Osteoprotegerin, C- Reactive protein and Fibroblast Growth Factor-23 in Stage (II-IV) CKD Patients and Their potential for Increased incidence of Cardiovascular Disorders

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Abstract

CKD is a public health problem and many studies had support the link between kidney dysfunction and cardiovascular risk. Whilst, Osteoprotegerin (OPG), after its capacity to protect bone , also serum OPG in patients with chronic kidney disease (CKD could predict the deterioration of kidney function, cardiovascular, vascular events and all-cause mortality. On the other hand, fibroblast growth factors (FGFs), in patients with CKD, seem to increase progressively as kidney function worsens. Furthermore, renal insufficiency was independently associated with an increased level of C-reactive protein (CRP), which indicates a vital pathway mediating the increased cardiovascular risk in those patients.

Our aim is to study the correlations between Osteoprotegerin, CRP and fibroblast growth factor-23 serum levels in patients with chronic kidney disease stage (II-IV) and its possible relation with cardiovascular events.

The study enrolled fifty-nine patients with chronic kidney disease and according to CKD-EPI Creatinine/ 2009 equation were allocated as stage (II-IV), those patients were divided into three groups: Group1 (29 patients) with chronic kidney disease stage (II-IV) with cardiovascular events.Group2 (30 patients) with chronic kidney disease stage (II-IV) without cardiovascular event, to be compared with Group 3 (23 apparently healthy subjects .Serum obtained from their blood specimens to estimate; glucose, urea, creatinine, calcium, phosphate, sodium, potassium, aldosterone, FGF-23, Osteoprotergen& C-reactive protein.

Data analysis shows that OPG levels is significantly higher in patients at stage III from that of controls ,as well as from those in stage IV-CKD patients,While CRP levels were significantly elevated in stage III CKD patients with values by about 300% greater than that of controls.

Keywords: *Cardiovascular Disorders; CKD Patients; C- Reactive protein*

Introduction

CKD is a public health problem , that results from decrease in renal function represented by GFR for a period of three months or more which is associated with severe cardiac outcomes and high mortality rate [1]. Data on mortality for CKD adjusted for comorbidity, race, gender, age and previous hospitality for CKD patients indicate an obvious decline in rates since 1995 may reflect to some extent the increased recognition of CKD as this confirmed by increasing number of patients carrying the diagnosis. [2]

Osteoprotegerin (OPG) is a glycoprotein, after its capacity to protect bone. It is produced in many tissues including lung, bone, kidney, vasculature, heart and placenta. [3] It was shown that serum OPG is elevated in patients with non-diabetic and diabetic chronic kidney disease (CKD). [4]

On the other hand, fibroblast growth factors (FGFs) that signal through FGF receptors (FGFRs) can regulate a wide range of biological functions, including cell proliferation, survival, migration, and differentiation. Among the signal pathways, RAS/MAP kinase is known to be predominant in the case of FGFs. [5] As

the central target organ of FGF-23 appears to be the kidney, where tubular phosphate reabsorption and 1-alphahydroxylase expression are suppressed. These features raised the question which role FGF-23 might play in dialysis patients (CKD stage V), the CKD stage where end-stage kidney failure is firmly established and neither substantial phosphaturic effect can occur. [6] By the time patients reach end-stage renal disease, where FGF-23 concentrations are often increased 100-to 1000-fold above the normal range, whereas serum phosphate concentrations are only modestly increased or even normal. [7]Whist, CRP is an acute phase reactant [8] that is found in plasma in trace amounts, its concentrations rises quickly and markedly with tissue inflammation. [9] In the large Cardiovascular Health Study, renal insufficiency was independently associated with an increased level of CRP, which may indicate a vital pathway mediating the increased cardiovascular risk in persons with kidney disease. [10]

Epidemiological data show documented associations between C-reactive protein (CRP), and cardiovascular disease in the general population. [11] Our aim was to assess the associations between CRP, Osteoprotergin and fibroblast growth factor -23 with the incidence of cardiovascular disorders in CKD (stages II-IV) patients.

Subjects and Methods

This study was carried out at Baghdad teaching hospital at Baghdad Medical city, for the period from October 2017 to February 2018, which enrolled fifty-nine patients with chronic kidney disease from stage II-IV, under supervision of a specialized physician and the diagnosis was based on serum creatinine, according to CKD-EPI Creatinine/ 2009 equation [12]. Those patients were divided into three groups:

- Group1: 29 patients with chronic kidney disease stage (II-IV) with cardiovascular events.
- Group2: 30 patients with chronic kidney disease stage (II-IV) without cardiovascular event.
- Group 3:23 apparently healthy subjects (age and sex matched to that of patients).

Subjects were enrolled in this study after excluding CKD patients with a chronic liver disease, or a thyroid disorder, or having a history of endocrinopathy, or patient on dialysis or had at least one session of dialysis. Baseline characteristics of subjects are illustrated in table -1. The study was confirmed by The Local Research Ethics Committee and all subjects were provided with a written informed consent to participate in this study.

Analysis of serum creatinine, urea, calcium, phosphate and alkaline phosphatase were performed using the specific Flex® reagent cartridge /Dimension (RxL Max) / SIEMENS (USA).Whereas,. ,human fibroblast growth factor-23 ELISA kit [13] and human osteoprotegerin ELISA Kit were purchased by Cusabio Biotech Co.,LTD (China) [14],while CRP we used turbilatex by Accent 200, Poland [15]. Statistical analysis of data is presented as means ± SD. Significance was set at $p < 0.05$.Cases and controls were compared using either the t-test for independent samples, and Pearson's coefficient for correlations among normally distributed variables.

Results

According to serum Ca levels, patients with CKD were presented with significantly lowered values of serum Ca levels (table -1) Data analysis according to the presence or CV disorders in patients compared to that of controls showed that fasting serum glucose levels of CKD patients (with and without CV disorder) had significantly higher values as compared to the controls (76.5% and 29% respectively). Furthermore, patients with CV disease were even presented with greater fasting serum glucose as compared to those without CV disorder (Table -2). GFR values of CKD- patients were significantly lower than that of the control subjects considering both with and without CV disorders. Meanwhile, serum urea levels were significantly elevated as compared to the controls, irrespective to the presence or not of CV disease. But serum creatinine concentrations showed no significant variation between patients with CV disorder than those without CV disorder. Serum Aldosterone, FGF-23, OPG levels were presented with no significant variation among studied patient groups comparing patient with or without CV disorder (Table -2). Serum CRP levels were significantly elevated in stage III CKD patients with values by about 300% greater than that of controls (figure-1), but no difference between patients with or without cardiovascular events. While serum ALP levels is positively ($r=0.409$) correlated with serum CRP level at significant level of ($p=0.002$), but, serum CRP levels is negatively ($r=0.428$) correlated with serum calcium level in CKD patients at significant level of ($p=0.001$) in CKD patients [not shown]. While, serum FGF-23 levels

according to CKD stages (figure-2) showed no significantly variations among different CKD stages, nor from that of the control group.

Table (1) Basic Criteria for Participant in the study

Parameter	Group				P-value
	Patient(59)		Control(23)		
	Mean	SD	Mean	SD	
Age* (years)	60.54	12.49	50.70	16.21	P=0.013
Gender	37/22	-	17/6	-	P=0.337
Weight (Kg)	80.56	16.42	83.85	13.31	P=0.466
Height* (Meter)	167.33	5.94	171.75	7.84	P=0.033
BMI	28.66	5.18	28.37	3.72	P=0.835
FSG* (mg/dl)	157.50	68.49	99.83	23.54	P=0.005
Urea* (mg/dl)	72.52	29.25	28.70	9.54	P=0.005
Creatinine* (mg/dl)	1.98	1.81	0.66	0.16	P=0.001
GFR* (ml/min)	42.83	16.28	111.26	13.69	P=0.005
Hypertension*	26	-	-	-	P=0.015
Phosphate (mg/dl)	4.20	0.79	4.32	0.55	P=0.513
Calcium* (mg/dl)	8.64	1.03	9.31	0.52	P=0.005
Potassium	4.32	0.86	4.53	0.75	P=0.231
Sodium	140.72	6.31	142.08	4.29	P=0.293
Cardiovascular disorder (No.21)	21	-	-	-	P=0.005
Smoker (No.12)	7	-	5	-	P=0.979

*=Significantly different from control

Table-2 Variation in Some Studied Parameters in Patients Compared to the Control Subjects According to Presence of CV Disorder or Not

Groups / Parameters	Patient				Control		P value
	Cardiovascular event				No Cardiovascular event		
	Yes(No.29)		No(No.30)		No. 23		
	Mean	SD	Mean	SD	Mean	SD	
Glucose (mg/dl)	176.29 ab	59.09	128.81 a	47.09	99.83	23.54	0.005
ALP (µkat/l)	115.21	42.30	129.33	70.91	82.70	44.74	0.444
CRP (mg/dl)	15.56	11.80	16.91	13.63	3.91	11.43	0.748
GFR (ml/min)	41.62 a	13.79	48.19 a	18.98	111.26	13.69	0.005
Urea (mg/dl)	78.64 ab	26.11	63.16 a	23.93	28.70	9.54	0.005
Creatinine (mg/dl)	1.76	0.49	1.65	0.62	0.66	0.16	0.359
OPG (pg/ml)	105.65	122.66	93.39	118.90	22.79	46.37	0.233
FGF-23 (pg/ml)	50.7	165	105	408	7.8	3.9	0.613
Aldosterone (pg/ml)	44.80	3.87	53.95	32.42	48.09	20.63	0.317
Calcium (mg/dl)	8.60 a	0.83	8.49 a	1.03	9.31	0.52	0.005
Phosphate (mg/dl)	4.20	0.86	4.12	0.79	4.31	0.55	0.697
Sodium	140.60	6.89	142.38	6.98	142.07	4.28	0.655
Potassium	4.35	0.44	4.03	0.73	4.53	0.74	0.077

a: significantly different from control, b: significantly different from patient with no CVD,

N: Number of subjects

Table (3) Serum level of Osteoprotegerin in of CKD Patients Compared to The Control

Groups / Parameters	Stage								P-value
	II(N=11)		III(N=36)		IV(N=12)		Control(N=23)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
OPG* pg/ml	77.11	94.40	112.07 ab	121.52	107.97	130.69	22.79	46.37	0.006

a: significantly different from control, b: significantly different from stage IV

c: significantly different from stage III

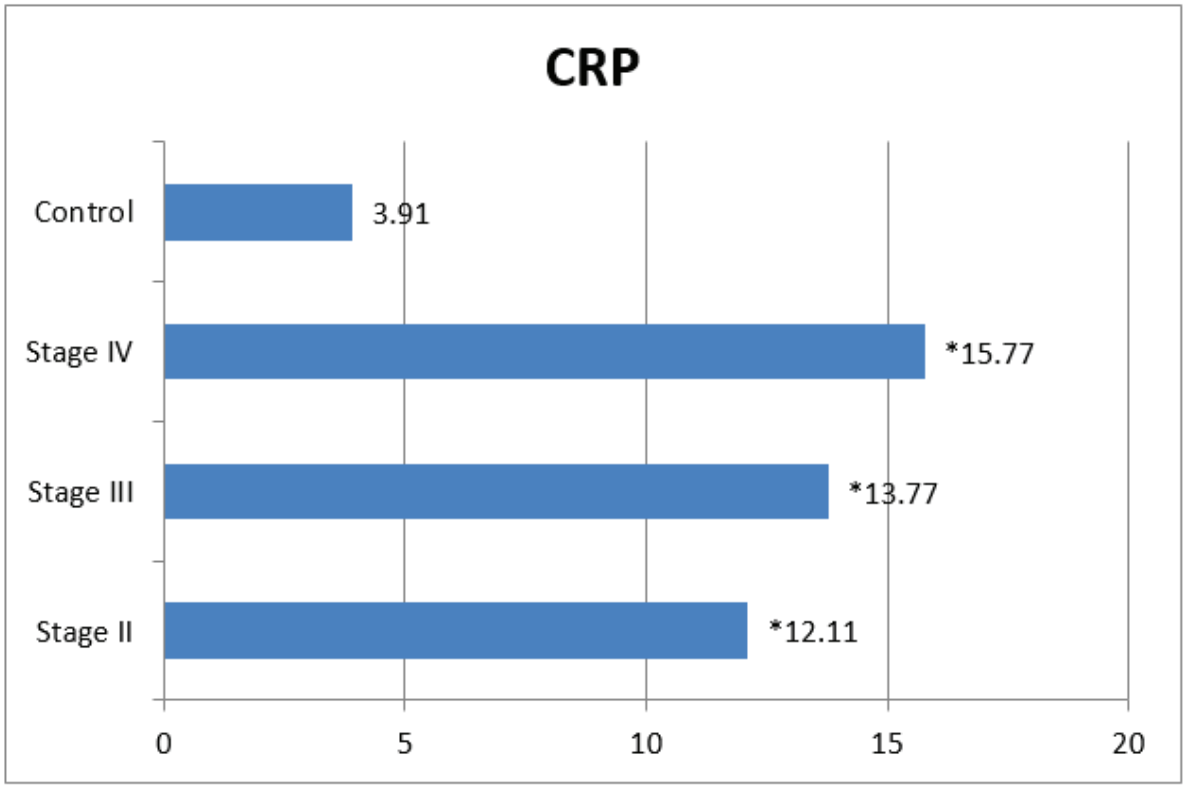

Figure (1) Serum C - Reactive Protein Mean Levels(mg/dl)

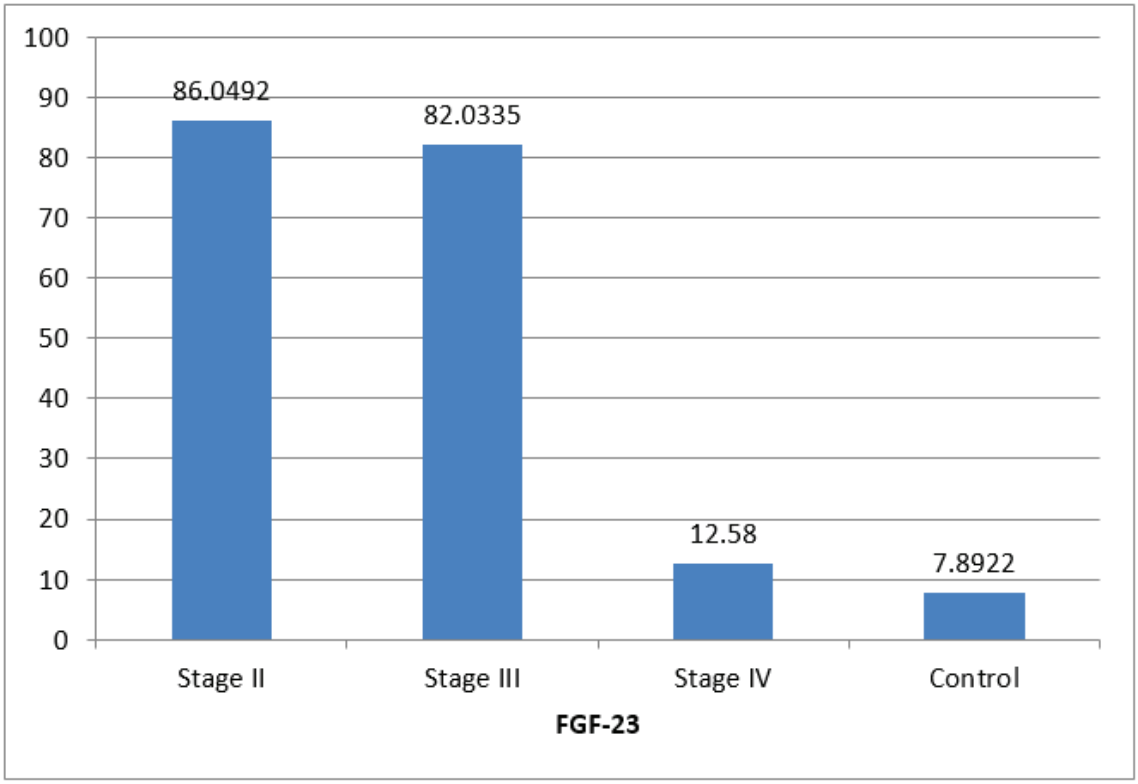

Figure (2) Serum FGF-23 Mean levels Among Various Stages of CKD Patients Compared to The Control Group

Discussion

Considering OPG levels only patients in stage III showed significantly higher levels from controls as well as from those in stage IV as shown in Table 3, and this is consistent with what was shown in studies as serum OPG is elevated in patients with chronic kidney disease where it could predicts the deterioration of kidney function, despite the fact that direct effects of OPG on kidneys are still largely not discovered. [4].Serum OPG activity levels were presented with no significant variation between studied groups (table -2); those results were not in agreement with a study referenced and say that in patients with chronic kidney disease, vascular calcification contributes to increased cardiovascular (CV) morbidity and mortality, [16] this results may be contributed to the small number of patients present in each group.

Whereas, serum CRP levels were significantly elevated in stage III CKD patients with values by about 300% greater than that of controls (figure- 1) and this is consistent the large Cardiovascular Health Study, as it demonstrates that renal insufficiency was independently associated with elevation of CRP, which may indicate an important pathway mediating the increased cardiovascular risk in persons with kidney disease[10], and this lead to improving the opinion says that inflammation is an essential part of chronic kidney disease (CKD), there has been an exponential growth of interest in inflammation in CKD and end-stage renal disease (ESRD), which led to the evolution in our perception of inflammation as not any longer a novel but rather a well-established, if not traditional risk factor of morbidity and mortality in CKD. [17,18]

The negative correlates serum CRP levels with serum calcium level in CKD patients at significant level, indicates that serum calcium level decreases in CKD patient and this comply with the fact that in patients with CKD there have marked disruption lead to decrease in calcium level called CKD-mineral bone disorder [19], CRP serum levels were significantly elevated in stage III CKD patients [10] but in studies another kind of relation

was found, atherosclerosis considered as inflammatory disease and the amount of coronary Calcium is thought to be an indicator atherosclerosis in uremic and non-uremic patients, so a positive correlation between CRP and vascular calcification can be hypothesized, if this will be a true fact that means inflammation might be considered as a trigger for calcium deposition in the arteries of dialysis patient, in addition we can find that increased body burden of Calcium may or may not be reflected in elevated serum calcium level as normal serum calcium can be maintained in many cases despite a considerable increase in total body burden of calcium due to calcium deposition in the blood vessels and other extra skeletal tissues. [20]

Our result did not show significant variations among studied groups for serum FGF-23 levels, which may be elaborated by the fact that there was a wide difference between minimum and maximum readings that affect statistics significance despite the fact that when we consider figure -2, for the 1st time you will have an impression of confirmed significant difference between patients and controls data, the reason for that wide difference between minimum and maximum reading can be explained by adopting "In patients with CKD, FGF-23 concentrations are constitutively elevated and increase progressively as kidney function worsens, likely as an appropriate compensation to help maintain normal serum phosphate concentrations in the face of declining nephron mass. By the time patients reach end-stage renal disease, where FGF-23 concentrations are often increased 100-to 1000-fold above the normal range, whereas serum phosphate concentrations are only modestly increased or even normal." [7] Several FGF-23 studies found that increased FGF-23 level is associated with left ventricular hypertrophy, fat mass, and dyslipidemia in elderly patients [21].This positive correlation between FGF-23 and mortality also is found in the general population with coronary artery disease. [22] FGF-23 is regulated by phosphate (albeit in an indirect manner) and phosphate is correlated with mortality and heart failure, it is possible that this association is not causative. [23]

Conclusion

Serum FGF-23, Aldosterone, Phosphate, Na, K, levels were presented with no significant variation between studied groups. By, considering OPG levels only patients in stage III showed significantly higher levels from controls as well as from CKD patients at stage IV. Meanwhile , serum CRP levels were significantly elevated in stage III CKD patients with values of about 300% greater than that of controls.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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