Quantitative Estimation of Osteoprotegerin biomarker of Metabolic Syndrome Patients in Najaf city, Iraq

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Abstract
Objective: The prime objective of this investigation was to find a new method for the diagnosis of Metabolic Syndrome disease (MetS) by estimating the level of Osteoprotegerin (OPG) in the serum of patients. **Subjects and Methods:** The study included 106 subjects divided into two groups, (61) with Metabolic Syndrome and (45) in the control group, the mean of its age was 50. Most of the serum assays were measured by colourimetric methods, and ELISA Technique evaluated serum levels of OPG. **Results:** The clinical findings confirm that OPG is expressed in cells involved in atheroma plaque development and progression, such as arterial smooth muscle cells these leads showed that OPG levels were more elevated compared to the levels of the control group ($p < 0.00001$). Others outcomes the Pearson's correlation study illustrated that the OPG level is positive relationships with fasting blood glucose (FBG) and Hemoglobin A1C (HBA1C) ($r = 0.919$, $p < 0.00001$), ($r = 0.628$, $P=0.002$) respectively. Also, strong positively correlation with insulin level and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ($r = 0.759$, $p = 0.010$) ($r = 0.628$, $P< 0.00001$) respectively. Furthermore, the OPG level denoted a significant positive correlation with the obesity index is the ratio of waist circumference to hip circumference (WHR) ($r =0.594$, $P< 0.00001$) and Triglycerides (TG) ($r =0.475$, $P< 0.00001$). **Conclusion:** These results support a strong correlation between serum OPG level and most indicators of metabolic syndrome disease, so we can conclude that it is a great biomarker for predicting Metabolic Syndrome disease (MetS) in individuals who are likely to be infected with this disease, such as hypertension patients and obese people.

Keywords: OPG, ELISA, MetS, HOMA, Insulin.
Source of support: Nil.
Conflict of interest: Non

1. Introduction

OPG is a soluble glycoprotein that can be found as either a 120-kDa dimer linked by disulfide bonds or a 60-kDa monomer. The dimerization of OPG is important for the Receptor activator of nuclear factor-kappa-B (RANK) - Receptor activator of nuclear factor-kappa-B ligand (RANKL) inhibition as dimerization increases the association of OPG for RANKL (from 3μM KD as a monomer to 10nM as a dimer) as a monomer, OPG would have an insufficient affinity for RANKL to vie with RANK and effectively inhibit RANK-RANKL interactions [1]. The molecule of OPG contains 401 amino acids. However, the splitting of a 21-amino acid single peptide leads to the forming of a ripe 380 amino acid form which comprises seven functional domains. Domains 1-4 are amino-terminal cysteine-rich domains that are structural as the extracellular fractions of others related in the TNF receptor superfamily and essential for dimerization and Osteoclastogenesis. The carboxyterminal mixes sections five and six that are death domain symmetrical areas. Domain 7 is a C-terminal heparin binding domain ending with a cysteine (Cys-400) that even plays an essential role in the dimerization of OPG. It is up-regulated in calcified coronary plaques and associated with illness intensity. Furthermore, OPG has been specified as insulin sensitivity and glucose homeostasis. Metabolic syndrome (MetS) is a significant worldwide public health burden with an estimated 25% prevalence worldwide. It is a risk factors constellation that includes increased waist circumference, hypertriglyceridemia, diminished high-density lipoprotein, hyperglycemia and increased blood pressure. It is significantly progressing the risk of type 2 diabetes (T2DM) and cardiovascular diseases (CVD) [2].

2. Subjects and Methods

Subjects

This research was conducted on 61 (33 female & 28 male) MetS patients without other diseases such as cardiovascular disease and also no history of drinking or smoking as well as 45 (23 female & 22 male) control individuals with old range (35 to 65) years. Controls were selected as non-diabetic, clear from acute diseases, Patients were determined in Al-Sadder

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Teaching Hospital in Najaf, Iraq, every participant should agree about participating in this research, written consent was obtained from them, during the period from November 2019 until May 2020. All anthropometric measures that involved weight, height, sex, age, BMI, and WHR will be registered.

3. Methods

The blood samples were assembled before drug administration and after overnight fasting of fully 12 hours. BMI has been calculated by the following equation BMI (kg/m²) = weight (kg)/height (m²). Routine parameters (glucose, HBA1C, urea and creatinine, Uric acid, and Lipid profile) were assayed on UV-Vis Spectrophotometer using the colorimetric method. Whereas LDL concentration was calculated using the Fried Ewald formula, Fasting human insulin was estimated via Cobas e 411 instruments. By HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) Insulin resistance was calculated [insulin (μIU/mL) x glucose (mg/dL) / 405] [3]. Albumin has been estimated using I ChromaII, ACR is calculated via the ratio of the concentration of urine albumin (milligrams) to urine creatinine concentration in grams, by the following equation: [GFR (mL/min/1.73 m2) = 186 × Serum Cr-1.154 × age-0.203 × 1.212 (if the patient is black) × 0.742 (if female)] GFR was estimated [4] while Sandwich ELISA technique was applied to estimate the concentration of sRAGE (enzyme-linked immunosorbent assays).

Statistical Analysis

Statistical Package (SPSS-24) was used to analyze results, data were described as "mean ± standard deviation (SD). The significance of the difference in the mean was assessed through an independent t-test. P-value < 0.01, is considered highly significant, statistically significant when p<0.05 and non-significant when (p>0.05), using Pearson's correlation to estimate the correlation between variables.

4. Results

Description of the group of MetS and healthy control

The characteristics of all volunteers who participated in the current research were given in Table1, the mean of age, WHR, Bp diastolic, Bp systolic, and weight were high positively significant (p>0.01) for the MetS patients group compared with the health group. However positive significant (p>0.05) in a mean of duration of disease and BMI.

Table 1. The demographic characteristics of the present study

Parameters		MetS (m±SD) (n=61)	Control(m±SD) (n=45)	P-value
Age (years)		46.49± 10.94	40.02±4.62	< 0.00001
(35-44 years) No. (%)		26 (42.6%)	34 (75.5 %)	a
(45-55 years) No. (%)		26 (42.6%)	11(24.5 %)	
(56-65 years) No. (%)		9 (14.8%)	0 (0 %)	
sex	27 (44%)	21(49%)	22 (49%)	a
	34 (56%)	24 (51%)	23 (51%)	
Duration of Disease (years)		6.86±5.142	0.00	< 0.05
BMI (kg/m²)		32.51±6.11	28.15±12.1	< 0.05
WHR		0.983±0.188	0.895±0.05	< 0.00001
Bp (systolic) mmHg		141.36±22.59	116.51±9.3	< 0.00001
Bp (diastolic) mm Hg		97.10±17.017	74.91±5.70	< 0.00001
Weight Kg		88.92±18.31	73.05±11.8	< 0.00001

Also, the outcomes of the study illustrated that the body mass index (BMI) was high in (35-44) old group, then (45-55) age group higher than the (56-65) old group in patients.

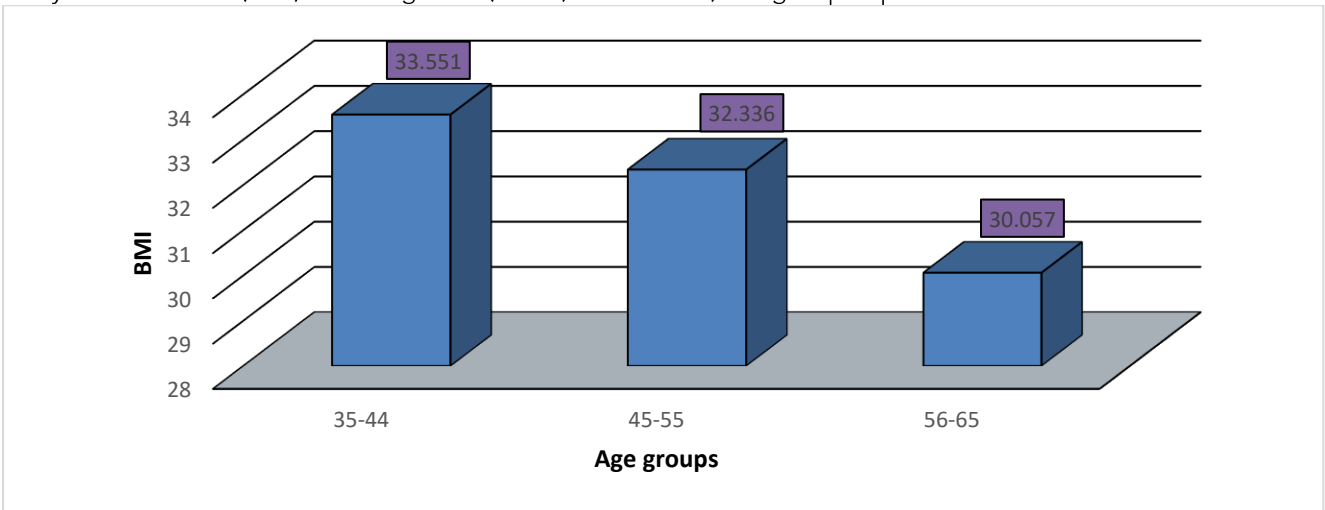

Figure (1): Distribution BMI according to the Old groups

Glycemic State of study

It was observed in levels of insulin, and HOMA a significant raise (p<0.00001) compared to healthy subjects as shown in table 2.

Table (2): Mean ±SD values of FBG, insulin, HOMA, and HbA1c for the studied groups.			
Parameters	MetS (m±SD) (n=61)	Control (m±SD) (n=45)	P-value
FBG (mg/dl)	109.74±22.04	99.61±13.9	NS
Insulin (µIU/ml)	16.724±5.361	10.791±4.29	< 0.00001
HOMA-IR	4.604±1.903	2.65±1.395	< 0.00001
HbA1c (%)	5.063±0.457	4.889±0.524	NS

Insulin relationship with different parameters of the study

Table 3 explained insulin relation with other parameters in the current study, since showing the presence of positive and negative associations by analysis of bivariate statistical.

Table (3): The Pearson correlation and P-value of Insulin with other parameters in the study.				
Parameter	Controls		T2DM	
	r	P- value	r	P-value
Age	-0.179	0.293	0.292	0.060
Duration	a	a	0.130	0.318
WHR	0.483**	0.001	0.541**	< 0.00001
BMI	-0.027	0.859	0.026	0.844
SYS	0.031	0.840	0.087	0.506
DIA	-0.248	0.100	0.116	0.371
Glucose	0.488**	0.002	0.289**	0.010
HOMA	0.947**	< 0.00001	0.875**	< 0.00001
HbA1c	0.209	0.169	0.329**	0.010
Urea	0.332*	0.026	0.082	0.530
Creatinine	0.325*	0.029	0.231	0.073
Uric acid	0.254*	0.018	0.449**	< 0.00001
TG	-0.104	0.497	-0.087	0.503
CHOL.	0.001	0.993	0.484**	< 0.00001
HDL	0.409**	0.005	0.011	0.935
LDL	0.068	0.656	-0.004	0.977
VLDL	-0.103	0.501	0.214	0.098
eGFR	-0.075	0.625	-0.268**	0.010
ACR	0.204	0.179	0.321**	0.010
OPG	0.104	0.502	0.759**	0.002
Correlation is significant at the 0.05 level (2-tailed).		a. Cannot be computed because at least one of the variables is constant		

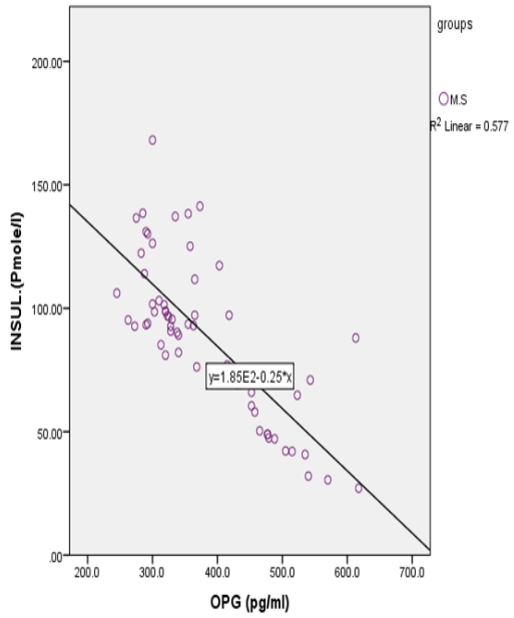

Figure (2): The correlation between insulin and OPG

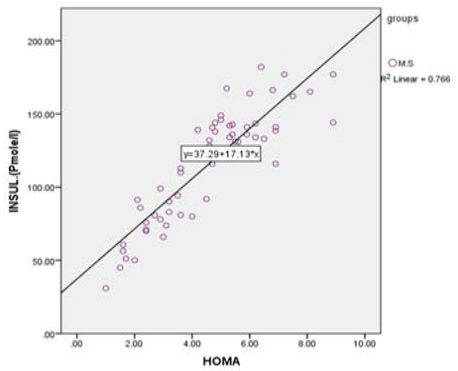

Figure (3): The correlation between insulin and HOMA

Lipid profile

All outcomes of the lipid profile are revealed in table 4, there was a high significantly rise in the concentration of Cholesterol, HDL, TG, and VLDL (p>0.00001). While it is positive significant difference in LDL levels.

Table (4): Mean ±SD of lipid profile.			
Parameters	MetS (m±SD) (n=61)	Control (m±SD) (n=45)	P value
TG (mg/dl)	212.55±98.73	83.080±18.72	< 0.00001
Chol.(mg/dl)	166.138±49.7	134.82±36.60	< 0.00001
HDL (mg/dl)	41.48±10.258	48.719±7.45	< 0.00001
LDL (mg/dl)	82.718±41.23	70.873±36.20	< 0.005
VLDL(mg/dl)	41.213±19.44	16.614±3.748	< 0.00001

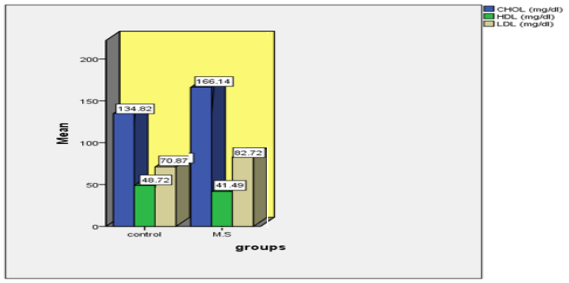

Figure (4): Mean values of Cholesterol, HDL and LDL of the studied groups

Serum OPG level

The results have been shown (mean + standard deviation) of OPG (pg/ml) (321.081±67.2) of patients and (187.00 ± 23.6) of normal subject, As well as, OPG correlation with others parameters illustrated in Table 5.

Table (5): The correlation of OPG and other parameters.

Parameter	OPG			
	Controls		MetS	
	r	P- value	r	P-value
Age	0.017	0.914	0.086	0.512
Duration	a		0.186	0.150
BMI	0.098	0.523	0.100	0.442
WHR	0.084	0.581	0.594**	<0.00001
SYS	0.100	0.513	0.010	0.936
DIA	0.037	0.811	0.019	0.884
Glucose	0.911	0.365	0.919**	<0.00001
Insulin	0.104	0.502	0.759**	0.010
HOMA	0.149	0.198	0.628**	<0.00001
HbA1c	0.170	0.786	0.268**	0.002
Urea	0.173	0.256	0.060	0.643
Creatinine	0.235	0.121	0.100	0.441
Uric acid	0.098	0.521	0.251**	0.010
TG	0.080	0.599	0.475**	<0.00001
CHOL.	0.028	0.857	0.059	0.650
HDL	0.500	0.744	0.214	0.098
LDL	0.073	0.635	0.194	0.134
VLDL	0.080	0.601	0.456**	<0.00001
U. Creatinine	-0.018	0.906	0.274	0.063
Protein	0.067	0.664	0.137	0.292
Albumin	0.157	0.302	0.137	0.292
eGFR	-0.192	0.207	-0.147	0.258
ACR	0.046	0.762	0.011	0.933

Correlation is significant at the 0.05 level (2-tailed).
a. Cannot be computed because at least one of the variables is constant.

ROC area under the curve

The finding of ROC analysis as described in Figure (5) with sensitivity = %100, specificity =%97.3, AUC=0.889. This also confirms that OPG is a good predictor of MetS disease.

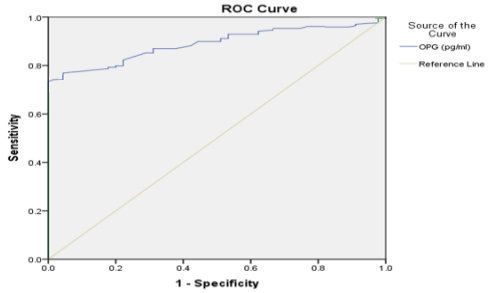

Figure (5): ROC for the different parameters of MetS and control group.

5. Discussion

Description of the group of MetS and healthy control

The determines fat sedimentation in the body from the energy imbalance outcome between the fuel that is depleted through comes from feed and daily activities, obesity is a multifactorial a etiology in the adipose tissue, rather than only being a place to store excess energy, operates as an endocrine organ with vasoactive impacts that are involved in the evolution of metabolic diseases [5, 6]. In addition, obesity is a higher risk of the potential incidence of metabolic syndrome. It can be determined by body mass index (BMI) which is a simple index estimated from (weight and height) and waist-hip ratio (WHR) therefore, it is very important to measure it [7, 8]. This study observed that the means of BMI, and WHR, are more in the MetS patients and suggests that one of the genes plays an important function in regulating body fat. This investigation is designed to find the association between MetS and specific genotypes [9].

Also, Bp systolic and Bp diastolic have highly significant in the patient group, this is associated with the level of insulin since hyperinsulinemia appears to influence the sympathetic nervous system leading to water and sodium retention and vasoconstriction that raise blood pressure [6]. The outcomes of a study reflect that BMI was increased in an age range (35-44) then an age range (45-55) ultimately an age range (56-65) in the patient because of lifestyle changes with food. Also, the anabolism process is slower, as people get older [10, 11].

Glycemic State of study

All participant's results of glycemic state are revealed in the table (2), comprise fasting Insulin (µIU/ml), HOMA-IR, fasting Serum glucose (mg/dl), HbA1c (%) and p values which showed strongly significance in fasting Insulin (µIU/ml), HOMA-IR of MetS group using independent sample t-test.

The high level of insulin in the body because of insulin resistance leads to it converting glucose into fat that collects around the abdominal areas and liver, which leads to an raised in the waist circumference and a high mean of WHR in the patient [12]. Additionally, one of the functions of insulin is to present potassium to the cells. In the case of insulin resistance, this ion is not submitted into the cell, and consequently, the proportion of sodium became high, which leads to high pressure, and this demonstrates the raised in SBP and DBP pressure in the MetS patients [13, 14].

Insulin relationship with different parameters of the study

The results show a good significantly positive correlation of insulin with HOMA and WHR in MetS patients ($r = 0.875$, $p<0.00001$), ($r = 0.541$, $p<0.00001$) respectively where the amount of insulin secretion is affected by obesity and insulin resistance. As well as, Insulin shows a positive correlation with U.A ($r=0.449$, $p=0.00001$) and TG and VLDL($r=0.484$, $p<0.00001$) and ($r=0.447$, $p>0.00001$) respectively, these factors are one of the causes of metabolic syndrome.

Finally, OPG level is similar in that it has a positive significant correlation with insulin levels in MetS patients ($r=0.759$, $p<0.002$) this indicates the relationship of insulin with the means of OPG concentration

Lipid profile

From the findings of estimating the lipid profile in the serum, it was found that the mean of TG, LDL, VLDL, HDL and cholesterol was the highest in the patients which MetS is a cluster of conditions that occur together from these conditions including excess body fat [15, 16].

Serum OPG level and its relationship with other various variables in the study

The current study found that OPG media has a significant increase in MetS patients compared to normal subjects ($p < 0.00001$), and this is due to the fact that OPG concentrations are associated with obesity-induced inflammation, hyperinsulinemia, and insulin resistance. The oxidative stress inducers that transform vascular smooth muscle cells into osteoblast-like cells that form nodules, it spontaneously mineralizes and eventually produces hydroxyapatite. It seems likely that bone mineralization and vessel calcification are active processes involved in common pathophysiological and biochemical properties. In this sense, it has been suggested that Osteoprotegerin molecules participate in bone homeostasis, in addition to having a pivotal role in vascular calcification [17, 18].

From the analysis of the relationship between OPG and the other variables, it was found that it is associated with the causes of the disease, which is the high level of insulin and the increase in its resistance in the body, as well as the high level of triglycerides, which was ($r = 0.759$, $p = 0.010$), ($r = 0.628$, $p<0.00001$), and ($r = 0.475$, $p<0.00001$) respectively. These results in agreement with the results of a study conducted on Egyptian obese women [19].

ROC area under the curve

The result of the ROC is described in Figure (5) showing that the biomarker is a good indicator for the diagnosis of metabolic syndrome.

6. Conclusion

From this study is concluded that OPG level is raised in Metabolic Syndrome patients, hence OPG is believed to be useful to denote people at risk of MetS thus decreasing morbidity and mortality. Furthermore, the ROC analysis of the sensitivity and specificity shows that of biomarker OPG can be presented as a potential marker for the earlier designation of Metabolic Syndrome.

7. Acknowledgments

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