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RESEARCH ARTICLE

Association of Plasma Neutrophil Elastase and Interleukin 1 Beta Levels with Metabolic Syndrome in Obese Premenopausal Women

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Abstract

Introduction: Metabolic syndrome (MS) is the state of chronic low grade inflammation. Plasma neutrophil elastase (NE) might be a critical marker associated with several choric diseases. However, relation of NE, interleukin 1 beta (IL-1ß) and MS and its complications in Egyptian obese women has not been yet investigated. The aim of this study is to determine the relationship between levels of NE, IL-1ß with MS and metabolic components in obese premenopausal women. Methods: This cross-sectional study was conducted on 150 obese women with MS and 150 non obese healthy women matched in age. Inflammatory markers including NE and IL-1ß were measured by ELISA. Blood pressure (BP), blood glucose, lipid profile and insulin sensitivity were studied. Insulin resistance was assessed by the homeostasis assessment model (HOMAIR) and insulin sensitivity by quantitative insulin sensitivity. Body fat % was assessed by Body composition analyzer. Results: MS patients showed significant higher levels of NE, IL-1ß, fasting insulin, glucose and HOMA-IR and markers of serum lipid parameters (increase of triglycerides, low density lipoprotein, total cholesterol and decrease of high density lipoprotein), elevated levels of SBP and DBP than non obese controls. In addition, significant positive correlations were observed between NE and metabolic components of MS. Partial correlations revealed significant positive relation between NE, IL-1ß and HOMAIR, body fat % in obese MS cases after adjustment of BMI and age. Conclusion: NE and IL-1ß and body fat % are elevated in obese women with MS, suggesting their critical role in MS complications in obese Egyptian women and emphasized that these biomarkers might be used as good indicators for severity of the disease.

Keywords: *Metabolic syndrome, Interleukin 1 beta, Neutrophil elastase, Insulin sensitivity.*

Introduction

Adipose tissue rearrangement occurs in obesity, with increase in size of fat cells, augmented macrophages invasion that change into proinflammatory pattern too. Substances produced by macrophages change fat cell role, including; suppressing adipose tissue synthesis, triggering inflammatory reply thus decreasing insulin sensitivity [1]. IL-1b, an important cytokine secreted macrophages, and involved in insulin resistance enhancement linked to obesity [1].

IL-1b disturbs adipose tissue sensitivity to insulin by insulin signal down regulation. Therefore, stopping the action of IL-1b, its receptor attaching and formation makes signaling of insulin better inside human fat cells.

That occurs in concordance with pro-inflammatory profile and lipolysis decrease triggered by macrophage. Thus, IL-1b is essential to protect from insulin resistance associated obesity. Although, IL-1b is produced by adipose tissue, it is mainly secreted by non adipose cells, its production is increased in obesity [2, 3]. A new research showed;IL-1b of human fat cells in minimal dose (2 ng/ml) suppressed insulin signal production via decreasing the expression of glucose transporter (GLUT4) proteins [4].

It was shown before that production of matrix metalloproteinase 1 and 3 by preadipocy test imulated by macrophage is helped by IL-1b [5].

These data propose that IL-1b might play a cornerstone part at macrophage-adipocyte pathway that stops insulin effect on human adipose tissue. Neutrophils produce NE that digests extracellular matrix. It has been shown that high NE level/activity coexisted with many diseases including chronic obstructive pulmonary disease, type2 diabetes mellitus and atherosclerosis [6]. NE elevation leads to inflammation of adipose tissue and insulin resistance [7, 8]. Clarifying the association between inflammatory markers and MS might lead to new therapeutic strategies and identification a predictive marker for its prognosis.

Materials and Methods

Study Population

Sample size: This study is a cross sectional study. Sample size was calculated based on the estimated prevalence of the disorder, population size and the confidence level was 1.96, which corresponds to a 95% confidence interval. Sample size justification Based on the assumption of least expected prevalence of MS of 30% and highest expected prevalence of 57%, alpha error 1% and power of study 90%, the required sample size would be of 250 subjects. All statistical analyses were performed applying SPSS 20.0package for Windows (SPSS Inc., Chicago, IL).

Inclusion Criteria: The study included 25-35 years old, 150 premenopausal obese women with MS and 150 non-obese healthy controls. Obese women were patients at the obesity clinic at the National Research Centre (NRC], Egypt. Metabolic syndrome was defined as having three or more criteria according to the modified NCEP ATP III definition[9].

Exclusion Criteria: We excluded women with history of cardiovascular disease or diabetes, hypothyroidism, pregnancy or under any medication known to affect glucose levels, insulin secretion, or insulin sensitivity and smoking.

Ethical Approval

The research was approved by the Ethical Committee of NRC (No: 16361) and followed the World Medical Association's Declaration of Helsinki. Furthermore, each participant in the study signed a written consent after a full description of the study.

Anthropometric and Clinical Measurements

All patients and controls were subjected to full medical history and clinical examination. All anthropometric measurements were taken 3 times on the left side of the body and the mean of the 3 values was used. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with the patients standing with their backs leaning against a stadiometer scale.

BMI was calculated as weight in kilograms divided by height in meters square (kg/m^2). WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. Body fat % was assessed by Tanita Body Composition Analyzer (SC-330).

Biochemical Measurements

The blood samples were collected after an overnight fasting and stored at 80ºC until further analysis. Enzymatic colorimetric analysis was carried out using Hitachi auto-analyser 704 (Roche Diagnostics Switzerland). Fasting plasma glucose, serum insulin concentration and serum lipids have been measured as per protocol previously described by Zaki et al., 2016 [10].

Insulin resistance was estimated using homeostasis model assessment (HOMA-IR). Enzyme linked immunosorbent assay (ELISA) kits provided by Glory Science were used to measure serum IL-6 and NE.

Results

Table 1 revealed that MS patients had significantly higher levels of NE, IL-1ß, total cholesterol, triglyceride, low-density lipoprotein-cholesterol (LDL-L), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG) and HOMA-IR, compared to the control group.

Table 2 shows positive correlations between NE, clinical and metabolic parameters in obese MS women including BMI, body fat %, WC, FBG, HOMA-IR, lipid parameters and blood pressure levels. Table 3 shows partial correlation of NE, IL-1ß and HOMA-IR. After adjustment of BMI and age, significant positive correlations between these parameters were observed ($p<0.001$).

Table 1: Comparison of clinical and biochemical characteristics of the study groups

	MS	Controls
NE(ng/ml)	23.81± 9.38**	9.48 ± 2.42
FPG(mg/dL)	125.32 ± 12.54*	74.00 ± 8.124
TC (mg/dL)	226.74 ± 35.546*	174.18 ± 29.749
HDL-C(mg/dL)	40.15 ± 10.19*	48.09 ± 9.85
LDL-C(mg/dL)	190.48 ± 20.12*	116.18 ± 43.155
SBP(mmHg)	133.03 ± 14.22*	101.10 ± 9.11
DBP (mmHg)	89.37 ± 11.04*	67.50 ± 9.20
HOMA-IR	5.6 89 ± 1.97**	2.6 8 ± .98
IL-6 (pg/ml)	41.71± 22.81**	22.61±12.13
Body fat percentage	45.4±21.61**	25.4± 11.01**

*Significantly elevated as compared with healthy controls
*p < 0.05 ** p<0.001

Table 2: Correlations between serum NE and clinical and metabolic parameters in obese MS women

Adiposity indices	NE(ng/ml)	
	r	p
BMI (kg/m^2)	0.298	0.01
WC (cm)	0.26	0.02
FPG(mg/dL)	0.31	0.05
HOMA-IR	0.34	0.03
TC (mg/dL)	0.56	0.02
TG (mg/dL)	0.45	0.01
LDL-C(mg/dL)	0.55	0.02
HDL-C(mg/dL)	-0.34	0.03
SBP(mmHg)	0.36	0.02
DBP(mmHg)	0.48	0.01
Body fat percentage	0.49	0.04

Table 3: Partial correlation of NE, IL-1 β and HOMA-IR adjusted for BMI and age

Variables	R	P
HOMA-IR	0.737	0.001
IL-1ß	0.837	0.0001

Discussion

Obesity and its consequent insulin resistance is one of the main constituents of metabolic syndrome. It is well established that inflammation is the main underlying part of obesity and it plays a role in causing resistance to insulin [11]. Macrophage in adipose tissue has been known as the main cells leading to inflammation in obesity [12].

Adipose tissue shows early infiltration by Neutrophils after the initiation of obesity course induced by diet at mice [13] in addition to human obese cases [14]. The dramatic aggregation of neutrophils in adipose tissue was reported recently.

The increased production of neutrophil-specific protease; neutrophil elastase, has been confirmed also in the course of diet rich in lipids in the studied mice [15]. The association of insulin resistance or dyslipidaemia with inflammatory markers has been previously searched in obese or overweight middle-aged patients [8,11,16,17].

Moreover, it has been investigated in subjects susceptible to diabetes mellitus [18]; or metabolic syndrome cases [16, 17] in addition to cases with type 2 diabetes [19, 20]. However, the number of cases was small in these studies.

Obesity, inflammation, insulin resistance, and fatty liver is caused by disturbance in balance between an enzyme known as neutrophil elastase; which is secreted by neutrophils and its inhibitor. It is involved in immunity against bacteria[21].

Moreover, elastase was suggested by [22], as a non-specific biomarker of infection and inflammation. However, the relation of NE and interleukin 1 beta (IL-1ß) with MS in Egyptian premenopausal obese women has not been yet investigated. Therefore, we decided to conduct our study to detect the relation between neutrophil elastase, IL-1ßand metabolic syndrome in Egyptian obese premenopausal women. The current study revealed that serum neutrophil elastase level was significantly higher in obese premenopausal women than normal controls.

In a study done previously [6] there was significant elevation of elastase in serum of prehypertensive obese than obese females with average blood pressure and normal subjects. This is in agreement with the results of the current study. Moreover, our study showed that serum neutrophil elastase was correlated significantly with BMI, waist circumference, plasma insulin, plasma glucose, HOMA-IR, SBP, DBP and lipid profile in our cases.

There was also association between neutrophil elastase level and MS components. These findings also coincide with the previous results reporting similar results [6]. The present study results are parallel to those of previous study detected significant higher levels of elastin-derived protein (EDP) in obese children with high blood pressure and positive family history of hypertension with significant elevation in was increased significantly than in obese normotensive children and normal controls.

In addition, other studies reported significantly increased levels of elastin peptides in obese children with high blood pressure and children with diabetes than normal healthy children [23]. Other study [24]detected that plasma elastase level was significantly elevated in cases with obesity and diabetes than in non-obese normal controls.

In contrast other study [25] concluded that the levels of plasma elastase in obese subjects had no significant difference than those in non-obese normal individuals. Hypertension associated with obesity has been basically attributed to inflammation; which enhances insulin resistance, resulting in obesity and predisposing to diabetes, hypertension, and dyslipidemia [26]. Disturbance in plasma level of inflammatory markers including; CRPs, interleukin-6 and TNF-a is more encountered in subjects with high blood pressure than those with normal blood pressure [27, 28].

This is in agreement with the present study which detected significant positive correlation between NE and IL-1ß in obese MS cases. Interestingly, an important study was conducted on a large scale of 1,400 cases complaining of cardiovascular disease illustrated that the effect of serum elastase was positively correlated with body mass index, plasma level of glucose, while negatively correlated with triglyceride level of [29].

Also previous studies [30, 31] reported positive correlations between elastase level and BMI, systolic and diastolic blood pressure. In addition, other study [6] detected that serum elastase level was significantly negatively correlated with HDL, again this is in agreement with our results.

Our results agree with other studies [32] observed significant negative correlations between HDL, HDL2-c and elastase inhibitory capacity in atherosclerosis patients and the control group. Furthermore, the activity of elastase type and inhibitory capacity of elastase have been detected in atherosclerotic cases sera, who complain of ischemic cardiovascular disease and the control group [33].

It has been reported that, LDL and HDL, had an effect on release of NE from neutrophils that exerts proteolytic action. Previous studies [34] showed also a significant positive correlation between neutrophil elastase and CRP as an inflammatory marker in obese females with prehypertension. These results coincide with results reported previously [35] confirming the association of the activity of serum elastase with fibrinogen and CRP after follow-up of 859 cases aged (59-71) years for 4 years. Consistent with these findings, our study showed significant positive correlation between NE and IL-1ß as an inflammatory marker in obese MS cases.

Previously, extracellular neutrophil elastase was detected in the intracellular space; accompanied with insulin receptor substrate-1 (IRS-1) breakdown [36]. This could be the mechanism which explains that neutrophil elastase might enhance insulin resistance and support our results[37]. Another mechanism might be that, the NE can influence insulin sensitivity through elevating serum high molecular weight adiponectin which stimulates the liver AMPK pathway [38].

Furthermore, coinciding with our results; another study [39] showed significantly higher NE levels. They demonstrated an elevation of NE which was strongly associated with glucose level, insulin resistance and lipid profile. However, on the contrary to the results of our study, their study showed no correlation between NE-A1AT and high blood pressure. The above discussion demonstrates obviously high neutrophil elastase serum level in obese females which can reflect a condition of inflammation could be used as a significant participant of MS pathogenesis. Conclusion: NE and IL-1ß are elevated in obese women with MS and associated with its complications. Therefore, it can be concluded that these biomarkers might be good indicators for the severity of disease.

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