Endothelial dysfunction in obese females with and without polycystic ovary syndrome: role of vascular endothelial growth factor

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Abstract: Background and Objective: Obesity is the key determinant of insulin resistance. Hyperinsulinemia plays a central role in the pathogenesis of both metabolic syndrome and polycystic ovary syndrome (PCOS). Adipose tissue expresses and releases some bioactive molecules which may have a potential role in the development of obesity associated metabolic disorders and cardiovascular diseases. Vascular endothelial growth factor (VEGF) is an important angiogenic paracrine factor secreted from adipose tissue and plays a fundamental role in pathological neovascularization that observed in atherosclerosis. Nitric oxide (NO) is a cell signaling molecule that plays important role in regulating and increasing arterial blood flow and any endothelial dysfunction that impairs its secretion may be a key risk factor for the development of micro- and macrovascular diseases. In this study we aimed to evaluate the serum concentrations of VEGF and NO in obese females with and without PCOS and to explore the relations between both of them and different components of the metabolic syndrome in such patients. **Subjects and methods:** Forty female patients were included in this study. They were categorized into 3 groups; group (1): comprised 18 obese non PCOS females; group (2): comprised 12 obese females having PCOS, group (3): comprised 10 non obese females having PCOS, in addition, to 10 normal-weight healthy females as a control group. Serum VEGF, NO, fasting insulin, free testosterone, blood glucose, lipid profile were measured in addition to different clinical and anthropometric parameters. **Results:** Serum VEGF concentrations were significantly higher in obese females with and without PCOS compared to non obese PCOS Patients (P=<0.02, <0.01, respectively). Serum NO concentrations were significant1y lower in obese females with and without PCOS compared to non obese PCOS patients (P<0.001, <0.001 respectively). Serum free testosterone concentrations were significantly higher in obese and non obese females having PCOS compared to obese females without PCOS (P=<0.01, <0.05 respectively). Serum VEGF concentrations were positively correlated with BMI (r=0.4, P=<0.05), waist circumference (r=0.7, P=<0.001), fasting insulin (r=0.5, P=0.01), HOMA-IR (r=0.6, P=<0.001) and cholesterol (r=0.45, P=0.02). While, serum NO concentrations were negatively correlated with BMI (r=-0.7, P=<0.001), waist circumference (r=-0.4, P=<0.05), VEGF (r=-0.38, P=0.05), cholesterol (r=-0.5, p = <0.01) and LDL-C (r=-0.4, P < 0.05) and positively correlated with HDL-C (r=0.6, P=0.001). Serum free testosterone concentrations were positively correlated with waist circumference (r=0.42, p=<0.05) and HOMA-IR (r=0.48, P = < 0.05). Mean blood pressure, fasting glucose, fasting insulin, cholesterol, triglycerides, and LDL-C were significantly higher, while HDL-C was significantly lower in obese females with PCOS compared to non obese females with PCOS (P=< 0.01,<0.001,<0.01,< 0.01,<0.001,< 0.01, <0.05 respectively). **Conclusion:** It can be concluded that, obesity is associated with increased level of serum VEGF and decrease level of serum NO, and there is strong correlation between their concentrations and some components of the metabolic syndrome. In female patients with PCOS, the metabolic abnormalities (Hyperinsulinaemia, impaired glucose tolerance and dyslipidemia) that act as a cardiovascular risk are mainly due to the accompanying obesity, and increase serum level of testosterone in such patients is additional risk. Increased level of VEGF in obesity could induce endothelial dysfunction, impairing NO secretion and may have a potential role in progression of atherosclerosis and cardiovascular complications associated with obesity.
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Key words: Obesity, Polycystic ovary syndrome, Vascular endothelial growth factor, Nitric oxide, Metabolic syndrome.

Introduction:

Obesity is becoming a world wide problem and a major risk factor for developing type 2 diabetes mellitus (DM), cardiovascular diseases (CVD), dyslipidemia and shortening life expectancy [1,2]. Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting approximately 5-8% of reproductive aged women [3]. It is characterized by chronic anovulation and hyperandrogenism with variable clinical manifestations that include oilgomenorhea, infertility, hirsuitism and acne [4]. Obesity is the key determinant of insulin resistance which plays a central

role in the pathogenesis of both PCOS and metabolic syndrome [5,6] .

Adipose tissue expresses and releases various biologically active molecules such as leptin [7], TNF-α [8], plasminogen activator inhibitor-1(PAI-1) [9] and interleukin-6 [10].These molecules may have a potential role in the development of metabolic disorders and CVD resulting from obesity [11].

Vascular endothelial growth factor (VEGF) also, known as vascular permeability factor (VPF) is an important angiogenic paracrine factor secreted by adipose tissue [12] and serves a fundamental role in both physiological and pathological neovascularization [13] . The pathogenic neovascularization plays a major role in the development of atherosclerosis, tumour growth and various retinopathies [11,14] .

Insulin Increases the secretion of VEGF from adipocytes and such effect of insulin is a dose dependant and omental fat tissue has a relatively higher rate of VEGF secretion compared with other depots [12].

Nitric Oxide (NO) also known as the endothelium-derived relaxing factor is a cell signaling molecule that plays an important role in dilating blood vessels and increasing arterial blood flow and any endothelial dysfunction that impair its secretion may be a key risk factor for the development of micro-and macro-vascular diseases [15].

Aims of the study:

1- Evaluate serum level of VEGF (as an important angiogenic paracrin factor which may have a role in atherosclerosis) in obese non diabetic females with and without PCOS.

2- Determination of serum Nitric Oxide (NO) concentration as a good marker of vascular endothelial integrity in such patients.

3- To explore the relations between these two factors (VEGF & NO) and different components of the metabolic syndrome.

Subjects and Methods:

Forty female patients were included in this study. They were categorized into 3 groups; group (1): comprised 18 obese non PCOS females; group (2): comprised 12 obese females having PCOS, group (3): comprised 10 non obese females having PCOS. They were recruited from the attendants of Endocrinology Clinic at Specialized Medical Hospital of Mansoura University. Their ages ranging from 22 to 32 years, in addition to 10 normal weight healthy females of matched age as a control group. Written informed consents were taken from all subjects (patients and controls). All of them were subjected to through history taking, complete clinical examination with special stress on anthropometric measures. Each subject had height and weight recorded at the initial visit while wearing light clothes and no shoeses. Body mass index (BMI) is calculated as: weight (kg)/height (m^2).

While in the supine position waist circumference was measured at the level of the umblicus (at the highest point of iliac crest parallel to the floor) and hip circumference was measured at the level of symphysis pubis crossing the outermost part of greater trochanter. Blood pressure was measured three times after the patient was seated and at rest for a minimum of 5 minutes. The systolic and diastolic measurements reported represent the mean of the three readings. 12 leads resting ECG and pelvic ultrasound were done to all subjects. The diagnosis of PCOS was made when the patient meeting 2 of the following 3 criteria: (1) Oligomenorhea or anovulation, (2) Clinical and/or biochemical signs of hyperandrogenism as acne and hirsutism and (3) Polycystic ovaries by plevic ultrasound [16].

All participants were defined as non diabetic according to the WHO criteria for fasting glucose <126 mg/dl (7 mmol/L) and post glucose load level <200 mg/dl (<11.1 mmol/L). Patients with recent history of acute coronary syndrome or malignancy were excluded.

Sample collection:

After an overnight fast (12 hours) venous blood samples were withdrawn into plain tubes from every subject in the study. Clear non haemolyzed sera were separated into aliquots. One aliquot was used for estimation of fasting glucose and lipid profile using commercially available kits supplied by Human (Germany). LDL-C was calculated according to Friedewald Formula [17]. LDL-C = Total Cholesterol - (TG/5 + HDL-C).

The 2nd aliquot was kept frozen at -20°C for analysis of:

Fasting insulin by immunoenzymatic assay using Medgenix Ins ELISA supplied by Biosource (Belgium) [18]. HOMA was calculated by the formula of Matthews et al. (1985) [19]. HOMA INDEX= Fasting glucose (mmol/l) X Fasting insulin (uU/ml)/ 22.5

Serum nitric oxide was assayed by conversion of nitrate to nitrite using reductase enzyme, then total nitrite was determined as a colored azo-dye product of Griess reaction [20]. The material supplied by R and D system (USA). Serum VEGF was assayed using solid phase sandwich ELISA assay supplied by Biosource International Inc (Belgium) [21]. Serum free testosterone was assayed by competitive RIA using DSL 4900 Active Free Testosterone coated tube radioimmunoassay kit supplied by Diagnostic System Laboratories Inc (Texas) [22].

Statistical Methods:

Statistical analysis was done by using SPSS program "statistical package for social science' version 10, 1999. The data were parameteric by using Kolinogrov-Smirnov test. The parameteric data was presented in the form of mean, standard deviation and range. Student t test was used for comparison of quantitative data of two groups. Person correlation coefficient was done to study relation between items in each group. Significance was considered when P value equal to or less than 0.05. Insignificance was considered when P value more than 0.05.

Results:

Our study included 40 female patients (18 of them were obese, 12 of them were obese having PCOS and 10 non obese having PCOS). Their ages ranged from 22 to 32 years and their BMI values ranged from 24 - 43 kg/m² (Table 1).

Serum VEGF concentrations were significantly higher in obese females without PCOS and obese female having PCOS compared to non obese PCOS Patients (P= <0.02, <0.01 respectively) (Table 4). Serum VEGF concentration were significantly correlated with BMI and waist circumference P= <0.05, <0.001 respectively. Waist circumference was the most important determinate factor for serum VEGF in these variables (r = 0.7, P<0.001) (Table 5). These results indicate that the subjects with high visceral fat accumulation tend to have higher serum VEGF concentrations. Also, serum VEGF concentration had a significantly positive correlation with fasting insulin as well as with HOMA index P= <0.05, <0.00 1 respectively (Table 5).

Serum NO concentrations were significantly lower in obese females without PCOS and obese females having PCOS compared to non obese PCOS patients (P= <0.001, <0.001) (Table 4). Nitric oxide (NO) had significant negative correlation with VEGF, BMI, waist circumference, T-cholesterol and LDL-C (P = 0.05, <0.001, <0.01, <0.01, <0.05 respectively) and significant positive correlation with HDL-C (P <0.05) (Table 5).

Fasting insulin concentrations were significantly higher in obese patients with and without PCOS compared to non obese PCOS (P<0.01 and < 0.05 respectively). Fasting blood glucose was significantly higher in obese with PCOS when compared to obese non PCOS and non-obese PCOS females (P= <0.01, <0.001 respectively) (Table 2).

Obese women having PCOS had a trend toward a higher values for waist circumference and free testosterone compared to obese women without PCOS (P= <0.05, <0.01 respectively) (Tables 1, 4).

Mean blood pressure, fasting glucose. fasting insulin, T. cholesterol, triglycerides, LDL-C were significantly higher while HDL-C was significantly lower in obese females with PCOS compared to non obese females with PCOS (P= < 0.01 , <0.001, <0.01, < 0.01, <0.001, <0.01, <0.05 respectively) (Tables 1,2,3).

Table (1): Clinical characteristics of studied groups

	Age (Y) X±SD	Wt (Kg) X±SD	Waist (cm) X±SD	W/H X±SD	BMI (kg/m²) X±SD	MBP (mmhg) X±SD
Control (n=10)	24.1±7.0	70.5±7.3	75.9±4.8	0.7±0.1	22.5±2.1	95±6.6
Group (1) (n=18)	29.0±7.4	103.6±17.5	102.5±8.4	0.85±0.1	40.2±3.4	98±7.0
Group (2) (n=15)	25.7±3.7	96.3±11.4	111.6±14.9	0.9±0.2	38.0±4.2	104±6.8
Group (3) (n=10)	27.5±5.0	75.3±10.0	88.4±5.2	0.82±0.2	26.0±2.5	97±4.3
P1	>0.05	<0.001	<0.0001	<0.0001	<0.0001	>0.05
P2	>0.05	<0.001	<0.0001	<0.001	<0.0001	<0.05
P3	>0.05	>0.05	<0.001	<0.05	<0.05	>0.05
P4	>0.05	>0.05	<0.05	>0.05	>0.05	<0.01
P5	>0.05	<0.001	<0.0001	>0.05	<0.001	<0.01
P6	>0.05	<0.001	<0.001	>0.05	<0.0001	>0.05

P1, P2, P3 patient groups # control; P4 group 1#2; P5 group 2#3; P6 group 1#3.
Significant P :< 0.05

Table (2): Glycemic status of studied groups

Parameter / Group	Fasting glucose mmol/L	Fasting insulin uU/ml	HOMA-IR
Control (n=10)	4.5±0.3	18.7±3.7	3.7±2.1
Obese non PCOS (n=18)	5.3±0.6	28.3±8.3	6.6±2.4
Obese with PCOS (n=12)	5.9±0.5	30.4±5.4	7.9±3.2
PCOS non obese (n=10)	5.0±0.8	23.5±4.8	5.2±2.8
P1	<0.0001	<0.0001	<0.0001
P2	<0.0001	<0.0001	<0.0001
P3	0.05	<0.01	<0.05
P4	<0.001	>0.05	>0.05
P5	<0.0001	<0.01	<0.01
P6	>0.05	<0.05	>0.05

P1, P2, P3 patient groups # control; P4 group 1#2; P5 group 2#3; P6 group 1#3.
Significant P :< 0.05

Table (3): Statistical data of lipid profile in studied groups

	T.Cholesterol mg/dl	Triglycerides mg/dl	HDL-C mg/dl	LDL-C mg/dl
Control (n=10)	165±30.7	92±16.4	52±18	95.7±20
Group (1) (n=18)	220±32	213±39	38±12.6	137±25.4
Group (2) (n=12)	230±36	225±48	32±10.3	147±30.4
Group (3) (n=10)	186±25	113±22	48±11.4	115±20.6
P1	<0.0001	<0.0001	<0.05	<0.001
P2	<0.0001	<0.0001	<0.05	<0.001
P3	<0.05	<0.01	>0.05	<0.05
P4	>0.05	>0.05	>0.05	>0.05
P5	<0.01	<0.001	<0.05	<0.01
P6	<0.01	<0.001	0.05	<0.05

P1, P2, P3 patient groups # control; P4 group 1#2; P5 group 2#3; P6 group 1#3.
Significant P :< 0.05

Table (4): Statistical data of VEGF, nitric oxide and free testosterone in studied groups

	VEGF pg/ml	NO mmol/L	Free testosterone pg/ml
Control (n=10)	113±26.2	20.5±3.7	2.1±0.5
Group (1) (n=18)	176.5±36.7	10.8±2.9	2.4±0.8
Group (2) (n=12)	179.5±48.5	±	5.2±2.0
Group (3) (n=10)	144±29.4	17.6±4.2	4.0±1.6
P1	<0.0001	<0.0001	>0.05
P2	<0.0001	<0.0001	<0.0001
P3	<0.02	>0.05	<0.001
P4	>0.05	>0.05	<0.01
P5	<0.01	<0.001	>0.05
P6	<0.02	<0.001	<0.05

P1, P2, P3 patient groups # control; P4 group 1#2; P5 group 2#3; P6 group 1#3. Significant P :< 0.05

Table (5): Correlation coefficient between studied parameters in whole

Parameter	VEGF		NO		Free testosterone	
	r	p	r	p	r	p
Age	0.2	>0.05	0.6	>0.05	0.3	>0.05
BMI	0.4	<0.05	-0.7	<0.001	0.27	>0.05
Wt	0.5	<0.01	-0.5	<0.01	0.31	>0.05
Waist	0.7	<0.001	-0.4	<0.05	0.42	<0.05
MBP	0.19	>0.05	0.3	>0.05	0.11	>0.05
Fasting glucose	0.25	>0.05	0.25	>0.05	0.09	>0.05
Fasting insulin	0.5	0.01	0.21	>0.05	0.15	>0.05
HOMA-IR	0.6	0.001	0.23	>0.05	0.48	<0.05
T-cholesterol	0.45	0.02	-0.5	<0.01	0.2	>0.05
TG	0.1	>0.05	0.25	>0.05	0.17	>0.05
HDL-C	0.08	>0.05	0.6	0.001	0.28	>0.05
LDL-C	0.36	>0.05	-0.4	<0.05	0.13	>0.05
Nitric Oxide	-0.38	0.05	-	-	0.14	>0.05
Free testosterone	0.38	>0.05	-	-	-	-

Significant P: <0.05

Discussion

It has been reported that various cytokines such as TNF-α, IL-6, and PAI-1 are secreted from adipose tissue and their concentrations are closely related to the accumulation of intra-abdominal fat [8,9,10]. These cytokines and hyperinsulinaemia are though to play a key role in the development of obesity associated atherosclerosis [2,9,23,24]. VEGF is an angiogenic paracrine factor that is involved in pathological neovascularization that is observed in atherosclerosis [11,13]. Impaired NO secretion due to endothelial dysfunction may be a key risk factor for the development of micro and macro vascular diseases [15].

The present study investigated the concentrations of serum VEGF and nitric oxide (NO) in obese women with and without PCOS and non obese women having PCOS, as well as exploring the relations between their concentrations in one hand and the degree of obesity and different components of metabolic syndrome on the other hand.

In our study, we found that circulating VEGF is increased in obesity and its concentration is shown to be positively correlated to BMI and visceral fat accumulation. Waist circumference was the most important factor for determination of the serum VEGF concentration. The same finding has been reported by Miazawa et al., [11], which found that VEGF enhanced expression of matrix metalloproteinase-3 (MMP-3). Matrix degradation enhances the ability of migration as well as tube formation in endothelial cells and could induce pathological neovascularization that is observed in atherosclerosis. These results suggest that the increase in serum VEGF concentration associated with central adiposity could accelerate vascular disorders associated with obesity.

In the present study, although serum VEGF concentration was high in non obese with PCOS compared to controls, its concentration was much higher in obese with or without PCOS denoting that obesity plays the fundamental role in its elevation. Also, we found that VEGF level was positively correlated with fasting plasma insulin concentration and HOMA Index and these results has also been found by Miyazawa et al. [11].

The vasculo-protective effect of adiponection is mediated by up-regulation and increased production of nitric oxide (NO) [25]. Impaired endothelium dependant vasodilatation can be directly linked to the decreased synthesis of the endothelium dependant NO [15]. In the present study, we found that NO concentration were significantly lower in obese compared to non obese and there were a significant negative correlation between NO in one hand and VEGF, BMI and waist circumference in the other hand. The same finding has been reported by Koukoglu et al. (2005) and Nacul et al. (2007) [26,27], they found that plasma NO concentrations were lower in obese diabetic compared to non-obese diabetic.

Many obese patients with PCOS have developed the metabolic syndrome usually before the end of their third decade [5] and are at increased risk for coronary heart disease, impaired glucose tolerance (IGT) and type 2DM [28]. In our study, mean blood pressure, fasting glucose, fasting insulin, total cholesterol (T. cholesterol), triglycerides (TG) and LDL-C were significantly higher, while HDL-C was significantly lower in obese females having PCOS compared to non obese females with PCOS. Similarly, these results have been found by Morrison et al. [29]. In addition, it has been reported that long standing obesity is associated with carotid intimal thickening [30.31]. These findings

denoting that, obesity plays the fundamental role in metabolic changes and vascular complications associated with POCS [32].

In our study, free testosterone was much higher in women having PCOS compared to those without PCOS and was positively correlated with waist circumference and HOMA index. This finding has been reported by Morrison et al. [33]. Increased free testosterone was associated with hyperinsulinaemia and IGT components of the metabolic syndrome [34]. Also, it has been found that increased free testosterone in women having PCOS is an additional risk beside obesity and is an independent risk factor for aortic calcification [35,36].

In the present study, it can be concluded that: (1) Obesity is associated with increased level of serum VEGF and decrease level of serum NO, and there is strong correlation between both of them and some components of the metabolic syndrome. (2) In female patients with PCOS, the metabolic abnormalities (Hyperinsulinaemia, impaired glucose tolerance and dyslipidemia) that act as a cardiovascular risk are mainly due to the accompanying obesity, and increase serum level of testosterone in such patients is an additional risk. (3) Increased level of VEGF in obesity could induce endothelial dysfunction, impairing NO secretion and may have a potential role in progression of atherosclerosis and cardiovascular complications associated with obesity.

All efforts should be directed towards early management of obesity by diet regimen, intensive weight loss program with life style modification. This will lead to prevention of endothelial dysfunction, decrease the risk of CVD, decrease morbidity and improve quality of life.

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