lower in neutrophilic than in eosinophilic asthmatics and healthy subjects ($p<0.05$ for both) [38].

In our study the relationship between asthma phenotype and serum IL-17 level was detected. A significant association between mixed granulocytic and neutrophilic asthma and increased serum IL-17 level was found in comparison to other asthma phenotypes Our study was in agreement with Miossec et al., (2009). This study concluded that IL-17 may play a role in the airway inflammation of asthmatics by recruiting neutrophils into the lung, no matter if they are suffering from allergic or non-allergic asthma [39]. In contrast to our study, Agache et al., (2010) reported that increased serum IL-17 did not correlate with asthma phenotype [40].

Conclusion:

Bacterial colonization and subsequent elevation of serum IL-17 levels are associated with neutrophilic asthma. Also, IL-17 can be used to predict asthma severity since it is increased in severe asthma compared to mild and moderate forms of the disease.

Table (1): Frequency of bacterial colonization in sputum samples among asthmatic patients (group I) and controls (group II).

	Asthmatic patients (group I) (N=30)		Controls (group II) (N=30)
Bacterial colonization	10 (33.3%)		0 (0%)
K. pneumoniae	7 (70%)	23.3% of cases	-
S. pneumoniae	2 (20%)	6.7% of cases	-
S. aureus	1(10%)	3.3% of cases	-

Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

Table (2): The median and interquartile range (IQR) of serum IL-17 level in the studied groups.

Serum IL-17 (pg/ml)	Asthmatic patients (group I)	Controls (Group II)	p-value
Median IQR	14.1 10.05, 20.1	10.9 9, 13.5	0.001 (HS)

Table (3): The median and *IQR* of serum IL-17 level in the asthmatic patients with and without bacterial colonization.

Serum IL-17 (pg/ml)	Asthmatic patients With bacterial colonization	Asthmatic patients Without bacterial colonization	p-value
Median	18.5	11.7	<0.003 (S)
IQR	14.6, 20.1	10.05, 13.1	

Table (4): Comparison between different asthma severity groups as regards asthma phenotypes, bacterial colonization and serum IL-17 levels.

	Mild	Moderate	Severe	p-value
Asthma phenotypes (N)				
Eosinophilic (12)	1 (8.3%)	11 (91.6%)	0 (0.0%)	0.008 (HS)
Neutrophilic (10)	0 (0.0%)	3 (30.0%)	7 (70.0%)	0.004 (HS)
Mixed granulocytic (3)	0 (0.0%)	0(0.0%)	3 (100%)	0.043 (S)
Paucigranulocytic (5)	4 (80.0%)	1 (20.0%)	0 (0.0%)	0.02 (S)
Bacterial colonization (10)	0 (0.0%)	0 (0.0%)	10 (100%)	0.001 (HS)
Serum IL-17 (pg/ml) Median	11.7±0.5	11.6±1	18.5±1.5	<0.001 (HS)

Table (5): Laboratory findings among different asthma phenotypes:

Laboratory findings	Eosinophilic N=12	Neutrophilic N=10	Mixed N=3	Pauci-granulocytic N=5	p-value
TLC(10^9/L) Mean±SD Range	2.3±0.6 1.45-3.3	2.53±1.07 10-3.8	1.47±0.37 1.05-1.75	1.36±1.13 0.95-3.3	0.076 (NS)
DLC Mean (range) Neutrophils% Eosinophils% Macrophages% Lymphocytes%	32.6 (14-38) 3.5 (1-5) 63.3 (49-78) 0.6 (0-1)	81.2 (61-93) 0.0 18.5 (5-29) 0.3 (0-1)	65.6 (62-88) 2.4 (1-4) 31.5 (22-45) 0.5 (0-1)	12.3 (10-29) 0.0 70 (60-88) 0.7 (0-1)	<0.001 <0.001 <0.001 <0.001
Bacterial colonization K.pneumoniae S.pneumoniae S.aureus	0 (0%) 0 (0%) 0 (0%) 0 (0%)	7 (70%) 4(57.1%) 2(28.5%) 1(14.2%)	3 (100%) 3 (100%) 0 (0%) 0 (0%)	0 (0%) 0 (0%) 0 (0%) 0 (0%)	* <0.001 <0.232 0.558
Serum Total IgE level (kU/l) Median	287.67	174.85	243.33	93.75	**
IQR	245.35, 390.18	138.65, 200.34	205.15, 294.28	55.25, 128.78	

*p-value > 0.05 (NS) was found when rate of bacterial colonization in neutrophilic asthma was compared with that of mixed granulocytic asthma, while p-value < 0.001 (HS) was found when rate of bacterial colonization in neutrophilic and mixed granulocytic asthma as one group compared with eosinophilic and paucigranulocytic asthma as separate groups.

**p-value > 0.05 (NS) was found when serum total IgE level in eosinophilic asthma compared with mixed granulocytic asthma, while p-value < 0.001 (HS) was found when serum total IgE level in eosinophilic and mixed granulocytic asthma as one group compared with neutrophilic and paucigranulocytic as separate groups.

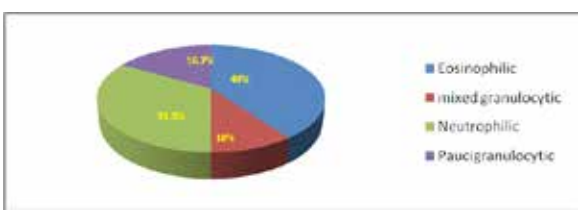
Table (6): Comparison among median and IQR of the serum IL-17 level in different phenotypes of asthma.

Serum IL-17	Eosinophilic N=12	Neutrophilic N=10	Mixed granulocytic N=3	Pauci-granulocytic N=5
Median	10.7	18.7	16.4	11.7
IQR	10.05, 13.1	16.1, 20.1	14.6, 17.8	11.05, 12.08

*p-value >0.05 (NS) was found when neutrophilic asthma compared with mixed granulocytic groups, while p-value < 0.001 (HS) was found when neutrophilic and mixed granulocytic groups (as single group) compared with eosinophilic and paucigranulocytic as separate groups.

Figure legend

Figure (1): Frequency of asthma phenotypes



REFERENCE

[1] Brussee JE, Smit HA, van Strien Corver K, et al.: Allergen Exposure in Infancy and the Development of Sensitization, Wheeze, and Asthma at 4 Years. J Allergy Clin Immunol 2005; 115 (5): 946-52. | [2] Pavord ID, Sterk PJ, Hargreave FE, et al.: Clinical applications of assessment of airway inflammation using induced sputum. Eur Respir J 2002; 37 (Suppl): 40s-3s. | [3] Haldar P and Pavord ID: Non-eosinophilic asthma: a distinct clinical and pathologic phenotype. J Allergy Clin Immunol 2007; 119 (5): 1043-52. | [4] Simpson JL, Scott R, Boyle MJ, Gibson PG.: Inflammatory subtypes in asthma: Assessment and identification using induced sputum. Respirology 2006; 11 (1): 54-61. | [5] Medzhitov R and Janeway CJ: Advances in immunology: Innate immunity. New Engl Med 2000; 343 (5): 338-44. | [6] Adcock IM and Ito K: Steroid resistance in asthma: A major problem requiring novel solutions or a non-issue? Curr Opin Pharmacoly 2004; 4 (3): 257-62. | [7] Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. PLoS One 2010; 5: e8578. | [8] Zhang Q, Illing R, Hui CK, et al.: Bacteria in sputum of stable severe asthma and increased airway wall thickness Respir Res 2012; (18): 13-35. | [9] Song C, Luo L, Lei Z, et al.: IL-17-producing alveolar macrophages mediate allergic lung inflammation related to asthma. J Immunol 2008; 181 (9): 6117-24. | [10] Global initiative for asthma (GINA) (2012): Available from: http://www.ginasthma.org. | [11] Scott H.A., Gibson P.G., Garg M.L. et al., (2011): Airway inflammation | is augmented by obesity and fatty acids in asthma.Eur.Respir.J.m;38:594– 602. | | [12] Gupta KB and Seema Garg: Sputum induction a useful tool in diagnostic respiratoty diseases. Lung India 2006; (23): 82-6. | [13] Pye A, Stockley RA and Hill SL: Simple method for quantifying viable bacterial numbers in sputum. J Clin Pathol 1995; 48 (8): 719-24. | [14] Merrill WW, Carter D and Cullen M: The Relationship between Large Airway Inflammation and Airway Metaplasia. Chest 1991; 101(1):131-5. | [15] Jung JW, Kim SH, Kwon JW, et al.: Clinical characteristics and long-term outcomes related to sputum eosinophilia in Korean asthmatics. Asia Pac Allergy 2011; 1 (1): 16-24. | [16] Collee JG and Marr W: Culture of bacteria in: Collee, J.G.; Marmion, B.p. and Simmons, A. (eds). Mackie and McCartney, Practical medical microbiology. 14th edition. Churchill Levingstone. London Ch. 6, p.113-30; 1996. | [17] Dixon JMS and Miller DC: Value of dilute inocula in cultural examination of sputum. Lancet 1965; 2 (7421): 1046-8. Cited from: Zaki ME and Goda T: Clinico-pathological study of atypical pathogens in community-acquired pneumonia: a prospective study. J Infect Developing Countries. 2009, 3 (3):199-205. | [18] Joseph NM, Sistla S, Dutta TK, Badhe AS, Rasitha D, Parija SC.: Ventilator-associated pneumonia: role of colonizers and value of routine endotracheal aspirate cultures; Int J Infect Dis 2011; 14 (8): e723–9. | [19] Wood LG, Simpson JL, Hansbro PM, Gibson PG.: Potentially pathogenic bacteria cultured from the sputum of stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia Free Radic Res 2009; 44 (2): 146-54. | [20] Baird D: Staphylococcus: Cluster forming gram positive cocci. In: Collee J. G. Fraser, A. G.; Marmion, B. P. and Simmons,A. (eds) Mackie and McCartney, Practical medical microbiology . edition . Churchill Levingstone. London. Ch. 11, p.245-62; 1996. | [21] Collee GJ, Miles RS and Watt B: Tests for the identification of bacteria. In Collee, J,G., Fraser, A. G.; Marmion, B.P. and Simmons,A.(eds). Mackie and McCartney, Practical medical microbiology . 14th edition. Churchill Levingstone. London. Ch. 7, p.131-50; 1996. | [22] Cheesbrough M: Biochemical tests to identify bacteria. In, District Laboratory Practice in Tropical Countries, Part 2, 1st edition. Cambridge University Press; 62-70; 2006. | [23] Collee JG and Marr w: Culture of bacteria in: Collee, J.G.; Marmion, B.p. and Simmons, A. (eds). Mackie and McCartney, Practical medical microbiology. 14th edition. Churchill Levingstone. London Ch. 6, p.95-112; 1996. | [24] http://www.ardsusa.org/acute-respiratory-distress-syndrome.htm | [25] Anderson GP: Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet; 372 (9643): 1107-19; 2008. | [26] Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R.: Sputum IgE and Cytokines in Asthma: Relationship with Sputum Cellular Profile. PLoS ONE 2013; 8 (3): e58388. | [27] Wang K, Wu Y, Li H, et al.: Synthesis, characterization and antimicrobial activity of silver nanoparticles: Ag (NALC) and Ag (GSHR) . RSC advances 2014; (4): 5130-5. | [28] Jounio U, Juvonen R, Bloigu A, et al.: Pneumococcal carriage is more common in asthmatic than in non-asthmatic young men. Clin Respir J 2010; 4 (4): 222-4. | [29] Hament JM, Aerts PC, Fleer A, et al: Direct binding of respiratory syncytial virus to pneumococci: a phenomenon that enhances both pneumococcal adherence to human epithelial cells and pneumococcal invasiveness in a murine model. Pediatr Res 2005; 58: 1198-203. | [30] Pericone CD, Overweg K, Hermans PW, Weiser JN. Inhibitory and bactericidal effects of hydrogen peroxide production by Streptococcus pneumoniae on other inhabitants of the upper respiratory tract. Infect. Immun. 200068: 3990-3997. | [31] Bar-On ES, Goldberg E, Hellmann S, Leibovi L (2012). Combined DTP-HBV-HIB vaccine versus separately administered DTP-HBV and HIB vaccines for primary prevention of diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzaeB (HIB). In Bar-On, Edna S. "Cochrane Database of Systematic Reviews". The Cochrane database of systematic reviews 4: CD005530. doi:10.1002/14651858.CD005530.pub3. PMID 22513932. | [32] Lu H, Liu L, Cheng H, Zhang Y, Hua S: Expression of interleukin 17 and IgE, and its significance in patients with bronchial asthma. African Journal of Pharmacy and Pharmacology 2012; 6 (40): 2828-31. | [33] Pukelsheim K, Stoeger T, Kutschke D, Ganguly K, Wjst M: Cytokine Profiles in Asthma Families Depend on Age and Phenotype; PLoS ONE 2010; 5 (12): e14299. | [34] Borch TS, Lobner M, Bendtzen K, Holmstrup P, Nielsen CH.: Decreased interleukin-2 responses to Fusobacterium nucleatum and Porphyromonas gingivalis in generalized aggressive periodontitis; J Periodontol2009; 80 (5): 800-7. | [35] Yong-chang S, Qing-tao Z and Wan-zhen Y: Sputum interleukin 17 is increased and associated with airway neutrophilia in patients with severe asthma, chinese med J (Engl) 2005; 115 (11): 953-6. | [36] Alyasin S, Karimi MH, Amin R. Babaei M, Darougar S: Interleukin-17 Gene Expression and Serum Levels in Children with Severe Asthma. Iran J Immunol. 2013; 10 (3): 179-87. | [37] Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG: Innate immune activation in neutrophilic asthma and bronchiectasis. Thorax 2006; 62 (3): 211-8. | [38] Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R. Sputum IgE in and cytokines asthma: relationship with sputum cellular profile. PLoS One. 2013;8(3):e58388. | [39] Miossec P, Korn T and Kuchroo VK: Interleukin-17 and type 17 helper T cells. N Engl J Med 2009; 361 (9): 888-98. | [40] Agache I, Ciobanu C, Agache C, Anghel M.: Increased serum IL-17 is an independent risk factor for severe asthma. Respir Med 2010; 104 (8): 1131-7. |