Innate Immunity in Obese Asthmatic Allergic and Non-Allergic Adults

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Obesity is characterized by activation of the innate immune responses and low grade systemic inflammation with increased levels of inflammatory cytokines. In the past two decades, the prevalence of both asthma and obesity has increased dramatically. The aim of the present study is to examine the relationship between innate immunity, obesity and asthma in allergic and non allergic obese persons by estimating interleukin-6 (IL-6) and C-reactive protein (CRP) levels as markers of innate immunity. The study included 2 groups of asthmatic patients; 50 obese asthmatic and 50 lean asthmatic patients. The obese asthmatic group included 25 allergic obese and 25 non-allergic obese asthmatics. Similarly the lean asthmatic group included 25 allergic and 25 non-allergic lean asthmatics. Body mass index (BMI), skin prick test, serum total IgE, peak expiratory flow rate (PEFR), interleukin-6 (IL-6) and C-reactive protein (CRP) levels were all assessed. A significant difference was found between allergic and non-allergic obese asthmatics and between allergic and non-allergic lean asthmatics as regards IgE, IL-6 and CRP (P=0.000). Comparison between allergic obese asthmatics and allergic lean asthmatics as regards BMI, IL-6 and CRP revealed high significant differences (P=0.000). In contrast no significant differences existed between them as regards IgE, PEFR (P=0.621, P=0.321 respectively). Comparison between non-allergic obese asthmatics and non-allergic lean asthmatics as regards BMI, IL-6 and CRP levels revealed highly significant differences (P=0.000). While no significant difference existed between them as regards IgE and PEFR (P=0.14, P=0.336 respectively). A significant negative correlation was found between PEFR and IgE in all groups (P=0.000) and negative correlation between PEFR and IL-6, CRP in all groups (P=0.000) except for allergic obese asthmatics group. Meanwhile, there was a significant positive correlation between BMI and IL-6 and CRP in both allergic and non-allergic obese asthmatics (P=0.000). In conclusion, obesity is associated with activation of the innate immune system leading to release of inflammatory cytokines more in non-allergic obese than allergic obese asthmatics. Control of obesity in such patients may lead to control of asthma.

The innate immune system represents a critical first line of host response to infectious, injurious and inflammatory insults (Mark, 2008). Asthma is characterized by chronic inflammation of the airways in which there is an overabundance of eosinophils, mast cells, and activated T helper lymphocytes. These inflammatory cells release mediators that include cytokines, chemokines and growth factors leading to bronchoconstriction, mucus secretion and remodeling (Hamid and Tulic 2009). Obesity represents a low – grade generalized systemic inflammation (Marchesini et al., 2003). The prevalence of obesity has increased considerably over the past 20 years because of increased caloric intake and reduced physical activity (James et al., 2004). Both asthma and obesity are important health issues. There is an increase body of evidence that obesity is an important determinant of asthma, particularly for adult, (Nystad et al., 2004) and both are characterized by the presence of inflammation. The mechanisms of the obesity-asthma association are not known and there are a number of possibilities (Weiss and Shore 2004). The European community respiratory health survey found that obesity was associated with an increased risk of wheeze with shortness of breath and other asthma-like symptoms. However body mass index (BMI) was not associated with hay fever or nasal allergies, specific IgE levels for house dust mite, grass, or cat dander, or with total IgE, suggesting that atopy may not be involved in the obesity-asthma association.
The link between obesity and asthma is unlikely to be explained by enhancement of the “classical” forms of airway inflammation resulting from the systemic inflammatory effects of obesity itself. Other mechanism, possibly related to innate immunity, may explain the relationship between obesity and asthma (Tim et al., 2008). It was explained that the innate immune system is primarily responsible for acute-phase response, a selflimiting process induced by a variety of stressors causing a number of cells to secrete cytokines (IL-6, TNF) which act on liver to synthesize acute-phase proteins (fibrinogen, C-reactive protein and others) (Pick up and Crook 1998). Since the role of adaptive immunity in the pathogenesis of asthma is unclear, the aim of the present study is to clarify the relationship between innate immunity, obesity and asthma in allergic and non-allergic obese persons by estimating IL-6 and CRP levels as markers of innate immunity.

**Patients and Methods**

**Patients**

This work was conducted on 100 patients selected from allergy and immunology outpatient clinic of Ain-Shams University Hospitals. All were matching age and sex.

They were divided as following:

- **Group I:** included 50 obese (BMI ≥30) asthmatic patients (diagnosed according to British thoracic society 1997). They were subdivided into two subgroups:
  - Subgroup a: Included 25 allergic patients (allergy defined as having history of allergy and positive skin test). They were 9 males and 16 females with mean age 40.28 ± 6.18.
  - Subgroup b: Included 25 non allergic patients (non allergic defined as having no history of allergy and negative skin test). They were 11 males and 14 females with mean age 40.28 ± 6.18.

- **Group II:** Included 50 lean (BMI=18-25) asthmatic patients. They were subdivided into two subgroups.
  - Subgroup a: Included 25 allergic patients with history of allergy and positive skin test. They were 10 males and 15 females with mean age 40.2 ± 6.22.
  - Subgroup b: Included 25 non allergic patients with no history of allergy and negative skin test. They were 12 males and 13 females with mean age 38.76 ± 6.39.

**Exclusion Criteria**

Patients were excluded from the study if they were receiving any drugs that might have modified the results of the study particularly systemic steroids. Also patients with concomitant acute illnesses or general medical diseases were excluded.

**Methods**

Patients were subjected to the following investigations after writing consents:

- History and thorough clinical examination.
- Calculation of BMI (body mass index) as follows: BMI = weight (in kilograms) / height (in meters²)
- Skin sensitivity test by prick method using standard allergen extracts (mite, mould, grass, mixed pollens and house dust), negative control (saline) and positive control (histamine) were included. All prepared in allergy laboratory of Ain-Shams University Hospitals of 1/10 concentration according to Neuman and Arman (1988). The test involves placing of small amount of the allergen on the skin (usually forearm or upper arm) and then pricking the skin surface. Signs of reaction includes swelling and redness of the site obtained within 20 minutes. Positive skin test was defined as a wheal 3mm in diameter greater than the wheal of negative control.
- Peak expiratory flow rate (PEFR) using the traditional peak flow meter. Results were expressed as percentage of the predicted values considering height, age and sex.
- 6 ml of venous blood were collected using aseptic veinpuncture technique. Serum was separated by centrifugation at 2760 rpm for 10 minutes and kept at -20°C till being tested for:
Serum total immunoglobulin E (IgE) in IU/L by ELISA utilizing a commercially available ELISA kit (Med’ Biotech, Inc., Agenzyme Company, Industrial Road, San Carlos, CA, USA).

Assessment of IL-6: the serum levels of IL-6 were determined by human IL-6 which is a quantitative microtitrative solid phase competitive enzyme immunoassay kit “Accucyte assay system”, IL-6 antibodies, biotinylated IL-6 conjugate and non biotinylated IL-6 (either in the standard or unknown sample) are mixed. The IL-6 antibodies bind specifically to the wall of the wells, the biotinylated IL-6 and the non tinylated IL-6 compete for the antibodies binding sites. By the end of the assay, after addition of a streptavidin alkaline phosphatase conjugate and a chromogenic substrate, a color was developed which is inversely proportional to the concentration of IL-6 in the sample. This concentration was determined by the reading of the OD at 490 nm using a spectrophotometer, then blotting the rating on the standard curve. This curve was plotted on a semi-log graph paper having sigmoidal shape that showed an inversely relationship between OD and IL-6 concentration.

Assessment of serum CRP using CRP-ELISA technique: microtiterstrips coated with anti CRP (from Diamed Eurogen) are incubated with diluted sera and patients samples. During incubation, CRP bound to the well. After removal of the unbound serum proteins the antigen antibody complex is detected with specific peroxidase conjugated antibodies. After removal of the unbound conjugate, the strips incubated with solution containing tetramethyl benzidin and hydrogen peroxide. The enzymatic reaction is stopped by addition of 2N H₂SO₄ and the absorbance values at 450 nm are determined.

Statistical Methods
SPSS statistical software package (V. 17, Echosoft Corp., USA, 2008) was used for data analysis. Data were expressed as Mean±SD for quantitative measures and both number and percentage for categorized data. Comparison between two independent mean groups for parametric data was done using Student t test. Pearson correlation test was used to study the possible association between each two variables among each group for parametric data. Chi-square test was used to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered significant.

Results
Group Ia (allergic obese asthmatics) showed high significant increase in IgE and decrease in IL-6 and CRP levels when compared to group Ib (non-allergic obese asthmatics) (P=0.000). While no significant difference existed between the two groups as regards PEFR and BMI (P= 0.898, P= 0.974 respectively) (Table 1).

Group IIa (allergic lean asthmatics) showed high significant increase in IgE and decrease in IL-6 and CRP levels when compared to group IIb (non-allergic lean asthmatics) (P=0.000). While no significant difference existed between the two groups as regards PEFR and BMI (P= 0.847, P= 0.936 respectively). (Table 1).
Group Ia showed high significant increase in BMI, IL-6 and CRP levels when compared to group IIa ($P=0.000$). While no significant difference existed between the two groups as regards IgE, PEFR. ($P=0.621$, $P=0.321$ respectively) (Table 2).
Group Ib showed high significant increase in BMI, IL-6 and CRP levels, when compared to group IIb \( (P=0.000) \). While no significant difference existed between the two groups as regards IgE, PEFR \( (P=0.14, P=0.336 \text{ respectively}) \). (Table 3).

**Table 3. Comparison between non-allergic obese asthmatics & non-allergic lean asthmatics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>non-allergic obese asthmatics</th>
<th>non-allergic lean asthmatics</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE(IU/L)</td>
<td>66.84±27.86</td>
<td>77.6±22.58</td>
<td>NS</td>
</tr>
<tr>
<td>PEFR(%)</td>
<td>82.44±8.08</td>
<td>84.4±6.68</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>36.8±4.09</td>
<td>21.16±1.88</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-6(pg/ml)</td>
<td>39.61±8.77</td>
<td>10.91±5.74</td>
<td>0.000</td>
</tr>
<tr>
<td>CRP(µg/ml)</td>
<td>139.70±20.28</td>
<td>56.99±9.32</td>
<td>0.000</td>
</tr>
</tbody>
</table>

NS= not significant

Among the patients of group Ia, there was highly significant negative correlation between PEFR and IgE \( (P=-0.000) \) and highly significant positive correlation between IL-6 and BMI \( (P=0.000) \), CRP and BMI \( (P=0.000) \) and CRP and IL-6 \( (P=0.000) \). While no significant correlation existed between BMI and IgE \( (P=0.163) \), PEFR \( (P=0.176) \), IL-6 and IgE, PEFR \( (P=0.222 \text{ for both}) \) CRP and IgE \( (P=0.084) \), PEFR \( (P=0.088) \).

Among the patients of groups Ib, there was highly significant negative correlation between PEFR and IgE \( (P=-0.000) \) and highly significant positive correlation between BMI and IgE, PEFR, BMI \( (P=0.000) \) and CRP and IgE, PEFR, BMI, IL-6 \( (P=0.000) \).

Among the patients of group IIa, there was highly significant negative correlation between PEFR and IgE \( (P=-0.000) \), IL-6 and PEFR \( (P=-0.000) \) and CRP and PEFR \( (P=-0.000) \) and highly significant positive correlation between IL-6 and IgE \( (P=0.000) \), CRP and IL-6 \( (P=0.000) \). While no significant correlation existed between BMI and CRP \( (P=0.493) \), IL-6 \( (P=0.517) \), IgE \( (P=0.213) \) and PEFR \( (P=0.283) \).

Among the patients of group IIb, there was highly significant negative correlation between IL-6 and PEFR \( (P=-0.000) \). Also between CRP and PEFR \( (P=-0.000) \) and highly significant positive correlation between CRP and IL-6 \( (P=0.000) \). While no significant correlation existed between PEFR and IgE \( (P=0.467) \), BMI and IgE \( (P=0.118) \), PEFR \( (P=0.661) \), IL-6 \( (P=0.813) \), and CRP \( (P=0.558) \), IgE and IL-6 \( (P=0.521) \).

### Discussion

Obesity is associated with an increased prevalence of asthma (Andrèa *et al.*, 2008). It’s sought that obesity may up regulate airway inflammation resulting in asthma (Rachel, 2007). Some observations suggest that asthma in obese subjects may differ from the classical phenotype of asthma (Bustos *et al.*, 2005). Asthma is characterized by chronic inflammation of the airway (Takemura *et al.*, 2006), and associated with increased levels of IL-6 (Yokoyama *et al.*, 1995) and CRP (Ford, 2003, Kony *et al.*, 2004; Miyoshi *et al.*, 2007) that may serve as a surrogate markers of airway inflammation in asthma. It was found that, sever obesity was associated with
asthma, but not with atopy and airway hyper-responsiveness (Schachter et al., 2001).

In the present study there is a significantly increased levels of IL-6 and CRP in all obese asthmatics, but significantly higher in obese non allergic asthmatics than obese allergic asthmatics. These results were in agreement with Yue and Robert (2006) who suggested that obesity may have greater effect on non allergic asthma than on allergic asthma. Ölafsdittir et al., (2005) concluded in their study that raised levels of CRP are significantly associated with respiratory symptoms and non allergic asthma but not allergic asthma. This also goes with Leonid et al. (2000) who reported that serum IL-6 is increased in obese persons. However, Sanchez et al. (1997) found that IL-6 levels did not show any significant difference either in non allergic or in allergic asthmatics (This is because the included patients aren’t all obese).

There is a growing support for the hypothesis that obesity is an inflammatory condition associated with chronic activation of innate immune system (Tamakoshi et al., 2003) leading to increased levels of inflammatory cytokines (Canoz et al., 2008). The results of the current study showed that IL-6 levels in obese asthmatics were significantly higher than that in lean asthmatics. In our study we found that CRP levels in obese asthmatics were significantly higher than that in lean asthmatics. These results agree with Christina et al. (2008) who showed that obesity is associated with higher CRP concentration. Both findings explained by Shore and Johnston (2006) who stated that obesity is characterized by low-grade systemic inflammation with increased levels of inflammatory cytokines, adipokines, and acute phase proteins including IL-6, TNF- and CRP. Our study confirms a previous results conducted by Douwes et al. (2002) who found that allergic asthma in both obese and non obese is associated with increase in IgE levels. Our results also revealed significant positive correlations between BMI and inflammatory cytokines (IL-6, CRP) in obese asthmatics. This was supported by Marjolien et al. (1999) who reported that human adipose tissue expresses and release the proinflammatory cytokines IL-6 and CRP, potentially inducing low-grade systemic inflammation in persons with excess body fat, and concluded that higher BMI is associated with higher CRP concentrations. Also, another positive correlation which was non significant in obese allergic asthmatics and highly significant in obese non allergic asthmatics between BMI and IgE levels. This disagree with Canoz et al. (2008) who revealed that no association was found between allergy test results and obesity. Furthermore, Jarvis et al. (2002) suggested that BMI not associated with specific IgE levels or with total IgE. On the other hand, significant negative correlations existed between PEFR and IL-6, CRP in all studied groups. This was consistent with Savykoski et al. (2004) who suggested that there is association between CRP levels and severity of asthma. Moreover, Jousilahti et al. (2002) found that serum CRP levels correlated negatively with pulmonary function tests. Based on our results, we concluded that obesity is a state of chronic inflammation associated with chronic activation of innate immune system leading to increased levels of inflammatory cytokines. This inflammatory state plays a great role in the pathogenesis of asthma in non allergic obese asthmatics as atopy is not the major cause of asthma in obese. Control of obesity, will reduce the inflammatory state and may cure asthma in such patients.

References


