Serum osteoprotegerin as an early marker of chronic kidney disease in hypertensive patients

Khaled A. Elhefnawy[a], Ezzat M. Mohamed[a], Athar M. Abou Yousef[c], Abeer A. Fikry[b], Hala M. Allam[a]

Departments of [a]Internal Medicine, [b]Clinical Pathology, Faculty of Medicine, Zagazig University, Zagazig, [c]Department of Internal Medicine, Shebin Elkom Fever Hospital, Shebin Elkom, Egypt

Correspondence to Khaled A. Elhefnawy, MD, Department of Internal Medicine, Faculty of Medicine, Zagazig University, Zagazig, 44519, Egypt. Mob: +20100779790;
e-mail: kelhefnawy@gmail.com

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Background
Serum osteoprotegerin (OPG) is a marker of cardiovascular disease. The deterioration of renal function in diabetic patients could be predicted by OPG concentrations, and it was suggested that OPG could be used as a risk marker for chronic kidney disease (CKD) in patients with hypertension.
Objective
The aim was to investigate the role of serum OPG as an early marker of CKD in hypertensive patients.
Patients and methods
A total of 144 hypertensive patients were included and classified into two main groups: group I, which included 72 hypertensive patients without CKD [estimated glomerular filtration rate (eGFR) $\geq$60 ml/min/1.73 m^2], and group II, which included 72 hypertensive patients with CKD (eGFR$<$60 ml/min/1.73 m^2). All participants of the study were subjected to detail clinical examination and investigations including complete blood count, fasting and random blood glucose, glycosylated hemoglobin, urine analysis, serum creatinine, liver function tests, eGFR, and serum OPG assays.
Results
Serum OPG levels were significantly higher in hypertensive group with CKD than in hypertensive group without CKD. A significant positive correlation was found between OPG and diastolic blood pressure, duration of hypertension, serum creatinine, and albumin creatinine ratio, whereas there is a negative correlation between OPG and both weight and eGFR. The best cutoff value of serum OPG in prediction of CKD among hypertensive patients is greater than 1.9 ng/ml with sensitivity of 100% and specificity of 100%.
Conclusion
Serum OPG can serve as an early marker for CKD in hypertensive patients.

Keywords:
chronic kidney disease, hypertension, marker, osteoprotegerin

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Introduction

Chronic kidney disease (CKD) is a major health problem all over th world, is associated with increased morbidity and mortality, and is defined as abnormalities of kidney structure or function, present for greater than 3 months. Because the initial stages of CKD can be asymptomatic, its early diagnosis is difficult [1].

Undiagnosed and untreated CKD may gradually progress to end-stage renal disease, and necessitate renal replacement therapy to maintain the patient's life. Earlier stage CKD can lead to several complications, such as anemia, bone mineral metabolism disorders, and cardiovascular events [2].

Hypertension is a chronic medical condition and is the most common preventable risk factor for cardiovascular disease (CVD) [3].

Osteoprotegerin (OPG) is a circulating glycoprotein that acts as a cytokine decoy receptor and antagonizes receptor activator for nuclear factor κB ligand (RANKL) and tumor necrosis factor-related apoptosis-inducing ligand [4]. Initially, owing to its ability to block RANKL and to inhibit bone reabsorption, OPG was considered as one of the key regulators of bone turnover. Since then, it has become increasingly clear that OPG exerts also other actions, involving the immune and the cardiovascular system [5].

OPG is a risk marker of CVDs [6]. OPG levels are positively correlated with markers of vascular damage,

such as endothelial dysfunction, vascular stiffness, and coronary calcification, as well as with the presence and severity of coronary artery disease. In addition, OPG is associated with the risk of future coronary artery disease and heart failure and with the incidence of cardiovascular and all-cause mortality in patients with coronary artery disease and also in the general population [7].

Current laboratory markers for CKD such as proteinuria, creatinine clearance, and serum creatinine are not ideal for early diagnosis. There is an increasing interest in identifying other markers that might give sensitive and rapid means of early diagnosis of CKD in hypertensive patients. So we conducted this study to investigate the role of serum OPG as an early marker of CKD in hypertensive patients.

Patients and methods

Study design

A case–control study was carried out among patients with hypertension attending the Nephrology Unit of Zagazig University Hospitals. The study was approved by the local Institutional Ethics Committee of Faculty of Medicine, Zagazig University, and conformed to the Helsinki Declaration and has been conducted in the Departments of Internal Medicine and Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt.

Participants and groups

A total of 144 hypertensive patients were included after their written and informed consents and classified into two main groups: group I, which included 72 hypertensive patients without CKD [estimated glomerular filtration rate (eGFR)≥60 ml/min/1.73 m^2], and group II, which included 72 hypertensive patients with CKD (eGFR<60 ml/min/1.73 m^2). Patients with hepatitis or liver cirrhosis, patients with diabetes mellitus, patients with history of nephrotoxic exposure, patients with congenital or intrinsic renal diseases, patients with systemic illness associated with renal damage, patients with chronic infections or malignancy, and pregnant or lactating women were excluded from the study.

Physical examination and measurements

All participants of the study were subjected to the following: first, full history taking, second, thorough physical examination, and third, investigations (to verify the inclusion and exclusion criteria of studied subjects), including first, routine investigations such as complete blood count (by Sysmex KX21N, Sysmex America, Inc., One Nelson C. White Pkwy, Mundelein, IL 60060, USA), fasting and random blood glucose, glycosylated hemoglobin, serum creatinine, liver function tests, and calculation of eGFR using modification of diet in renal disease equation: eGFR (ml/min/1.73 m^2)= $175\times(Scr)^{-1.154}\times(Age)^{-0.203}\times(0.742$ if female) [8]. Complete urine analysis by uriscane analyzer, determination of urinary albumin excretion (UAE) and creatinine then measuerment of albumin creatinine ratio (ACR), and urinary albumin were determined by Immunoturbidimetric assay, these parameters were measured by Cobas 8000 (Roche Diagnostics, California, USA). Second, Specific investigations included serum OPG, which was measured by ELISA Kit that was provided by Bioassay Technology Laboratory(Shanghai, China) (http://www.bt-laboratory.com).

Ethical issues

The study was approved by the local Institutional Ethics Committee of Faculty of Medicine, Zagazig University and conformed to the Helsinki Declaration. The aim of the study was explained, and informed consents were obtained from the patients.

Statistical analysis

All data were collected, tabulated, and statistically analyzed using SPSS 24.0 for windows (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean±SD (Standard deviation). Analysis of variance by ANOVA and post hoc analysis with LSD tests were applied for comparing differences among groups. Qualitative data were expressed in the form of numbers and percentages, and comparison between data was performed by using the χ^2-test. The correlation between variables was calculated using the Pearson's and the Spearman correlation tests. Receiver operating characteristic curve analysis was used to identify optimal cutoff values of serum OPG with maximum sensitivity and specificity for prediction of the disease. Accuracy was measured by the area under the receiver operating characteristic curve. The criterion for statistical significance was set at P less than 0.05.

Results

There is no statistically significant difference between the two study groups regarding sex distribution, age, and systolic blood pressure (SBP), whereas there are statistically significant differences between both groups regarding frequency of albuminuria, CKD stages, weight, diastolic blood pressure (DBP), duration of

Table 1 Basic characteristics of the study patients

Basic characteristics	Without CKD (*N*=72) [*n* (%)]	With CKD (*N*=72) [*n* (%)]	Test	*P*
Sex				
Male	44 (61.1)	34 (47.2)	1.894[a]	0.434
Female	28 (38.9)	38 (52.8)		
Albuminuria				
Absent	72 (100)	20 (27.8)	50.143[a]	<0.001
Present	0	52 (72.2)		
CKD stage				
Stage 0	72 (100)	0		
Stage 3	0	38 (52.8)	77.010[a]	<0.001
Stage 4	0	22 (30.6)		
Stage 5	0	12 (16.7)		
Age (years) (mean±SD)	51.08±9.58	51.04±8.18	–0.198[b]	0.706
Weight (kg) (mean±SD)	74.34±8.52	62.12±9.41	4.862[c]	<0.001
SBP (mmHg) (mean±SD)	129.5±11.87	131.61±14.9	–0.886[b]	0.321
DBP (mmHg) (mean±SD)	80.31±8.42	86.10±9.32	–2.567[b]	<0.01
HTN duration (years) (mean±SD)	5.21±2.11	13.11±7.12	–5.978[b]	<0.001
Serum creatinine (mg/dl) (mean±SD)	0.82±0.12	2.67±1.79	–6.985[b]	<0.001
ACR (mg/g) (mean±SD)	0±0	5.65±3.73	–5.982[b]	<0.001
eGFR (ml/min/1.73 m^2) (mean±SD)	82.92±16.20	30.59±15.32	14.653[c]	<0.001
Osteoprotegerin (ng/ml) (mean±SD)	0.912±0.492	5.132±3.304	–7.276[b]	<0.001

ACR, albumin-creatinine ratio; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HTN, hypertension; SBP, systolic blood pressure. [a]χ^2-test. [b]Mann–Whitney *U*-test. [c]Independent samples Student *t*-test. $P<0.05$ is significant.

Table 2 Correlation between serum osteoprotegerin and different variables

Variables	Osteoprotegerin (ng/ml)	
	r	*P* value
Age (years)	+0.005	0.845
Weight (kg)	-0.530	<0.001
SBP (mmHg)	+0.178	0.125
DBP (mmHg)	+0.330	0.005
HTN duration (years)	+0.812	<0.001
Serum creatinine (mg/dl)	+0.961	<0.001
eGFR (ml/min/1.73 m^2)	-0.990	<0.001
ACR (mg/g)	+0.701	<0.001

ACR, albumin-creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HTN, hypertension; *r*, Spearman's rank correlation coefficient; SBP, systolic blood pressure. $P<0.05$ is significant.

hypertension, serum creatinine, ACR, eGFR, and osteoprotegerin level (Table 1).

A significant positive correlation is found between OPG and DBP, duration of hypertension, serum creatinine, and ACR, whereas there is a negative correlation between OPG and weight and eGFR (Table 2).

A non-statistically significant difference is found regarding SBP between hypertensive patients without CKD and hypertensive patients with CKD with stage 3, stage 4, and stage 5, whereas there is a statistically significant difference found regarding DBP, duration of hypertension, ACR, and serum OPG between hypertensive patients without CKD and patients with CKD with stage 3, stage 4, and stage 5 (Table 3).

Table 4 revealed diagnostic performance of weight (kg), DBP (mmHg), duration of hypertension (years), and OPG (ng/ml) in predication of CKD among hypertensive patients, where weight less than or equal to 65 kg had 64% sensitivity and 80% specificity, DBP greater than 80 mmHg had 58.3% sensitivity and 69.2% specificity, duration of hypertension greater than 7 years had 86% sensitivity and 91.58% specificity, and OPG greater than 1.9 ng/ml had 100% sensitivity and 100% specificity. OPG showed the highest predictive value for CKD development in hypertensive patients, as they obtained the highest areas under the curve.

Discussion

Hypertension is one of the main health problems affecting a large number of population and associated with increased cardiovascular morbidity and mortality [9].

Intensive research studies have been done to identify the interplay between hypertension and CKD. Molecular genetic analyses have clarified the relationships between mild and moderate essential hypertension and the initiation of CKD in nondiabetic patients. Hypertension is a common reported cause of end-stage renal disease requiring

Table 3 Comparison of different variables between hypertensive patients without chronic kidney disease and chronic kidney disease group with different stages

Variables	Without CKD (*N*=72) (mean±SD)	With CKD (*N*=72) (mean±SD)			Test[a]	*P*
		Stage 3 (*N*=40)	Stage 4 (*N*=22)	Stage 5 (*N*=10)		
SBP (mmHg)	129.5±11.87	131.21±15.1	132.57±14.94	133.32±15.05	1.003	0.789
DBP (mmHg)	80.31±8.42	86.74±9.55	87.30±6.46	82.62±12.21	7.972	0.032
HTN duration (years)	5.21±2.11	13.3±6.80	13.2±6.32	18.15±8.48	47.002	<0.001
ACR (mg/g)	0±0	5.04±3.8	5.8±3.61	1.9±1.60	40.053	<0.001
OPG (ng/ml)	0.912±0.492	2.701±0.452	4.801±2.162	11.076±0.980	59.984	<0.001

ACR, albumin-creatinine ratio; CKD, chronic kidney disease; DBP, diastolic blood pressure; HTN, hypertension; OPG, osteoprotegerin; SBP, systolic blood pressure. [a]Kruskall–Wallis *H* test. $P<0.05$ is significant.

Table 4 Diagnostic performance of different variables and osteoprotegerin in predication of chronic kidney disease among hypertensive patients

Cutoff values	SN (%) (95% CI)	SP (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Accuracy (95% CI)	AUC (95% CI)	*P*
Weight ≤65 kg	64% (46.2–79.2)	80% (64–91.8)	77% (57.3–90.3)	69% (52.9–82.4)	72% (55.1–85.5)	0.755 (0.641–0.850)	<0.001
DBP >80 mmHg	58.3% (40.8–74.5)	69.2% (51.9–83.7)	65.4% (46.8–81.4)	62.5% (45.6–77.5)	63.7% (46.4–79.1)	0.671 (0.551–0.778)	0.005
Duration >7 years	86% (70.5–95.3)	91.58% (77.5–98.2)	91.19% (76.3–98.1)	86.71% (71.7–95.7)	88.8% (74–96.8)	0.959 (0.891–0.994)	<0.001
OPG >1.9 ng/ml	100% (90.3–100)	100% (90.3–100)	100% (90.3–100)	100% (90.3–100)	100% (90.3–100)	1.000 (0.950–1.000)	<0.001

95%CI, 95% confidence interval; AUC, area under curve; NPV, negative predictive value; PPV, positive predictive value; SN, sensitivity; SP, specificity. $P<0.05$ is significant.

renal replacement therapy. Thus, the 'hypertension-induced' nephropathy has gained great concerns by clinicians, as proper control of hypertension can delay or stop the progression of nephropathy in nondiabetics [10].

Routine screening for CKD in hypertensive patients at diagnosis and regularly is essential to perform the proper interventions that can delay or prevent the progression of CKD to advanced stages and to decrease the risk of CVD and other complications of renal function loss [11].

Many hypertensive patients with CKD stay undiagnosed and have no benefits from the proper interventions. Moreover, the diagnosis of CKD is by the elevated levels of blood urea and serum creatinine, which have low predictive values; therefore, there is a need for other biomarkers with high diagnostic performance in the diagnosis of renal diseases [12].

OPG is a circulating glycoprotein that acts as a cytokine decoy receptor and antagonizes receptor activator for RANKL and tumor necrosis factor-related apoptosis-inducing ligand. Initially, owing to its ability to block RANKL and to inhibit bone reabsorption, OPG was considered as one of the key regulators of bone turnover. Then, it has become increasingly clear that OPG exerted also other actions, involving the immune and the cardiovascular system [6].

It has been reported that OPG can predict the decrease of kidney function, vascular events, and cardiovascular disorders [13] and was increased in diabetic patients [14].

The current published literature showed inconsistencies regarding the role of OPG in the prediction of CKD among different types of population. Therefore, we conducted this study to evaluate the relationship between OPG and CKD in patients with essential hypertension and its possibility as an early marker of CKD.

Our findings showed that there was no significant difference between the two study groups regarding sex distribution, age, and SBP, whereas there were significant differences between both groups regarding frequency of albuminuria, CKD stages, weight, DBP, duration of hypertension, serum creatinine, ACR, eGFR, and OPG levels. These results were in agreement with previous studies [15,16].

There was a significant positive correlation found between OPG and DBP, duration of hypertension, serum creatinine, and ACR, whereas there was a negative correlation between OPG and weight and eGFR. These findings were also reported in other studies [15,17].

Such significant correlations can be linked through the severity of CKD. The severity of weight loss and high

blood pressure increased steadily with the progression of CKD. On the contrary, OPG showed a significant trend of the increasing level with more severe forms of CKD. Thus, it may be reasonable to assume that the correlations among OPG, hypertension, and weight loss are due to their associations with the severity of CKD.

The exact mechanisms of this possible association between OPG and CKD are not yet fully understood. Possible explanations for increased serum concentration of OPG with CKD may include decreased clearance of OPG or increased inflammatory activity. Inflammatory mediators are known to promote the production of OPG. As renal failure is associated with increased inflammatory activity, it is likely that inflammatory responses may partly explain increased serum OPG in CKD.

The diagnostic performance of weight (kg), DBP (mmHg), duration (years), and OPG (ng/ml) in predication of CKD among hypertensive patients revealed that weight less than or equal to 65 kg had 64% sensitivity and 80% specificity, DBP greater than 80 mmHg had 58.3% sensitivity and 69.2% specificity, duration of hypertension greater than 7 years had 86% sensitivity and 91.58% specificity, and OPG greater than 1.9 ng/ml had 100% sensitivity and 100% specificity. OPG showed the highest predictive value for CKD development in hypertensive patients, as they obtained the highest areas under the curve. Previous studies have demonstrated similar results [15–17].

Conclusion

This study showed that serum OPG was significantly associated with the presence of CKD in hypertensive patients, independently from other variables, where it might have a higher predictive value than that of hypertension for CKD development. So it could be suggested as an early marker for hypertension-induced CKD.

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Conflicts of interest

There are no conflicts of interest.

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