Original Article

Circulatory Level of Serum Osteoprotegerin (OPG) in Patients with Type 2 Diabetes Mellitus (T2DM) and Its Association with Peripheral Arterial Disease (PAD)

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Abstract

Background: Peripheral arterial disease (PAD) is a very common problem in diabetic population. It constitutes a major economic and social burden. Early diagnosis and prevention is very important to reduce this burden. Osteoprotegrin (OPG) is thought to be closely associated with the presence of PAD and its severity.

Objective: The aim of the current study was to test this hypothesis among Egyptian patients with T2DM.

Patients and Methods: The study included 180 subjects equally divided into 3 groups; Group I consisted of 60 type 2 diabetic patients with evidence of PAD, group II consisted of 60 type 2 diabetic patients without evidence of PAD, group III consisted of healthy adults who were age- and gender-matched to the studied patients. OPG in addition to lab investigations that included (lipid profile, HbA1c, HOMA IR) were compared in the three groups.

Results: The differences between all groups were significant as regards to HOMA IR level. The differences were also significant between the all three groups according to lipid profile except for triglycerides which were significant only when comparing three groups but not Group I and group II together. With respect to osteoprotegrin level, group I was significantly higher than group II which was significantly higher than group III. The mean level was 0.32 ± 0.23, 0.14 ± 0.05 and 0.05 ± 0.05 for group I, II and III respectively. ($p<0.001$) (Table 6) The results of group I as regards to Doppler were as follows; percentage of monophasic, biphasic and triphasic was 33.3%, 60.0% and 0.0% for right foot respectively while for left foot the percentage was 23.3%, 66.7% and 6.7% respectively.

Conclusion: OPG level is closely related to the presence of peripheral arterial disease and it may have the merit of being an early marker for the disease.OPG level may have a correlation with duration of diabetes, age and ankle brachial index (ABI).The relation between ABI and OPG level may reflect a role of the later as a marker of not only the presence but also the severity of PAD.

Keywords: Serum Osteoprotegerin (OPG), T2DM, PAD.

Introduction

Over the last years, our knowledge on the mechanisms involved in the pathogenesis of cardiovascular disease has been enriched by the discovery of new molecules emerging as novel risk factors. Osteoprotegerin (OPG) is a soluble glycoprotein, member of the tumor necrosis factor (TNF)-related superfamily, involved in bone resorption. It was first described as a key regulator of bone homeostasis and vascular calcification in mice.[(1)] Clinical studies have suggested that serum OPG is associated with vascular calcification in humans.[(2)] The role of OPG in the development of macroangiopathy in diabetes is not yet clear.[(3)] It is possible that the increased OPG levels in diabetes reflect a compensatory response to arterial injury and that it is not involved in the pathogenesis of atherosclerosis.[(4)] Whether harmful or not, determination of serum OPG levels has been suggested as a prognostic biomarker of cardiovascular disease. In addition, increased OPG levels have been reported in diabetic patients with microvascular complications.[(3)] The potential of

OPG administration for therapeutic reasons is challenging for future investigators.

The mechanism underlying the effect of OPG on the development of vascular disease in diabetes is subject to controversies. TNF, a proinflammatory cytokine, is closely related to the development of vascular calcification and atherosclerotic plaque. TNF-a, which has been found to be increased in the plasma of patients with both type 1[(5)] or type 2 diabetes, induces OPG production from the arterial wall of diabetic patients.[(6)] Peroxisome proliferator –activated receptor (PPAR), found predominantly in adipose tissue, is an important nuclear transcription factor with anti-inflammatory and anti-atherogenic effects.[(7)] Several reports have demonstrated that PPAR ligands inhibit proliferation and migration of VSMCs and activation of macrophages, both functions involved in the progression of atherosclerosis. [(8)] PPAR has recently been shown to down regulate OPG expression in human aortic smooth muscle cells. CRP level has intensively been studied as a marker of atherosclerosis, especially in diabetic patients. The positive association between OPG, CRP and CAD has been reported in many previous studies. Whether elevated OPG levels represent simply a new marker of inflammation or part of the atherosclerotic process is unclear. [(9)]

The aim of this study was to test the association of OPG with the presence and/or severity of peripheral arterial disease in Egyptian patients with type 2 diabetes.

Patients and Methods

The study included 180 subjects classified as: **Group I:** 60 type 2 diabetic patients with peripheral arterial disease recruited from the Diabetes Clinic in Alexandria Main University Hospital. **Group II:** 60 type 2 diabetic patients without peripheral arterial disease recruited from the Diabetes Clinic in Alexandria Main University Hospital. **Group III:** 60 non- diabetic healthy control group with matched age and sex with group **I** and group **II**. All participants gave a written informed consent after explaining the nature and the aim of the study. The healthy control group included those without any chronic cardiovascular or metabolic disease and not receiving any long-term medication for both conditions.

All patients with the following criteria were excluded from the study; patients who had arteriovenous grafts/shunts, with recent vasculitis, patients known to have chronic kidney disease, previous cerebral infarction, coronary artery disease, malignancies, osteoporosis or those receiving systemic glucocorticoids or immune-suppressants.

All participants were subjected to full history taking, complete physical examination including the average of the right and left brachial artery pressures. PAD is defined as an ABI of <0.9 in at least one leg. In addition, full routine Laboratory investigations Blood drawn for metabolic, biochemical and hematological parameters after a 10-12 hours overnight fasting and estimate the following:[(10-13)]Fasting serum glucose, Homeostasis Model Assessment 2 (HOMA2) calculator was used to estimate steady state beta cell function (%B) and insulin resistance (%S) (HOMA-IR) according to the updated computer based HOMA2 mode in subjects with normal or impaired glucose tolerance, Glycated haemoglobin (HbA1C), Serum uric acid, Serum triglycerides, Total serum cholesterol, Low density lipoprotein cholesterol (LDL- Cholesterol), High density lipoprotein cholesterol (HDL-Cholesterol), Serum Osteoprotegerin level by using ELISA. In addition to serum Osteoprotegerin level by using ELISA.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** **(**Armonk, NY: IBM Corp**)** [(14)] Qualitative data were described using number and percent. 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 , F-test (ANOVA): For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons , Mann Whitney test: For abnormally distributed quantitative variables, to compare between two studied groups , Kruskal Wallis test: For abnormally distributed quantitative variables, to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons and Regression: To detect the most independent/ affecting factor for

interpretation.

Results

The studied population included 46.7% males (n=84) and 53.3% females (n=96). The largest age category was people aged from 50 to 60 years which represented 38.9 The mean age for the whole study population was 52.28 ± 9.30.(TableI)

When comparing the three studied groups according to gender distribution, the percentage of males was 50.0%, 43.3% and 46.7% for group I, II, and III respectively.(p=0.764) with no significant statistical differences between the studied groups for gender distribution. The mean age for group I (59.37 ± 5.99) was significantly higher than group II (49.37 ± 7.41) and group III (48.10 ± 9.71) (p<0.001).

The percentage of hypertensive patients was 76.7% and 43.3% for group I and II respectively while the percentage of smokers was 33.3% and 16.7% respectively the p value was <0.001 for both and the differences were statistically significant. Nevertheless, no patient in group III was smoker or hypertensive.

In group I, 56.7% were on insulin alone and 43.3% were on oral medications alone while in group II 13.3% were on insulin and 83.3% were on oral medications. It is worth mentioning that no patients in group I was on both insulin and oral medication while in group II the percentage was 3.3%, patients in group I were more likely to be on insulin while patients in group II were more likely to be on oral medications with p value <0.001. With respect to statins, 56.7% were on this medication in group I while in group II the percentage was 50.0%, p=0.646 The mean duration of illness was 13.80 ± 4.94 and 6.90 ± 3.74 for group I and group II respectively (p<0.001), so duration of illness was significantly higher in group I.

There were significant statistical differences between the three studied groups as regards to BMI, Waist circumference, systolic and diastolic pressure. The mean BMI was 27.17 ± 4.65, 30.57 ± 4.15 and 23.23 ± 0.89 for group I, II and II respectively while the waist circumference was 96.07 ± 7.32, 98.73 ± 5.35 and 90.93 ± 7.24 respectively. The mean systolic BP was 138.0 ± 13.63, 126.7 ± 14.57 and 115.0 ± 5.67 for group I, II and II respectively while the diastolic BP was 89.67 ± 9.56, 81.0 ± 8.38 and 74.83 ± 5.29 respectively. (p<0.001) for all parameters.

While group I was significantly lower as regards to the monofilament score (6.65 ± 1.27) than group II (7.80 ± 1.34) and group III (9.0 ± 0.0), also it was significantly lower as regards to VPT score (22.08 ± 4.01) than group II(17.77 ± 4.) and group III(8410.45 ± 0.91). (Table II) There were also significant statistical differences between the two groups as regards to right, left and average ABI. The mean ABI was 0.63 ± 0.21, 1.12 ± 0.07 and 1.03 ± 0.03 for group I, II and III respectively. (Table III)

There were no significant statistical differences between group I and II with respect to FBG, HbA1c and Uric acid, however, when comparing the three groups together the differences were statistically significant. The differences between all groups were significant as regards to HOMA level. The differences were also significant between the all three groups according to lipid profile except for triglycerides which were significant only when comparing three groups but not Group I and group II together. The mean values are mentioned in details in tables IV and V.

With respect to osteoprotegrin level, group I was significantly higher than group II which was significantly higher than group III. The mean level was 0.32 ± 0.23, 0.14 ± 0.05 and 0.05 ± 0.05 for group I, II and III respectively. (p<0.001)(Table VI) The results of group I as regards to Doppler were as follows; percentage of monophasic, biphasic and triphasic was 33.3%, 60.0% and 0.0% for right foot respectively while for left foot the percentage was 23.3%, 66.7% and 6.7% respectively.

Nine parameters were significantly related to osteoprotegrin when univariate analysis was used namely, age, duration of illness, BMI, blood pressure, monofilament score, VPT score, average ABI, uric acid and LDL. However, only three parameter were significant when multivariate analysis was used which were age, duration of illness and average ABI. (Table VII)

ROC curve analysis of OPG to predict Diabetes with PAD showed that the area under the curve was 89% with 95% confidence intervals (0.835–0.945) the cutoff point was more than 0.1 IU/ml, the sensitivity was 100%, the specificity was 63.33%, the positive predictive value was 73.2%, and the negative predictive value was 100. (Figure 1)

Table (I): Distribution of the studied cases according to demographic data (n = 180)

	n	%
Sex		
Male	84	46.7
Female	96	53.3
Age (years)		
<40	14	7.8
40 – <50	48	26.7
50 – <60	70	38.9
≥60	48	26.7
Min. – Max.	35.0 – 72.0	
Mean ± SD.	52.28 ± 9.30	
Median	54.0	
HTN	72	40.0
Smoker	30	16.7

Table (II): Comparison between the three studied groups according to average of monofilament and VPT

Average	**Group I (n = 60)**	**Group II (n = 60)**	**Group III (n = 60)**	F	**P**
Monofilament					
Min. – Max.	4.0 – 9.0	5.0 – 9.0	9.0 – 9.0		
Mean ± SD.	6.65 ±1.27	7.80 ±1.34	9.0 ±0.0	72.867	<0.001*
Median	6.0	8.0	9.0		
Significance between groups	p1<0.001*,p2<0.001*,p3<0.001*				
VPT					
Min. □Ma□	□□.□□□□.□□	□□.□□□□.□	□.□□□□.□□		
Mean ± SD.	22.08 ±4.01	17.77 ±4.84	10.45 ±0.91	154.50*	<0.001*
Median	22.50	16.50	10.50		
Significance between groups	p_1<0.001*,p_2<0.001*,p_3<0.001*				

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

Group I: Diabetes with PAD Group II: Diabetes without PAD Group III: Healthy control

Table (III): Comparison between the three studied groups according to ankle brachial index (ABI)

ABI	Group I	Group II	Group III	H	P
Right	(n = 56)#	(n = 60)	(n = 60)	143.253*	<0.001*
Min. – Max.	0.20 – 0.84	1.01 – 1.30	1.01 – 1.12		
Mean ± SD.	0.61 ±0.22	1.11 ±0.07	1.02 ±0.03		
Median	0.73	1.12	1.01		
Significance between groups	p1<0.001*,p2<0.001*,p3<0.001*				
Left	(n = 58)#	(n = 60)	(n = 60)	147.874*	<0.001*
Min. – Max.	0.20 – 1.0	1.02 – 1.26	1.0 – 1.13		
Mean ± SD.	0.67 ±0.21	1.13 ±0.07	1.03 ±0.03		
Median	0.75	1.14	1.02		
Significance between groups	p1<0.001*,p2<0.001*,p3<0.001*				
Average				151.388*	<0.001*
Min. – Max.	0.20 – 0.86	1.02 – 1.28	1.01 – 1.13		
Mean ± SD.	0.63 ± 0.21	1.12 ± 0.07	1.03 ± 0.03		
Median	0.75	1.13	1.02		
Significance between groups	p1<0.001*,p2<0.001*,p3<0.001*				

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

Group I: Diabetes with PAD Group II: Diabetes without PAD Group III: Healthy control

Table (IV): Comparison between the three studied groups according to laboratory investigations

Laboratory investigations	Group I (n = 60)	Group II (n = 60)	Group III (n = 60)	Test of Sig.	p
FBS					
Min. – Max.	71.0 – 288.0	80.0 – 289.0	73.0 – 99.0		
Mean ± SD.	159.8 ±60.58	148.8 ±50.12	88.93 ±5.96	H= 99.189*	<0.001*
Median	145.5	141.0	89.0		
Significance between groups	p1=0.599,p2<0.001*,p3<0.001*				
HOMA2					
Min. – Max.	0.50 – 5.50	0.35 – 5.13	0.75 – 0.90		
Mean ± SD.	2.22 ±1.30	1.50 ±1.0	0.83 ±0.05	H= 77.762*	<0.001*
Median	1.93	1.20	0.80		
Significance between groups	p1=0.030*,p2<0.001*,p3<0.001*				
HbA1c					
Min. – Max.	6.10 – 7.90	6.30 – 8.40	1.90 – 5.80		
Mean ± SD.	11.28 ±12.87	11.10 ±13.79	5.03 ±0.66	H= 119.919*	<0.001*
Median	8.30	7.80	5.10		
Significance between groups	p1=0.519,p2<0.001*,p3<0.001				
Uric acid					
Min. – Max.	3.10 – 11.50	2.20 – 7.40	2.40 – 6.0		
Mean ± SD.	5.64 ±1.72	5.16 ±1.29	4.33 ±1.16	F= 13.273*	<0.001*
Median	5.75	5.45	4.35		
Significance between groups	p1=0.160,p2<0.001*,p3=0.004*				

□□□ for AN□□A □es□□Pair□ise □om□arison be□ea□h □gro□□s □as done □sing Pos□Ho□□es□□□□ey□H□H for Kr□s□al □ allis □es□□Pair□ise □om□arison be□□een ea□h □gro□□s □as done □sing Pos□Ho□□es□□D□nn□s for m□1i□le □om□arisons □es□□ □□□ □al□e for □om□aring be□□een □he □hree gro□□s □□□□ □al□e for □om□aring be□□een gro□□I and gro□□II □□□□ □al□e for □om□aring be□□een gro□□I and gro□□III □□□□ □al□e for □om□aring be□□een gro□□II and gro□□III

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Table (V): Comparison between the three studied groups according to lipid profile

Lipid profile	Group I (n = 60)	Group II (n = 60)	Group III (n = 60)	Test of Sig.	p
Cholesterol					
Min. – Max.	81.0 – 286.0	126.0 – 250.0	109.0 – 198.0		
Mean ± SD.	172.6 ±50.64	192.3 ±32.85	171.1 ±20.99	F= 6.208*	0.002*
Median	181.5	200.0	175.0		
Significance between groups	p1=0.011,p2=0.973,p3=0.005*				
Triglycerides					
Min. – Max.	73.0 – 314.0	74.0 – 422.0	49.0 – 161.0		
Mean ± SD.	144.7 ±65.59	145.1 ±75.25	100.5 ±26.19	H= 18.802*	<0.001*
Median	130.5	111.5	98.50		
Significance between groups	p1=0.905,p2=0.973,p3=0.005*				
LDL					
Min. – Max.	24.0 – 174.0	50.0 – 184.0	56.0 – 132.0		
Mean ± SD.	97.73 ±40.10	120.1 ±30.81	106.6 ±20.37	H= 11.316*	0.003*
Median	104.5	124.0	108.5		
Significance between groups	p1=0.002*,p2=0.544,p3=0.010*				
HDL					
Min. – Max.	12.0 – 187.0	28.0 – 66.0	39.0 – 52.0		
Mean ± SD.	45.67 ±29.16	43.20 ±8.35	44.30 ±3.56	H= 4.990	0.082
Median	42.0	41.50	44.50		

Group I: Diabetes with PAD Group II: Diabetes without PAD Group III: Healthy control

Table (VI): Comparison between the three studied groups according to osteoprotegerin

	Group I (n = 60)	Group II (n = 60)	Group III (n = 60)	H	p
Osteoprotegerin **Min. – Max.** **Mean ± SD.** **Median**	 0.20 – 1.30 0.32 ±0.23 0.20	 0.10 – 0.20 0.14 ±0.05 0.10	 0.0 – 0.10 0.05 ±0.05 0.05	 131.693*	 <0.001*
Significance between groups	p1<0.001*,p2<0.001*,p3<0.001*				

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the three groups p1: p value for comparing between group I and group II p2: p value for comparing between group I and group III p3: p value for comparing between group II and group III

Group I: Diabetes with PAD Gro□□II□Diabe□es □i□ho□□PAD Gro□□III□Heal□hy □on□rol

Table (VII): Univariate and multivariate linear regression analysis for the parameters affecting osteoprotegerin for total cases (n = 120)

Osteoprotegerin	Univariate		#Multivariate	
	B(95%C.I)	p	B (95%C.I)	p
Age (years)	0.007*(0.003 – 0.011)	<0.001*	-0.005*(-0.009 - -0.001)	0.013*
Duration	0.021*(0.016 – 0.026)	<0.001*	0.017*(0.007 – 0.027)	0.001*
BMI (kg/m2)	-0.011*(-0.018 – -0.004)	0.002*	-0.001 (-0.008 – 0.005)	0.649
Waist circumference	-0.001 (-0.006 – 0.004)	0.724		
Systolic	0.003*(0.001 – 0.005)	0.004*	0.003 (-0.002 – 0.007)	0.233
Diastolic	0.005*(0.001 – 0.008)	0.008*	-0.004 (-0.011 – 0.002)	0.181
Monofilament	-0.054 (-0.076 - -0.032)	<0.001*	0.008 (-0.024 – 0.040)	0.617
VPT	0.015 (0.009 – 0.021)	<0.001*	-0.003 (-0.012 – 0.007)	0.591
ABI (Average)	-0.421 (-0.510 - -0.332)	<0.001*	-0.319*(-0.473 - -0.165)	<0.001*
FBS	0.000(0.00 – 0.001)	0.476		
HOMA2	0.002 (-0.027 – 0.030)	0.902		
HbA1c	0.00 (-0.002 – 0.003)	0.757		
Uric acid	0.034*(0.013 – 0.056)	0.002*	0.015 (-0.002 – 0.031)	0.080
Cholesterol	-0.001 (-0.002 – 0.00)	0.056		
Triglycerides	0.000 (0.00 – 0.001)	0.352		
LDL	-0.001*(-0.002 – 0.000)	0.015*	0.00 (0.00 – 0.0001)	0.405
HDL	0.000 (-0.002 – 0.001)	0.837		

Beta: Standardized Coefficients C.I: Confidence interval #: All variables with p<0.05 was included in the multivariate

*: Statistically significant at p ≤ 0.05 Both Group I& II Group I: Diabetes with PAD Group II: Diabetes without PAD

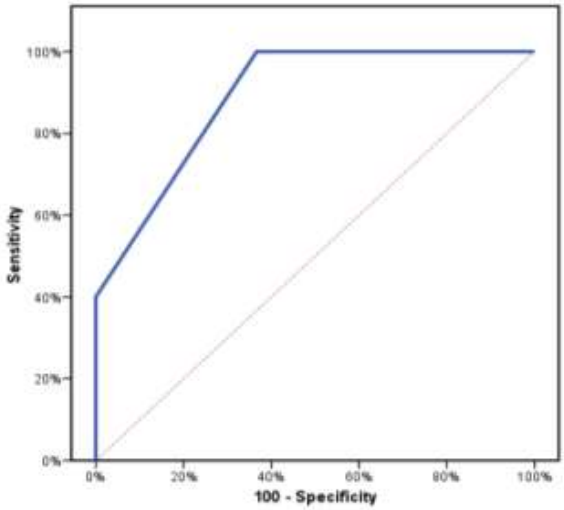

Figure (1): ROC curve for Osteoprotegerin to predict Diabetes with PAD cases vs without PAD (group I vs II)

Discussion

The main finding of the current study is that OPG is significantly higher in diabetic patients with PAD than in patients with DM without PAD.

The first evidence of an involvement of OPG in vascular calcification was derived from genetically altered laboratory animals, which in addition to osteoporosis displayed calcification of the large artery system, resembling vascular lesions of patients, suffering from atherosclerosis. [15, 16] The development of calcification of vessels was completely prevented by restoration of the OPG gene in these animals [15]. The hypothesis of a link between osteoporosis and vascular calcification was suggested also by the fact that in humans low bone mineral density often coincidences with vascular calcification. [17] Subcutaneous injection of OPG was shown to inhibit osteoclastic bone resorption in postmenopausal women. [18] Furthermore, in an animal model of artery calcification induced by Vitamin D intoxication, administration of OPG was able to prevent vascular lesions. [19] A recent clinical study on humans demonstrated elevated serum OPG levels to be an independent risk factor for progressive atherosclerosis and cardiovascular disease. [20] Schoppet et al.[21] and Jono et al.[22] observed a positive correlation of OPG serum levels with the severity of CAD.

Our results are in complete accordance to the study of Ziegler et al [23] which aimed to study the significance of OPG as a marker of PAD. They conducted their study on 67 patients who were undergoing percutaneous transluminal angioplasty (PTA) because of advanced symptomatic PAD of the lower extremities and same number of healthy controls. They found that OPG was significantly higher in patients with PAD than the age matched control. Their study has been different from ours in that they correlated between the severity of PAD and the level of OPG according to PTA unlike the current study which correlated between OPG level and severity of PAD according to ABI and Doppler pattern whether monophasic or biphasic. They found that in patients with PAD, plasma OPG concentrations were significantly higher in subjects with ischemic ulcerations than in those without and were positively correlated with higher severity grade of disease. Therefore, Ziegler et al [23] hypothesized that alterations of the OPG cytokine system may be related also to severe PAD, which represents a further manifestation of generalized artery occlusive disease. However, the origin and the molecular mechanisms by which OPG concentrations are elevated in patients with severe PAD were elusive.

To date, very few studies commented on a potential interaction between both Coronary artery disease (CAD) and PAD on serum OPG. Avignon et al published a study primarily designed to evaluate a number of biomarkers for silent myocardial ischemia (SMI) in asymptomatic patients with diabetes, Out of 6 serum markers of vascular inflammation measured, only OPG was independently predictive of SMI, but this predictive power was lost when the presence of PAD was controlled. [24] This suggests that the co-existence of both conditions could affect OPG levels. Given the established findings of high levels of OPG in patients with diabetes,[23,24] Avignon and his colleagues hypothesized that the combination of diabetes and PAD would be associated with significantly higher serum levels of OPG than either condition alone.[24]

One of the most recent studies that aimed to examine the association between serum OPG levels and both the presence as well as the severity of lower extremity arterial disease in patients with type 2 diabetes is Demková et al. [25] The study was conducted in 2018 and included 165 consecutive patients with T2DM. They differed from our study in that PAD was diagnosed by measurement of the toe-brachial index (TBI) not ABI. Their conclusion was similar to our conclusion which was that serum OPG level is significantly associated with both the presence and severity of PAD in patients with T2D and it might be a biomarker for the presence of atherosclerotic disease in patients with T2DM.

On the other hand, the results of Pennisi et al [26] are not in accordance with our findings. They found no difference in OPG concentrations in 36 patients with ultrasound-defined atherosclerosis of the carotid or femoral artery compared to a group of 30 age and gender matched controls. The reason for this apparently contradictory finding may be that the definition of atherosclerotic disease in this

study was on the basis of ultrasound defined disease in the carotid and femoral arteries; no patient in this study had ABI measurement performed. It is possible that atherosclerotic disease of different vascular beds may not have similar effects on serum OPG.

Elevated OPG serum levels in osteoporosis have been interpreted to result as a counter-regulatory mechanism to protect against bone loss. [27] Similarly, the paradox of increased OPG concentrations in vascular disease could also be interpreted as a compensatory self-defensive mechanism against other factors that promote vascular damage. Therefore, it could be assumed that raised OPG in human atherosclerosis is a response to, rather than a cause, of atherosclerosis, obviously in an attempt to prevent further vascular lesions. On the other hand, cell culture experiments suggested that the protective effect of OPG on the vascular phenotype starts already during the prenatal period, [28] suggesting a later role as a defense against progression of atherosclerosis.[29] Vascular diseases are known to be promoted by immune mediated mechanisms [30] OPG also was shown to be involved in immune responses, i.e. in B-cell maturation or the generation of efficient antibody responses. [31] A further important aspect of the immunological function of OPG is to act as a survival factor for endothelial cells due to its ability to inhibit TNF-related apoptosis inducing ligand (TRAIL)-induced apoptosis of cells.[32] Endothelial cells have an essential barrier function and altered endothelial cell survival may contribute to endothelial dysfunction, which precedes atherosclerosis.[33] It can be hypothesized that elevated OPG concentrations in patients with PAD may well represent an increased production of this molecule by endothelial cells and smooth muscle cells in order to repair advanced plaque calcification.

Hyperglycemia observed in DM modulates OPG release by endothelial cells via a tumor necrosis factor alpha-dependent pathway. [34,35] However, increased OPG concentrations in patients with diabetes are not accompanied by a concomitant increase in its ligand (receptor activator of nuclear factor kappa-B ligand [RANKL]).[36] An imbalance between OPG /RANKL, resulting from an isolated increase in OPG, but not its ligand, might explain why, despite increased concentrations in diabetes, additional vasculo-protective benefits are not seen. In fact, the United Kingdom Prospective Diabetes Study (UKPDS) indicated that tight glycaemic control does not reduce the risk of amputations due to diabetic foot. [37]

Another finding in our study is that OPG was significantly related to age, duration of DM when measured in Patients with DM with or without PAD (120 patients in our study)

As regards the correlation between OPG level and age, our results are in accordance with Ziegler et al [23] who found level of OPG was significantly correlated with the age of the diabetic patients. Szulc et al [38] also agreed with our results and they also suggested that OPG may be an important paracrine mediator of bone metabolism in elderly men. Another study that agreed with the current results i.e. the correlation between age and OPG was that of Pammer et al [39]who found that high OPG not only correlates with old age but also may be a predictor of pathological fracture in elderly. Nevertheless, O'Sullivan et al [40] found that OPG was independent of age in all patients.

With respect to correlation between OPG level and ABI, O'Sullivan et al [40] agreed with the current results and found that OPG correlated negatively with ABI, independent of age, gender, glycaemic status, hs-CRP and IL-6. Esteghamati et al[41] also found that OPG was a significant predictor of disease severity, determined by ABI and percentage of vessel occlusion in univariable and multivariable models. Alkac et al[42] found a weak and inverse correlation between OPG and atherosclerosis as measured by ABI in type 1 diabetics only, but not in type 2 diabetics.

We found that OPG levels correlated with the duration of DM when computing only diabetic subjects, and correlated with FBS. This was consistent with the study conducted by Secchiero and his colleagues[34] as they found a significant correlation between OPG and both FBS and HbA1c. Alshock et al[43] also agreed with our results and found that OPG significantly correlates with duration of DM and FBS. However, Alkac et al [42] found that OPG did not correlate with DM

duration or FBS. Atile et al[44] not only denied a correlation between OPG and FBS or DM duration but also suggested that OPG has no role in the pathogenesis of PAD in diabetic patients.

The explanation of these different results may be that the other studies enrolled a different population with different inclusion criteria than our study like Ziegler et al [23] who enrolled patients with severe symptomatic PAD and Avignon et al[24] whose study material was subjects with both CAD and PAD . They also had different definitions of atherosclerosis diagnosis by ABI. Moreover, the difference in the sample size might have its impact in the statistical significance of the results.

Our ROC curve analysis of OPG to predict Diabetes with PAD showed that the area under the curve was 89% with 95% confidence intervals (0.835–0.945) the cutoff point was more than 100 pg/mL, the sensitivity was 100%, the specificity was 63.33%, the positive predictive value was 73.2%, and the negative predictive value was 100.

Another study evaluated the relationships between vascular risk biomarkers (including classic risk factors and OPG) and coronary artery calcification (CAC) extent in chronic kidney disease (CKD) patients and to establish within the markers the appropriate cut-off value to predict coronary artery calcifications. After adjustment for age, diabetes, smoking and gender, among biological markers, ROC curve analysis showed that the OPG best cut-off value predicting CAC was 757.7 pg/mL. [45]

Another study investigated whether plasma OPG levels were associated with the presence and severity of cerebral atherosclerosis. In multivariate ordinal logistic regression using the number of arteries with cerebral atherosclerosis as dependent variable, plasma OPG > 229.9 pg/mL was a significant predictor for the severity of cerebral vessel atherosclerosis (adjusted OR [95%CI] was 3.20 [1.26–8.12], p = 0.014). [46] Others conducted study aiming to determine whether plasma markers of bone remodeling osteoprotegerin and osteopontin (OPG, OPN) are in relation to endothelial dysfunction, which results in hypertension. They determined optimal cut-off for osteopontin OPN and osteoprotegerin OPG (19.7 ng/mL, 2.7 pmol/L, respectively) for differentiating between the hypertensive and asymptomatic subjects, but the sensitivity and specificity of these tests are not sufficient to use OPG and OPN as sole makers for recognizing endothelial dysfunction. [47]

To the best of our knowledge, the present study is the first to discuss the correlation between the OPG level and severity of PAD among the Egyptian population.

Strengths and Limitations

Our study has a main limitation, which is the relatively small number of studied patients as this may constrain the significance of multivariate adjustment analysis and decrease the strength of the evidence. Other limitation in the study is that the assay used in the present study, unlike some of the previously reviewed studies, [35,40] measures the total amount of OPG and therefore cannot discriminate between the free fraction of OPG and that complexed to RANKL. Ziegler et al [23] used angiography to define patients with PAD which is considered the gold standard investigation, contrary to the current study, which used Doppler that considered a safer procedure for diabetic patients.

The main strength points in the current study is the prospective design and the homogeneity of demographic data of the studied cohort that prevented any bias in the results.

Conclusion:

The current study found that osteoprotegrin (OPG) level was significantly higher in diabetic patients with peripheral arterial disease than both diabetic patients without peripheral arterial disease and healthy adults, so the study had the following conclusions:

1- OPG level is closely related to the presence of peripheral arterial disease and it may have the merit of being an early marker for the disease.

2- OPG level have a correlation with duration of diabetes, age and ankle brachial index ABI

3- The relation between ABI and OPG level may reflect a role of the later as a marker of not only the presence but also the severity of PAD.

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