Osteopontin, Malondialdehyde and Interleukin-1β Levels in Patients with Insulin Resistance and Dyslipidemia in Obese Egyptian Women

Moushira Zaki¹, Sanaa Kamal¹, Hala El-Bassyouni², Mona Abd Elmotaleb A. Hussein³, Hend M. Tawfeek⁴, Mina Wassef Girgis⁵, Nadia Mohamed Ahmed⁶, Hisham A. Aziz Orban⁶ and Eman R. Youness⁶*

¹Biological Anthropology Department, National Research Centre, Giza, Egypt.  
²Clinical Genetics Department, National Research Centre, Cairo, Egypt.  
³Internal Medicine National Institute of Diabetes and Endocrinology, Cairo, Egypt.  
⁴Internal Medicine Faculty of Medicine, Al-Azhar University- Girls.  
⁵Medical Department, Medical Research Division, National Research Centre, Giza, Egypt.  
⁶Medical Biochemistry Department, Medical Research Division, National Research Centre, Giza, Egypt.  
*Corresponding Author E-mail: hoctober2000@yahoo.com

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Osteopontin (OPN) is a pro-inflammatory cytokine implicated in immune processes regulation and mediating chronic inflammation. Interleukin-1β (IL-1β) is the key mediator of the inflammatory response and malondialdehyde (MDA) reflects oxidative stress status. Dyslipidemia may contribute to the increase in serum MDA. Chronic inflammation plays a principal role in the development of insulin resistance (IR). Our goal was to investigate the correlation between circulating OPN, IL-1β and MDA in obese women with IR and dyslipidemia subgroup. This interaction has not yet been studied in Egyptian obese subjects. The study included 200 obese women with IR and 100 healthy controls. Patients with IR were divided into two subgroups according to presence of dyslipidemia. OPN and IL-1β were measured using an enzyme-linked immunosorbent commercial assay. MDA was assessed spectrophotometrically. The anthropometric measurements were assessed for each participant. OPN, total cholesterol, LDL-C, fasting glucose, MDA and IL-1β were significantly increased in obese women with IR in comparison with control group. The dyslipidemic group presented significant higher levels of serum lipids, OPN MDA and waist circumference compared to the non-dyslipidemic group. This study highlights the increase of serum OPN, MDA and IL-1β levels in obese cases with IR. OPN might be important biomarker for IR and for the early diagnosis of dyslipidemia in IR patients. Therefore, the pharmacological inhibition of OPN levels might be a novel approach for the treatment of these cases.

Keywords: Interleukin-1β; Insulin Resistance; Malondialdehyde; Obesity; Osteopontin.

OPN is a pro-inflammatory cytokine that acts by the modulation of immune cell response. It triggers several chronic inflammatory diseases and might play a critical role in the insulin resistance development and inflammation of adipose tissue1. OPN is considered a paradigm of the intricate inflammatory processes underlying metabolic syndrome2. OPN is dramatically augmented...
in visceral adipose tissue in obesity and its insufficiency protects against the development of inflammation and insulin resistance. Expression of OPN in human macrophages is upregulated via a diversity of pro-inflammatory mediators, comprising IL-6, oxidized LDL, and TNF-α, recognized to be raised in obesity, cardiovascular disease and type 2 diabetes. OPN increases the risk of atherosclerosis by increasing endothelial cell migration and the inflammatory processes associated with coronary artery disease. OPN is also expressed and released from Kupffer cells into the stellate cells, circulation, hepatocytes, and macrophages in non-alcoholic fatty-liver disease. Thereby, it may also partake to the augmented risk of cardio-metabolic diseases observed in non-alcoholic fatty-liver disease. Consistently, redundant OPN is correlated with increased systolic dysfunction and left ventricular stiffness in patients with heart failure and hypertensive heart disease. Also OPN is highly expressed in human atherosclerotic plaques. Decisively, deficiency of OPN not only cause reduced adipose tissue inflammation, but also amended whole-body glucose tolerance and diminished insulin resistance. Therefore, the pharmacological repression of OPN expression might be a novel approach for the treatment of type 2 diabetes and combat obesity. Moreover, OPN is a pro-fibrotic molecule, whose expression is upregulated by interleukin (IL-1β) through the liberation of its downstream cytokines. It has been reported that OPN levels in serum are in correlation with obese state and may be reduced by loss of fat mass. Interleukin (IL-1β), an inflammation-associated cytokine, is generally secreted from epithelial cells and macrophages. Dyslipidemia is the most common metabolic abnormality in obese cases. Antioxidant defenses impairment resulting in augmented oxidative stress (OS) contribute to the progression and development of lipid complexity. Some studies reporting augmented levels of plasma MDA as an indicator of raised lipid destruction. Although several previous studies have shown higher OPN and MDA levels in obese cases with IR, some others showed the opposite. However, the pathophysiological role of these biomarkers is not completely explicated in various complications in relation to obesity.

The aim was to evaluate OPN concentrations in serum and assess the correlations between IL-1β, MDA in obese and normal weight women and in IR cases with and without dyslipidemia.

**Patients and Methods**

The processes altogether utilized in the current work were in agreement with the rules of the Helsinki Declaration on Human Experimentations. This work was approved by the National Research Centre ethical committee (No: 16361); tenacity of the protocol was elucidated to the women, and informed written consent was gotten prior starting the research. The current case-control study included 200 unrelated women with IR and 100 age matched healthy controls, their age ranged between 25 and 40 years. IR group was divided into two subgroups: dyslipidemic and non-dyslipidemic. IR cases were collected from obesity clinics of the National Research Centre, Egypt when visiting clinics for metabolic evaluation. The exclusion criteria for the study were as follows: (i) patients with type 1 diabetes, gestational diabetes, or other specific types of diabetes; (ii) patients with renal dysfunction, thyroid dysfunction infectious diseases, or other endocrine disorders, severe cardiovascular diseases any other disease which may alter insulin resistance and BMI.

The inclusion criteria for participants were obese women with IR, aged 25-40 years, attending obesity Clinic of National Research Centre, Egypt.

The control group consisted of normal weight healthy women who were not using any medications.

**Blood Lipids and HOMA-IR Measurements**

Blood samples were collected after an overnight fast (minimum 12 h). Fasting serum lipids (total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and plasma glucose were evaluated by enzymatic colorimetric methods. Low density lipoprotein cholesterol (LDL-C) was calculated via the equation (LDL-C= Total cholesterol – Triglycerides/5+HDL-C). Participants were considered with dyslipidemia if have elevations of LDL-C, triglycerides, non-HDL-C, and decreases in plasma HDL-C.

Insulin concentration in serum was examined by chemiluminescent immunoassay (Immuli2000, Siemens, Germany). Insulin resistance was determined by (HOMA-IR) that is calculated as the product of the fasting plasma
insulin level (IU/mL) and the fasting plasma glucose level (mg/dl), divided by 22.511. A clinical history and physical examination was done for all patients. The cut-off level of HOMA-IR was >3.0 to specify insulin resistance.

Dyslipidemia was considered in women with high levels of low density lipoprotein cholesterol (LDL-C) (=130 mg/dl), total cholesterol (=200 mg/dl), and triglycerides (TG) (=150 mg/dl), or low levels of high density lipoprotein cholesterol (HDL-C) <50 according to Friedewald equation.

MDA, the end product of lipid peroxidation was evaluated spectrophotometrically as thiobarbituric acid reactive substances (TBARS).

**Measurements of Serum OPN and IL-1β**

Serum OPN and IL-1β were measured by the ELISA (enzyme-linked immunosorbent assay) method using an ELX-800 system (RayBiotech, Norcross, GA, USA).

**Anthropometry and Blood Pressure**

The anthropometric measurements and tools followed the International Biological Program (IBP). Fat mass was measured by Tanita body composition analyzer SC–330.

**Statistical Analysis**

Data were expressed as the means and standard deviations.

The statistical analysis was done with SPSS 16.0 for Windows (SPSS Inc). The P<0.05 was considered statistically significant. The differences between the two groups were evaluated with an unpaired t test or the Mann–Whitney U test, as appropriate.

**RESULTS**

Table 1 shows the anthropometric and metabolic features of the IR and control groups. No statistically significant differences in the mean age, TG and HDL between cases and controls. Subjects of the IR group had higher BMI, fat mass, WC, serum LDL, total cholesterol, IL-1β, glucose, HOMA-IR, MDA and OPN than those in the control group.

Table 2 shows comparison of anthropometric, biochemical and metabolic parameters between non-dyslipidemic and dyslipidemic groups in patients with IR. WC, OPN, HOMA-IR, total-cholesterol, LDL, and IL-1β were higher in dyslipidemic patients than non-dyslipidemic patients.

**DISCUSSION**

OPN has been explicate as a multifunctional protein that is upregulated in a diversity of chronic and acute inflammatory conditions. The chief role of OPN throughout inflammation is to activate different leucocytes eliciting a functional response and prompting cytokine secretion, in order to form the complete immune response. Overweight and obesity are
Table 2. Comparison of anthropometric, biochemical and metabolic parameters between non-dyslipidemic and the dyslipidemic sub groups of IR group

| Variable                | Non - Dyslipidemic subgroup | Dyslipidemic subgroup | P-value |
|-------------------------|-----------------------------|-----------------------|---------|
| Age (year)              | 37.09±7.73                  | 36.81±9.70            | 0.46    |
| BMI Kg/m²               | 31.29±6.91                  | 33.26±6.73            | 0.44    |
| Systole (mmHg)          | 107.23±17.21                | 106.06±14.12          | 0.52    |
| Diastole (mmHg)         | 76.92±10.32                 | 71.52±9.05            | 0.44    |
| Fat mass (Kg)           | 30.29±12.44                 | 32.73±13.35           | 0.65    |
| Waist circumference (cm)| 95.43±15.125                | 102.79±14.624         | < 0.001 |
| Serum Osteopontin (ng/mL) | 43.37±17.99                | 58.20±13.54          | < 0.001 |
| HOMA-IR                 | 3.88±1.99                   | 6.88±1.99             | < 0.001 |
| MDA(nmol/mL)            | 2.89±0.79                   | 4.57±1.79             | < 0.001 |
| Total-cholesterol(mg/dl)| 177.91±32.23                | 251.62±30.89          | < 0.001 |
| Triglycerides(mg/dl)    | 94.25±30.34                 | 120.22±32.92          | < 0.001 |
| HDL(mg/dl)              | 50.35±13.16                 | 47.46±11.79           | < 0.001 |
| LDL(mg/dl)              | 108.44±26.04                | 180.05±39.03          | < 0.001 |
| IL-1β(pg/ml)            | 23.63±8.99                  | 26.96±8.51            | < 0.001 |

risk factors for chronic diseases as type 2 diabetes, insulin resistance and cardiovascular diseases. The stimulus and cause of enduring inflammatory activation in obesity remain mainly unknown. OPN is an important regulator in inflammatory diseases. Several studies have highlighted the significance of the pro-inflammatory cytokine interleukin (IL-1β) in adipose tissue, predominantly in insulin resistance and obesity-associated inflammation. The correlation between high-sensitivity C-reactive Protein (hsCRP) and OPN was previously reported in some studies, confirming the significance relation of OPN with vascular inflammation.

In the current work, we delineated that concentrations of OPN were significantly augmented in individuals with obese IR. Previous study reported that the major source of OPN in murine and human genetic and diet-induced obesity were adipose tissue macrophages, and its expression was extremely upregulated by 40- and 80-folds in adipose tissue of genetically obese mice and diet-induced. Concentrations of OPN associate with body fat. OPN protein and mRNA expression elevated in adipose tissue in obese persons and in obesity-associated T2DM, however, moderate diet-induced weight loss was complemented by a significant decrease in the levels of OPN in plasma. T2DM and Obesity are phenotypes of metabolic syndrome and are related to low-grade chronic inflammation. The associations between and OPN and inflammatory markers has been previously reported. Therefore, these relations may participate, at least in part, to the development of obesity-associated T2DM.

Dyslipidemia secondary to IR is a idiosyncratic conclusion in T2D. Dyslipidemia is the greatest mutual metabolic abnormality in obese individuals, although its type and extent is variable. Furthermore, dyslipidemia is characterized by significantly lower HDL, a lipoprotein that is thought to be the powerful metabolic predictor of coronary heart disease. Inflammation is obviously involved in the initiation and progression of atherosclerosis and there is a close association between oxidative stress and chronic inflammation in the atherosclerotic pathological process.

In the present study we observed elevated MDA levels in obese patients with IR. Few authors suggested that insulin resistance correlates with obesity. Oxidative stress is increased in obese subjects. Previous studies have proposed that oxidative stress may play an imperative role in the development and pathogenesis of obesity-related co-morbidities and the close relationship between the development of insulin resistance and oxidative stress and atherosclerosis. Previous studies reported that serum MDA levels were higher in cases with hyperlipidemia and in animal.
models\textsuperscript{10}. The increase of MDA levels could be imputed to augmented reactive oxygen species production and/or lack of antioxidant defense system.

In conclusion, our findings confirmed the correlation of serum OPN levels with insulin resistance and dyslipidemia. Additionally, elevated levels of OPN, MAD and IL-16 in IR patients indicated that altered lipid metabolism might be associated with oxidative stress and the chronic inflammation in obese women.

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