Study of Osteoprotogerin in Chronic Kidney Disease Patients with Vascular Calcification

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Abstract: Objective: The aim of this work is to assess osteoprotogerin level in chronic kidney disease patients to determine its role in the occurrence of vascular calcification. **Background**: Vascular calcification is common in CKD. OPG levels are higher in patients with chronic kidney disease. **Patients and methods:** patients with chronic kidney disease underwent OPG levels assessment and determine its relation to vascular calcification. Serum OPG levels was compared to those of healthy individuals used as controls. An OPG levels, CCIMT and densitometry scans were measured. The effects of age, BMI, MBP were analyzed. **Results**: The osteoprotogerin levels of eighty patients were analyzed; there was a significant increase in its levels. However, OPG levels remained higher compared to controls. There was no significant impact of age alone. **Conclusion**: There is a considerable increase of osteoprotogerin levels in hemodialysis patients compared to those on conservative therapy. Several factors may have specific effects on osteoprotogerin levels.

[Ahmed Rabee Elarbagy, Hany Said Elbarbary, Ahmed Ragheb Tawfeek and Aya Ali Abdulla Elhanafy. **Study of Osteoprotogerin in Chronic Kidney Disease Patients with Vascular Calcification.** *Stem Cell* 2017;8(3):68-73]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 10. doi:10.7537/marsscj080317.10.

Keywords: chronic kidney disease, osteoprotogerin, vascular calcification.

1. Introduction:

Chronic kidney disease is a worldwide public health problem and is recognized as a common condition that is associated with an increased risk of cardiovascular disease and chronic renal failur**e (1).**

Osteoprotogerin (OPG) is a glycoprotein that acts as a decoy receptor for receptor activator of nuclear factor _B ligant (RANKL) and tumor necrosis factor – related apoptosis-inducing ligant. The OPG\ RANKL\ receptor activator of nuclear factor axis plays an important regulatory role in skeletal, immune, and vascular system **(2).**

The researchers reported association between levels of circulating OPG and vascular calcification. Such an association with the presence of abdominal aortic calcification, a known risk factor in the development of abdominal aortic aneurysms **(3).**

OPG levels are significantly higher in patients with chronic kidney disease compared with age and sex matched controls and increasing OPG levels have a liner relationship with worsening renal function **(4).**

The aim of this work was to study the relationship between the serum osteoprotogerin level and arterial stiffness in patients with chronic kidney disease on conservative treatment versus chronic kidney disease on regular hemodialysis therapy.

2. Patients and methods

All subjects gave written informed consent before inclusion into the study. Eighty subjects diagnosed to have chronic kidney disease in our hospital (outpatient clinic of Internal medicine and hemodialysis unit-Menoufyia university hospital) was offered osteoprotogerin assessment as a prospective evaluation.

We were included in our analysis the subjects who was treated conservatively and for whom on hemodialysis.

Controls: Twenty subjects of age and sex matched (but nonsmoking) healthy volunteers without any known diseases were used as control.

Subjects were excluded because of one or moreof the following reasons:

Acute infection, Connective tissue disease, Malignancy, History of thyroid gland dysfunction, Recent myocardial infarction, Recent major trauma less than six months and oral anticoagulants. From each subjects, under complete aseptic technique, five ml venous blood samples were collected. Samples were allowed to clot, then were centrifuged at one thousand xg for ten minutes within one hour after collection. A part of separated serum is aliquot and stored frozen at minus twenty c for subsequent determination of serum osteoprotogerin. The kit assay Human OPG in the sample, use purified Human OPG anti body to coat micro titer plate wells, make solid – phase antibody, then add OPG to wells, combined OPG antibody which with enzyme labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, add substrate, substrate becomes blue color. At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of forty hundred and fifty nm. The concentration of Human OPG in the samples is then determined by comparing

the optic density of the samples to the standard curve. Use a serum separator tube and allow samples to clot for ten to twenty minutes at room temperature before centrifugation for twenty minutes at the speed of two thousands to three thousands r. p. m. remove serum and assay immediately or aliquot and store samples at minus twenty centigrade. Centrifuge the sample again after thawing before the assay. Measurement of intimal_medial thickness of carotid arteries through ultrasonographic examination of carotid arteries using duplex probe (eleven L) of G E Divide nine ultrasound imaging system. This was done for the right and left common carotid arteries. Longitudinal views of the layers of the normal carotid wall demonstrate two nearly parallel echogenic lines separated by a hypo echoic to anechoic region. The first echo, bordering the vessel lumen, represents the lumen intima interface, the second echo caused by the media adventitia interface. The media is the anechoic hypo echoic zone between the echogenic lines. The distance between these two lines represents the combined thickness is up too.6mm**.** Bone densitometry was performed with a discovery W model Hologic densitometer with fan beam technology, emitting 100 and 140 kV X-rays with 128 detectors and 2mm resolution.

Statistical analysis

All data were collected, tabulated and statistically analyzed using personal computer using Microsoft Excel 2010 and SPSS v.20 for Microsoft Windows 7.

Two types of statistics were done:

Descriptive statistics [e.g. mean (x) and standard deviation (SD)],

Analytic statistics: which include the following tests.

Student's t – test: is a test of significance for comparison between two quantitive variables.

f test (ANo VA- analysis): is a test of significance for comparison between three quantitative variables.

The MANN-Whitney one of two samples of independent observations tends to have larger values than the other.

Correlation coefficient test (person test): is a test of significance for correlation between two quantitative variables. Correlation coefficient test (person test) results may be positive (+) correlation (reverse) or negative (-) correlation (inverse).

3. Results

Table (1) shows that**:** Clinical characteristics of the studied groups: There is no significant increase as regard age of patients in conservative group compared to other groups (P<o.1). There is highly significant increase of disease duration of patients in conservative group compared to HD group. There is significant increase of the numbers of hypertensive patients in HD group compared to other groups.

Table (1): Personal data of studied groups (N=1oo):

Studied variables	Studied groups						χ^2	P value
	Control (n=2o)		CKD (n=4o)		dialysis (n=4o)			
	No.	%	No.	%	No.	%		
Age /years: $\bar{X}$ ±SD Range	39.1±2.26 24- 6o		4o.7±12.7 23 - 7o		45.6±13.5 23 - 7o		2.34#	o.1o1
Gender:								
- Male	11	55	29	72.9	25	62.5	1.98	o.371
- Female	9	45	11	27.5	15	37.5		
Diabetes mellitus								
- Positive	o	o.o	4	1o.o	5	14.3	2.36	o.269
- Negative	2o	1oo	36	9o.o	35	87.5		
Hypertension								
- Positive	o	o.o	5	12.5	5	12.5	2.77	o.249
- Negative	2o	1oo	35	87.5	35	87.5		
IHD								
- Positive	o	o.o	4	1o	3	7.5	2.o7	o.355
- Negative	2o	1oo	36	9o	37	92.5		
Duration on dialysis $\bar{X}$ ±SD					43.6±17.1			

X=mean, SD=standard deviation, P=paired t test

Table (2) shows that There is no significant of serum calcium in the control group (P1:o.997). There is highly significant increase as regard serum phosphorus in HD group compared to control group (P2:o.o13). There is highly significant decrease as regard alkaline phosphatase in the control group compared to other groups (P:o.oo1).

Table (3): There is significant negative correlation between blood urea and serum creatinine in conservative group with arterial stiffness (r: -o1oo,-o.258 respectively) (P:o.o21). There is significant negative correlation between eGFR and serum OPG in conservative group with arterial stiffness (r:-o.25o) (P: o.o25). There is no significant correlation between serum OPG and ca, phosphorus, alkaline phosphatase, albumen and intact PTH. There is highly significant positive correlation between mean CCMT and serum OPG among the conservative group with arterial stiffness (r:o.558)

Table (4): There is highly significant increase as regard serum OPG in HD group compared to other groups (P2:o.oo1). There is highly significant increase of mean CCIMT in HD group (P: o.oo1). There is highly significant increase of abdominal aortic calcification in HD group (P: o.oo1).

Table (5): There is significant negative correlation between eGFR and mean CCMT in studied groups with arterial stiffness (r:-o.354) (P: o.oo1). There is highly significant positive correlation between mean CCMT and serum OPG among the studied groups with arterial stiffness (r:o.558) (P:o.oo1).

Table (2): Laboratory investigation of studied groups (N=1oo):

Studied variables	Studied groups			K	Post hoc test
	Control (n=2o)	CKD (n=4o)	dialysis (n=4o)		
	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD		
Calcium	8.91±o.53	9.1o±6.54	8.68±o.9o	14.1	P1:o.997 P2:o.536 P3:o.971
Phosphate	3.94±o.52	4.59±1.o8	6.57±7.84	15.2	P1:o.118 P2:**o.o13*** P3:o.322
Alkaline phosphatase	8o.o±25.6	216.6±143.4	245.o±131.3	39.5	**P1:o.oo1**** **P2:o.oo1**** P3:o.737
Albumin	4.24±o.41	4.o6±2.98	3.54±o.47	2o.6	P1:o.976 P2:**o.oo1**** P3:o.629
Intact Parathyroid	28.o±12.3	98.1±6o.6	112.5±79.2	31.5	**P1:o.oo1**** **P2:o.oo1**** ***P3:o.*oo1**

*Significant ** highly significant
X=mean, SD=standard deviation, P=paired t test

Table (3): Correlation between serum Osteoprotogerin and laboratory investigations of cases (N=8o):

Studied variable	Osteoprotogerin	
	r	P value
Urea	-o.1oo	o.377
Creatinine	o.258	**o.o21***
Calcium	o.133	o.24o
Phosphate	-o.o13	o.9o6
Alkaline phosphatase	o.oo9	o.938
Albumin	o.o76	o.5o5
Intact Parathyroid	-o.136	o.23o
Estimated GFR	-o.25o	**o.o25***
Mean CCIMT	o.558	**o.oo1****

*Significant, ** highly significant, (**r**) confident , X=mean, P=paired t test
Mean CCIMT: Mean common carotid intima media thickness

Table (4): Osteoprotogerin and mean CCIMT of studied groups:

Studied variables	Studied groups			K	Post hoc test
	Control (n=2o)	CKD (n=4o)	dialysis (n=4o)		
	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD		
Osteoprotogerin	136.3±21.8	22o.7±59.1	3o2.8±165.1	37.9	**P1:o.oo7*** **P2:o.oo1**** P3:**o.oo1****
Mean CCIMT	o.48±o.o7	o.67±o.15	o.82±o.13	54.7	**P1:o.oo1**** **P2:o. oo1**** **P3:o.oo1****
Length of AAC	No calcification	1-2 vertebra	More than 3 vertebra	48.6	**P1:o.oo1**** **P2:o. oo1**** **P3:o.oo1****

*Significant, ** highly significant, X=mean, SD=standard deviation, P=paired t test, Mean CCIMT: Mean common carotid intima media thickness, AAC= abdominal aortic calcification

Table (5): Correlation between mean CCIMT and laboratory investigations of cases (N=8o):

Studied variable	Mean CCIMT	
	r	P value
Urea	-o.o44	o.7oo
Creatinine	o.164	o.145
Calcium	o.o17	o.882
Phosphate	-o.15o	o.184
Alkaline phosphatase	-o.o73	o.52o
Albumin	-o.o62	o.584
Intact parathyroid	-o.1o4	o.361
Estimated GFR	-o.354	**o.oo1****
osteoprotogerin	o.558	**o.oo1****

*Significant, ** Highly significant, **(r)** confident,
Mean CCIMT: Mean common carotid intima media thickness

4. Discussion

It is worth noting to point out that the results of our study have shown that the plasma OPG concentrations were elevated early in CKD patients with further increase in HD patients.

We also demonstrated a positive correlation between OPG and CCIMT a marker of vascular calcification.

We also demonstrated a positive correlation between OPG and abdominal aortic calcification as a marker of vascular calcification.

Therefore, it can be concluded that the plasma OPG may be used as a simple, rapid, easy to perform and interpret test for predication of vascular calcification in patients with chronic kidney disease.

In the current work, there was a highly significant increase of OPG levels in both conservative and HD groups compared to control group.

In a cross sectional study by **Nishiura R. (5)** performed in one hundred and two hemodialysis patients, investigating serum osteoprotogerin levels, serum osteoprotogerin level were higher in patients on hemodialysis when compared with other populations.

Lieb (6) reported that OPG was higher in women compared with men and positively related to age, smoking, diabetes, systolic blood pressure, serum glucose, and prevalent CVD. Inverse associations were observed with diastolic blood pressure.

Kosaku (7) show that serum OPG is positively correlated with age, suggesting that the factors related to aging may regulate serum osteoprotogerin in dialysis patient.

In the current work, there were significant negative correlations between eGFR and intact PTH and OPG, While There were significant positive correlations between serum creatinine and CCIMT and

OPG. There was no significant correlation between serum OPG and calcium, phosphorus, alkaline phosphatase or albumin.

Significant negative correlations were found between serum OPG and PTH levels. PTH has been found to stimulate OPGL and reduce OPG secretion in vitro in osteoplastic_ like cells **(8).**

The serum OPG levels were significantly correlated with age and there was no correlation between serum OPG levels and the duration of dialysis. In univariate analysis were positively correlated with intact PTH. Serum OPG tended to be greater in patients with severe aortic calcification than in those with mild calcification Aaron ***R*** **(9)** documented that noninvasive measures of atherosclerosis such as CCIMT are associated with positively and strongly future incidence of cardiovascular disease **(7).**

CIMT has been correlated to cardiac risk factors and established as a cardiac screening tool in studies on high risk adults. Both age and gender are identified as non_modifiable atherosclerotic risk factors in adults **(1o).**

The results obtained by densitometry are statistically equivalent. Thus the authors suggest that densitometry equipment can be utilized to investigate abdominal aortic calcification **(17).**

OPG was associated with an increased eGFR in younger subjects with normal renal function and with a decreased eGFR in older subjects with reduced renal function ***(11).*** Inverse association was found in participants with reduced renal function (eGFR< 9o.ml\min\1.73m). The modest changes in eGFR across OPG are probably of questionable clinical relevance. Subjects with serum OPG greater than the median serum concentration had five percent lower eGFR compared with those with lower serum OPG**.**

Ali Momeni (12) showed CIMT greater than 0.9 mm in HD patients and the mean CIMT was 0.8 mm in CKD patients on conservative treatment.

Gentile M (13) studiedon one thousands and three patients older than fifty years and found that the estimated GFR was adversely correlated with the CIMT.

Bulent (14) was evaluating the relationship between serum osteoprotogerin levels and carotid artery intimae-media thickness and carotid plaque formation. CCIMT was positively correlated with presence of plaque, age and OPG. These studies suggest that elevated levels of serum OPG is associated with CCIMT and may play role in the pathogenesis of atherosclerotic disease.

Martin L., (15) shown a relationship between OPG and aortic stiffness in the patients with CKD stage three and four patients using the gold standard measurement of arterial stiffness.

Carotid intima media thickness and aortic calcifications predict the risk of vascular calcification independently of each other. similar independence of the predictive power of AAC and CCIMT was seen for the prediction of cardiovascular mortality **(18).**

Other studies such as **Sigrist MK., (16)** had found association between OPG and adverse outcome in patients both with and without renal dysfunction. osteoprotogerin and age were independently associated with aortic stiffness. This relationship remained significant after sensitivity analyses for diabetic status, calcium and vitamin D supplement.

Conclusion

Our study concluded that:-

The prevalence of arterial stiffness as estimated by CCIMT, AAC and serum OPG was one hundred percent, eighty percent respectively in the studied HD patients and was eighty percent, fifty five percent respectively in patients on conservative therapy.

Serum OPG levels are significantly increased in HD patients compared to patients on conservative therapy with arterial stiffness.

In HD patients, serum OPG levels era significantly correlated with the disease duration and significantly correlated to serum calcium, serum phosphorus, alkaline phosphatase, intact parathyroid hormone and CCIMT.

In patients on conservative therapy with arterial stiffness serum OPG level did not significantly correlated with the clinical parameters (age, sex, BMI, disease duration and MBP) but significantly negative correlated with serum creatinine, blood urea, eGFR and intact PTH and significantly positive correlated with CCIMT.

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5/31/2017