ORIGINAL RESEARCH

The relationship of inflammatory cytokines with asthma and obesity

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Abstract

Objectives: Obesity is considered a risk factor for asthma. However, the mechanism that connects the two is not well understood. In this study we investigated the relationship between inflammatory cytokines and acute phase reactants in obesity, and asthma.

Method: Asthmatic and control subjects were divided into 2 sub-groups: obese and non-obese. Anthropomorphic parameters, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), leptin, tumour necrosis factor-alpha (TNF- α), and interleukin-6 (IL-6) were compared between obese, asthmatics and control subjects of normal weight. Respiratory function tests and allergy skin tests were also performed in the patients with asthma.

Results: ESR, CRP, TNF- α , IL-6, and leptin levels in obese asthma patients were higher than in the healthy controls ($P < 0.01$). TNF- α , IL-6, and leptin levels were higher in obese asthma patients than in non-obese asthma patients ($P < 0.01$). Inflammatory markers were related to parameters of obesity. No association was found between allergy test results and obesity ($P > 0.05$).

Conclusion: We identified a relationship between acute phase reactants and inflammatory cytokines, and the criteria for obesity in obese asthma patients. Inflammation markers were at their highest levels in obese asthma patients. Leptin levels were considerably higher in obese patients than in normal weight controls. Like obesity, leptin is suggested to play a role in the pathogenesis of asthma.

Obesity is a serious health problem worldwide. It has been on the increase during the last decade due to changes in eating habits and a decrease in physical activity.^{1,2} Asthma is also on the increase as a result of industrialization and constant exposure to irritants and allergens, which result from urbanization.³

There is epidemiological data indicating a causal relationship between obesity and asthma. Several studies suggest that weight loss and weight gain may have an effect on the clinical course of asthma.^{2,4-9}

Prospective studies show that obesity is a risk factor for asthma, though the mechanisms linking the two are not fully understood.¹⁰⁻¹³ Obesity is a disease associated with systemic inflammation, and an increase in inflammatory mediators, which may also result in airway hyperreactivity, has been observed in obese individuals.⁶

Adipose tissue is an important source of cytokines and contributes to the inflammatory milieu. Apart from general obesity, visceral adipose tissue is the key factor in the formation of low-grade chronic inflammation in obese individuals.¹⁴ High levels of interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α) are observed in individuals with visceral obesity. They induce the production of TNF- α , IL-6,

and some acute phase reactants. On the other hand, IL-6 is the primary mediator in acute phase response and leads to the synthesis of C-reactive protein (CRP), which exacerbates the inflammatory response.¹⁵

Asthma is a chronic inflammatory disease of the airways.¹⁶ Obesity acts as a risk factor for asthma through various mechanisms. Static and dynamic factors may play primary roles. The secondary mechanism may be the role played by obesity in increasing remodeling, which is a characteristic of asthma. Consequently, they may work by creating an inflammatory micro-environment in the airways. TNF- α is expressed in the airways and TNF- α may increase contractility against airway constrictor agents; in other words they may increase airway hyperreactivity.² TNF- α and IL-6 are expressed by adipocytes and are associated with total fat mass. Similarly, a pro-inflammatory substance, leptin, derived from adipose tissue also stimulates the release of inflammatory cytokines, such as IL-6 and TNF- α . These cytokines contribute to IgE production, subepithelial fibrosis, and airway remodeling, which are primary in the pathogenesis of asthma.^{15,17}

In this study we investigated two inflammatory conditions, asthma and obesity, with respect to proinflammatory cytokines and acute phase reactants. We compared obese and non-obese asthmatic patients with non-asthmatic controls and, subsequently, compared obese asthma patients with those of normal weight, with respect to inflammatory markers.

Patients and methods

Informed consent was obtained from all participants. The study included 54 female asthma patients who were being followed-up at the allergy clinic of the internal medicine department. Forty-four control subjects were selected from the healthy relatives of our hospitalized patients. Individuals with a known infectious disease and those with cardiovascular, rheumatismal, malignancy, and liver and kidney disorders, breast-feeding and pregnant women, and individuals

with obesity due to secondary factors were excluded from the study. Physical examination, routine hematological and biochemical tests of all participants were normal suggesting normal nutritional status, and they were not taking a special diet. Asthmatic patients were receiving medication with varying dose inhaled steroid in combination with short or long acting β -agonists according to the severity of asthma. None of the patients or control subjects smoked tobacco or received systemic steroids for 3 months before the study.

BMI was calculated as weight (kg)/height (m²). Waist circumference (WC) was measured in cm between the lower border of the ribcage and mid line of the iliac crest. Hip circumference (HC) was measured in cm from the widest point of the hip, then the waist-hip ratio (WHR) was calculated.

Fasting venous blood samples were collected at 09.00 h. After centrifuging at 4 °C, the blood was stored at -70 °C until analyzed. Plasma from blood collected in sodium citrate tubes was separated within 1 h for fibrinogen sampling. It was then centrifuged at 3000 g for 20 min and was stored at -70 °C until analyzed.

Serum CRP levels were measured with commercial kits (Dade Behring, Marburg, Germany), using the nephelometric method. Plasma fibrinogen levels were measured on an autoanalyzer (Dade Behring, Multifibren U, Marburg, Germany), using the von Clauss method, which is essential for determining clotting time.

Serum TNF- α , IL-6, and leptin levels were measured using the immunometric method (Quantikine high sensitivity TNF- α , Quantikine IL-6, and Quantikine leptin, R&D Systems, Minneapolis, USA), in accordance with the manufacturer's guidelines. All tests were performed in duplicate and values above the standard curve were re-tested using appropriate dilutions. All measurements were carried out under blinded conditions. The minimum measurable levels of TNF- α , IL-6, and leptin were 0.12 pg/ml, 0.7 pg/ml, and 7.8 pg/ml, respectively. No significant cross-

TABLE 1. Comparison of antropometric variables and biochemical parameters of the groups.

	mean±SD		<i>P</i>	mean±SD		<i>P</i>
	Nonobese controls n: 20	Obese controls n: 22		Nonobese asthmatics n: 24	Obese asthmatics n: 30	
Age (yer)	33.25±9.50	38.77±11.91	0.10	34.92±10.28	45.27±6.56	0.001
BMI	23.03±2.41	35.68±3.55	0.001	24.165±2.80	36.71±4.93	0.001
WC	71.30±6.70	98.80±9.72	0.001	81.29±9.91	109.40±13.45	0.001
HC	96.35±4.70	117.23±9.32	0.001	100.67±8.57	123.53±12.82	0.001
WHR	0.73±4.31	0.84±5.82	0.001	0.80±6.70	0.88±4.17	0.001
ESR (mm/h)	12.80±4.44	20.59±9.53	0.002	11.08±4.89	19.80±13.61	0.04
CRP (mg/dl)	0.21±0.10	0.57±0.33	0.001	0.32±0.28	0.75±0.46	0.001
Fibrinogen (mg/dl)	231.30±33.54	292.00±62.50	0.001	289.00±78.87	355.43±117.21	0.021
IL-6 (pg/ml)	3.43±0.52	11.12±1.54	0.001	22.50±3.12	26.69±3.99	0.001
TNF-α (pg/ml)	2.10±0.45	9.72±1.11	0.001	15.49±1.65	16.90±2.90	0.039
Leptin (pg/ml)	9.75±1.59	34.95±3.18	0.001	24.38±5.63	40.87±5.24	1

reactivity or interference was observed in association with TNF-α, IL-6, or leptin.

Statistical analysis was performed using SPSS v.13.0 for Windows. Data are expressed as mean ± SD. Comparison of parameters between the groups was performed using one-way ANOVA, Tukey's HSD, Student's *t*, Kruskal-Wallis, and Mann-Whitney *U* tests. Pearson's correlation analysis was used to evaluate the relationship between parameters. The level of significance was accepted as $P < 0.05$.

Results

The asthma group consisted of 54 women (30 obese and 24 non-obese). Among the 42 women in the control group, 20 were of normal weight, while 22 were obese. Mean age of the obese asthma patients ($n = 30$) was 38.4 ± 11.2 yr, whereas mean age of the non-obese asthma patients ($n = 24$) was 42.8 ± 7.7 yr. The control group consisted of 20 women of normal weight (mean age: 33.3 ± 9.5 yr) and 22 obese women (mean age: 38.8 ± 11.9 yr). (P :NS). Mean age of the asthma patients was 40.7 ± 9.8 yr, whereas that of the control group was 36.1 ± 11.1 yr (P :NS). Obese asthma patients had a mean body mass index (BMI) > 30 , while the healthy control group and non-obese asthma patients had a mean BMI < 25 .

Anthropomorphic data are shown in Table 1. As expected, BMI, WC, and WHR of the obese women in

the control group were higher than those of the non-obese asthma patients ($P = 0.001$ for every subject). BMI was higher in obese asthma patients and obese controls than in healthy controls ($P < 0.01$ for each). Comparison of obese asthma patients and obese controls to healthy controls demonstrated that WC, HC, and WHR values were higher ($P < 0.01$ and $P < 0.001$, respectively) in the obese subjects. No correlation was observed between BMI and asthma.

Acute phase reactant, cytokine, and leptin values are shown in Table 1. The erythrocyte sedimentation rate (ESR) and CRP levels were higher in obese asthma patients than in healthy controls ($P < 0.05$ and $P < 0.01$, respectively); however, there were no differences in these levels between the obese asthma patients and obese controls. The CRP values of the patients are shown in Table 1 and comparisons in Figure 1. Plasma fibrinogen values were higher in obese asthma patients than in non-obese asthma patients and the control groups ($P < 0.01$ for each). IL-6, TNF-α, and leptin values were higher in obese asthma patients when compared with obese patients without asthma and the controls ($P < 0.01$ for each) (Figures 2,3,4). Leptin values were higher in obese asthma patients than in those of normal weight ($P < 0.001$).

The relationships between biochemical markers and obesity parameters: CRP, TNF-α, IL-6, and leptin values were positively correlated with BMI, WC, and

FIGURE 1. Comparison of CRP between groups.
NOC: Non-obese control, OC: Obese control, NOA: Non-obese asthma, OA: Obese asthma

FIGURE 2. Comparison of IL-6 between groups.
NOC: Non-obese control, OC: Obese control, NOA: Non-obese asthma, OA: Obese asthma

FIGURE 3. Comparison of TNF-α between groups.
NOC: Non-obese control, OC: Obese control, NOA: Non-obese asthma, OA: Obese asthma

FIGURE 4. Comparison of Leptin between groups.
NOC: Non-obese control, OC: Obese control, NOA: Non-obese asthma, OA: Obese asthma

WHR in all obese participants; TNF-α and IL-6 correlated with CRP in obese asthma patients.

There were no differences between obese and non-obese asthma patients with regards to eosinophil, PEF, and PEF₂₅₋₇₅ values, or duration of asthma. The allergy skin test was positive in 36 (66.7%) of the 54 asthma patients; *P*:NS between the obese asthma patients and those of normal weight.

Discussion

In the present study, although higher WC and WHR were noted in asthma patients than in the control group, there was no relationship between BMI and asthma. Abdominal obesity was seen in association with an increase in acute phase response. ESR and CRP levels were higher in the obese groups. Plasma fibrinogen values were also higher in obese asthmatic patients than in non-obese asthmatic patients and normal controls. TNF-α, IL-6, ESR, and CRP levels

correlated with each other, and to indices of obesity, such as BMI, WC, and WHR. Serum leptin levels were higher in all the obese participants than in the healthy controls, and were correlated with BMI, WC, and WHR. Leptin levels, together with TNF-α and IL-6 were higher in obese asthma patients than the other groups. No differences were observed between obese asthma patients and those of normal weight, with regards to allergy tests, eosinophil values, and the severity of asthma.

The relationship between obesity and asthma has been demonstrated in many studies.^{4,5,7,10,11,13,18} BMI has a strong, independent and positive association with asthma.⁶ Unlike in men, there is a relationship between BMI and asthma in women^{10,11,13,18} although the results of some studies have demonstrated that obese men and women are at equal risk with regards to asthma.^{4,5,19} Due to the small size of our study

group, the patient and control groups consisted only of women in order to avoid gender bias.

Although an increase in BMI has been suggested as a factor associated with the increased prevalence of asthma^{10,12,13,17}, Appleton²⁰ emphasized the importance of central obesity in asthma, whereas Kronander²¹ stressed the relationship between increased body weight and WC with asthma. In contrast, Bustos et al²² proposed that there is a relationship between BMI and asthma but they also suggested that there was no relationship between WC and asthma. We found a relationship between asthma and WC and WHR rather than BMI. Visser²³ identified higher CRP values among obese children, and noted that there was a small degree of inflammatory activity in obese children and that the concentration of CRP increased as BMI increased in adults.²⁴ The results of studies concerning plasma fibrinogen are inconclusive. Although Jousilasti²⁵ found plasma fibrinogen to be high in asthma patients, Büyüköztürk²⁶ could not determine any relationship between allergic asthma, and CRP and fibrinogen values. In our research obesity was associated with inflammatory markers such as ESR, CRP and fibrinogen levels. Dandona²⁷ and Ronne-
ma²⁸ observed the relationship between obesity and TNF- α and IL-6 levels. Studies of the relationship between leptin and TNF- α report inconclusive results. Tsigos²⁹ demonstrated that there was no correlation between serum leptin and TNF- α levels in obese individuals and TNF- α was higher in cases of abdominal obesity than in peripheral obesity. On the other hand, Bastard³⁰ demonstrated that obese individuals had increased levels of TNF- α , IL-6, CRP, and leptin. These biochemical markers are correlated with each other and with parameters of obesity.^{14, 31} We observed the highest levels of TNF- α , IL-6 and leptin levels in obese asthmatics. Chen³² suggested that the relationship between asthma and obesity was greater in non-allergic individuals than in allergic subjects. We did not find a relationship with obesity and allergic status.

Pro-inflammatory conditions observed in obese individuals may contribute to airway reactivity. There

may also be a common inflammatory process involved in both diseases.⁶ Obesity-related changes in adipose-derived hormones, including leptin and adiponectin may participate in asthma.³³ Leptin can also play a crucial role in allergic inflammation by activating eosinophils.³⁴ Güler et al.³⁵ showed that leptin levels were higher in obese asthmatic children than in controls but this was observed only in male children. Dixon et al.³⁶ reported that obese asthmatics had increased IL-6 levels which correlated with asthma severity. They suggested that IL-6 along with leptin, adiponectin and TNF- α could also contribute to the pathogenesis of asthma in the obese subjects. Dandona²⁷ and Bastard³⁰ demonstrated that TNF- α , leptin and IL-6 levels increased in obese individuals and decreased with weight loss. In a 15-year observational study conducted by Mai et al.³⁷ it was shown that the risk of asthma in children increased with obesity and high leptin levels. Güler³⁵ and Mai³⁷ also suggested that inflammation induced by leptin might play a role in the pathogenesis of asthma.

The present study has some limitations. In previously published studies the relationship between asthma and obesity was greater in women. In our study only women were enrolled in both the patient and control groups and, therefore, we cannot generalize the results that were obtained. All our asthmatic patients were receiving inhalation steroids to control asthma. We did not create subgroups with regard to asthma severity that could make the groups smaller. As a result, no relationship was established between respiratory function tests, which were almost normal in these patients, and the inflammatory markers. Although not obvious, the presence of illnesses such as sub-clinical rheumatism and cardiovascular diseases, which could potentially be a confounder, was not known. Cytokine concentration was measured in serum and, we did not gather information concerning inflammatory markers at the tissue level. Moreover, we did not perform any investigations to determine if it was obesity that caused asthma in our patients, as this was a cross-sectional study. At the beginning of

the study we expected to find very high cytokine concentrations in obese asthmatics compared with other groups. We questioned if two plus two was more than four. But at the end, we observed this was not true. We think that IL-6 and TNF- α are the most important cytokines of obese asthmatics derived mainly from asthma; whereas CRP and leptin were fundamentally originating from obesity. Our study did demonstrate that serum TNF- α , IL-6, and leptin levels were higher in obese asthma patients, and that they were related to obesity parameters and inflammatory markers. With regards to the obesity parameters' associations with asthma, WC and WHR were found to be more significant than BMI. IL-6 and TNF- α may reflect inflammatory activity better in obese asthmatic subjects.

In conclusion, it is obvious that there was a strong association between asthma and obesity, with regards to inflammation. A higher degree of inflammatory substances were present in the asthma patients than in the controls. Similarly, a higher degree of inflammatory substances were observed in the obese participants compared to those with normal weight. These values were the highest in obese asthma patients; as a result these individuals should be encouraged to lose weight. Further study is required in order to better demonstrate and clarify the relationship between asthma and obesity.

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