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Research Article

RISK FACTORS THAT ARE ASSOCIATED WITH INTERLEUKIN-8 LEVEL IN IRAQI ASTHMATIC PATIENTS

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ABSTRACT

Obesity is associated with increased morbidity related to cardiovascular disease and asthma. Cytokines and chemokines have been implicated in the pathogenesis of asthma. This study tries to clear the correlation and association between asthma, obesity related parameters and IL-8 levels. This is a case control study conducted on (38) asthmatic patients and (20) healthy control who were closely similar by age, gender and BMI. The main statistical tests used were student *t* test, linear regression test and linear correlation test. Significance was set at ($P < 0.05$). Sampling method used for this study was convenience sampling method. The main results of this study show a significant increase in IL-8 level in asthmatic patients than healthy control, significant association and positive correlation between IL-8 and BMI in Iraqi asthmatic patients since ($P< 0.05$). The results of this study revealed that IL-8 may play an important role in the pathogenesis of obesity related disease like asthma.

Keywords: Interleukin-8, Asthmatic patients, Iraqi, BMI, Lipid profile.

INTRODUCTION

Asthma is a chronic disease of the airways associated with severe inflammation caused by inflammatory cells and potent proinflammatory mediators. The asthmatic inflammatory response is orchestrated by Th2-type cytokines and small-molecular-weight cytokines called chemokines. Chemokines are involved in the recruitment of cells to the site of inflammation, and these chemokines, including eotaxin, IL-8, IFN-g–producing protein (IP-10), and monocyte chemotactic proteins (MCPs) 1 to 4, are thought to be involved in the pathology of asthma. Airway epithelial cells are thought to be the major producers of chemokines; however, inflammatory cells themselves have also been shown to be a source of these chemokines [1].

Interleukin-8 (IL-8) was the first member identified of a growing family (currently more than 40 members) of proinflammatory chemokines belonging to the C-X-C subfamily that attract and activate immune and inflammatory cells [2].

A diverse variety of biological effects are attributed to IL-8, including several involving neutrophils: inflammatory cell activation and chemotaxis, production of reactive oxygen species, calcium translocation, shape change ,actin polymerization, degranulation, increased expression of the integrin CD11b–CD18, enhancement of cell adhesion to endothelial cells, promotion of angiogenesis, modulation of histamine release and the release of azurophil granules. IL-8 activates a number of inflammatory cells in addition to human neutrophils, for example T cells, B cells and basophils [2,3].

The predominant form of IL-8 is a nonglycosylated 72-amino-acid protein that is produced by many cells, including neutrophils, monocytes, tissuemacrophages, mast cells, vascular endothelial cells, stromal cells, peripheral blood, T lymphocytes, hepatocytes, mesangial cells, airwayepithelial cells, airway smooth muscle cells and plasma cytoid dendritic cells [2,4].

IL-8 has been shown to be involved in the pathology of a wide range of disorders such as rheumatoid arthritis, gout, severe trauma, psoriasis, acute inflammation, infection, sepsis syndrome, cancer, systemic lupus erythromatosis, nephritis and number of pulmonary disorders, including chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), wheeze, chronic asthma, pulmonary fibrosis and bacterial pneumonia. The measurement of IL-8 in these conditions has been shown to be useful not only in understanding the basis of these disorders, but also as a prognostic and diagnostic marker of disease. Numerous investigations are underway attempting to inhibit the activity of IL-8 in these diseases as well as exploring the possibilities of using IL-8 clinically to attract immune cells into tumors and to re-correct neutrophil function in myelodysplasia [2,3,5].

IL-8 has also been suggested to play a role in asthma, where it is reported to be involved in lymphocyte, eosinophil and basophil activation and migration to the inflammatory site [6].

Obesity is associated with increased morbidity related to cardiovascular disease and asthma [7]. These morbidities are thought to result, in part, from the production of pathogenic mediators by adipose tissue, therefore the current study has been set to clarify the relationship between IL-8 and obesity-related parameters, which are known to be elevated in obesity, such as body mass index (BMI) and lipid profiles, in asthmatic patients which could logically support their significant association.

MATERIALS AND METHODS

Subjects

This is a case control study conducted on (38) asthmatic patients and 20 healthy control who were closely similar by age, gender and BMI but the only difference that the control were not suffering from asthma. Those asthmatic patients were admitted to AL-KHADHIMEIA Teaching Hospital and AL-YARMOOK Teaching Hospital in the period between 23th November 2010 to 30th December 2010. Clinical data that related with patients age, gender, BMI and waist to hip ratio were collected from patients' files by specific data sheet form designed for this study. The study was approved by the Ethics Committee of the two hospitals, Ministry of Health Iraq (MOH), and consent was obtained from all subjects before the start of the study.

Methods

Blood samples were drawn between 9:00 AM and 12:00 PM. All specimens were centrifuged at 4000 rpm within 2 hour of collection and serum stored at -20°C until analysis. After preparation of serum, the levels of interlukin-8 were measured with (ELIZA) method using (Biological Company/ United States)kit while the levels of lipid profile were measured with (Enzymatic) method by using from BioMaghreb Company – Tunisia, Kit, VLDL level calculated from the equation (VLDL=TG/5) and LDL calculated from Friedewald's equation[LDL=TC-(HDL+TG)]. All of the patients who were involved

in this study fulfilled the inclusion criteria. The inclusion criteria include adult asthmatic patient ≥ 18 years old, male or female and were admitted for treatment of asthma. The exclusion criteria include smokers, alcoholism and chronic obstructive pulmonary disease (COPD).

The following anthropometric measurements were obtained: weight, height, BMI (body mass index), and waist and hip circumferences. Weight was measured after calibration the scale before each weight measurement. Height was obtained with an aluminum cursor stadiometer graduated in millimeters. The subject was barefoot, with heels, back, and head in contact with the stadiometer in horizontal plane. Body mass index (BMI) had been estimated from person's weight &height; it was calculated by dividing weight (in kilograms) by height (in square meters).

BMI≤18.5 (Underweight).

BMI=18.5-24.9(Normal weight).

BMI=25-29.9(Over weight).

BMI≥ 30(Obese) [8].

Waist and hip circumferences (WC and HC, respectively) were measured with a tape measure to the nearest 0.5 cm. The waist-to-hip ratio (WHR) was calculated by dividing waist measurement (in centimeters) by hip measurement (in centimeters). The cutoff points of risk for WHR were ≥0.8 for women and ≥1.0 for men; WC cut-off points were ≥102 cm for men and ≥88 cm for women [9].

Statistical analysis

Since the type of data collected were continues type, therefore the statistical tests used were Linear Correlation test and linear regression test the main reasons for selecting these two tests are the data were normally distributed and this linear correlation test will help us to detect the type of correlation depending on (*r*) (*r* value range +1 to −1) whether it is positive or negative correlation but these results will mean nothing only when *P* value < 0.05 (significant result). While Student *t*-test was used to find if there is a differences between the measured IL-8 values and the control values, significance was set at $P < 0.05$. Sampling method used for this study was convenience sampling method which is a type of non-probability sampling which involves the sample being drawn from that part of the population which is close to hand. That is, a sample population selected because it is readily available, convenient and within inclusion criteria. It may be through meeting the person [10]. The results were expressed as mean ± standard deviations (SDs).

RESULTS

Clinical characteristics of asthmatic patients are summarized in Table 1. The mean values for age, weight, height, WHR and BMI did not show any difference between the groups of asthmatic patients and healthy control since all *P* values > 0.05. The result of student *t* test show IL-8 level for the total asthmatic patients is significantly higher than the healthy control .While for lipid profile the results do not show significant difference between asthmatic patients and control, only VLDL show significant difference.

In Table 2 the results show that insignificant correlation between IL-8 and all lipid profile since (P>0.05).

Association and correlation of asthmatic patients IL-8 with BMI will be shown in Table 3. According to the results which are shown in this table we notice that normal weight, overweight and obese have significant correlation with IL-8 level in asthmatic patients since (P<0.05) and obesity show the highest one while in underweight there is insignificant correlation since (P>0.05).

TABLE 1:-ASTHMATIC PATIENTS AND HEALTHY CONTROL VARIABLES.

DEMOGRAPHIC DATA			
VARIABLES	**ASTHMATIC PATIENTS**	**CONTROL**	***P* VALUE***
n	38	20	
Gender: male/female	22/12	10/10	
Age (years)	52.7±14.4	50.4±12.7	0.274
Weight (kg)	75.94± 21.53	73.7±19.83	0.649
High (cm)	158.92±9.78	159.77±9.48	0.359
Waist (cm)	107.29±19.74	106.73±17.45	0.802
Waist-Hip ratio (WHR)	0.97±0.14	0.95±0.13	0.074
BMI (km/m^2)	30.48±8.89	28.79±6.92	0.182
Lipid Profile			
HDL	64.22±13.22	70.19±11.37	0.334
LDL	138.29±14.37	98.22±14.02	0.089
TG	165.05±14.06	92.51±15.73	0.162
TC	212.59±11.92	159.48±13.29	0.115
VLDL	33.01±12.16	18.50±13.81	0.059
IL-8 total (pg/ml)	52.46.25±9.67	27.33±7.20	0.027

***t* test is used**

TABLE 2:-ASSOCIATION AND CORRELATION OF ASTHMATIC PATIENTS IL-8 WITH LIPID PROFIL.

Lipid profile	**Correlation***	***P* value****
HDL	0.44	0.211
LDL	0.29	0.156
TG	0.11	0.732
TC	0.58	0.398
VLDL	0.32	0.173

*** Linear correlation, ** Linear regression**

TABLE 3:-ASSOCIATION AND CORRELATION OF ASTHMATIC PATIENTS IL-8 WITH BMI.

BMI	**Correlation***	***P* value****
Underweight	0.26	0. 181
Normal weight	0.31	0.046
Overweight	0.79	0.022
Obese	0.85	0.000

*** Linear correlation, ** Linear regression**

DISCUSSION

The aim of this study was to investigate serum IL-8 levels and its relationship with the main risk factors which play role in its elevation within Iraqi asthmatic patients. The results of student *t* - test show that there is a significant increase in IL-8 level among asthmatic patients than healthy control in spite of no difference in age, weight, high, waist-hip ratio and BMI levels .

IL-8 might participate in development of airway inflammation and airway hyper responsiveness in asthma [11].The reason of increases IL-8 level may be due to the association of IL-8 in neutrophil recruitment with lung injury. Recruitment of inflammatory cells in response to tissue injury is a normal physiological response to fight infection, remove damaged or dead cells and stimulate healing; however, excessive recruitment of these cells results in tissue damage and slows the rate of healing. In a number of pulmonary disorders, including chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), asthma, pulmonary fibrosis and bacterial pneumonia, IL-8 appears to be important for the recruitment of neutrophils and T cells into the lung. The accepted mechanism for neutrophil extravasations initially involves an E-selectin-mediated rolling interaction between neutrophils and the vascular endothelium. Subsequently, neutrophils are stimulated by inflammatory mediators, such as IL-8, which may be synthesized by endothelial cells through a process involving tumor necrosis factor α (TNFα-induced activation of p38 mitogen-activated protein kinase (MAPK), resulting in integrin activation. Firm adhesion is thus established through integrin–endothelium interaction, followed by migration through the endothelial lining [2].

Since IL-8 is one of the most potent chemoattractantand activators of neutrophils and neutrophils can release substantial quantity of IL-8, they may form a positive feedback circle , in which IL-8 induce neutrophil accumulation and release of IL-8, and then released IL-8 cause further accumulation of neutrophils [4].

Excessive release of IL-8, which is confirmed in the present study consistent with what was observed in numerous previous studies [11,12,13]. IL-8 was elevated in patients with cystic fibrosis and asthma .They hypothesized that IL-8 acts directly on airway smooth muscle cells in a way that may contribute to the enhanced airway responsiveness and any way remodeling observed in cystic fibrosis and asthma [14].

In agreement with other report [15] we observed that the levels of lipid profile were insignificantly correlated to IL-8 level since (P>0.05), (Table 2). Possible explanation for this is the limited sample size of patients. As it's well known, HDL cholesterol is a strong predictor of cardiovascular disease, and the chemokines may be implicated in the development of vascular complications such as dyslipidemia.

In this study we also found IL-8 is increased in proportion to BMI and this because in the obese state, adipose tissue is a metabolically active tissue consisting of adipocytes produces multiple proinflammatory cytokines, including tumor necrosis factor- α (TNF-α), interleukin-1 b (IL-1 b), IL-6, and IL-8, and it is believed they may have systemic effects mediating many of the other medical complications of obesity. Thus, our data suggests that obesity represents a chronic inflammatory state [7,15].

Our findings support previous studies that showed the association of elevated serum levels of IL-8 with obesity-related diseases, including diabetes, atherosclerosis and impaired glucose tolerance, suggesting that IL-8 may play an important role in the pathogenesis of these diseases by causing inflammation and tissue injury [16,17],and are inverse to those found in other literature which showed there is no correlation between IL-8 and BMI in asthmatic patients [18].

In conclusion, the circulating levels of chemokine like IL-8 were significantly higher in asthmatic patients compared with healthy control. The levels ofIL-8 are correlated to the obesity related parameters such as BMI, lipid profile. Taken together, we suggest that the high circulating levels of IL-8 may play an important role in the pathogenesis of obesity related disease like asthma. Future studies are needed to understand how obesity may alter the inflammatory response in patients with asthma. These investigations should include both animal and human studies comparing neutrophil trafficking, cell signaling, and differences in innate and adaptive immune responses in asthmatic patients who are obese and of normal weight.

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