

Effects of Aldosterone, Osteoprotegerin and Fibroblast Growth Factor-23 and Some Biochemical Markers in Chronic Kidney Disease Patients (Stage II-IV) among Patients with or without Cardiovascular Events

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Abstract

Chronic Kidney Disease (CKD) is a public health problem and many studies support the link between kidney dysfunction and cardiovascular events. Aldosterone has been shown for decades that a plasma aldosterone concentration is elevated in CKD. Whilst, Osteoprotegerin (OPG), after its capacity to protect bone, also osteoprotegerin is elevated in patients with chronic kidney disease (CKD), where it 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, its levels seem to increase progressively as kidney function worsens. The aim of the present study is to assess the correlations between serum osteoprotegerin, aldosterone and fibroblast growth factor-23 levels in patients with chronic kidney disease stage (II-IV) with and without cardiovascular events.

The study includes fifty-nine patients with chronic kidney disease (CKD) and according to CKD-EPI /Creatinine/ 2009 equation to be allocated as stage II-IV, patients were divided into three groups: Group1 (29 patients) with chronic kidney disease (CKD) 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), age and sex matched to that of patients. Serum obtained from their blood specimens to measure; glucose, urea, creatinine, calcium, phosphate, sodium, potassium, aldosterone, FGF-23, Osteoprotegerin.

Data analysis 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). Serum Aldosterone, FGF-23, OPG levels were presented with no significant variation among studied groups with CV events or those without CV events.

Keywords: CKD, Aldosterone, Osteoprotegerin, FGF-23.

دراسة تأثير الألدوستيرون، الأوستيوبروتكترين وعامل النمو الليفي - ٢٣ على الأصابة

بالأحداث القلبية الوعائية في مرضى العجز الكلوي المزمن

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الخلاصة

مرض اعتلال الكلية المزمن هو مشكلة صحية عامة عززت دراسات عديدة وجود ربط بين العجز الكلوي مع الاصابات القلبية الوعائية. ان الألدوستيرون هو هرمون يفرز من الغدة الكظرية ولقد تم التأكد وعلى عقود من الزمن ان تركيز الألدوستيرون في البلازما يرتفع في مرض الكلية المزمن. الأوستيوبروتكترين هو هوة كلايكتوبروتين يحمي العظام، لقد تم ملاحظة زيادة الأوستيوبروتكترين في المصل في مرضى اعتلال الكلية المزمن و هذا يمكن أن يتنبأ بانحلال وظيفة الكلى، القلبية الوعائية، وزيادة حالات الوفاة. على الجانب الآخر عامل النمو الفايبروبلاستي-٢٣ والذي يرتفع عند مرضى العجز الكلوي بصورة متصاعدة كلما ساءت وظيفة الكلى. ان الهدف من هذا البحث هو دراسة العلاقة بين ايض العظام (ممثلة بتركيز الأوستيوبروتكترين في المصل)، الألدوستيرون وعامل النمو الليفي-٢٣ في مرضى العجز الكلوي للمرحلة الثانية والثالثة والرابعة وعلاقتها المحتملة بالأحداث القلبية. سجل في الدراسة تسعة وخمسون مريض مصابون بالعجز الكلوي وطبقا لمعيار ٢٠٠٩ CKD/ EPI لتحديدهم ضمن درجة العجز الكلوي من المرحلة الثانية الى الرابعة، حيث تم تقسيم المرضى الى ثلاثة فئات: اولاً: ٢٩ مريض مريض بالعجز الكلوي من المرحلة الثانية الى الرابعة عانى من احداث قلبية. ثانياً: ٣٠ مريض بالعجز الكلوي من المرحلة الثانية الى الرابعة بدون احداث قلبية. ثالثاً: ٢٣ أناس اصحاء ظاهرياً، يماثلون عمر وجنس المرضى. المصل المستخرج من عينات دماء المرضى لقياس الكلوكرز، اليوريا، الكرياتينين، الكالسيوم، الفوسفات، الصوديوم، البوتاسيوم، الألدوستيرون، عامل النمو الليفي ٢٣ و الأوستيوبروتكترين. تحليل النتائج اوجدت تركيز الكلوكرز في المصل لمرضى العجز الكلوي للمرضى الذين يعانون أو لا يعانون من احداث قلبية بان هناك فرق مؤكد بالمقارنة مع الأصحاء (٢٦,٥٪ و ٢٩٪) على التوالي. بالنسبة للأوستيوبروتكترين فقط مرضى المرحلة الثالثة أظهروا فرق مؤكد عن الأصحاء وكذلك عن مرضى المرحلة الرابعة للعجز الكلوي.

الكلمات المفتاحية: مرضى الفشل الكلوي ، الألدوستيرون ، الأوستيوبروتكترين ، عامل النمو الليفي ٢٣.

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Introduction

CKD is a public health problem, that results from the 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⁾.

Mortality of CKD adjusted for comorbidity, race, gender, age and previous hospitalization 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⁾.

Aldosterone is an adrenal hormone that plays a pivotal role in electrolyte and fluid homeostasis and thus control of blood pressure. It has been shown for decades that a plasma aldosterone concentration is elevated in CKD⁽³⁾.

Regulatory systems that are normally involved in homeostasis, can take on maladaptive roles. This paradoxical situation has been convincingly illustrated in heart failure. The evidence is more obvious with respect to the beneficial effects of sympathetic and renin-angiotensin-aldosterone system blockade⁽⁴⁾. Although the success of medications such as angiotensin-converting enzyme (ACE) inhibitors was initially attributed to hemodynamic actions unloading the left ventricle, additional direct cellular effects on cardiac remodeling have been discovered⁽⁵⁾. In the case of chronic progressive renal disease, substantial evidence attests to the efficacy of ACE inhibitors and, more recently, angiotensin receptor blockers in slowing the progression of both experimental and clinical renal disease⁽⁶⁾.

Osteoprotegerin (OPG) is a glycoprotein, in addition to its capacity to protect bone. It is produced in many tissues including lung, bone, kidney, vasculature, heart, and placenta^(7, 8). It was shown that serum OPG is elevated in patients with non-diabetic and diabetic chronic kidney disease (CKD), where it could predict the deterioration of kidney function, cardiovascular, vascular events and all-cause mortality⁽⁹⁾. Consistent to these results, it is recently reported that elevated OPG is combined with elevated risk of rapid renal decline, renal disease hospitalization, and/or deaths in elderly women⁽⁹⁾.

In CKD patient vascular calcification (VC) is recognized as a strong predictor of cardiovascular mortality⁽¹⁰⁾.

On the other hand, fibroblast growth factors (FGFs) that signal through FGF receptors (FGFRs) can regulate a wide domain of biological functions⁽¹¹⁾. As the central target

organ of FGF-23 appears to be the kidney, where tubular phosphate reabsorption and 1-alpha-hydroxylase expression are suppressed. These features raised the question which role FGF-23 might play in dialysis patients (CKD stage V)⁽¹²⁾. 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. At the level 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⁽¹³⁾. FGF23 is associated with heart failure and mortality in patients with or without chronic kidney disease, Because FGF23 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⁽¹⁴⁾.

The aim of this study is to assess the correlation between serum aldosterone, osteoprotegerin and fibroblast growth factor - 23, glucose levels in patients with CKD (stages II-IV) in relation to cardiovascular events.

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⁽¹⁵⁾.

Fasting subjects were enrolled in this study after excluding CKD patients with a chronic liver disease, a thyroid disorder, or patient on dialysis or had at least one session of dialysis. 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.

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).

Analysis of serum creatinine, urea nitrogen, calcium, phosphate and alkaline phosphatase were performed using the specific

Flex® reagent cartridge /Dimension (RxL Max) / SIEMENS (USA). Whereas, serum aldosterone was measured by ELISA kit, purchased by DRG Instruments GmbH, Germany⁽¹⁶⁾. While, human fibroblast growth factor-23 ELISA kit⁽¹⁷⁾ and human osteoprotegerin ELISA Kit was purchased by Cusabio Biotech Co, LTD (China)⁽¹⁸⁾. Statistical analysis of data is presented as means \pm SD. Significance was set at $p < 0.05$. Cases and controls have been compared using either the t-test for independent samples, The Pearson coefficient for normally distributed variables.

Results

Pooled data of patients when compared to controls indicated that fasting serum glucose (FSG) , urea , creatinine and GFR were elevated except for serum calcium levels were lowered significantly (table 1). According to CKD stages of studied group of patients , serum alkaline phosphatase (ALP) levels tend to be significantly elevated through stages II to IV compared to controls (table 2) , but statistically only values of patients with stage III showed significant elevation in serum

OPG. However, data analysis according to the presence of 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 -3). Meanwhile, serum urea levels, as well as GFR values, were significantly elevated as compared to the controls. While, serum calcium levels were significantly reduced irrespective to the presence or not of CV disease. But serum creatinine concentrations showed no significant variation between patients and controls. Serum Aldosterone, FGF-23, OPG levels were presented with no significant variation among studied groups (table 3).

Serum aldosterone levels were not altered among different studied groups according to the stage of CKD when compared to controls (figure 1). Serum FGF-23 levels were not significantly different among the different groups of patients nor from that of the control subjects, as shown in figure 2.

Table1. Subjects characteristic

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(M/F)	37/22	-	17/6	-	P=0.337
Weight (Kg)	80.56	16.42	83.85	13.31	P=0.466
Height* (cm)	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
Alkaline Phosphatase (μ kat/L)	122.34	56.39	82.70	44.74	P=0.474
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
Sodium (mEq/L)	140.72	6.31	142.08	4.29	P=0.293
Potassium (mEq/L)	4.32	0.86	4.53	0.75	P=0.231
Cardiovascular disorder	21	-	-	-	P=0.005
Smoker	7	-	5	-	P=0.979

Table 2. Serum level of ALP and Osteoprotegerin through different stages of CKD patients compared to the control.

Parameters	Stage								P-value
	II(No.11)		III(No.36)		IV(No.12)		Control (No.23)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
ALP μkat/l	87.63 c	24.25	126.27 a	63.51	121.09 a	40.79	82.70	44.74	0.010
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

Table3 .Variation in some studied parameters in patients compared to the control subjects according to presence of CV disorder or not.

Parameters	Patient				Control		P-value
	Cardiovascular event				No CV event		
	Yes (No=29)		No (No=30)		(No=23)		
	Mean	SD	Mean	SD	Mean	SD	
Glucose (mg/dl)	176.29 ab	59.0 9	128.81 a	47.09	99.83	23.54	P=0.005
GFR (ml/min)	41.62 a	13.7 9	48.19 a	18.98	111.26	13.69	P=0.005
Urea (mg/dl)	78.64 ab	26.1 1	63.16 a	23.93	28.70	9.54	P=0.005
Creatinine (mg/dl)	1.76	0.49	1.65	0.62	0.66	0.16	P=0.359
OPG (pg/ml)	105.65	122. 66	93.39	118.90	22.79	46.37	P=0.233
FGF-2 (pg/ml)	50.7	165	105	408	7.8	3.9	P=0.613
Aldosterone (pg/ml)	44.80	3.87	53.95	32.42	48.09	20.63	P=0.317
Calcium (mg/dl)	8.6 a	0.83	8.49 a	1.03	9.31	0.52	0.005
Phosphate (mg/dl)	4.2	0.86	4.12	0.79	4.31	0.55	0.697

a: significantly different from control

b: significantly different from a patient with no CVD

N: Number of subjects

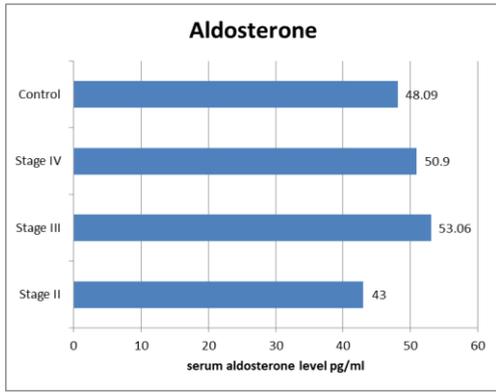


Figure 1. Serum aldosterone levels (pg/ml) among various stages of CKD patients compared to the control group

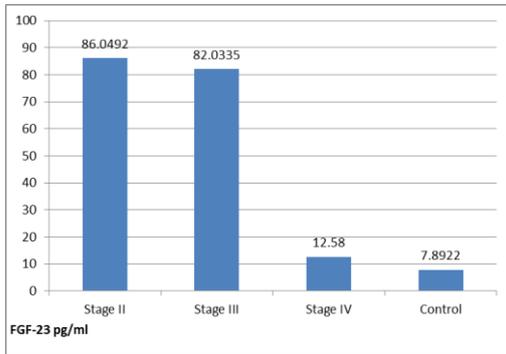


Figure 2. Serum FGF-23 levels (pg/ml) among various stages of CKD patients compared to the control group

Correlation studies for studied parameters among various groups

As shown in figure -3, mean serum sodium levels are negatively ($r = -0.353$) correlated with serum OPG levels in CKD patients at a significance level of ($p = 0.019$). In figure 4 the mean of serum FGF-23 levels is positively ($r = 0.482$) correlated with serum OPG level in CKD patients at a significance level of ($p = 0.0001$). While figure 5 showed that the mean of serum FGF-23 levels is positively ($r = 0.313$) correlated with serum phosphate levels in CKD patients at a significance level of ($p = 0.032$). Aldosterone levels in CKD patients are correlated with serum urea levels ($r = 0.328$), at a significance level of $p = 0.015$ (figure 6).

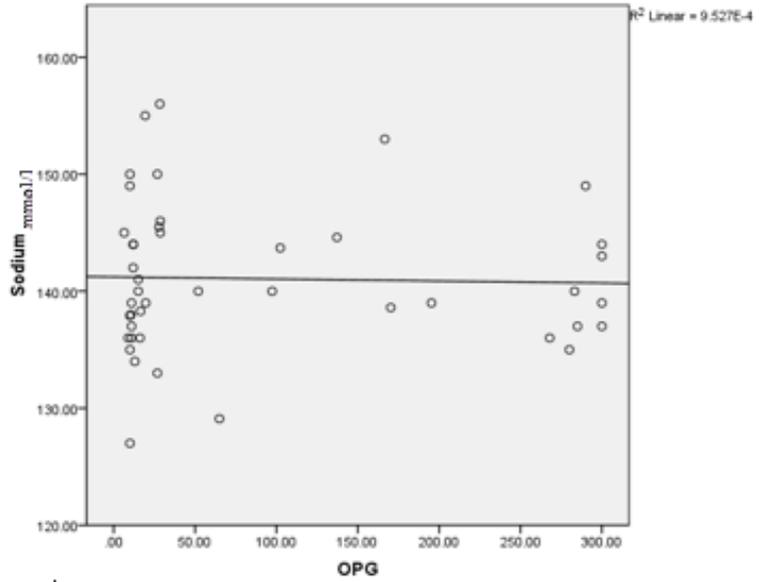


Figure 3. Correlation between Serum Sodium and OPG Levels in CKD Patients (with and without CV events) ($r = -0.353$, $p = 0.019$)

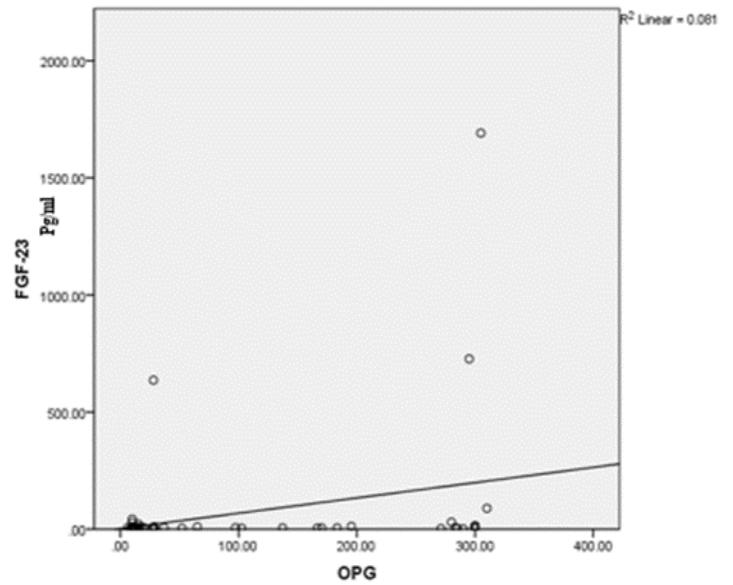


Figure 4. Correlation between Serum OPG and FGF-23 Levels in CKD Patients with or without CV events ($r = 0.482$, $p = 0.0001$).

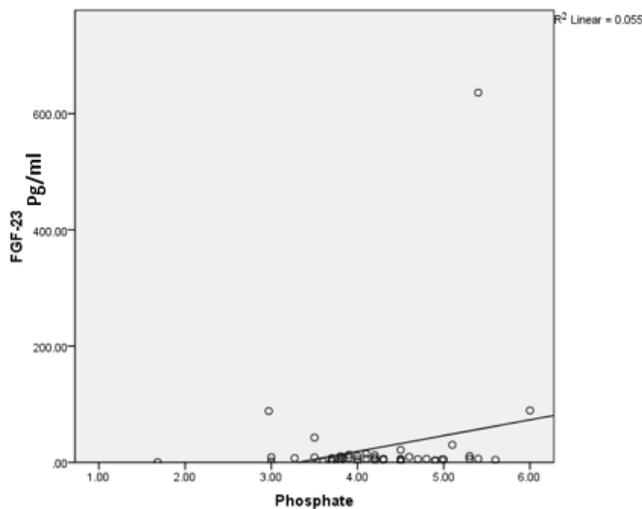


Figure 5. Correlation between Serum FGF-23 and phosphate levels in CKD Patients with or without CV events ($r=0.313$, $p=0.032$).

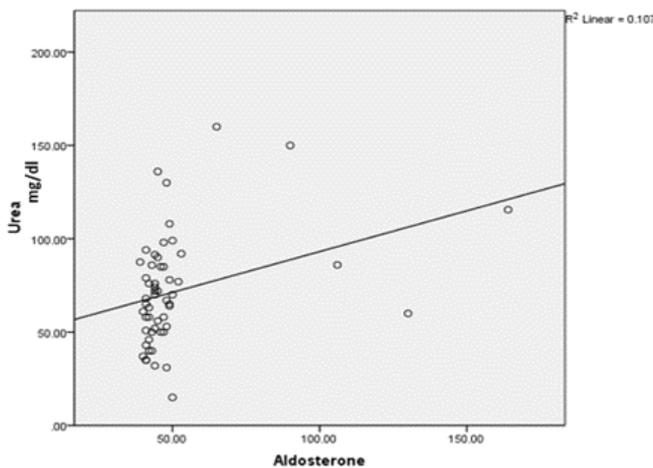


Figure 6. Correlation between serum aldosterone and urea levels in CKD patients with or without CV events ($r=0.328$, $p=0.015$).

Discussion

Effect of studied parameters on cardiovascular events

Glucose

Data analysis according to the presence of CV disorders in patients compared to that of controls showed that fasting serum glucose levels of CKD patients (with and without CV disorder) were 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 -3) and this indicates that diabetes is the main risk factor for CKD and cardiovascular disease, so the increase in glucose level will expose patients to the

additional risk of cardiovascular event independent of baseline CKD present ⁽¹⁹⁾.

Osteoprotegerin

Considering OPG levels only patients in stage III showed significantly higher levels from controls as well as from those in stage IV and this is consistent with what was shown in study in which serum OPG is elevated in patients with chronic kidney disease where it could predict the deterioration of kidney function, despite the fact that direct effects of OPG on kidneys are still largely not discovered ⁽⁹⁾.

Serum sodium levels were negatively correlated with serum OPG level in CKD patients and this may be explained by correlation of CKD progression linked to increasing OPG serum level to hyponatremia as in large cohort study of 655,000 veterans with a mean GFR of 50 ml/min/1.73 m², hyponatremia was seen in 13.5% of patients and this may be due to CKD patients are at additional risk of compromised capacity to dilute or concentrate urine (Figure 3) ⁽²⁰⁾.

Serum OPG levels showed no significant variation between studied groups (table -3); these results disagreed with a study found that in patients with chronic kidney disease vascular calcification contributes to increased cardiovascular (CV) morbidity and mortality, ⁽²¹⁾ In the present study, the results may be contributed to the small number of patients present in each group.

Aldosterone

Serum aldosterone levels in patients compared to the control levels showed no significant variation among patients with different stages of CKD and with that of the controls (Figure -1), and this is inconsistent with what has been known for decades that a plasma aldosterone concentration is elevated in CKD ⁽²²⁾.

From our point of view, this was expected because all patients whether only have CKD or CKD with cardiovascular disorders are taking anti-hypertensive medications to treat either hypertension (diuretics) or other related conditions like heart failure (ACE-I), myocardial infarction (Valsartan), angina (Amlodipine) or even protecting kidney function by ACE-I or ARBs and all of which are well known that these medications affect the aldosterone serum levels ⁽²³⁾.

Renin-angiotensin-aldosterone system which is known to be activated in patients with CHF, and in fact plasma renin activity also has been shown to correlate directly with mortality besides that angiotensin II is known to cause myocardial remodeling and aldosterone may increase myocardial fibrosis and necrosis in the heart, ⁽²⁴⁾ and this was not found in this study as

there was no significant difference in aldosterone level between patients with or without CV disease, this may be contributed to all patients whether only have CKD or CKD with cardiovascular events are taking anti-hypertensive medications to treat either hypertension (Diuretics) or other related conditions like heart failure (ACE-I), myocardial infarction (Valsartan), angina (Amlodipine) or event protecting kidney function by ACE - I or ARBs and these medications affect the aldosterone serum levels⁽²³⁾, so the result of the aldosterone level may not reflect reality and because this study is not an intervention study and not interfere with medications the patient is taking.

FGF-23

Serum FGF-23 levels were not significantly different among the different groups of patients nor from that of the control subjects, as shown in Figure -2 and likely as an appropriate compensation to help maintain normal serum phosphate concentrations in the face of declining nephron mass, in the situation where patients reach end-stage renal disease, where FGF-23 concentrations are often increased, whereas serum phosphate concentrations are only modestly increased or even normal⁽¹³⁾. The present study found that increased FGF-23 level is associated with left ventricular hypertrophy, fat mass, and dyslipidemia in elderly patients⁽²⁵⁾. This positive correlation between FGF23 and mortality also is found in the general population with coronary artery disease⁽²⁶⁾.

FGF-23 is associated with heart failure and mortality in patients with or without chronic kidney disease, Because FGF23 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⁽²⁷⁾.

While in the present result, this was not proven and this 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, there is 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."⁽¹³⁾ Regarding urea, there was a significant difference between patients with CV disease and patients without CV disease (Table -3) and this can comply with another interesting observation in the POTIME-CHF study which is the significant rise in jugular venous pressure as quartile BUN values rose. The associated increase in renal venous pressure would increase renal interstitial pressure and activate the Renin angiotensin aldosterone system(RAAS), in addition to increase in cardiac preload and cardiac dilatation is known to be important risk factors for increased death rate in heart failure patients⁽²⁸⁾.

Alkaline Phosphatase

Stage III and IV patients Serum ALP levels were significantly higher than that of controls (Table -2), whereas, patients with stage II serum ALP values were significantly lower than that of stage III patients and this match the new evidence states that serum ALP increases in patients with CKD, also in CKD patients without liver disease, alkaline phosphatase can be elevated in high-turnover bone disease, however measuring this readily available and inexpensive biomarker has not been considered as an individual therapeutic target of CKD-MBD⁽²⁹⁾.

Correlation studies

Serum sodium levels were negatively correlated with serum OPG level in CKD patients ($r=0.353$), ($p=0.019$), and this may be explained by correlation of CKD progression linked to increasing OPG serum level to hyponatremia as in large cohort study of 655,000 veterans with a mean GFR of 50 ml/min/1.73 m², hyponatremia was seen in 13.5% of patients and this may be due to CKD patients are at additional risk of compromised capacity to dilute or concentrate urine (Figure -3)⁽²⁰⁾.

While serum FGF-23 levels are positively correlated with serum OPG level in CKD patients at a significant level ($p=0.0001$) (Figure -4), this supports the theory that both OPG and FGF-23 are linked with cardiovascular mortality and this link may be mediated by direct action on the myocardium rather than by indirect effect such as arterial stiffening⁽³⁰⁾.

Serum FGF-23 levels are positively correlated with serum Phosphate level in CKD patients at a significant level ($p=0.0001$) (Figure -5) and this can be explained by its relation to hyperphosphatemia, a metabolic disorder that is almost universal in CKD which stimulates the release of the hormone FGF23

from skeletal osteocytes as a role of a negative feedback loop⁽³¹⁾.

Serum urea levels are positively correlated with serum aldosterone level in CKD patients at significant level ($p=0.015$) (as shown in Figure-6), which could indicate an association between the decline in kidney function and corresponding elevation in aldosterone levels, and this may be due to increase in Sodium and water reabsorption correspondent to increase in aldosterone level which lead to increased reabsorption of urea and this can be seen mainly in heart failure patients, so increase sodium and water reabsorption in proximal tubule leads to increase in Urea concentration and that promote the passive reabsorption of Urea⁽³²⁾.

Conclusion

Osteoprotegerin was significantly increased in stage III CKD patients as compared to control group, but no significant difference was shown between patients with or without cardiovascular events. Meanwhile, there was no significant difference between patients and controls regarding both serum aldosterone and FGF-23 levels.

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