Osteoprotegerin Expression and Serum Values in Obese Women with Type 2 Diabetes Mellitus

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Abstract

Obesity and diabetes prevalence are increasing worldwide. We aimed by this work to detect the possible association of osteoprotegerin (OPG) gene expression with visceral adiposity indices and cardiometabolic risk factors among obese patients. This is research enrolled 150 controls and 150 obese cases subdivided into two subgroups non-diabetic (n = 70) and 80 patients with type 2 diabetes mellitus (T2DM). Circulating OPG expression levels were determined by RT-PCR. Serum OPG concentrations were assessed by ELISA.Our results explored that OPG serum levels were higher in the control group compared to obese women and obese diabetics had higher serum levels of OPG in comparison to obese non-diabetic patients. In obese group, regarding expression levels of OPG were higher than controls in diabetic obese patients, the blood expression levels of OPG were higher than non-diabetics patients. We found positive correlations between parameters of MetS and obesity indices, among them, the highest positive correlation found between VAI. After adjustment of the traditional risk factors, stepwise linear regression analysis test revealed that, OPG expression levels were independently correlated with HbA1c, HDL-cholesterol, and WHR. ROC analysis demonstrated that cutoff values of OPG expression levels and serum levels were 2.226 and 7.5; the AUC were 0.833 (95% CI = 0.821–0.946) and 0.981, respectively. Additionally, the sensitivities and the specificities of OPG expression were (90% and 86.2%), and OPG serum levels (98% and %94), respectively.OPG mRNA and serum levels are useful diagnostic biomarkers discriminate metabolic risk among obesity with or without T2DM.

1. Introduction

The obesity pandemic is considered a major public health problem, as in this disorder is there's increased risk of medical comorbidity leading to a significant rise in death rate. Obesity is a common and a major risk factor for metabolic syndrome (MetS) [1, 2]. Obesity and Insulin resistance are key phenotype contributing to atherogenic and diabetogenic profiles [3–6].

The worldwide prevalence of obesity is rising in association with an increasing frequency of obesogenic factors in Egypt has the prevalence of obesity is increasing (22%) of adult men and (48%) of adult women [7]. This is a growing concern as obese subjects are at increased risk of developing diabetes, high blood pressure, dyslipidemia, in addition to coronary heart disease, stroke and many examples of cancers [8, 9].

A common argument against BMI as an anthropometric test for measurement of obesity is that BMI is not discriminating between the lean and fat body mass. Therefore, it is easily established that we indeed to consider other clinical tests for accurate assessment of obesity. To address this demand, significant research endeavors are being steered toward this target [10].

Notably, the recent obesity index; body adiposity index [BAI] [11], based on both hip circumference and height. Thus, it is to be expected that BAI overcomes the limitation of BMI [11]. Nonetheless, visceral

adiposity index (VAI), based on both anthropometric; BMI and WC) in combination with triacylglycerol levels [TAG] and high-density lipoprotein cholesterol [HDL-c] [12].

That is an enormously important clinical consideration that obesity is a condition of low-grade inflammation [13]. Noteworthy, increasing clues showed that low grade inflammation, which engages obesity leads to the continuous activation of the immune system [14]; with the leading of circulatory immune cells to a pro-inflammatory state [15].

Osteoprotegerin (OPG) is a glycoprotein cytokine which acts in cooperation with the receptor activator of receptor activator of nuclear factor κB ligand [RANKL] and tumor necrosis factor [TNF] -related apoptosis-inducing ligand. The OPG gene is approximately 29 kB of the genome on chromosome 8 and constitutes 5 exons [16]. Mounting evidence explored that OPG is expressed in vascular smooth muscle cells [VSMCs], osteoblasts and endothelial cells [ECs] [16].

Two incontrovertible conclusions can be derived that EC triggering by pro-inflammatory cytokines are one of the expected sources of circulating OPG in patients showing active atherosclerosis [17]. At that place were strong associations between OPG levels and ischemic heart disease, insulin resistance [IR], obesity, stroke as well as decompensated heart failure [18, 19–23].

More and more, modern pieces of knowledge into genetic basis of obesity have been plucked up from genome wide association [GWAs], but the pathological connection between obesity, metabolic syndrome, and inflammation is complicated and till now not clearly so, further studies are needed for better clarification of the bounds of this kinship. Regarding the investigation of OPG relative expression in obesity, according to our knowledge, it is the first study explored the association between OPG relative expression and obesity as well as cardio metabolic risk factors. But few studies have studied serum OPG levels in obesity. To address this need, the objective of the present work was to investigate the expression and serum levels of OPG in relation to new obesity indices [BAI and VAI] as well as cardio-metabolic risk factors among Egyptian obese patients.

2. Subjects And Methods

2.1. Subjects

This study included 300 irrelevant subjects. One hundred fifty obese females (BMI > 30) enlisted from diabetes and endocrinology outpatient clinic of Internal Medicine, Department of Zagazig University Hospitals and 150 healthy, lean controls, who were matched to cases by age, and cultural considerations.

Obese women were categorized into two subgroups according to their fasting blood glucose [FBG] based criteria reported by the American Diabetes Association [ADA] in 2015 into: non-diabetic patients [FBG < 126 mg/dl] ($n = 70$) and 80 cases with T2DM [FBG ≥ 126 mg/dl]. All individuals were submitted to careful history taking and complete clinical assessment including blood pressure, waist circumference [WC] and

ng BMI [kg/ meters ^{2}], WHR = waist

circumference (cm) /hip circumference (cm), and WHtR was calculated as the waist (cm) /height (cm). Moreover, VAI in women was calculated as follows: Its normallyhealthy, nonon healthy non-obese subjects with normal adipose distribution and normal levels of triacylglycerol [TAG] and high-density cholesterol [HDLc] [12]. Finally, BAI which is approximately equal to the percentage of body fat, It was calculated as $\frac{\text{Hipcircuferance (cm)}}{\text{height (m)}^{1.5}} - 18$ [11].

The MetS was diagnosed depending on International Diabetes Federation criteria. Patients diagnosed with the MetS if she has a WC (≥ 80 cm) with any two of the following risk factors: (a) FBG ≥ 100 mg/dl or previously diagnosed impaired fasting glucose (b) blood pressure [BP] ≥ 130/85 mmHg or treated for hypertension; (c), TAG ≥ 150 mg/dl; (d) HDLc < 50 mg/dl or taking medical treatment for low HDL cholesterol [LDLc].

Patients with cancer, stroke, or liver, kidney, thyroid, parathyroid, and cardiovascular or any active inflammatory diseases have been excluded from this study. None of the participants had history of bariatric or liposuction surgery, as well as receiving medications for weight reduction and osteoporosis for example strontium ranelate which may affect osteoprotegerin levels. No concurrent minor infection was detected during the study or in the month preceding the study. Ethical committee, Faculty of Human Medicine; Zagazig University approved this research protocol, and enrolled participants assigned informed written consent.

2.2. Biochemical analysis

A trained nurse collected the blood samples were at the outpatient clinic by after a 10–12 hour fasting period for measurement of serum TC, HDL-c, TAG, and the LDL–c level was determined using the Friedewald formula [24]. Glucose concentrations were estimated using the glucose oxidase method (Spinreact, Spain).

2.3. Measurement of serum osteoprotegerin concentration

Serum OPG concentration was quantified utilizing the enzyme-linked immunosorbent assays (ELISA) (RayBiotech, USA). Fasting serum insulin concentration was determined by human insulin ELISA kit (Biosource, Belgium). Homeostasis model were calculated Homeostasis model insulin resistance assessment [HOMAIR] and β-cell function [HOMA-β] were calculated.

2.5. RNA extraction and complementary DNA [Cdna]

Total RNA was extracted from the whole blood using QIAamp RNA [Qiagen; USA] according to the manufacturer's protocol. And so the extracted RNA had been reversely transcribed using QuantiTect [Qiagen; USA] according to the manufacturer's guides.

2.6. Estimation of OPG expression in blood by real time polymerase chain reaction (RT-PCR)

The OPG mRNA gene expression in blood was done by RT-PCR using StepOne™ system (Applied Biosystems). Primers of OPG were as follows: forward primer: 5'- TGCTGTTCCTACAAAGTTTACG-3'; reverse primer: 5'- CTTTGAGTGCTTTAGTGCGTG-3'; β-*Actin* forward primer: 5'-CGTGACATTAAGGAGAAGCTGTGC-3' and reverse primer: 5'-CTCAGGAGGAGCAATGATCTTGAT-3', as a housekeeping gene [25]. The PCR was done in a final volume (25 µl) containing 12.5 µl 2X QuantiFast SYBR Green PCR Master Mix, 1 µM of each primer and 7.5 µl cDNA with the this protocol: 95°C for 5 min, 40 cycles of denaturation at 95°C for 10 sec, annealing and extension at 56°C for 30 sec. OPG The expression was reported as the Delta cycle threshold (Δ Ct) value. The relative expression of mRNA was calculated utilizing the comparative CT method and OPG expression levels were normalized to β-Actin mRNA using the relative expression using the Δ CT method [26]. All kits were purchased from QIAGEN (Valencia, USA).

2.7. Statistical analysis

Descriptive statistics included means and SDs for continuous variables, and numbers and percentages for categorical variables. Group comparisons were performed by a $\chi 2$ test or analysis of variance as appropriate. Pearson correlation coefficient was used to assess the association between obesity indices and other studied metabolic parameters in obese women. Receiver operating characteristic (ROC) analysis was performed to assess the potential accuracy of OPG; the area under the curve (AUC), and the cutoff values for diagnosis of T2DM among obese patients. Stepwise multiple linear regression analyses were utilized to detect the main predictors of OPG mRNA and serum values in the obese group. Logistic regression analysis was performed to assess the predictor's powers of expression as well as serum levels of OPG in the prediction of T2DM among obese patients. SPSS V.21.0 (SPSS, USA) was applied for all analyses. P Value < 0.05 was considered statistically significant.

3. Results

3.1. Anthropometric and biochemical data of study groups

They were shown in **Table 1**. As expected obese patients had significantly higher values of SBP, DBP, FPG, HbA1c, fasting serum insulin, HOMA-IR, TC, LDL-c and TAG levels compared to lean controls. Moreover, all obesity indices and parameters (WC, BMI, WHR, WHtR, BAI and VAI) were significantly higher in obese women compared to leans. On the contrary, obese patients had significant lower levels of HOMA-β than in healthy lean individuals *(P* < 0.001).

Table 1: Anthropometric and biochemical characteristics of the studied groups.

	Lean healthy control (n = 150)	Obese patients (n = 150)	P value
Age (years) Sex (male/female)	44.58 ± 11.4 15/35	43.64 ± 8.2 42/108	0.15 0.93
Body mass index (kg/m^2)	21.4 ± 1.15	32.8 ± 13.95	< 0.001*
Waist circumference (cm)	91.4 ± 14.71	108.01 ± 13.8	< 0.001*
Waist/hip ratio	0.94 ± 0.013	1.05 ± 0.176	< 0.001*
Waist/height ratio	0.54 ± 0.09	0.64 ± 0.089	< 0.001*
Body adiposity index (BAI)	22.97 ± 8.7	35.86 ± 15.4	< 0.001*
Visceral adiposity index (VAI),	0.89 ± 0.14	5.92. ±1.34	< 0.001*
Systolic blood pressure (mmHg)	116.2 ± 4.79	134.5 ± 16.2	< 0.001*
Diastolic blood pressure (mmHg)	75.3 ± 4.21	84.03 ± 9.13	< 0.001*
Total cholesterol (mg/dl)	166.1 ± 7.85	221.2 ± 75.2	< 0.001*
Triglycerides (mg/dl)	129.5 ± 12.3	189.9 ± 34.6	< 0.001*
LDL cholesterol (mg/dl)	91.8 ± 0.98	135.3 ± 73.9	< 0.001*
HDL cholesterol (mg/dl)	48.4 ± 4.41	46.6 ± 8.95	0.178
Fasting blood glucose (mg/dl)	86.8 ± 5.08	147.9 ± 55.9	< 0.001*
Fasting serum insulin (μU/ml)	7.4 ± 1.82	13.18 ± 6.37	< 0.001*
HbA1c (%)	5.36 ± 0.41	7.56 ± 1.76	< 0.001*
HOMA-IR	1.57 ± 0.40	5.7 ± 4.14	< 0.001*
HOMA-B	118.6 ± 37.1	85.8 ± 27.9	< 0.001*

HOMA-IR: homeostasis model assessments of Insulin resistance; HOMA-β: an index of β-cell function; OPG: osteoprotegerin.* P < 0.05 when compared with control group.

3.2. Circulating OPG levels (pmol/L) and relative OPG expression levels in study groups

Regarding OPG serum levels, healthy lean individuals had significantly higher values compared to obese patients (6.56 ± 0.82 vs.5.06 ± 0.876). However, obese diabetics had higher serum values of OPG compared to those obese non-diabetic patients (5. 6 ± 0. 515 vs. 4.4 ± 0.739) (Fig. 1A). In obese group, the blood expression levels of OPG were significantly higher than control group (2.3 ± 0.732 vs. 1. 53 ± 0. 32).

Additionally, in diabetic obese patients, the blood expression levels of OPG were significantly higher than non-diabetics patients (2.83 ± 0.61 vs. 1. 53 ± 0. 32) (P < 0.001) (Fig. 1B).

3.3. General characteristics of obese patients stratified by FPG diabetic and non-diabetic obese patients

We found statistically significant higher values of FBG, serum insulin, HbA1c, HOMA-IR, SBP, DBP, LDL, TC levels, OPG expression levels, serum OPG level as well as VAI, BMI, WHtR and BAI in obese T2DM patients than in non-diabetic obese *(P* < 0.001). In contrast, obese patients had significantly lower levels of HOMA-B than in healthy lean individuals, *(P* < 0.001). On the other hand, there were no statistically significant differences between obese non-diabetic and T2DM patients as respect to other parameters *(P* > 0.05) (Table 2).

Table 2
Laboratory and anthropometric parameters in non-diabetic obese and T2DM obese groups.

	Obese non-diabetic patients (n = 70)	Obese diabetic patients (*n* = 80)	*P* value
Age (years)	42.3 ± 5.8	41.5 ± 8.02	0.49
Sex (male/female)	22/48	20/60	0.49
Body mass index (kg/m^2)	33.304.29	32.38 ± 3.6	0.103
Waist circumference (cm)	113.2 ± 15.35	103.8 ± 10.22	< 0.001*
Waist/hip ratio	1.02 ± 0.15	1.079 ± 0.19	0.050
Waist/height ratio	0.67 ± 0.09	0.6 ± 0.065	< 0.001*
Body adiposity index (BAI)	26.6 ± 4.4	43.9 ± 11.4	< 0.001*
Visceral adiposity index (VAI)	6.98 ± 1.28	8.74 ± 0.96	< 0.001*
Systolic blood pressure (mmHg)	119.1 ± 4.77	148.12 ± 8.39	< 0.001*
Diastolic blood pressure (mmHg)	77.2 ± 5.32	90 ± 7.46	< 0.001*
Total cholesterol (mg/dl)	245.9 ± 95.96	200.7 ± 41.55	< 0.001*
Triglycerides (mg/dl)	193.12 ± 46.7	187.9 ± 17.4	0.359
LDL cholesterol (mg/dl)	159.9 ± 93.7	114.6 ± 42.11	< 0.001*
HDL cholesterol (mg/dl)	47.4 ± 8.94	45.9 ± 9.02	0.324
Fasting blood glucose (mg/dl)	89.3 ± 12.37	199.1 ± 9.24	< 0.001*
Fasting serum insulin (μIU/ml)	7.78 ± 2.98	17.9 ± 4.46	< 0.001*
HbA1c (%)	5.77 ± 0.67	9.13 ± 0.3	< 0.001*
HOMA-IR	2.9 ± 0.45	5.9 ± 0.67	< 0.001*

HOMA-IR: homeostasis model assessments of Insulin resistance; HOMA-β: an index of β-cell
ared with obese non-diabetic patient's group.

	Obese non-diabetic patients (n = 70)	Obese diabetic patients (n = 80)	P value
HOMA-B	96.5 ± 10.03	60.4 ± 12.07	< 0.001*

HOMA-IR: homeostasis model assessments of Insulin resistance; HOMA-β: an index of β-cell functions. OPG: osteoprotegerin. * P < 0.05 when compared with obese non-diabetic patient's group.

3.4. Correlations between anthropometric measures with parameters of MetS in obese patients.

Our results demonstrated significant positive correlations between parameters of MetS including WC, SVP, low HDL as well as TAG with all anthropometric measures in obese cases (BMI, WHR, WHtR, BAI and VAI). Interestingly, among obesity indices, the highest positive correlation found between VAI and parameters of MetS (P < 0.001) (Table 3).

Table 3
Pearson correlation coefficient between anthropometric indices and parameters of metabolic syndrome among obese patients.

		Body mass index (kg/m^2)	Waist/hip ratio	Waist/height ratio	BAI	VAI
Waist circumference (cm)	*r*	0.747	0.121	0.983	0.125	0.228
	P	< 0.001*	0.139	< 0.001*	0.128	< 0.001*
Systolic blood pressure (mmHg)	*r*	0.255	0.125	0.235	0.527	0.326
	P	< 0.01*	0.062	0.004	< 0.001*	0.062
Diastolic blood pressure (mmHg)	*r*	0.140	0.122	0.103	0.139	0.471
	P	0.089	0.138	0.212	0.091	< 0.001*
Triglycerides (mg/dl)	*r*	0.833	0.389	0.425	0.230	0.804
	P	< 0.001*	< 0.001*	< 0.001*	0.01*	< 0.001*
HDL cholesterol (mg/dl)	*r*	-0.383	-0.356	-0.020	-0.383	-0.592
	P	< 0.001*	< 0.001*	0.811	< 0.001*	< 0.001*
Fasting blood glucose (mg/dl)	*r*	0.355	0.362	0.144	0.539	0.243
	P	< 0.001*	< 0.001*	0.080	< 0.001*	0.01*

BAI: body adiposity index; VAI: visceral adiposity index

3.5. Multiple stepwise linear regression analyses in obese patients to assess the main independent parameters associated with OPG expression levels

Stepwise linear regression analysis test revealed that, OPG expression levels were independently correlated with HbA1c, HDL-c, and WHR ($P < 0.001$) (Table 4).

Table 4
Multiple stepwise linear regression analysis to test the influence of the main independent variables on OPG expression levels (dependent variable) among obese patients.

		Model	Unstandardized Coefficients		Standardized Coefficients	t	P value	95.0% Confidence Interval for B	
			β	Std. Error	Beta			Lower Bound	Upper Bound
1	**Model 1**	(Constant)	0.304	0.194	---	1.567	0.119	− .079	0.688
		HbA1c (%)	0.277	0.025	0.672	11.031	< 0.001*	0.227	0.326
2	**Model 2**	(Constant)	1.156	.293	---	3.943	< 0.001*	0.577	1.735
		HbA1c (%)	0.290	.024	0.705	11.941	< 0.001*	0.242	0.338
		Waist/hip ratio	0.905	.241	0.222	3.759	< 0.001*	-1.381	− .429
3	**Model 3**	(Constant)	1.536	0.330	---	4.658	< 0.001*	0.884	2.188
		HbA1c (%)	0.291	0.024	0.706	12.156	< 0.001*	0.244	0.338
		Waist/hip ratio	0.651	0.260	0.160	2.506	0.03*	1.165	0.138
		HDL cholesterol	− .014	0.006	-0.150	-2.382	0.02*	− .025	-0.002

3.6. Logistic regression analysis evaluating the association of OPG expression and serum values with T2DM *among obese patients*

After adjusting for the traditional risk factors, the logistic regression analysis test was done to evaluate the predictors of obesity among studies subjects. Serum OPG levels were statistically significant predictor of obesity ($P < 0.001$) with odds ratio was 10.668, 95% CI = 3.349–33.985. Regarding OPG expression, the odds ratio was 7.57, 95% CI = 2.894–19.081 (Table 5). Among anthropometric measurements the only tested measures were WHR ratio with odds ratio was 0 .003, 95% CI = 0.007–0.089

Table 5
Logistic regression analyses of OPG expression and serum OPG levels as well as anthropometric measures in obese versus lean subjects

	B	S.E.	t	P value	Odds ratio	95% C.I.	
						Lower	Upper
OPG (pmol/L)	2.367	.591	16.036	< 0.001	10.668	3.349	33.985
OPG expression	2.024	.491	17.020	< 0.001	7.570	2.894	19.803
Waist/height ratio	-8.668	3.191	7.379	0.007	0.003	.007	0.089
Waist/hip ratio	2.442	1.888	1.672	0.196	11.495	.284	465.360

3.7. Accuracy of OPG expression and serum levels *for discriminating obese from lean control by ROC analyses*

We further looked into the possible diagnostic value of OPG expression and OPG serum levels by ROC curves (Fig. 2A & B, respectively). For discrimination of obese among from lean subjects, the cutoff values of OPG expression levels and serum levels were 1.943 and 5.883 and the AUC were [0.859 (95% CI = 0.806–0.911) and 0.901 (95% CI = 0 .841-0.962), respectively, P < 0.001]. Additionally, the sensitivities and the specificities of OPG expression levels were (92% and 70.7%), and OPG serum level (90% and %99. 7), respectively. Thus, OPG expression and serum OPG levels could be useful diagnostic biomarkers discriminating obese from lean subjects.

3.8. Accuracy of OPG expression and serum levels *for discriminating type 2 diabetes mellitus among obese by ROC analysis*

We further looked into the possible diagnostic value of OPG expression (A) and OPG serum levels (B) by ROC curves were presented in (Fig. 3**).** In obese patients, when we discriminate T2DM among non-diabetic patients, the cutoff values of OPG mRNA levels and serum levels were 2.2267 and 5.263 and the AUC were [0.833 (95%CI = 0.821–0.946) and 0.950 (95%CI = 0.908–0.991), respectively, P < 0.001 for each]. To boot, the sensitivities and the specificities of OPG expression were (90% and 86.2%), and OPG serum levels (95.7% and %96. 4), respectively. Thus, OPG mRNA & serum values could be useful diagnostic biomarkers discriminate T2DM from non- diabetic obese patients

4. Discussion

The obesity pandemic is considered a major public health problem, as in this disorder is there's increased risk of medical comorbidity leading to a substantial rise in mortality. One of those commodities linked to obesity, MetS and T2DM are particularly preoccupying [6].

Considerable evidence implicates inflammation as a decisive component in the pathophysiology of obesity and diabetes as OPG is a member of the TNF receptor superfamily. OPG also a receptor of TNF-

related apoptosis-inducing ligand [TRAIL] playing a role in immune regulation and cell survival [28, 29]

TNF-α, interleukin-1 (IL-1), IL-18, transforming growth factor-β (TGF- β), and 17 β-estradiol are known to up-regulate OPG mRNA levels [28]. On the other hand, glucocorticoids, parathormone hormone [PTH], and prostaglandin E2 [PG-E_2] can inhibit the expression of OPG [29, 31–33]. Emerging evidence demonstrated that estrogens both raise BMD and increase the production of OPG. On the contrary, PTH stimulates a receptor activator of the NF-B ligand and suppresses OPG expression. Based on these findings, we include on women only and excluded any patients with parathyroid disorders or receive medication affect osteoprotegerin mRNA or serum levels.

In spite of the growing knowledge, the exact mechanism by which OPG, diabetes and cardiovascular disease are linked hasn't been found yet and the precise role of OPG in obesity remains speculative. The results presented herein are innovative; as this study, investigate for the first time the possible association of OPG gene expression with new obesity indices; VAI, BAI as well as parameters of cardiometabolic risk factors in obese patients.

Noteworthy, our results explored that the controls had significantly higher values of serum OPG levels compared to obese patients. Interestingly, obese diabetics had higher serum levels of OPG compared to those obese non-diabetic patients. Regarding to expression OPG mRNA values, there were significantly higher values in the obese group compared to lean group. Moreover, in T2DM the level OPG of expression levels were significantly higher than non-diabetics patients.

Here reported data are supported by a study conducted by Holecki et al they found that the serum OPG concentration has significantly lower levels in obese patients in relation to lean controls. In a later study, among patients with morbid obesity the production and expression of OPG was decreased by PTH [34]. Similar results confirmed by Toffoli et al. who found that in diabetic group, OPG mRNA levels were significantly increased as compared to other groups [35].

In line with our results, an Egyptian study conducted by Ahmed et al. found that the serum OPG had significantly higher levels in diabetic patients than in the healthy group and more in diabetic patients with MetS than in those without MetS [36].

Similar to our findings, Chang et al results demonstrated that OPG expression was increased in hyperglycemic rat aortic VSMCs, while RANKL expression was decreased [37]. In contrast, a study by Ayina et al. found that OPG showed higher levels in obese than in normal subjects. They explained that this finding led to different sizes of the study populations and different methodologies [38].

Other studies also have shown that serum OPG levels increased in obesity. Xiang et al. [39], found that OPG levels were high in obesity and hey suggested that this high level of OPG could be led to inflammatory biomarkers such as C- reactive protein [CRP] [40, 41].

Given the existing controversy in this results study by Alharbi et al. found that serum OPG level was significantly elevated in obese with insulin resistance patients compared to control subjects [42]. Moreover, other authors couldn't find any statistical difference of OPG among subjects with or without MetS [43].

Interestingly, we found that HbA1c and HDL-c is the main predictors of OPG gene expression among obese subjects'. In accordance with our findings, Toffoli et al. showed that both dyslipidemia and T2DM can affect OPG expression levels in the cardiovascular system and these changes could contribute to atherosclerosis [35].

To elucidate the relationship between anthropometric measures with the parameters of MetS, we found significant positive correlations between parameters of MetS including WC, SBP and DBP, low HDL-c as well as TAG with all anthropometric measures in obese cases (BMI, WHR, whiter, BAI and VAI). Interestingly, among obesity indices, the highest positive correlation found between VAI and parameters of MetS.

In conformity with our finding, Amato et al. among an age-stratified a Caucasian Sicilian population, cutoff points of VAI were proved to be strongly related to MetS, they explained that, VAI represents physical (BMI and WC) and metabolic parameters (TAG and HDL-c), may, indirectly show other non-classical risk factors, i.e. cytokines and plasma-free fatty acids [12]. Likewise, other researchers showed that OPG was correlated with HbA1c and the level of FBG in diabetes [44, 45].

In the current study, we detected significant positive correlation between BAI and all parameters of MetS except WC and DBP among obese cases. Similar to our results, Bergman et al. reported that BAI is a useful parameter to evaluate adiposity in Caucasian population. Furthermore; they reasoned that it is more practical as well as less demanding than other complex mechanical strategies [11].

Our previous studies explored the connection between obesity and inflammatory biomarkers [46, 47]. Interestingly, the present work is the initiative to concentrate on an OPG expression level in obesity and its correlations with obesity indices; BAI, VAI as well as parameters of MetS. The main finding of the present work is the independent correlation between OPG expression level and anthropometric measures as well as parameters of MetS. Multiple stepwise linear regression analysis test showed that, OPG expression levels were independently correlated with HbA1c, HDL-c, and WHR. Regarding OPG serum levels only HbA1c independently correlated with it.

These results matched with those described in other study conducted by Gannage-Yared MH et al. [23], they unveiled a positive correlation between OPG and HOMA-IR. There are noteworthy useful impacts of OPG on vascular tissues as it's associated with endothelial dysfunction and IR. These effects were backed up previously by other studies; they documented an association between high serum OPG levels and clinical cardiovascular disease [48–51].

In contrast, a study by conducted by Erol et al. found that the serum OPG values were negatively correlated with HOMA-IR ranges [52]. Together, these results may emphasize that there is no consensus on the association of OPG levels with cardiometabolic risk factors [23, 41]. In the study conducted by Ayina et al., they observed that only age significantly correlated with OPG levels. However, in obese group, OPG levels were correlated with both HDL-C, and HOMA-IR. Regarding the impact of independent correlated factors with OPG of in lean and obese groups Ayina and his colleagues found that by multiple linear regression analyses, age was an important determinant of OPG levels in both lean and obese women. However, only HDL-c was related to OPG levels in obese females [38].

Most significantly, the biological connections that link between OPG and inflammation could lead to its anti-inflammatory and anti-apoptotic impacts as well as its roles in the regulation of bone homeostasis [53]. Thus, special consideration must be paid when evaluating the role that the OPG- RANKL system plays in metabolic diseases [54].

Precocious, reliable, and early diagnosis of T2DM is particularly important. Interestingly, we analyzed our data by ROC to evaluate the sensitivity and specificity of OPG expression as well as its serum level for differentiating T2DM from non- diabetic. Our results detected that the power of the OPG serum level was higher than its expression levels. Thus, the main finding in the current work after adjusted for the traditional risk factors by logistic regression analysis test that serum OPG levels was a significant predictor of T2DM among obese patients' ($P < 0.001$). Therefore, serum OPG level could be used as biomarkers of T2DM.

In accord with our findings, Duan et al. detected increased circulating OPG levels in Chinese diabetic and prediabetic postmenopausal women with DM and prediabetes that circulating OPG concentrations were higher in Chinese postmenopausal women in relation to controls [55]. It is proposed that inflammation could link OPG to IR, the hallmark of obesity and T2DM, which considered a state of low grade chronic systemic inflammation [56]. It has been sugessted that OPG/RANK/RANKL pathway may play a role in the regulation of inflammatory and immune responses and directly regulate the production of proinflammatory cytokines from macrophages [57]. Furthermore, it has been suggested that this pathway plays a role in activation of NF-κB pathways and its downstream players which has a role in IR [58]. Recent reports demonstrated that this pathway could have a role in DM pathogenesis [59]. Putting these data together blocking this pathway may improve hepatic insulin resistance and prevent T2DM development [60].

In conclusion, the increased circulating OPG expression in obese women, especially diabetic obese patients could approve the link between inflammation, obesity indices (BAI and VAI) and MetS parameters. The identification of optimum cutoff of OPG expression and serum levels among obese patients could help in evaluating obesity and its comorbidities in an attempt to decrease health hazards related to obesity. Further future multicenter studies with a bigger sample size are needed to validate our findings.

Declarations

Conflict of interest: None

Funding: None

Contribution of all the authors: All authors contribute in study design, writing, methods, collection of data and its analysis.

Data Availability: The data used to support the findings of this study are included within the manuscript.

Consent to Participate (Ethical approval):

Written consent was obtained from all participants. The study was approved by the research ethics committee of our institutes. The work was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Consent to Publish (Ethics):

All authors approved the current state of this manuscript.

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Figures

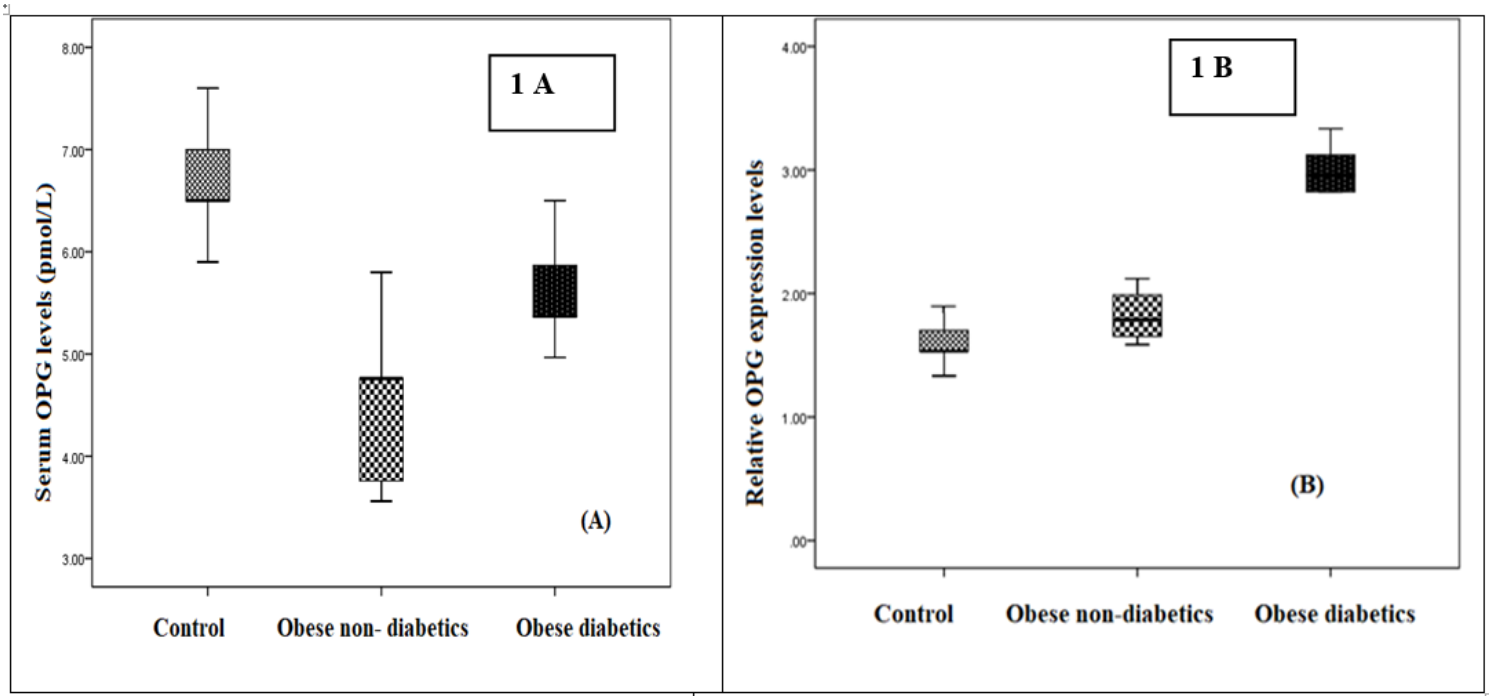

Figure 1

(A): Serum OPG levels (pmol/L) in studied groups; (B): relative OPG expression levels in studied groups.

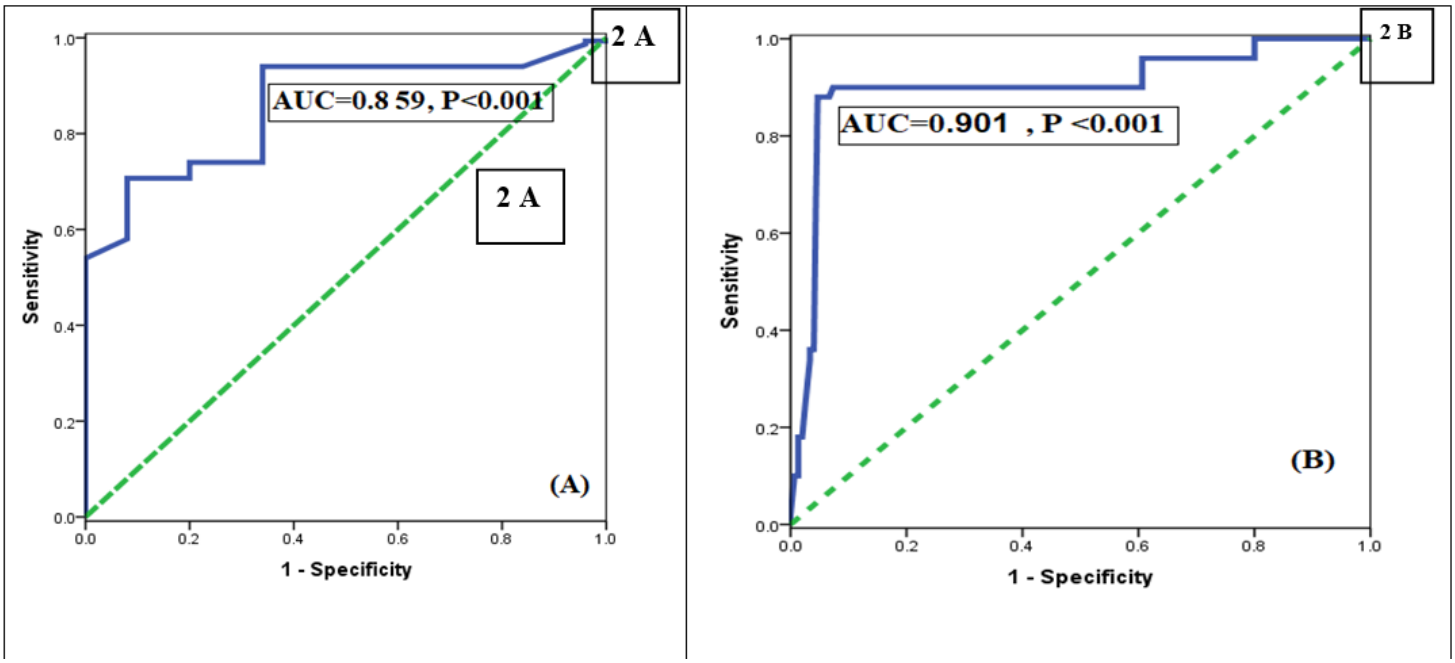

Figure 2

(A) : ROC curve for utility of for OPG expression levels in discriminating lean control from obese patients; (B): ROC curve for utility of for serum OPG levels in discriminating lean control from obese women.

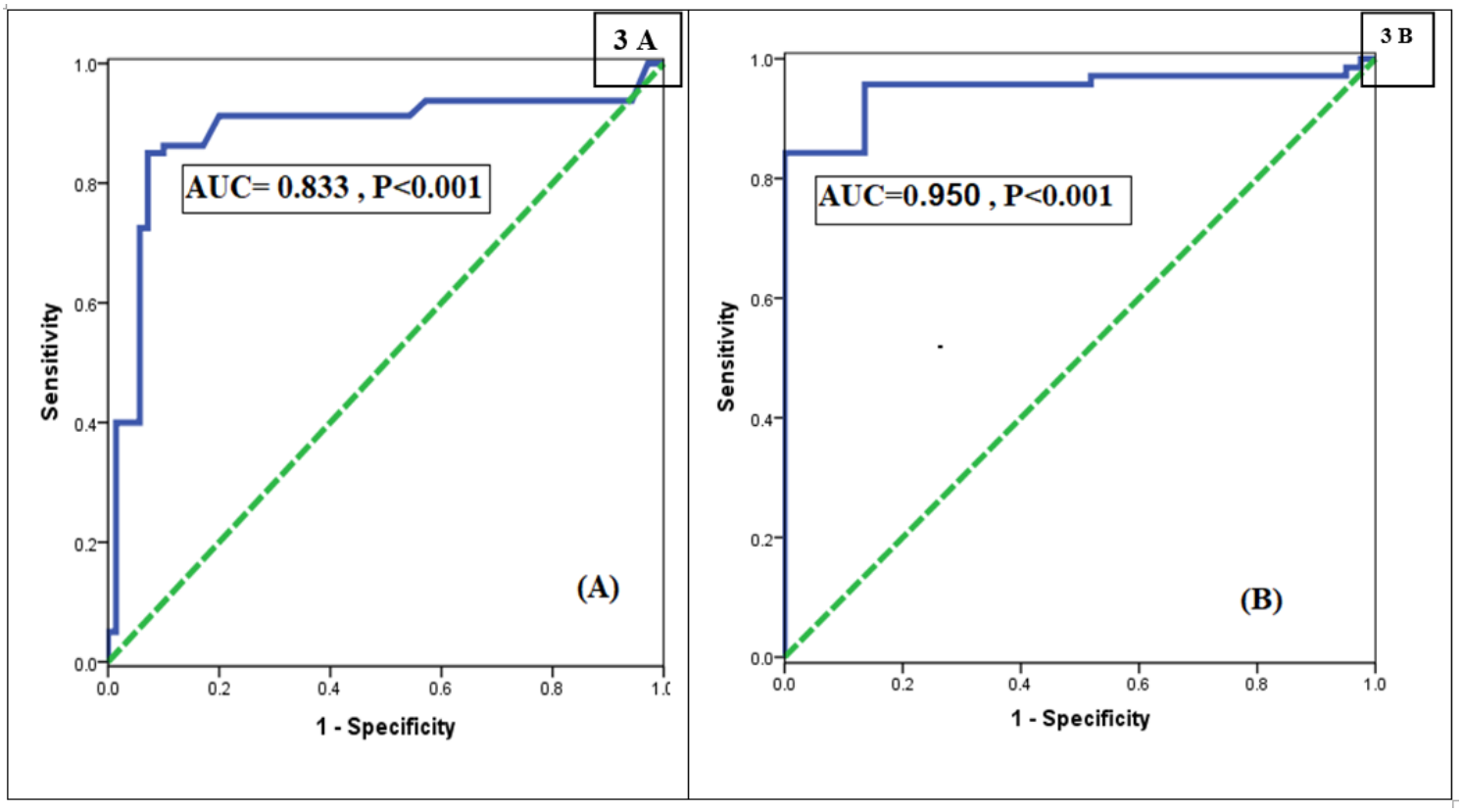

Figure 3

(A) ROC curve for utility of OPG expression in discriminating obese women with T2DM from those without diabetes; (B): ROC curve for utility of serum OPG levels in discriminating obese patients with T2DM from those without diabetes.