Original Article

Osteoprotegerin, High Sensitive C-Reactive Protien, Insulin Resistance and Their Relation to Carotid Intima Media Thickness in Type2 Diabetic Subjects.

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Abstract:

Background: The role of Osteoprotegerin (OPG) as a marker of atherosclerosis in (T2DM) is not well established. Moreover, the relationship between OPG and insulin resistance in T2DM remains to be elucidated. **Aim:**To determine the role of serum OPG in the prediction of atherosclerosis and insulin resistance in T2DM males. **Subjects and Methods:** Cross-sectional study with 80 males, 40 with T2DM and 40 subjects without diabetes and (ACVD) was excluded in both groups. We measured: serum OPG by immunoassay, FBG, HbA1C, HOMA-IR, lipid profile, Albumin/creatinine ratio, hs-CRP, carotid intima- media thickness (IMT) and ankle brachial index (ABI). The presence of ACVD was excluded by a negative stress test and/or coronary angiography. **Results:** The mean serum OPG level was statistically significantly higher in the diabetic group compared to the control group (p =0.029*),the mean serum OPG level was 506.45±488.48 ng/ml in the diabetic group and 266±272.73 ng/ml in the control group.OPG was statistically significantly positively correlated to age (p=0.047) also with HbA1c (p=0.009) and DM duration (p=0.041). However, OPG was not related to smoking, IMT, ABI or level of albuminuria. **Conclusions:** Our study showed that T2DM patients had higher OPG serum levels with respect to the non-diabetic population, butthe relationship of OPG and atherosclerosis in T2DM patients needs further investigations.

Keywords: Osteoprotegerin, Hs CRP, IR, T2 DM

Introduction:

Diabetes mellitus is a complex metabolic condition primarily defined by the level of chronic hyperglycemia giving rise to risk of vascular complications that may be microvascular or macro vascular.

Diabetes is a chronic illness and needs continuous medical care with multifactorial risk reduction strategies beyond glycemic control.(1) Atherosclerotic cardiovascular disease (ACVD) is the major cause of morbidity and mortality in T2DM patients.T2DM patients have (2-4) times increased risk of cardiovascular events with respect to subjects without diabetes.(2) (OPG) is a promising marker for atherosclerosis in patients with T2DM and insulin resistance. (OPG) is a glycoprotein that acts as a decoy receptor for receptor activator of nuclear factor B ligand (RANKL) and TNF-related apoptosis-inducing ligand. The OPG/RANKL/ receptor activator of nuclear factor B axis plays an important regulatory role in the skeletal, immune, and vascular systems.At the vascular level OPG is expressed in vivo by endothelial cells (ECs), vascular smooth muscle cells (VSMCs). RANKL is expressed in vivo by stromal cells and T- lymphocytes.(3)

Abbreviations: ABI: Ankle/brachial index, **ACVD:** Atherosclerotic cardiovascular disease**, BMI:** Body mass index, **CAD:** coronary artery disease, **CVS:** cerebrovascular disease, **DM:** Diabetes mellitus, **ECs:** Endothelial cells, **HbA1c:** Haemoglobin A1c, **HDL-C:** High Density Lipoproteins, **HOMA-IR:** Homeostasis Model Assessment insulin resistance, **hs-CRP:** high sensitive-C-reactive protein**, I-Kb:** Inhibitory kappa B, **IMT:** intima media thickness, **LDL-C:** Low Density Lipoproteins cholesterol, **NSTEMI:** non-ST segment elevation myocardial infarction, **PAD:** peripheral arterial disease, **RANK:** Receptor activator of nuclear factor k-B, **RANKL:** Receptor activator of nuclear factor k-B ligand, **STEMI:** ST segment elevation myocardial infarction, **VSMCs:** Vascular smooth muscle cells.

RANK is expressed on the surface monocytes, macrophages and dendritic cells.[4,5] Interaction of RANKL with RANK activates nuclear factor I-kB (a transcription factor), degradation of I-kB protein by I-kB kinase; frees the nuclear factor-kB complex, which then translocate to the nucleus initiating transcription of specific genes required for differentiation of macrophages.[6]

There is association between OPG levels and traditional cardiovascular risk factors.[6, 7] The major consistent direct association seems to be with increasing age and duration of diabetes. [7]The association of serum OPG levels with markers of endothelial dysfunction has been reported in patients with T1DM and T2DM. [8, 9]

In a general population serum OPG is associated with future risk of myocardial infarction, ischemic stroke and total mortality independent of other cardiovascular risk factors.[10] It has also been shown that OPG serum levels are related to increased cardiovascular mortality in accelerated atherosclerosis related condition conditions as advanced age[11] and CAD.[4]

Avignon et al[12] demonstrated the significant association of OPG levels and silent ACVD, diagnosed by myocardial perfusion imaging in patients with T2DM. The increase in OPG in T2DM has been linked with higher risk of coronary artery calcification [13] and silent CAD. [12] Serum OPG concentrations were predictive of increased risk of cardiovascular events in T2DM during an 18-month follow-up.[7] Reinhard et al[14] demonstrated that elevated serum OPG is a strong predictor of all-cause mortality in T2DM patients. This effect is independent of conventional cardiovascular risk factor.

Patients and Methods:

This cross-sectional study was conducted on 80 male subjects divided into two groups. Group I: 40 T2DMpatients without CAD.Group II: 40 Healthy control subjects matched for age, sex and socioeconomic status.

CAD was excluded in group I by a negative stress test and/or coronary angiography. Subjects of group I were recruited from those undergoing coronary angiography and/or stress test for a justified indication at the cardiology department, Alexandria University, and proved to be free of CAD. Patient categorization was based on history, clinical, laboratory, stress test and or coronary angiography.Patients with the following associated conditions were excluded from the study: history of acute coronary syndrome, unstable angina, NSTEMI and STEMI, history of infection with in the last 2 months, endocrinal, metabolic diseases or immunological diseases other than DM and T1DM.After giving their signed informed consent; all the participants were subjected to the following:full history taking in which personal history included name, age and occupation, history of cigarette smoking: according to smoking status, participants were classified into:

- Non-smoker: those with a lifetime exposure to no cigarettes.
- History of smoking: Including current and ex-smokers.

History of different cardio-metabolic risk factors: DM, hypertension, dyslipidaemia, drughistory and family history: participants were asked about family history of CAD, CVS, PAD, hypertension, dyslipidaemia and DM.

Complete physical examination with special stress on: body weight and height, BMI and waist circumference, vitalsigns: (heart rate and arterial blood pressure), andassessment of ABI.

Laboratory Assessment Including:

FBG,CBC, Fasting serum insulin level, HbA1C, Total serum cholesterol, HDL-C,LDL-C, Serum triglycerides, hs-CRP and serumOPG level using ELISA assays kit. Venous blood samples (10 ml from each patient) were collected from antecubital vein into vacuum tubes with appropriate additives. Sampling was done in the morning (8.00 - 10.00 am) of the same day of the Cardiac Catheterization and coronary angiography after an overnight fast of 12 hours. All subjects were asked to refrain from smoking and strenuous exercise during the fasting period. The collected venous samples were divided into 2 parts, one part in plain vacutainer tube left to clot at 37◦C: sera were separated by centrifugation and divided into 2 parts, one used for immediate assay of fasting glucose, insulin level, lipid profile and

hs-CRP. The other was kept at -70◦C for assay of OPG. The other part of blood sample was collected on EDTA tube for complete blood count and HbA1c.

Other investigations: Resting ECG, coronary angiography and Doppler Carotid Artery to determine the carotid IMT. Ultrasonographic scanning of the carotid arteries was performed using Philips ClearVue 350 at a transducer frequency of 7-12 MHz at Radiology Department, Alexandria Main University Hospital. Extracranial carotid arteries in the neck were scanned bilaterally in the longitudinal and in the transverse plane. This provided images of the common carotid artery, the carotid bulb, and parts of the internal and external carotid arteries. Scanning was performed by experienced ultrasonographers.

Statistical Analysis of the Data:

Data were fed to the computer and analysed using IBM SPSS software package version 20.0. Qualitative data were described using number and per cent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. The used tests were **Chi-square test:** for categorical variables, to compare between different groups, **Fisher's Exact or Monte Carlo correction:** correction for chi-square when more than 20% of the cells have expected count less than 5, **Student t-test:** for normally quantitative variables, to compare between two studied groups and **Mann Whitney test:** For abnormally quantitative variables, to compare between two studied groups.

Results:

Anthropometric measures: Table (I)

The mean waist circumference wasstatistically significant much higher in the diabetic group than the control group, (p= 0.015). Also, there was a statistically significant difference regarding the waist to hip ratio being higher in the diabetic group than in the control group, (p=0.005).

Arterial blood pressure: Table (II):

The mean value of the systolic blood pressure was statistically significant higher in the diabetic group than the control group, (p=0.009). The mean value of the diastolic blood pressure was statistically significant higher in the diabetic group than the control group, (p=0.039). The mean value of the ABI was statistically significant lower in the diabetic group than the control group, (p=0.001).

Lipid profile: Table (III)

The mean value of HDL-C was statistically significant lower in the diabetic group than in the control group, (p=0.049).

Fasting plasma glucose, HbA1c and HOMA2-IR: Table (IV):

The mean fasting plasma glucose was higher in the diabetic group than in the control group, (P=0.000). The mean HbA1c percentage was higher in the diabetic group than the control group, (P<0.001).The mean HOMA2-IR value There were no statistically significant differences between both groups regarding the mean HOMA2-IR value, (p=0.946).

Hs-CRP, OPG: (Table V, figures1):

The mean serum hs-CRP level was higher in the diabetic group than in the control group, (p<0.001). The mean serum OPG level was higher in the diabetic group than in the control group, (p=0.029).

Carotid (IMT):

The mean IMT was higher the diabetic group than in the control group, (p<0.001).

Serum OPG was statistically significantly positively correlated to age (p=0.047), also with HbA1c (p=0.009) as shown at **Figure (2)**, and DM duration (p=0.041) as shown at **Figure (3)**.

There were no statistically significant correlations between serum OPG and carotid (IMT). (p=0.581). There were no statistically significant correlations between serum OPG and hs-CRP. (p=0.114).There was no statistically significant difference between the mean serum OPG levels in the diabetic patients with and without albuminuria, (p=0.613).There was no statistically significant difference between the mean serum OPG levels in those with or without history of smoking in the studied groups, (p=0.137).

Table (I): Comparison between the two groups as regards anthropometric measurements

	Group I (n = 40)	Group II (n = 40)	T	P
Waist circumference (cm)				
Range	92.0 – 108.0	89.0 – 115.0	2.487*	0.015*
Mean ± SD	100.60 ± 4.61	97.50 ± 6.39		
Waist/hip ratio				
Range	0.93 – 1.04	0.85 – 0.99	2.912*	0.005*
Mean ± SD	0.97 ± 0.02	0.95 ± 0.03		

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (II): Comparison between the two groups as regards SBP, DBP & ABI

	Group I (n = 40)	Group II (n = 40)	t	P
Systolic blood pressure (mmHg)				
Mean ± SD	130.63 ± 12.31	123.12 ± 12.54	2.699*	0.009*
Diastolic blood pressure (mmHg)				
Mean ± SD	83.0 ± 10.61	78.50 ± 8.41	2.102*	0.039*
Ankle brachial index				
Mean ± SD	0.99±0.08	1.05±0.10	3.294*	0.001*

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (III): Comparison between the two groups as regards lipid profile

	Group I (n = 40)	Group II (n = 40)	t	P
HDL-C (mg/dl)				
Mean ± SD	36.53 ± 13.45	43.29 ± 16.44	1.969*	0.049*

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (IV): Comparison between the two groups as regards FPG, HbA1c, Serum insulin& HOMA2-IR

	Group I (n = 40)	Group II (n = 40)	t	P
Fasting blood sugar (mg/dl)				
Mean ± SD	140.65 ±76.08	92.45 ± 18.92	2.779*	0.000*
HbA1c (%)				
Mean ± SD	8.59±1.96	5.72±0.53	6.778*	<0.001*
HOMA2-IR				
Mean ± SD	1.67 ± 1.68	1.54 ± 1.21	0.067	0.946

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (V): Comparison between the two groups as regards hs- CRP and OPG

	Group I (n = 40)	**Group II (n = 40)**	**T**	**P**
Hs-CRP(mg/L) Mean ± SD	12.32 ± 11.24	5.29 ± 6.09	3.822*	<0.001*
OPG (ng/ml) Mean ± SD	506.45±488.48	266±272.73	2.180	0.029*

t: Student t-test

P≤ 0.05 = statistically significant

P> 0.05= statistically non-significant

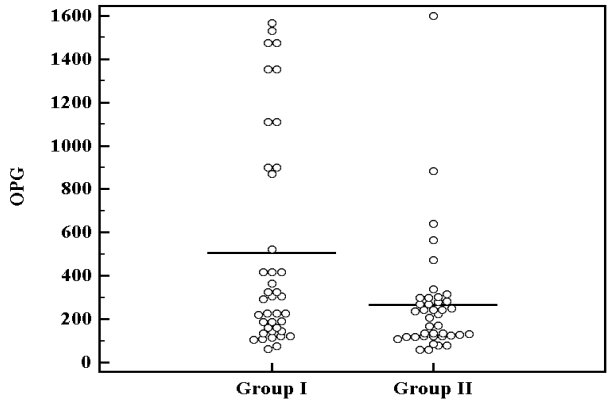

Figure (1): Comparison between the two groups as regards OPG

Table (VI): Comparison between the two groups regarding carotid intima media thickness

	Group I (n = 40)	**Group II (n = 40)**	**t**	**P**
IMT(mm) Mean ± SD	0.96±0.20	0.69±0.15	6.740	<0.001*

t: Student t-test

P≤ 0.05 = statistically significant

P> 0.05= statistically non-significant

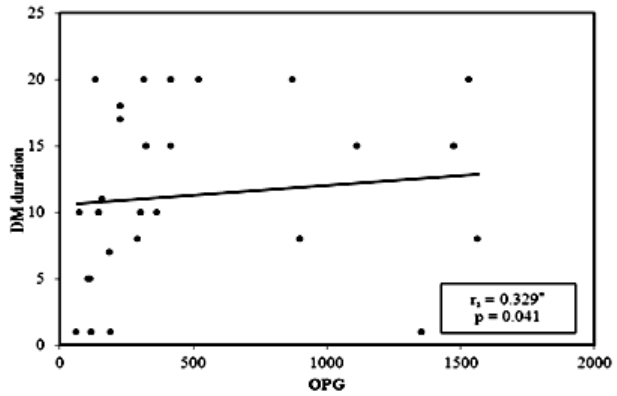

Figure (2): Correlation between OPG and HBA1c (%) in total sample.

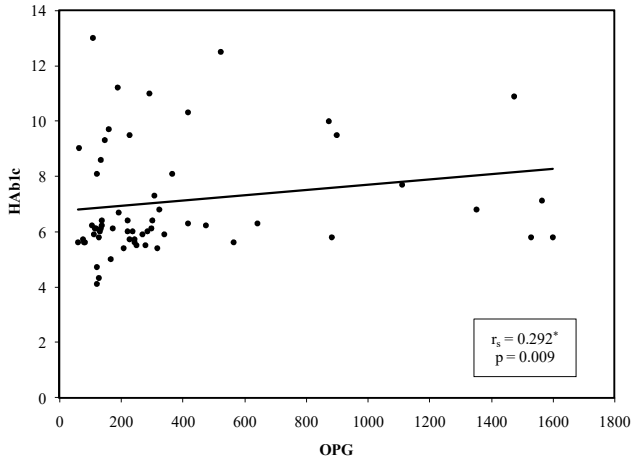

Figure (3): Correlation between OPG and diabetes duration (years) in total sample.

Discussion:

In the present study, the mean value of the waist circumference was statistically significantly higher in the diabetic group compared to the control group (p=0.015). Also, there was a statistically significant difference regarding the waist to hip ratio being higher in the diabetic group than in the control group, (p=0.005).Our results agree with published data by Björntorp et al that showed Visceral obesity is a strong predictor of T2DM and is associated with insulin resistance. Data suggest that visceral accumulation of fat contributes to increased circulating levels of FFA that cause insulin resistance and development of T2DM. [15]

The current study showed that. Both systolic and diastolic BP are much higher in T2DM than non-diabetic patients. Our results agree with data described by Buse et al [16] that showed the positive correlation between T2DM and hypertension, which was explained by the fact that hypertension is generally attributed to hyperinsulinemia resulting in increase in renal sodium retention and/or sympathetic nervous system activity [17]. Also, Insulin has an important role in the modulation of cellular calcium metabolism. Decreased insulin action on vascular smooth muscle cells may contribute both to hypertension and to accelerated atherosclerosis. [18]

In the present study, the mean value of the ABI was statistically significantly lower in the diabetic group compared to the control group (p=0.001). ABI is a non-invasive screening tool essential for diagnosing PAD; it measures clinical bedside atherosclerotic burden and risk of future CVD events. ABI is lower in the diabetic group than the control group which confirms that development of PAD is more common in patients with diabetes than non-diabetics, which agrees with Lüscher et al [19] who showed that PAD is a strong independent predictor of cardiovascular morbidity and all-cause mortality, and is a common manifestation of systemic atherosclerosis.

The results of the present study showed that there were no significant differences regarding total cholesterol, LDL-C and TG between both groups, however, the mean value of HDL-C was statistically significantly lower in the diabetic group compared to the control group, (p =0.049), which is in agreement with previous data described by Singh et al [20] and Barter P[21] who showed that HDL-C has several protective properties that are independent of their involvement in cholesterol metabolism, for example, they reduce oxidation, vascular inflammation and thrombosis, improve endothelial function, promote endothelial repair, enhance insulin sensitivity and promote insulin secretion by pancreatic beta islet cells.

In the present study, the mean carotid IMT was statistically significantly higher in the diabetic group than in the control group, (p=<0.001) which is in agreement with us, Ishiyama et al[22] who demonstrated that diabetic patients had much higher carotid IMT.

In the current study, the mean serum hs-CRP level was statistically significantly higher in the diabetic group compared to the control group (p<0.001*). This is in accordance with previous data prescribed by

Festa et al [23] who reported that increased hs-CRP is associated with insulin resistance. Hs-CRP has been proved to be a marker of chronic subclinical inflammation, to be a predictor of cardiovascular events, and to be independently related to Insulin resistance.

In the present study, the mean serum OPG level was statistically significantly higher in the diabetic group compared to the control group (p =0.029*) which agrees with the data from the previous study conducted by Knudsen et al [24] who showed that plasma OPG was significantly higher in diabetic patients.

In the present study, serum OPG level was statistically significantly positively correlated to age (p=0.047), that agrees with previous reports conducted by Gannagé-Yared et al [6] and Anand et al [7] that demonstrated serum OPG levels were significantly correlated with increasing age.

In the present study, serum OPG level was statistically significantly positively correlated to HbA1c (p=0.009) and DM duration (p=0.041) which agrees with previous reports described by Shin et al [9] demonstrated that serum OPG levels increase with age, diabetes and is positively correlated glycaemic status, and insulin resistance.

In the present study, serum OPG level was not correlated with carotid IMT. Our results agree with the study Vik et al [25] who demonstrated that increased serum OPG does not promote early atherosclerosis in younger subjects.

On the other hand, Akinci et al [26] demonstrated that elevated serum OPG levels have significant correlation with carotid IMT, which suggested that OPG might play a role in the pathogenesis of endothelial dysfunction.

In the present study, serum OPG level was not correlated to ABI, which is not in agreement with Ziegler et al [27] who reported a positive correlation between serum levels of OPG and severity of PAD. The explanation of those results is that Ziegler et al studied serum OPG level in advanced symptomatic PAD in lower extremities and their results demonstrated that serum OPG concentrations were significantly higher in subjects with ischemic ulcerations than in those without and were positively correlated with higher severity grade of disease and age .On the other hand, Pennisi et al [28] reported that there was no statistically significant difference in OPG levels between patients with documented PAD and healthy controls.

In the present study, serum OPG level was not correlated with hs-CRP which agrees with Anand et al [7] who showed that there was no significant correlation between hs-CRP and serum OPG level and it was explained by serum OPG level didn't predict cardiovascular mortality in short term.

In the present study, level of serum OPG not correlated with albuminuria.However, Wang et al [29] demonstrated that serum OPG level and the OPG expression in renal tubule cells of biopsy tissue were increased in nephropathy of T2DM.

The explanation of those results is that no patients in the current study had ACVD and the process of atherosclerosis is too early, so further investigations are needed to correlate the serum level of OPG to other atherosclerosis markers in patients with established ACVD.

On the other hand, our study demonstrated positive correlation between T2DM, duration of DM and elevated serum level of OPG.

Conclusion:

Our study showed that T2DM patients had higher OPG serum levels with respect to the non-diabetic population, but the relationship of serum OPG and atherosclerosis in T2DM patients needs further investigations.

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