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ASTHMA-OBESITY NEUTROPHILIC INFLAMMATION AS A DISTINCT PHENOTYPE

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ABSTRACT:

Background & objectives: Asthma in obese subjects has been suggested to differ from classical phenotypes of asthma. Obesity is associated with activation of innate immune response in which neutrophilic activation is a fundamental process. However, airway inflammation in obese asthmatics is not well characterized, but may involve neutrophils. The present work was designed to study the possible airway and systemic neutrophilic inflammation as a distinct feature of asthma in obese asthma phenotype.
Methods: Study included 30 obese asthmatics (BMI ≥ 30 kg/m2), 8 non-obese asthmatics (BMI< 25 kg/m2) and 7 normal non-obese subjects (BMI< 25 kg/m2). Anthropometric measurements and pulmonary flow rates were assessed for all subjects. Methacholine inhalation challenge was performed only in asthmatics. Blood and sputum samples were tested for neutrophilic and eosinophilic counts, as well as levels of interleukin-8 (IL-8)and myeloperoxidase (MPO) enzyme, as markers of neutrophilic activity.
Results: There was no significant difference between obese and non-obese asthmatics as regard the mean value of the provocative dose of methacholine (PD20-FEV1). Compared to non-obese asthmatics, obese asthmatics demonstrated significantly higher blood and sputum neutrophilic counts and significantly lower blood and sputum eosinophilic counts. However, on comparing IL-8 and MPO levels between the obese asthmatic group and non-obese asthmatic group, there were insignificant trends toward increased serum and sputum levels of IL-8 and serum level of MPO in the obese asthmatic group. In asthmatic subjects, there was a significant positive correlation between BMI and serum MPO levels. Conversely, BMI showed significant negative correlations with both sputum eosinophilic count and blood absolute eosinophilic count.
Conclusions: Findings suggest that neutrophil predominant airway inflammation is more likely to be a distinct inflammatory phenotype of asthma in obese asthmatic subjects, though further studies are needed to detect significant effects of obesity on markers of neutrophil activity in asthmatic subjects to shed more light on the possible role of neutrophilic inflammation in the association between obesity and asthma.

Keywords: *Obesity, Bronchial asthma, Asthma phenotypes.*

INTRODUCTION

The association between asthma and obesity is poorly understood. Several potential pathophysiologic mechanisms have been proposed to explain the link between obesity and *asthma, including* changes in pulmonary mechanics, common genetic determinants and hormonal influences.[(1,2)] Furthermore, it has been suggested that obesity, as a chronic inflammatory disorder, could affect asthma by enhancing airway inflammation.[(3)]

Some observations suggest that asthma in obese subjects may differ from classical phenotypes of asthma.[(4)] These include the finding that weight loss in obese subjects is associated with a significant improvement in asthma symptoms, either with [(5)] or without[(6)] a significant improvement of airway hyper-responsiveness (AHR). Moreover, obese asthmatics report poor asthma control in response to standard asthma medication. [(7)] suggesting that obesity produces a unique phenotype of asthma that requires a distinct therapeutic approach. However, the mechanisms by which obesity may enhance the occurrence of this distinct asthma phenotype need to be elucidated.

Obesity is associated with activation of innate immune response in which neutrophilic activation is a fundamental process.[(8)] However, the impact of this systemic neutrophilic activation on the pattern of airway inflammation in obese asthmatics is not well characterized.

Data obtained from a large population-based study(9) revealed that, although obesity was associated with asthma, there was no clear association with eosinophilic airway inflammation, suggesting that the obesity-mediated changes in airway inflammation may be more consistent with a predominant neutrophilic inflammation. In turn, it was theoretically possible that leptin, which is increased in

obesity, may indirectly enhance neutrophilic airway inflammation and if present, this might potentially explain why obese asthmatics require increased levels of anti-inflammatory treatment. In this context, leptin was found to be associated with higher neutrophil activation in peripheral blood.[10]

These observations together with the reported role of neutrophils in non-eosinophilic forms of asthma, may explain the severe and relatively resistant form of asthma to corticosteroid therapy[11,12] in the obese-asthma phenotype.

Assessing neutrophilic inflammation using markers of neutrophil activity, such as interleukin-8 (IL-8) and myeloperoxidase (MPO), is more likely to evaluate neutrophilic inflammatory state rather than neutrophil cell count alone.[13] IL-8 is a powerful chemoattractant and activator of neutrophils. In addition, IL-8 can be synthesized by neutrophils in response to various inflammatory mediators and therefore form a positive feedback loop that induces the accumulation of large numbers of neutrophils.[12,14] MPO is an enzyme stored in azurophilic granules of polymorphonuclear neutrophils and released by activated neutrophils in the setting of inflammatory process.[15]

Accordingly, the present work was designed to study the potential involvement of airway and systemic neutrophilic inflammation as a distinct feature of asthma in obese asthma phenotype, by measuring both the inflammatory cell counts and neutrophil activity markers (IL-8 and MPO) in the sputum and blood of obese asthmatic subjects comparing their levels with non-obese asthmatics and normal (control) subjects 2. Subjects and Methods

SUBJECTS

38 asthmatic patients (7 males, 31 females) as defined by the American Thoracic Society[16] recruited from patients attending to the Clinical Pulmonary Lab, Clinical Physiology Unit, Medical Research Institute, were included in this study. They were asymptomatic and their asthma was controlled and didn't have symptoms of respiratory infection within at least four weeks before the beginning of the study. Corticosteroids were withheld 2 weeks before the study. Other anti-asthma drugs were stopped 8 hours before the study.[17] Thorough history taking and clinical examination were performed. The patients were selected and grouped according to their body mass index (BMI) into non-obese group; BMI< 25 kg/m2 (n=8) and obese group; BMI ≥ 30 kg/m2 (n=30).
7 normal non-obese subjects; BMI < 25 kg/m2 (3 females, 4 males) were included in this study; none of them or their first degree relatives were suffering from asthma, or allergic diseases. They were non-smokers. None of them had symptoms of respiratory infection within eight weeks before the beginning of the study. An oral informed consent was obtained from each subject before performing the procedures according to the regulations adapted by the ethical committee of the Medical Research Institute.

METHODS

1-Anthropometric Measurements
Measurement of current body weight.
Measurement of body height.
Then BMI was calculated using the formula:

$$\text{BMI (in kg/m2) (18)} = \frac{\text{weight in kilogram}}{\text{square of the height in meters}}$$

Measurement of waist circumference (WC), according to the WHO(19) which specifies the measuring location at the circumference line half way between the lowest costal margin and the iliac crest.

2- Physiological Studies

A-Pulmonary function tests including, Forced vital capacity (FVC), Forced expiratory volume in one second (FEV1), FEV1/FVC%, Forced expiratory flow rate at 25%, 50% and 75% of FVC (FEF25%, FEF50%, FEF75% respectively) and maximum mid expiratory flow (MMEF) were measured using computerized dry spirometer (Jaeger, Germany) with automatic dosimeter for methacholine inhalation challenge. All measurements were performed according to the European Respiratory Society and American Thoracic Society protocol for spirometry standardization.(20)

B-**Determination of bronchial reactivity by methacholine inhalation challenge method (only in asthmatics)** using the five-breath dosimeter protocol according to the guidelines of the American Thoracic Society for methacholine inhalation challenge test.(17) If FEV1 fell more than 20% from baseline (or the highest concentration has been given), no further methacholine was given, inhaled salbutamol was administered and spirometry was repeated after 10 min. PD20- FEV1 was computed.

3 Sputum induction procedure [21]
Sputum induction was carried out in subjects with a baseline FEV1 > 1.0 L. If FEV1 > 1.0 L but < 1.2 L, sputum induction was performed with isotonic 0.9% saline. For subjects with FEV1 > 1.2 L, sputum induction was performed with 4.5% hypertonic saline.

Induced sputum processing
All sputum samples were processed as soon as they were collected. Mucus plugs were selected from saliva and dispersed with 0.1% dithiothreitol (DTT). For every mL of sputum, 4mL of DTT was added. The tube was capped and placed on a rotating mixer for 30 minutes at room temperature, to ensure optimal cell dispersion. The supernatant was aliquoted and stored at –80°C and the cell pellet for the cytospins was resuspended to a concentration of 1 x 106 cells/mL using phosphate buffered saline.

4 Sputum differential cell count [21]
Cytospins were fixed in methanol and stained with May and Grunwald stain and subsequently visualized with Giemsa. 400 non-squamous cells were counted, with the squamous cell proportion recorded separately. Cells were identified by their morphology and the differential cell count was expressed as a percentage of non-squamous

cells Chromotrope 2R staining was also performed to confirm the presence or absence of eosinophils. Sputum differential neutrophilic count was determined by light microscopy and absolute neutrophilic counts were

5 Absolute neutrophilic and eosinophilic count in peripheral blood

Peripheral venous blood samples were obtained from all subjects. One portion was allowed to clot in a serum separator tube (about 4 hours) at room temperature and then it was centrifuged at approximately 1000 X g for 15 min, aliquot and stored at -20°C for measurement of IL-8 and MPO. The other portion was taken into a glass tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant and stored at 4° C before measurement of neutrophilic and eosinophilic count within 12 hours. After Leishman's staining, percentage of neutrophil and eosinophil cells was computed relative to the total number of white blood cells *using* a *hemocytometer*. The absolute count was computed by multiplying the total leukocyte count by the percentage of the cells.

6 Determination of neutrophilic inflammatory biomarkers

A) Determination of IL-8 level in the supernatants of induced sputum and sera of all groups by ELISA technique using human IL-8/CXCL8 ELISA Kit (Boster Biological Technology Co., USA), according to the manufactures' instructions. Data were expressed as pg/ml.

B) Determination of myeloperoxidase (MPO) enzyme level in the supernatants of induced sputum and sera of all groups by ELISA technique using human Myeloperoxidase /MPO ELISA Kit (Boster Biological Technology Co., USA), according to the manufactures' instructions. Data were expressed as ng/ml.

7 Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver.20 Chicago, IL, USA). The distributions of quantitative variables were tested for normality and revealed that most of the data were normally distributed, so parametric statistics were applied. Quantitative data were described using minimum, maximum, mean and standard deviation. ANOVA was used for comparing the three studied groups. Kruskal Wallis test was used to compare different groups for abnormally distributed quantitative variables. Correlations between 2 quantitative variables were done using Pearson correlation test. In all statistical tests, level of significance of 0.05 was used, below which the results considered to to be statistically significant.

RESULTS

The anthropometric data of asthmatic patients (obese and non-obese) and controls are presented in table (1).

1-**Physiological Results**

1-***Pulmonary function parameters*** in asthmatic and control subjects are presented in table (2). There was a significant difference between the three groups as regard FEV1/FVC% (P=0.022), FEF25% (P=0.005), FEF50% (P=0.000), FEF75% (P=0.000) and MMEF (P=0.000). There was no significant difference between obese asthmatics and non-obese asthmatics as regard all pulmonary function parameters measured. (Data not shown)

2 Methacholine inhalation challenge

The provocational dose of methacholine that causes 20% reduction in FEV_1 ($PD_{20\text{-}FEV1)}$ in obese asthmatics and in non-obese asthmatics are presented in table (3). There was no significant difference in $PD_{20\text{-}FEV1}$ between obese asthmatics and non-obese asthmatics.

2-**Inflammatory biomarkers results (table 4, Figures 1, 2, 3, and 4)**

There was a significant difference between the three groups as regard absolute neutrophilic and eosinophilic count in blood, total cell count in sputum and neutrophils and eosinophils in sputum (P=0.000 for all).There was no significant difference between the three groups as regard levels of IL-8 in sputum and serum, as well as MPO enzyme in sputum and serum. On comparing obese asthmatics with non-obese asthmatics, obese asthmatics demonstrated significantly higher blood absolute neutrophilic count and sputum neutrophilic count (P= 0.044, 0.001 respectively) and significantly lower blood absolute eosinophilic count and sputum eosinophilic count (P= 0.000 for all) than non-obese asthmatics. In addition, there were trends, although not significant, towards increased neutrophilic inflammatory markers (serum IL-8, sputum IL-8 and serum MPO) in obese compared with non-obese asthmatics.

Table (1): Anthropometric data of control subjects and asthmatic patients:

Anthropometric data		Control subjects (n=7)	Asthmatics (n=38)		Test of significance P value
			Obese asthmatics (n=30)	Non obese asthmatics (n=8)	
Age (year)	Min. – Max. Mean ± SEM.	23 – 43 29.57± 2.57	22 –54 36 ± 1.66	24 –52 32.25± 3.69	0.197
Weight (kg)	Min. – Max. Mean ± SEM.	49 – 90 67.7± 5.40	75 –135 96.7± 3.10	53 –65 61.50± 1.88	0.000**
Height (cm)	Min. – Max. Mean ± SEM.	161 –190 168.8± 3.63	152 –182 162.23± 1.47	152 –168 163.75± 1.88	0.151

ata are expressed as mean ± SEM. p: is determined using ANOVA test comparing obese asthmatics vs. non obese asthmatics vs. controls. ** Denotes a p value < 0.001 that was considered highly statistically significant.

Table (2): pulmonary function parameters in control subjects and asthmatic patients:

Pulmonary function parameters		Control (n=7)	Asthmatics		
			Asthmatic obese patients (n=30)	Asthmatic non obese patients (n=8)	Test of significance P value
FEV_1	Min. – Max.	72 –109	70 –114	70 – 112	
	Mean ± SEM.	72±5.86	91.67±2.16	90.50 ± 4.26	NS
FVC	Min. – Max.	84 –102	61 – 107	80 – 106	NS
	Mean ± SEM.	84±1.99	86.87±2.40	90.63 ± 2.96	
FEV_1/FVC%	Min. – Max.	90 – 100	64.1 – 95.5	67.7 – 97.6	0.022*
	Mean ± SEM.	90±1.64	88.47 ±2.40	86.6 ± 3.86	
$FEF_{25\%}$	Min. – Max.	85 – 129	53 – 104	46 – 107	0.005*
	Mean ± SEM.	85±6.32	78.08 ±2.80	75.13 ± 7.12	
$FEF_{50\%}$	Min. – Max.	92 – 137	29 – 109	43 – 106	0.000**
	Mean ± SEM.	92±7.07	76.47± 3.83	74.33 ± 8.24	
$FEF_{75\%}$	Min. – Max.	91 – 197	17 – 113	33 – 110	0.000**
	Mean ± SEM.	91±15.84	74.05± 3.74	77.84 ± 11.21	
MMEF	Min. – Max.	90 – 157	46 – 103	41 – 105	0.000**
	Mean ± SEM.	90±9.77	80.03± 2.50	76.28 ± 9.32	

FEV_1: Forced expiratory volume after one second, **FVC**: Forced vital capacity, **FEV_1/FVC%** ratio between two values, **$FEF25_{\%}$**: Forced expiratory flow at 25% of FVC, **$FEF_{50\%}$**: Forced expiratory flow at 50% of FVC, **$FEF_{75\%}$:** Forced expiratory flow at 75% of FVC, **MMEF**: Maximum mid-expiratory flow rate. **All data are expressed as percent predicted except for FEV_1/FVC which is expressed as absolute value. Data are expressed as mean ± SEM. P: is determined using ANOVA test comparing obese asthmatics vs. non obese asthmatics vs. control.* Denotes a p value < 0.05 that was considered statistically significant. ** Denotes a p value < 0.001 that was considered highly statistically significant. **

Table (3): Provocational dose (mg) of methacholine that causes 20% reduction in FEV_1 ($PD_{20\text{-}FEV1}$) of asthmatic patients (obese, non-obese):

$PD_{20\text{-}FEV1}$(mg)	Obese Asthmatic patients (n=30)	Non obese Asthmatic patients (n=8)	Test of significance P value
Min. –Max.	0.0011- 0.19	0.0010- 0.13	0.477
Mean ± SEM	0.045± 0.01	0.034± 0.02	

Data are expressed as mean ± SEM. P: is determined using student t test comparing obese asthmatics vs. non- obese asthma

Table (4): Inflammatory biomarkers in control subjects and asthmatic patients

Inflammatory Biomarkers		Control (n=7)	Asthmatic patients		Test of significance P value
			Asthmatic obese (n=30)	Asthmatic non obese (n=8)	
IL-8 serum (pg/ml)	Min. – Max.	312 – 469	312 – 2361	312 – 558	NS
	Mean ±SEM.	334.43±22.30	543.32±98.73	353.87±30.31	
	Median	312	312	312	
IL-8 sputum (pg/ml)	Min. – Max.	312 – 787	65 – 5474	312 – 3586	NS
	Mean ± SEM.	427.71±70.15	908.46±264.8	735.25±407.5	
	Median	312	312	312	
MPO serum (ng/ml)	Min. – Max.	57.9 – 82.7	26.6 – 149.5	39.3 – 71.1	NS
	Mean ± SEM.	66.57±2.96	69.91±4.81	55.6±4.18	
MPO sputum (ng/ml)	Min. – Max.	30.7 – 239	15.20 – 239.4	21.1 – 277.5	NS
	Mean ± SEM.	89.1±25.82	77.51±13.34	80.81±28.89	
Absolute neutrophilic count in peripheral blood (cell/ml^3)	Min. – Max.	1200 –6300	1500 – 8300	1100 – 5600	0.000**
	Mean ± SEM.	3157.14±755.9	4420±409.4	2662.50±512.7	
Absolute eosinophilic count in peripheral blood (cell/ ml^3)	Min. – Max.	150 – 350	180 – 550	500 – 850	0.000**
	Mean ± SEM.	242.86±25.32	363.33±18.64	643.75±38.31	
Total cell count in sputum (10^6 cells/mL)	Min. – Max.	0.46 – 1.80	1.80 – 4.50	1.80 – 4.00	0.000**
	Mean ± SEM.	1.19±0.16	2.87±0.15	2.72±0.25	
Neutrophils in sputum (10^6 cells/mL)	Min. – Max.	22 –0.80	0.55 – 3.00	0.20 – 1.09	0.000**
	Mean ± SEM.	0.5±0.07	1.46±0.12	0.6±0.10	
Esinophils in sputum (10^6 cells/mL)	Min. – Max.	0.005 –0.020	0.050 – 1.800	0.70 – 1.9	0.000**
	Mean ± SEM.	0.010±0.002	0.46±0.08	1.18±0.14	

Data are expressed as mean ± SEM, P: determined using ANOVA test comparing obese asthmatics vs. non- obese asthmatics vs. controls. For IL-8: P determined using Kruskal Wallis test comparing obese asthmatics vs. non- obese asthmatics vs. controls. ** Denotes a p value < 0.001 that was considered highly statistically significant. NS: non-significant

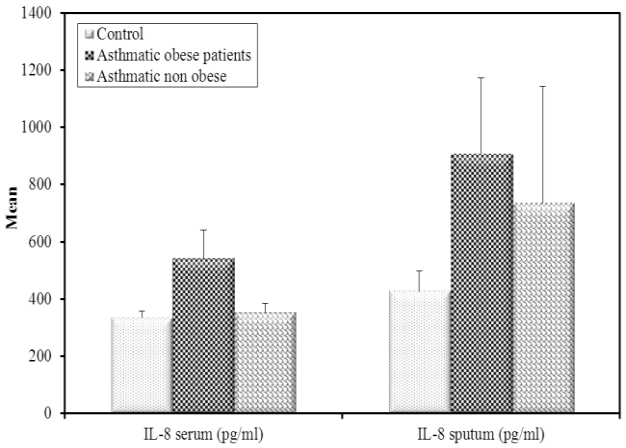

Fig. (1): Mean value of interleukin-8 (IL-8) (pg/ml) in sera and sputum of the three comparison groups

- There is no Significant difference between three groups
- There is no significant difference between obese asthmatics and non-obese asthmatics

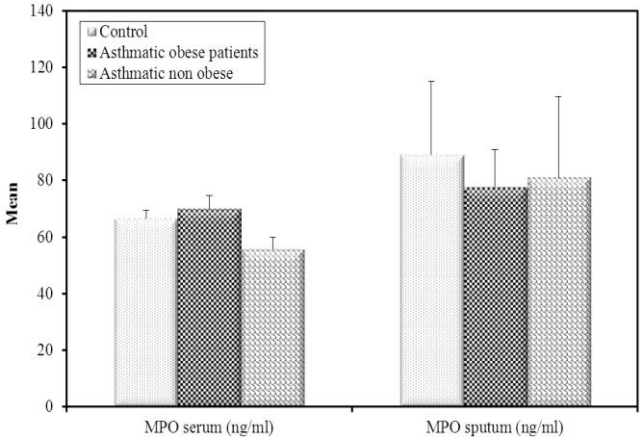

Fig. (2): Mean value of myeloperoxidase enzyme (MPO) (ng/ml) in sera and sputum of the three comparison groups

- There is no significant difference between three groups
- There is no significant difference between obese asthmatics and non-obese asthmatics

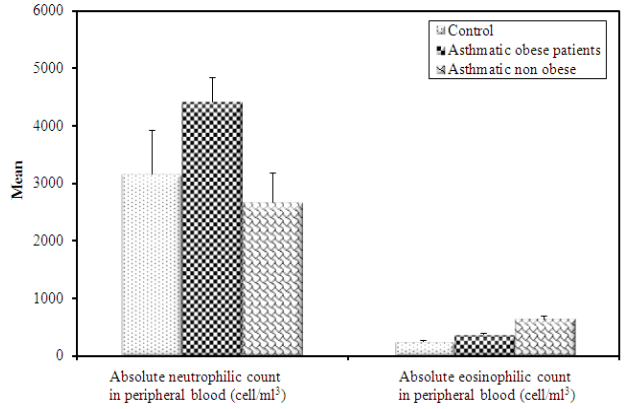

Fig. (3): Mean value of absolute neutrophilic count (cell/ml^3) and absolute eosinophilic count (cell/ ml^3) in peripheral blood of the three comparison groups

- Significant difference as compared obese asthmatics, non-obese asthmatics to control group
- Significant difference as compared obese asthmatics and non-obese asthmatics

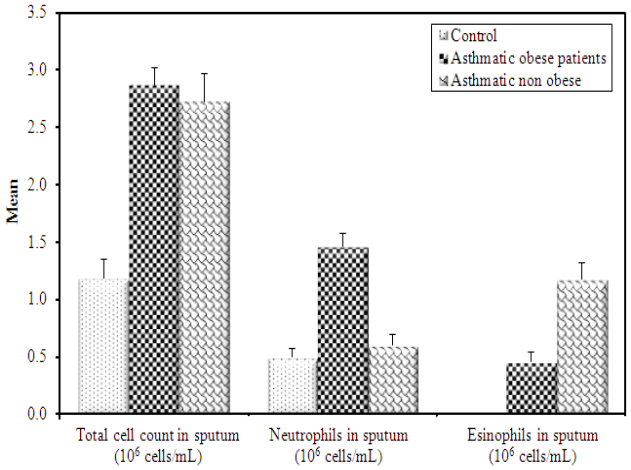

Fig. (4): Mean value of total cell count (10^6 cells/ml), neutrophils (polymorphs) (10^6 cells/ml) and eosinophils (10^6 cells/ml) in sputum of the three comparison groups.

- Significant difference as compared obese asthmatics, non-obese asthmatics to control group
- Significant difference as compared obese asthmatics and non-obese asthmatics

3 The obese asthmatic group was subdivided according to BMI into three classes:

Class I: 30.0 to 34.9 kg/m^2, Class II: 35.0 to 39.9 kg/m^2 and Class III: $\geq$ 40 kg/m^2. By comparing the data of these three groups considering physiological and inflammatory biomarkers, there was no significant difference between the three classes, except for weight, BMI and WC; meaning that there is no considerable difference could be detected between the different degrees of obesity as regard airway inflammation, or mechanical effect of obesity on asthma.

4 Significant correlations between body mass index and different variables among asthmatics (obese and non-obese): **(Figures 5-7)**

A. Body mass index (BMI) significantly positively correlated with myeloperoxidase enzyme (MPO) level in serum

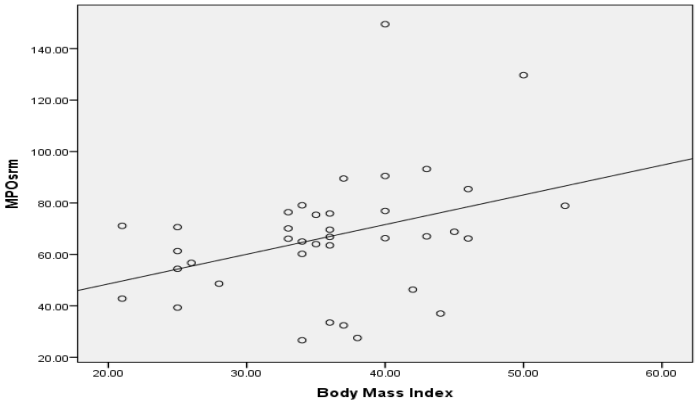

Fig.(5): Scatter plot diagram demonstrating the correlation between body mass index (BMI) (kg/m2) and myeloperoxidase enzyme (MPO) in serum (ng/ml)

- The line represents the line of identity
- The point represents a case among obese and non obese asthmatics

- **Body mass index (BMI) significantly negatively correlated with blood absolute eosinophilic count**

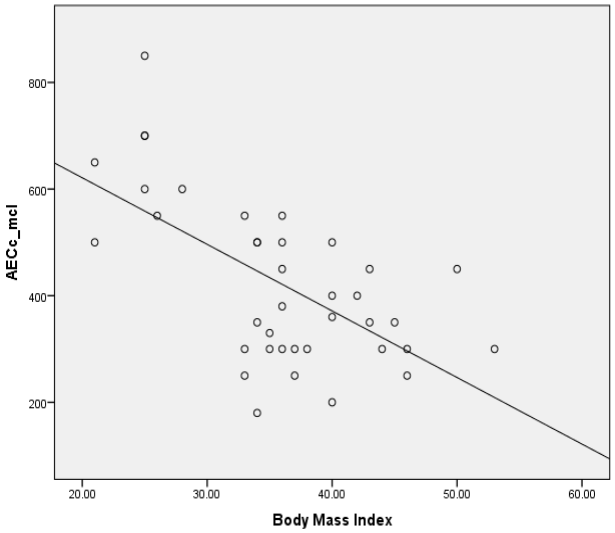

Fig.(6): Scatter plot diagram demonstrating the correlation between body mass index (BMI) (kg/m^2) and blood absolute eosinophilic count (cell/ ml^3)

- The line represents the line of identity
- The point represents a case among obese and non obese asthmatics

B-Body mass index (BMI) significantly negatively correlated with sputum eosinophils

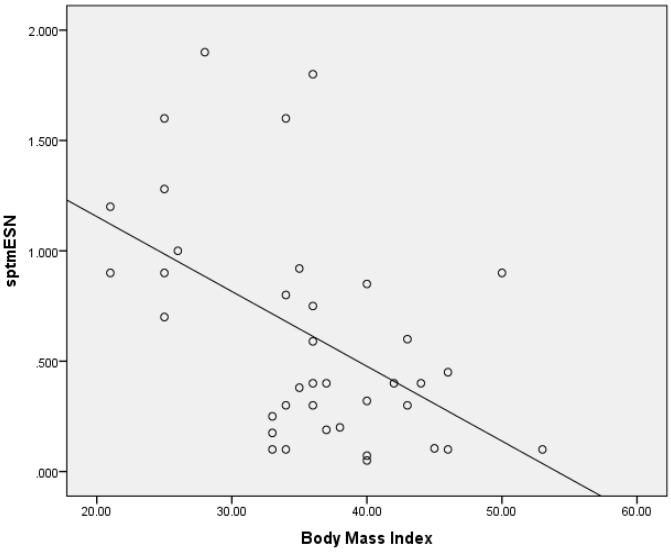

Fig. (7): Scatter plot diagram demonstrating the correlation between body mass index (BMI) (kg/m2) and sputum eosinophils (106 cells/ml)

- The line represents the line of identity
- The point represents a case among obese and non obese asthmatics

DISCUSSION

In the current study, obese-asthmatics demonstrated an increase in the mean level of neutrophils in both sputum and peripheral blood compared with non-obese asthmatics. In addition, there were trends, although not significant, towards increased neutrophilic inflammatory markers (serum IL-8, sputum IL-8 and serum MPO) in obese asthmatics compared with non-obese asthmatics. However, there was a significant correlation between BMI and serum levels of MPO. Taken together, these data could postulate that obese asthmatics have a more neutrophilic pattern of inflammation even in chronic stable asthma status and this may reflect persistent low-grade inflammation in obese asthmatics.

Neutrophilia has been associated with severe asthma and was generally seen in corticosteroid treated patients. Corticosteroids inhibit neutrophil apoptosis, and in some settings, contribute to neutrophilic activation, implicating that corticosteroid treatment itself is likely to have some relation to development of neutrophilia.[22] Lung neutrophilia in asthmatics has been associated with lower lung function, more trapping of air, thickened airway walls and greater expression of matrix metalloproteinases than are seen in people with non-neutrophilic asthma, but has not been associated with increased airway hyperresponsiveness.[23-25] Increased neutrophil level has been also reported in stable asthma.[26,27]

A cluster analysis performed by Halder *et al.*[28] identified a unique asthma phenotype: a population that is obese and predominantly female, with absence of eosinophilia. Furthermore, inverse relationships between either BMI or WC with sputum eosinophilia in asthmatics were found.[4] These findings are suggestive of obese asthmatics as a distinct phenotype characterized by non-eosinophilic airway inflammation. This non-eosinophilic phenotype could represent neutrophilic asthma.

Indeed, neutrophils have been shown to be more highly activated in obesity both in the circulation and adipose tissue vasculature[29,30], indicating that the characteristic low-grade inflammation seen in obesity appears to be the result of chronic activation of the innate immune response. It is possible that this inflammation extends to airways of susceptible individuals, leading to increased levels of airway neutrophils. This may be explained by the effect of leptin, as it promotes T-helper type 1 cell differentiation and increases activation of neutrophils via tumor necrosis factor alpha (TNF-α).[10] In a mouse pneumococcal pneumonia model[31], exogenous leptin stimulated the innate immune response leading to increased neutrophils in bronchoalveolar lavage fluid (BALF). Because leptin is strongly related to obesity, the relationship between circulating neutrophils and BMI would be expected to follow a similar pattern. However, it has been observed that there was no relationship between plasma leptin and airway neutrophils.[32] Assessment of airway leptin level may provide more insight.

The neutrophilic predominant airway inflammation in our asthmatics may suggest that the "obese-asthma" phenotype may be associated with alterations in airway leukocytes. This is in agreement with a couple of studies which demonstrated that obese asthmatics have a higher

level of neutrophilic inflammation, as reflected by both a higher percentage of sputum neutrophils and increased blood neutrophil counts; however, the increase in neutrophils was only seen in female obese asthmatics and not in male obese asthmatics.[32,33]

In the present study, we found significant negative correlations between BMI and eosinophilic counts, both in the sputum and peripheral blood in the asthmatic subjects. Yet we could not demonstrate a clear correlation between BMI and neutophilic counts; however, a larger sample size would probably have been needed to detect such effect.

It has been demonstrated that the concentration of IL-8 in sputum was higher in severe asthma [11] and there were correlations between sputum neutrophil numbers, IL-8 and MPO where the production of IL-8 by neutrophil can contribute to additional neutrophil recruitment and activation. The current study demonstrates relative elevations of IL-8 and MPO in obese asthmatics compared to non-obese asthmatics, inspite of similar severity of asthma as assessed by the measured pulmonary function and bronchial hyperreactivity. So, it could be supposed that obesity may independently activate innate immune response leading to a more neutrophilic pattern of airway inflammation. In agreement, IL-8 was reported to be increased in proportion to BMI.[34]

Additionally, in the present study, we found positive correlation between serum levels of MPO and BMI in the asthmatic subjects. MPO is a heme containing peroxidase expressed abundantly in neutrophils and released from secondary granules following neutrophil activation.[15] So our observations would suggest that obesity related neutrophilic inflammation has at least a synergistic effect on existing airway inflammation. Sustained release of these inflammatory products more than the capacity of their inhibitors implicates the role of neutrophils in airway injury and remodeling in chronic persistent asthma.

The present study demonstrated that obesity by itself is not sufficient to alter airway responsiveness to methacholine as there was no significant difference between obese and non-obese asthmatics as regard the mean value of $PD_{20\text{-}FEV1}$. There was no significant correlation between $PD_{20\text{-}FEV1}$ and the BMI or the inflammatory markers. This is in agreement with studies that reported no increase in airway responsiveness with increasing BMI[35,36] or serum IgE.[37] Our results could be explained by the finding that both asthmatic groups (obese and non-obese) in this study demonstrated similar baseline pulmonary function data despite having different degree of sub-acute airway inflammation.

In contrast, other studies have reported positive correlations between AHR and both BMI[38] and inflammatory markers.[39,40] One study[41] also noted a differing effect of obesity on AHR in female versus male subjects. Grouping subjects of both sexes together could thus obscure or attenuate any sex specific effects of obesity. Some studies describe a role for sex in the relationship between obesity and asthma.[42,43] So, studies utilizing advanced measurement of lung and airway mechanics may be necessary to fully understand the relationship between obesity and AHR.

CONCLUSIONS

In conclusion, neutrophil predominant airway inflammation is more likely to be a distinct inflammatory phenotype of asthma in obese asthmatic subjects. This stems from the finding that the mean value of neutrophil in peripheral blood and sputum was significantly higher in obese compared to non-obese asthmatics. Regarding neutrophilic inflammatory markers, there were insignificant trends towards increased serum levels of IL-8 and MPO and sputum level of IL-8 in obese asthmatic group associated with significant correlation between BMI and serum MPO. So, obesity likely results in a unique asthma phenotype that will require the development of a distinct therapeutic approach. Future studies, in both animal and human, might examine neutrophil trafficking, cell signaling and difference in innate and adaptive immune response in the obese asthmatics.

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