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Study The Association between Serum Osteoprotegerin and Diabetic Kidney Disease

Mohamed A. Elbana[*1], Nagwa S. Said[1], Ahmed M. Baraka[2], Mayada M. Mousa[1]
Departments of [1]Internal Medicine and [2]Clinical Pathology, Zagazig University, Sharkia, Egypt
***Corresponding author:** Mohamed A. Elbana, **E-Mail:** elbana73@gmail.com

ABSTRACT

Background: Osteoprotegerin (OPG) was first found as being an inhibitor of bone resorption; however, research on OPG in type 2 diabetes mellitus patients and its relationship to albuminuria in Diabetic Kidney Disease (DKD) has been insufficient.

Objectives: Study the association between serum osteoprotegerin and diabetic kidney disease in type 2 diabetic patients.

Patients and methods: The study recruited 45 participants who all had diabetes. Group 1 comprised 15 type 2 diabetic patients with albuminuria that ranged from normal to mildly elevated. The second group consisted of 15 type 2 diabetic patients with mildly elevated albuminuria. Group 3 consisted of 15 type 2 diabetic patients with an extremely high albuminuria level. In all participants, the following was carried out: history taking; clinical examination, including height and weight; ophthalmoscopy; kidney ultrasound; and evaluation of fasting blood glucose; glycosylated hemoglobin; lipid profile; serum creatinine and urea; urinary ACR; and serum osteoprotegerin levels.

Results: Our study showed statistically positive correlation between osteoprotegerin and degree of albuminuria in type 2 diabetic patients. We found that the best cutoff of serum OPG was ≥1.652 in prediction of severely increased albuminuria. **Conclusion:** A biomarker known as OPG may be effective in identifying diabetics with type 2 who are at high risk of developing severe albuminuria.

Keywords: Albuminuria, Diabetes, Osteoprotegerin (OPG).

INTRODUCTION

Twenty to forty percent of patients with diabetes have diabetic kidney damage, which normally arises after a 10-year period of diabetes, but may already be present at diagnosis in those with type 2 diabetes [(1)]. Type 1 and type 2 diabetics who have chronic kidney disease (CKD) are at significantly higher risk of cardiovascular disease and incurring significantly higher medical expenses. It is the primary cause of end-stage renal disease (ESRD) worldwide, necessitating dialysis or kidney transplantation. Cardiovascular disease, kidney disease progression, and mortality are all linked to an individual's level of albuminuria. A more thorough approach to CKD staging is recommended by Kidney Disease: Improving Global Outcomes (KDIGO), which includes albuminuria at all expected glomerular filtration rate (eGFR) levels [(2)].

There has been evidence that the immune system's release of OPG acts as a decoy receptor for the nuclear factor kB receptor activator and TNF-related apoptosis inducing ligand, respectively [(3)].

Serum OPG levels were found to be significantly correlated with insulin resistance, suggesting that OPG is a causative factor in the development of type 2 diabetes. Diabetes mellitus can be prevented or delayed by blocking the OPG/RANK/RANKL system, which has recently been developing. Diabetes mellitus is associated with hepatic insulin resistance [(4)].

The aim of the work was to study the association between serum osteoprotegerin and diabetic kidney disease in type 2 diabetic patients.

PATIENTS AND METHODS

Within Zagazig University Hospitals' Internal Medicine Endocrinology and Diabetes Clinic and Al-Ahrar Teaching Hospital's Nephrology Clinic from January 2021 to December 2021, the research was conducted. There were 45 individuals with type 2 diabetes in our study, divided into three groups of 15 each: The first group included six men and nine women, all of whom had normal to modestly elevated albuminuria (albuminuria less than 30 mg/g creatinine).

There were 15 patients in the second group, all of whom had moderately elevated albuminuria (30–299 mg/g creatinine) with type 2 diabetes. Group 3 included 15 diabetic patients (8 male and 7 female) with severely increased albuminuria (≥300 mg/g creatinine). End stage renal disease, neoplasms and infections were not allowed to participate in the trial. Patients having a history of type 1 diabetes mellitus and congestive heart failure were not allowed to participate in the study.

All participants had their medical histories reviewed, their physical examinations, including their BMI, their systolic and diastolic blood pressures, their hemoglobin levels, their fasting blood glucose levels, their hemoglobin A1c levels, their lipid profiles, their levels of calcium and phosphorus, their levels of parathyroid hormone (PTH), their levels of creatinine and urea, their eGFR using the modification of diet in renal disease (MDRD) equation, their levels of urinary albumin creatinine, serum OPG.

Sampling:

Blood samples: A total of 6 ml of venous blood was obtained and divided as follows under strict aseptic conditions following overnight fasting: 1 ml of whole blood was added to an EDTA-containing sterile tube for the determination of HbA1c and a total of five milliliters of whole blood were allowed to clot at a temperature of 37 degrees Celsius. Centrifugation was used to separate

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the serum into two sterile plastic tubes: Tube 1: serum of 3 ml blood for determination of fasting blood glucose (FBG), urea, creatinine, and lipid profile. Tube 2: serum of 2 ml blood for assay of OPG. Urine samples: 10 ml, second voided morning samples, were collected without preservative, utilized for the determination of the urine albumin creatinine ratio by centrifugation at 3000 rpm for 10 minutes.

Laboratory methods:

The human (OPG) ELISA kits provided by Shanghai Shanghong (SRB) Biotechnology Co. were used to estimate OPG utilizing an enzyme-linked immune sorbent assay.

Ethical consent:

An approval of the study was obtained from Zagazig University and Al-Ahrar Teaching Hospital's Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study.

This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Personal computers and SPSS version 20 were used to gather, tabulate, and analyse the data. For a normally distributed quantitative variable, data were presented as mean ± standard deviation (SD) and the one-way analysis of variance (F-test) was used to assess if there were statistically significant differences across groups. For the non-normal distributed quantitative variable, data were presented as median and interquartile range and Kruskal–Wallis tests were employed to assess if there was a statistically significant difference across many groups. Qualitative variables were presented as frequency and percentage and were compared using a chi-square test. P value < 0.05 was considered significant.

RESULTS

Gender, age, and BMI were all statistically insignificant in our research. The presence of concomitant hypertension differed statistically significantly between the groups investigated. 13.3% of individuals with mild to modesty elevated albuminuria, 40% of those with moderately elevated albuminuria, and 26.7 percent of those with significantly elevated albuminuria were smokers. Diabetic duration was statistically significantly different between the examined groups. There was a statistically significant difference among groups as regard complications **(Table 1)**.

Table (1): Comparison in terms of demographics, anthropometrics, and clinical data, as well as diabetes duration and macrovascular complications of the groups under investigation

	Groups			**P**
	Group 1 (N=15)	**Group 2 (N=15)**	**Group 3 (N=15)**	
Gender: (Frequency and %) **Female** **Male**	 9 (60.0%) 6 (40.0%)	 5 (33.3%) 10 (66.7%)	 7 (46.7%) 8 (53.3%)	0.343
Age (year) (mean ± SD)	56.67 ± 4.27	58.8±6.77	60.87±5.85	0.146
BMI (mean ± SD)	29.47 ± 3.32	28.17± 3.09	29.26 ± 3.87	0.545
SBP (mmhg) **(**mean ± SD)	132.0 ± 13.2	134.67±16.42	142.0±15.21	0.18
DBP (mmhg) **(**mean ± SD)	82.0 ± 6.76	82.87±11.53	84.0±8.9	0.836
Hemoglobin (mean ± SD)	11.09 ± 0.96	11.31 ± 1.87	11.01 ± 1.41	0.852
Diabetes duration (Median, (interquartile range))	4 (1 – 10)	4 (2 – 10)	9 (3 – 23)	<0.001**
Complication (Frequency and %) Diabetic foot IHD PVD None	 0 (0.0%) 0 (0.0%) 0 (0.0%) 15 (100.0%)	 0 (0.0%) 0 (0.0%) 0 (0.0%) 15 (100.0%)	 2 (13.3%) 10 (66.6%) 1 (6.7%) 2 (13.3%)	<0.001**

**: highly significant

Creatinine, UACR, eGFR, and calcium were statistically significantly different between the examined groups (Table 2).

Table (2): Comparison between the studied groups regarding kidney function test, electrolytes, eGFR and PTH

	Groups			p
	Group 1 (mean ± SD)	**Group 2 (**mean ± SD)	**Group 3 (**mean ± SD)	
S. creatinine (mg/dl)	0.8±0.11	1.0 ±0.28	1.1 ± 0.21	<0.001**
S. urea (mg/dL)	46 ± 3.5	49 ± 2.9	52 ± 4.8	0.109
UACR	20 ± 4.9	155 ± 30.3	575 ± 20.3	<0.001**
eGFR (ml/min)	74 ± 8.1	67 ± 6.8	46 ± 3.9	0.005*
Calcium (mg/dL)	8.89 ± 0.4	9.28 ± 0.51	8.66 ± 0.45	0.002*
Phosphorus (mg/dL)	3.69 ± 0.55	3.63 ± 0.38	4.41 ± 1.04	0.016*
PTH (pg/mL)	70 ± 5.6	66 ± 4.3	63 ± 4.1	0.149

*: Significant, **: Highly significant

All of glycosylated hemoglobin creatinine, urea, phosphorus, PTH, HbA1c, and OPG were found to be significantly positively correlated to UACR. On the other hand eGFR had a negative correlation **(Table 3)**.

Table (3): Correlation between UACR and kidney function test and electrolytes and PTH and glycemic profile and lipid profile serum osteoprotegerin

Parameter	UACR	
	r	p
Creatinine (mg/dl)	0.56	<0.001**
Urea (mg/dl)	0.439	0.003*
eGFR (ml/min)	-0.43	0.003*
Calcium (mg/dL)	-0.221	0.145
Phosphorus (mg/dL)	0.391	0.008*
PTH (pg/mL)	0.328	0.028*
RBS (mg/dl)	0.13	0.395
FBS (mg/dl)	0.083	0.5
2 hours postprandial glucose	-0.128	0.402
HbA1c (%)	0.324	0.02*
HDL cholesterol (mg/dl)	-0.032	0.835
LDL cholesterol (mg/dl)	0.074	0.63
Total cholesterol (mg/dl)	0.136	0.373
Triglycerides (mg/dl)	0.004	0.978
OPG (ng/mL)	0.847	<0.001**

There was a statistically significant difference among the studied groups as regard serum osteoprotegerin **(Table 4)**.

Table (4): Comparison between the studied groups regarding serum osteoprotegerin.

	Group1	Group 2	Group 3	p
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
OPG (ng/mL) (mean ± SD)	1.09 ± 0.23	1.43 ± 0.38	2.03 ±0.46	<0.001**

There were significant positive correlations between OPG and diabetes duration, creatinine, urea, UACR, phosphorus, and PTH. While there was a negative correlation between serum osteoprotegerin and eGFR **(Table 5 and figure 1)**.

Table (5): Correlation between serum osteoprotegerin and kidney function test, calcium, phosphorus and parathyroid hormone

Parameter	Serum Osteoprotegerin	
	r	p
Creatinine	0.515	<0.001**
Urea	0.437	0.003*
UACR	0.476	<0.001**
eGFR	-0.426	0.004*
Calcium	-0.289	0.054
Phosphorus	0.514	<0.001**
PTH	0.391$^{\infty}$	0.008*
Diabetes duration	0.474$^{\infty}$	0.001**
FBS	0.173	0.256
2 hours postprandial glucose	-0.051	0.741
HbA1c	0.286	0.383

UACR: Urinary albumin/creatinine ratio, eGFR: Expected glomerular filtration rate, PTH: Parathyroid hormone

The best cutoff of serum OPG was ≥1.652 in prediction of severely increased albuminuria and was ≥1.1975 in prediction of moderately increased albuminuria among diabetic patients **(Tables 6 and 7 and figure 2 and 3)**.

Table (6): Performance of OPG in prediction of severely increased albuminuria among diabetic patients

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≥1.652	0.987	93.3%	90%	82.4%	96.4%	91.1%	<0.001**

Table (7): Performance of OPG in prediction of moderately increased albuminuria among diabetic patients

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≥1.1975	0.889	93.3%	80%	82.4%	92.3%	86.7%	<0.001**

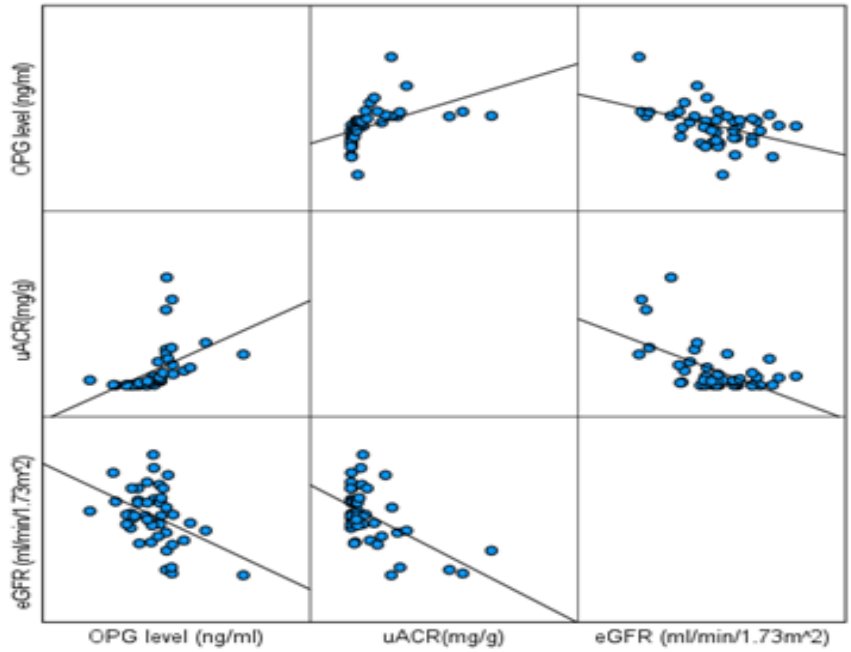

Figure (1): Scatter matrix showing significant positive correlation between serum OPG and UACR and significant negative correlation between eGFR and OPG

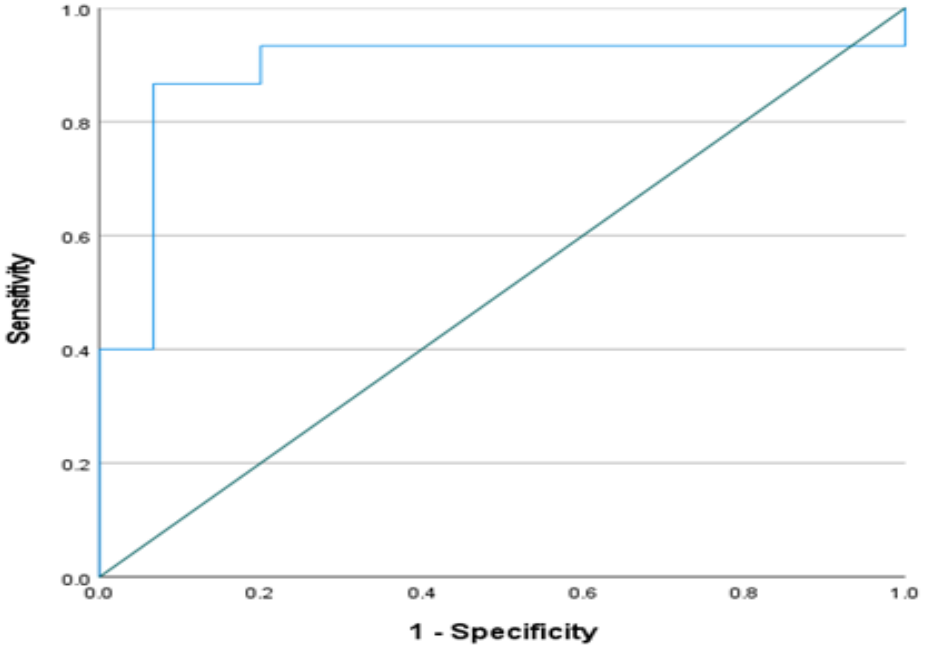

Figure (2): ROC curve showing performance of OPG of moderately increased albuminuria among diabetic patient

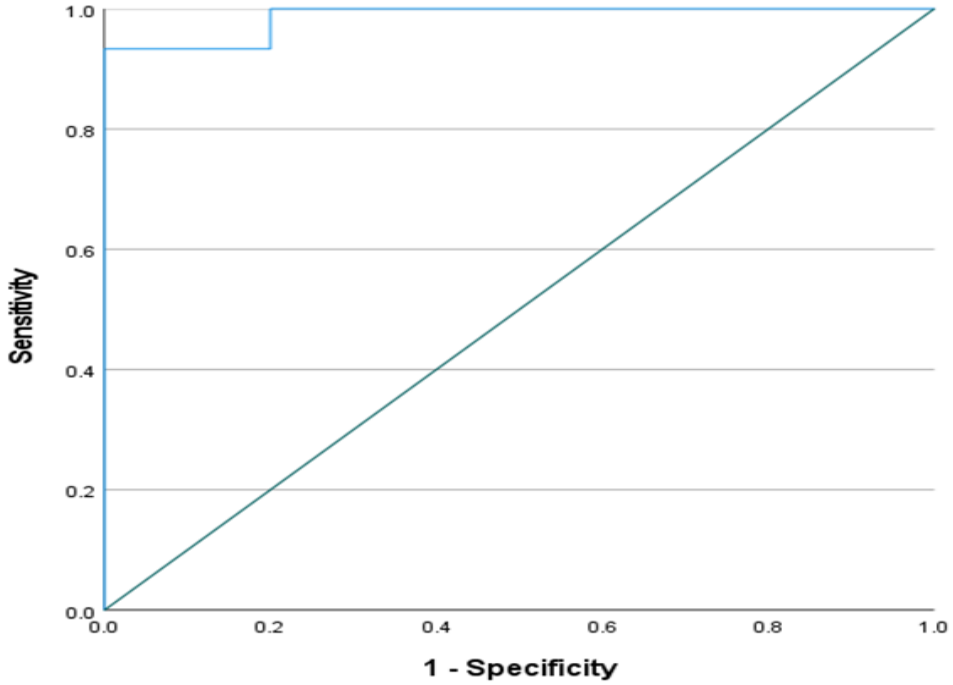

Figure (3): ROC curve showing performance of OPG of severely increased albuminuria among diabetic patients

DISCUSSION

This study has shown that there was a significant correlation between smoking and the degree of albuminuria. These findings were in agreement a study by **Kar *et al.*** [5] who found that there was, in fact, a 2.13 odds ratio between smokers and non-smokers, according to a meta-analysis of 20,056 participants from 13 studies; (95 percent CI 1.32, 3.45).

In our investigation, we found a statistically significant difference in the prevalence of concomitant hypertension among the groups. All patients in group 3 with severely increased albuminuria had comorbid hypertension and in agreement with our results a study by **Shigidi and colleagues**[6] who studied 372 DKD patients and 364 T2DM individuals with NKD and found a significant link between hypertension and albuminuria. Microalbuminuria develops as a result of elevated blood pressure and impaired vascular permeability. Angiotensin II stimulates efferent vasoconstriction and mesangial cell contraction, which favors the progressive emergence of microalbuminuria when the renin-angiotensin-aldosterone system (RAAS) is active, contributing to the development of microalbuminuria [7]**.**

A significant association was identified between albuminuria and diabetes duration in our study, In agreement with our findings a retrospective cohort study by **Wang *et al.*** [8] who followed-up patients with biopsy-proven DKD for at least 1 year. In this study, researchers found a link between diabetes duration and the likelihood of having albuminuria**.** There was a statistically significant difference in cardiovascular problems across the study groups in our research. In keeping with our findings, a research by **Lima *et al.*** [9] in 763 diabetic individuals with CAD found that even in the absence of proteinuria, coronary artery disease was 16 times more likely to occur in people with high blood pressure.

A significant difference was seen between the two groups studied in terms of eGFR and blood creatinine levels in our study. In agreement with our study a prospective cross-sectional study by **Rabbani *et al.*** [10] was conducted on two hundred and seven UAE nationals with type 2 diabetes mellitus, A substantial risk factor for renal disease development as measured by decreased GFR is albuminuria. An increase in urine albumin excretion causes an increase in glomerular filtration rate, as well as an enlargement of the mesangial cells due to the buildup of extracellular matrix (ECM) proteins [10]**.**

However, we found no connection between serum osteoprotegerin and fasting blood sugar, 2 hour postprandial glucose, or glycosylated hemoglobin in our investigation despite the fact that the two variables were statistically significant. In agreement with our study a study by **Moh *et al.*** [11] in the early stages of diabetes mellitus, blood OPG levels were shown to be elevated and to rise with time.

In disagreement with our study, a study by **Niu *et al.*** [12], which revealed a link between elevated serum OPG levels and an increased risk of diabetes in a large Chinese cohort of people aged 40 and older. Osteoprotegerin and creatinine, urea had a statistically significant positive link in our study, but eGFR had a statistically negative correlation. Chronic kidney disease patients had greater OPG levels than age- and gender-matched controls, and this association is linearly related to lower renal function, as demonstrated by **Krolewski *et al.*** [13] in their study of chronic kidney disease patients. Even so, **Reinhard *et al.*** [14] reported that OPG was not linked with serum creatinine in patients with Type 2 Diabetes Mellitus (T2DM).

OPG and hypertension had a strong, statistically significant positive relationship in our research, this was in agreement with **Tsioufis *et al.*** [15] who showed that since OPG levels were significantly higher in hypertensive patients than in normotensives, there is a correlation between hypertension and OPG levels, OPG values were higher in patients diagnosed and medically treated for hypertension compared to patients who were not diagnosed and were not being treated for hypertension. Patients with type 2 diabetes may have higher systolic blood pressure as a result of arterial stiffness and vascular calcifications, which may be related to the broad matrix changes, including OPG deposition in the arterial wall [16].

In our research, we identified a statistically significant link between serum osteoprotegerin levels and the degree of albuminuria, as well as a statistically significant difference among the three study groups. Mean OPG were 1.09 in group 1, 1.43 in group 2 and 2.03 ng/mL in group 3. We found that the best cutoff of serum OPG was ≥1.652 in prediction of severely increased albuminuria.

CONCLUSION

A biomarker known as OPG may be effective in identifying diabetics with type 2 who are at high risk of developing severe albuminuria.

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