ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study of Plasma Osteoprotegerin level and arterial stiffness in chronic kidney disease and hemodialysis patients.

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Manuscript Info

Manuscript History:

Received: 17 March 2016
Final Accepted: 20 April 2016
Published Online: May 2016

Key words:
Osteoprotegerin, CKD, ESRD, Arterial stiffness.

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Abstract

Background: chronic kidney disease (CKD) is a serious illness which seriously affects body systems. The dramatically increased cardiovascular risk of death of uremic patients is directly associated with the magnitude of vascular calcification (VC). Disturbances in mineral and bone metabolism are common complications in CKD. They are an important cause of morbidity and decreased quality of life and, as with the development of renal bone disease, are major risk factors for VC. Arterial stiffness is a reduction in arterial dispensability. Increased central arterial stiffening is a hallmark of the aging process and the consequence of many disease states such as diabetes, atherosclerosis, and chronic renal compromised patients. In ESRD patients, the extent of arterial stiffness was a strong predictor of all-cause and CV mortality.

Osteoprotegerin (OPG) is identical to osteoclastogenesis inhibitory factor (OCIF), a soluble member of the tumor-necrosis factor receptor family that inhibits osteoclastogenesis. OPG is considered to play an important role in the regulation of bone resorption by modifying osteoclast differentiation. Osteoprotegerin is an independent risk factor for the progression of vascular stiffness and onset of cardiovascular disease. **Aim of the work**: to assess plasma OPG levels and arterial stiffness in chronic kidney disease and hemodialysis (HD) patients.

Patients and methods: 66 chronic Kidney disease and HD patients were examined for evidence of arterial stiffness. we excluded patients with liver disease, autoimmune disease, malignant disease and inflammatory diseases, All patients subjected to full history taking: Through personal history taking with special stress on age, sex, hemodialysis duration, and presence of other systemic diseases especially cardiovascular diseases. Full clinical examination was done. Laboratory investigations: CBC, liver function tests, kidney function tests, calcium, phosphorus, parathyroid hormone and lipid profile. Specific investigations include plasma osteoprotegerin level. Radiological investigation: echocardiography and ultrasonography (Doppler on common carotid artery).

Results: In chronic kidney patients, plasma levels of osteoprotegerin (OPG) were associated with pulse wave velocity (PWV) which is the mean marker of arterial stiffness, but not with common carotid intima-media thickness (ccIMT). Arterial stiffness was associated with history of cardiovascular disease (CVD), hypertension and diabetes mellitus. Arterial stiffness also showed a strong significant positive correlation with OPG levels

In hemodialysis patients, plasma OPG levels were associated with arterial stiffness and showed that OPG levels can, in part, explain the association between coronary artery calcification and CKD.

Conclusion: high plasma OPG levels were associated with arterial stiffness and pulse wave velocity (PWV) in chronic kidney disease and HD patients, also arterial stiffness was strongly associated with cardiovascular disease

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Introduction:-

Chronic kidney disease (CKD) defined as abnormalities of kidney function or structure present for more than 3 months, with implications for health. This includes all people with markers of kidney damage and those with a glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m^2 on at least 2 occasions separated by a period of at least 90 days (with or without markers of kidney damage). It is common, frequently unrecognized and often exists together with other conditions (such as cardiovascular disease "CVD" and diabetes).[1]

Arterial stiffness is a reduction in arterial distensibility and may be quantified by the measurement of different parameters. Clinically, the gold standard parameter is the pulse wave velocity (PWV). Arterial stiffness is a growing epidemic associated with increased risk of cardiovascular events, dementia, and death. Decreased compliance of the central vasculature alters arterial pressure and flow dynamics and impacts cardiac performance and coronary perfusion.[2 3] in ESRD the extent of arterial calcifications was a strong predictor of all-cause mortality.[4]

Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor superfamily, is a soluble decoy receptor for the osteoclast differentiation factor receptor-activator of nuclear factor KB ligand (RANKL) that inhibits interaction between RANKL and its membrane- bound receptor RANK. The RANKL/OPG/RANK axis has been shown to regulate bone remodeling and was more recently found to be involved in carcinogenesis as Well as central thermoregulation.[5] .This system has also been linked to the development of atherosclerosis and plaque destabilization. RANKL exhibits several properties with relevance to atherogenesis, such as promotion of inflammatory responses in T cells and dendritic cells, induction of chemotactic properties in monocytes, induction of matrix metalloproteinase (MMP) activity in vascular smooth muscle cells (SMC), and RANKL has also been found to have prothrombotic properties.[6]

Expression of bone proteins resulting from transdifferentiation of vascular smooth muscle cells into osteoblasts suggests that vascular calcifications are a bioactive process. Regulating molecules such as osteoprotegerin and receptor activator of NF-KB ligand (RANKL) could play a key role in bone-vascular calcification imbalance associated with imbalances in mineral metabolism.[7] VC has intimate interactions with bone mineralization and enhanced bone resorption.[8]

Plasma OPG concentrations in serum from CKD patients were found to be independently associated with the serum potential to induce calcification of smooth muscle cells in vitro.[9]

Aim of the study:-

To assess plasma OPG levels and study its relation to arterial stiffness in chronic kidney disease and hemodialysis (HD) patients.

Subjects and Methods:-

This study has been performed in the period from Jan 2015 to Dec. 2015 in internal medicine and nephrology unit Zagazig university hospitals. Subjects were divided into two groups:

Group (A): 33 chronic kidney disease patients included 17 female and 16 male with age (Mean ±SD) 58.3±13 in different stages according to estimated GFR (eGFR) and albumin/creatinine ratio (ACR).

Group (B): 33 end stage renal disease patients included 14 female and 19 male with age (Mean± SD) 56 ± 6.1 on hemodialysis, Fresenius 4008S hemodialysis machine and polysalfon filters, three sessions per week 4 hours for each session with duration (Mean ± SD) 12.2± 2.3 hours.

Inclusion criteria:-
Chronic Kidney disease patients that diagnosed according to National Institute for Health and Care Excellence (NICE) clinical guidelines 2015 (Albuminuria "ACR" 2 3 mg/mmol, increasing serum creatinine above 1.5 mg/dl, glomerular filtration rate "GFR" > 60 ml/rnin/1.73 m^2 and abnormal renal ultrasound.

The End stage renal disease patients (GFR < 15 ml/min/1.73 m^2, ACR > 30 mg/mmol)
Exclusion criteria:-
1. Patients with liver disease
2. Patients with autoimmune disease
3. Patients with malignant disease .
4. Patients with inflammatory disease
All patients subjected to:

Clinical examination:-
Clinical examination includes arterial blood pressure (SBP, DBP, and MAP) measuring and detailed history taking with special stress on age, sex, hemodialysis duration, and presence of other systemic diseases especially cardiovascular diseases (mitral & aortic valve disease and coronary artery disease "CAD").

Laboratory investigations included:-
a) Routine laboratory investigations: CBC, Liver function tests, Parathyroid hormone measured by Quantitative, electro-chemiluminescence (ECLIA) assay, Serum calcium and serum phosphorus: measured by photometric test, Lipid profile (LDL, HDL, serum total cholesterol, serum triglyceride) by enzymatic colorimetric test for serum TC & serum TG and homogeneous enzymatic colorimetric test for LDL & HDL.

b) Special laboratory investigations:
Estimation of Serum osteoprotegerin: Kit full name Human OPG PicoKine TM ELISA Kit. Catalog No. EK0480. BOSTER BIOLOGICAL TECHNOLOGY Co., Ltd. I CA 94566 PLEASANTON.
Standard range of OPG = 0-20 pmol/L

Echocardiography and Doppler ultrasound:-
Echocardiography to determine presence of cardiovascular disease (mitral & aortic valve disease and coronary artery disease). Doppler ultrasound to determine arterial radius and intimal medial thickness.

PWV measurements:-
PWV measurements were performed with the Moens-Koiteweg equation, PWV=\/ (Eh/2pR), Where E is Young's modulus of the arterial wall, h is wall thickness, R is arterial radius at the end of diastole, and p is blood density by a trained operator unaware of the patient's clinical and laboratory parameters. Each subject was ultrasonographic (SonoAce R3 Ultrasound System@ Samsung Medison) examined for determination of equation parameters (common carotid artery radius and Wall thickness) of during a mid-Week non-dialysis day. PWV results were expressed in meters per second (m/s) ± SD.

IMT measurements:-
Ultrasonographic studies were performed in Radiology department of Zagazig University Hospitals by experienced operator (SonoAce R3 Ultrasound System@ Samsung Medison). Each subject was examined in the supine position in a semi-dark room during a mid-week non-dialysis day within one month from blood sampling. The common carotid artery was investigated bilaterally by the same trained operator, who was unaware of the patients' clinical and laboratory parameters. ccIMT was calculated as the distance between the leading edge of the lumen-intima interface and the media-adventitia interface on the far wall of the artery.

Results:-
A significant difference between the two groups as regard history of CVD, HD duration and presence of HTN & DM.

There is a highly significant difference between the two groups regards PWV, Pl. OPG, ccIMT, S. Creatinine, eGFR, total S. PTH, Hb, uric acid and S. TG & total S. Cholesterol ($p < 0.001$).

No significant difference between two groups as regard age, sex, smoking and BMI, Pl. Albumin, total S. Calcium and S. Phosphorus ($p > 0.05$).

There is a highly significant difference between the two OPG tertiles regards history of CVD, HTN & DM and HD duration, Hb, S. Creatinine, total S. Cholesterol & S. TG, total S. PTH, PWV, Pl. OPG, ccIMT and the two main groups ($p < 0.001$).

There is no significant difference between the two OPG tertiles regards age, sex, smoking, BMI, Pl. Albumin, total S. Calcium, S. Phosphorus, S. Uric acid ($p > 0.05$).

There is no significant correlation between Pl. OPG and age, BMI, Pl. Albumin, total S. PTH, S. Uric acid, and S. Creatinine, PWV, HD duration, total S. Calcium, S. Phosphorus & total S. Cholesterol, ccIMT, eGFR & S. TG in CKD group ($p > 0.05$).

There is highly significant positive correlation between Pl. OPG and S. Creatinine, total S. PTH, PWV, ccIMT & HD duration in ESRD group ($p< 0.001$).

There is highly significant negative correlation between Pl. OPG and eGFR considering ESRD group ($p < 0.001$)
There is no significant correlation between Pl. OPG and age, BMI, Pl. Albumin, total S. Calcium, S. Phosphorus, S. Uric acid, total S. Cholesterol, S. TG in ESRD group.

There is no significant correlation between PWV and S. Creatinine, total S. PTH, eGFR, Pl. OPG, ccIMT & HD duration, age, BMI, Pl. Albumin, total S. Calcium, S. Phosphorus, S. Uric acid, total S. Cholesterol, S. TG in CKD and ESRD group.

There is highly significant positive correlation between PWV and s. creatinine, total S. PTH, TG., cholesterol , Pl OPG, ccIMT & HD duration in ESRD group.

There is highly significant negative correlation between PWV and eGFR in ESRD group.
There is a highly significant positive correlation between ccIMT and S. Creatinine, total S. PTH, total S. Cholesterol, S. TG, Pl. OPG, PWV & HD duration and age considering ESRD group ($p < 0.001$).

Table 1: demographic distribution and clinical characteristics of the two main groups.

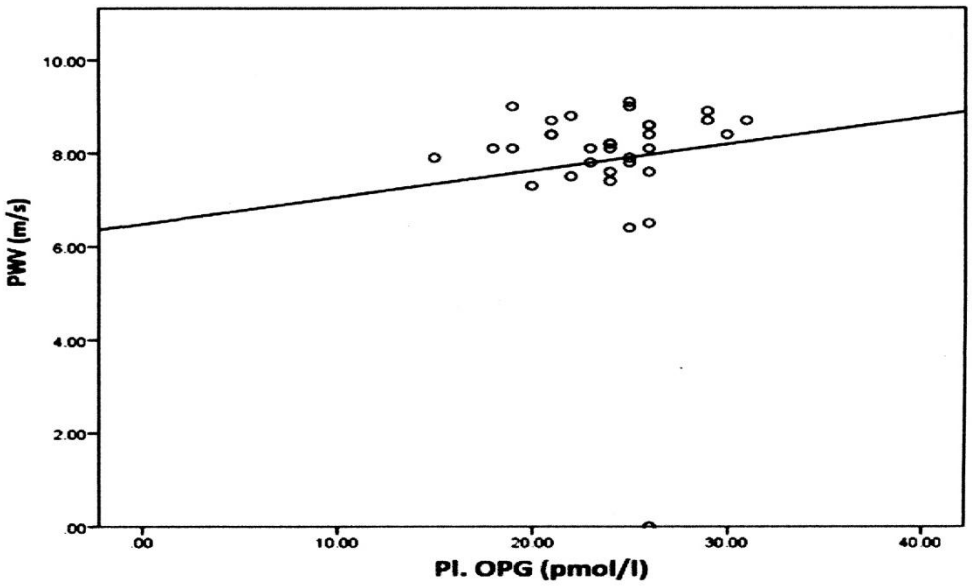
		CKD (n =33)	ESRD (n= 33)	t/x^2	Sig. (2 tailed)
sex	Female	17(51.5%)	14(42.4%)	0.5	0.456
	Male	16(48.5%)	19(57.6%)		
Age (years) (mean ±SD)		58.3 ±13	56± 6.1	0.4	.0523
BMI ($kg/m^{2)}$ (mean ±SD)		28±1.8	27.9±1.5	0.3	0.76
HD duration (years) (mean ±SD)		0	12.2±2.3	-31.0	0
History of CVD	Negative	27(81.8%)	13(39.4%)	13.0	<0.001
	Positive	6(18.2%)	20(60.6%)		
Smoking	Negative	23(69.7%)	23(69.7%)	0.0	1.000
	Positive	10(30.3%)	10(30.3%)		
DM & HTN	Negative	15(45.5%)	2(6.1%)	13.5	0.004
	DM	2(6.1%)	3(9.1%)		
	DM&HTN	3(9.1%)	6(18.2%)		
	HTN	13(39.4%)	22(66.7%)		

BMI: body mass index CVD cardiovascular disease DM: diabetes mellitus
HTN: hypertension HD. Hemodialysis

Table 2: Calcified area in ESRD group.			
Area			ESRD
Calcified	Aorta	Count	17
		% within group	51.5%
	Mitral	Count	16
		% within group	48.5%
total		Count	33
		% within group	100.0%

Table 3: lab. parameters of the studied groups.				
	CKD (n=33)	ESRD (n=33)	T	Sig.(2- tailed
Hb (g/dl) (mean ±SD)	11±0.9	9.2±0.5	10.3	0
Pl.Albumin (g/dl) (mean ±SD)	4.3±0.5	4.2±0.4	1.0	0.343
s.creatinine (mg/dl) (mean ±SD)	2.2±0.4	10.4±2.5	-18.8	0
Total s.calcium(mg/dl) (mean ±SD)	9.4±0.6	8.8±1.8	1.8	0.077
s. phosphorous(mg/dl) (mean ±SD)	4.3±0.6	4.6±1.4	-1.0	0.333
s.Total PTH(pg/ml) (mean ±SD)	67.7±14.6	667.1±304.8	-11.3	0
s. uric acid (mg/dl) (mean ±SD)	5.6±1.7	6.4±0.9	-2.3	0.024
Total s.cholesterol (mg/dl) (mean ±SD)	181±18.6	231.9±19.8	-10.8	0
s.TG (mg/dl) (mean ±SD)	171.6±24	227.8±18	-10.8	0
eGFR(ml/min/1.73m$^{2)}$ (mean ±SD)	52.6±4.3	5.6±1.5	59.2	0
Pl. OPG(pmol/l) (mean ±SD)	0.3±0.2	23.9±35	-83.9	0
PWV(m/s) (mean ±SD)	6.5±0.4	7.9±1.6	-5.1	0
ccIMT(mm) (mean ±SD)	0.7±0.1	1.1±0.2	-11.5	0
Hb: hemoglobin PTH: parathyroid honnone TG: triglyceride OPG: Osteoprotegerin PWV: pulse wave velocity S.: serum Pl.: plasma ccIMT: common carotid intima medial thickness eGFR: estimated glomerular filtration rate				

fig 1: correlation between OPG and PWV



There is a highly significant negative correlation between cclMT and total serum calcium and eGFR in ESRD group. Also there is no significant correlation between cclMT and BMI, Pl. Albumin, S. Uric acid & S. Phosphorus considering ESRD group($p > 0.05$)

Statistical analysis:-

Data are expressed as mean ± standard deviation (SD), median with range, or number of patients with percentage as appropriate. The associations between OPG, PWV, ccIMT, and demographic, clinical and laboratory parameters were assessed. The collected data and calculations were performed, summarized, tabulated and analyzed by using computerized 4 software statistical packages (SPSS for Windows® version 18).

Discussion:-

Our results were similar to **Lee et al. (2013)**[10] results that indicate that serum OPG levels were associated with arterial stiffness represented by PWV, and that higher OPG levels predict the onset of new CV events in HD patients.

Other studies have shown an independent positive association between OPG and carotid IMT in non-renal patients, such as healthy post-menopausal women, women with previous gestational diabetes, subjects older than 55, and males with type 2 diabetes, but not patients with acute or chronic coronary artery disease **(Ciccone et al., 2013).**[11]

We also observed that OPG levels were significantly associated with both PWV and ccIMT in elderly diabetic and HD patients similar to **Pateinakis et al., (2013)**[12] who demonstrated an independent positive association of serum OPG with carotid IMT in 61 peritoneal dialysis patients.

In our study, we found a significant difference between the two studied groups regarding history of CVD, HD duration and presence of HTN & DM, but there no significant difference between the two studied groups regarding age, sex, smoking and body mass index (BMI) in agreement with **Sigrist et al. (2009)**[13] who said that elevated serum OPG was associated with a negative outcome in stages four and five CKD patients..

In addition to presence of significant difference between the two studied groups regarding PWV, OPG, ccIMT, Creatinine, eGFR, PTH, Hb and Triglyceride & Cholesterol and no significant difference between the two studied groups regarding Albumin, Calcium, Phosphorus & Uric acid similarly to **Pateinakis et al. (2013)**.[12] Echocardiographic study showed that calcified aorta is 51.5% and calcified mitral is 48.5% in all 33 patients of ESRD group went with results proved by **Pateinakis et al. (2013).**[12]

We also observed that OPG is strongly associated with history of cardiovascular disease, HD duration and hypertension & diabetes mellitus similarly to **Nakashima et al. (2011)[14]** and **Kim et al., (2013**)[15] who stated that high serum OPG levels were associated with the progression of carotid atherosclerosis and coronary calcification in the general population, and a similar trend has been noted in HD patients.

Our results went with **Nakashima et** al. **(2011)[14]** study results that documented an independent association was observed between PWV and OPG. As two other studies, also demonstrated an independent association between PWV and OPG.

In our present study, we found a significant positive correlation between OPG and PWV & ccIMT in hemodialysis patients as **Pateinakis et al. (2013)**.[12]

But it should be noted that in our study, OPG levels were significantly associated with both PWV and ccIMT only in non diabetic patients.

We found no significant correlation of serum OPG with ccIMT has been documented in HD patients as **Pateinakis et al. (2013)[12]** and **Scialla et al. (2011)[16]** who found no evidence correlating serum OPG levels and mineral metabolism factors, such as calcium, phosphate, and PTH. These findings suggest that circulating OPG levels might not be indicative of active bone resorption. We also observed no significant correlation between OPG with Calcium and Phosphorus but positive significant correlation with PTH in hemodialysis patients.

In addition to OPG has a significant correlation with Creatinine & eGFR and HD duration and no significant correlation with age, BMI, Albumin, Uric acid, total serum Cholesterol and Triglyceride in hemodialysis patients as **Kim et al. (2013)** results and against **Pateinakis et al. (2013).[12]**

In HD patients, we found a positive association of PWV with ccIMT, even though it was not independent of age, which appeared, as expected, the strongest predictor of ccIMT and significant correlation with HD duration, creatinine, PTH but not with calcium and phosphorus.

We also observed no significant correlation between ccIM.T with age, PTH, creatinine, HD duration and calcium but not with BMI, albumin and phosphorus as **Scialla et al. (2011).[16]**

Our study proved that in chronic kidney patients, serum levels of the osteoclast inhibitor osteoprotegerin (OPG) are independently associated with pulse wave velocity (PWV) which is the mean marker of arterial stiffness, but not with common carotid intima-media thickness (ccIMT) **Pateinakis et al., (2013).[12]**

Arterial stiffness was associated with history of cardiovascular disease (CVD), hypertension and diabetes mellitus.

In hemodialysis patients, serum OPG levels were associated with arterial stiffness and showed that OPG levels can, in part, explain the association between coronary artery calcification and CKD as assessment of OPG in CKD patients help in explain vascular calcification and predicted cardiovascular disease development similar to **Pateinakis et al., (2013)[12]** and **Kim et al., (2013).[15]**

Conclusion:-

High plasma OPG levels were associated with arterial stiffness and increased pulse wave velocity (PWV) in chronic kidney disease and HD patients, also arterial stiffness is strongly correlated with cardiovascular diseases.

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