

Fibroblast growth factor 23 (FGF23) is an early specific marker in the diagnosis and staging of chronic kidney disease (CKD) in human

Running title: Early Diagnosis of CKD by FGF23

Abstract

Objective: To evaluate the clinical utility of serum FGF23 as an early specific biomarker in the diagnosis and progression of chronic kidney disease (CKD) patients.

Methods: A number of 120 male patients with CKD who were classified according to the eGFR into four stages (n=30 for each stage), in addition to 30 healthy control men were included. **Results:** Patients in stage 2 of CKD did not show any significant difference in serum levels of urea and creatinine, and lactate dehydrogenase (LDH) activity. With the progression of CKD from stage 3 to stage 5, there were linear increases in the serum urea and creatinine levels, and LDH activity. There was a significant decrease in serum albumin and a significant elevation in creatine kinase in all CKD stages. There was a significant decrease in serum Ca^{2+} level in stages 2-4. Only patients in CKD stage 5 showed a significant elevation in serum phosphorus level. There were significant elevations in serum aminotransferases, C-reactive protein, and parathyroid hormone levels in stages 4 and 5. Serum testosterone level was significantly reduced in stages 3 and 4 as compared to control. With the progression of CKD stages from stage 2 to 5, there were linear significant elevations in serum $\text{TNF-}\alpha$ and FGF23 levels. **Conclusions:** FGF23 was the most sensitive indicator in the early diagnosis and staging of CKD. Other biomarkers were elevated in the late stages in addition to their low specificity. Therefore, FGF23 could be used in the diagnosis and prognosis of CKD patients.

Keywords: Calcitropic hormones, CKD, FGF23, inflammation, PTH, testosterone

1. Introduction

The incidence of chronic kidney disease (CKD) is reaching an epidemic proportion worldwide. The number of patients diagnosed at the early stages of CKD exceeds those reach the end-stage renal disease by more than 50-folds [1]. The severity of CKD is classified into five stages starting with stage 1, being the mildest and with few symptoms, to stage 5, being the most severe and life-threatening if untreated [2]. Although there are promising intervention tools and medications available for the treatment of CKD at early stages, the poor and late diagnosis makes these interventions useless [1]. Therefore, the nephrology community needs an early sensitive and reliable marker of CKD.

In humans, fibroblast growth factor 23 (FGF23) encoded by the FGF23 gene [3], is mainly responsible for phosphate metabolism and regulation of plasma phosphate level [4]. The elevated level of calcitriol is the main inducer of osteocytes which in response secrete FGF23. The latter reduces the expression of sodium-phosphate cotransporter (NPT2) in the proximal tubules, resulting in diminished phosphate reabsorption and enhanced of phosphate excretion with a net reduction in plasma phosphate level [5,6].

The aim of the present study is to evaluate the clinical utility of serum FGF23 as a novel specific and early marker of diagnosis and progression of renal disease in non-diabetic patients of CKD.

2. Subjects and Methods

2.1. Subjects

This study was conducted at Ain Shams Specialized Hospital (ASUSH) and involved known and diagnosed CKD patients who were having follow up at the dialysis inpatient and outpatient clinics. Estimation of glomerular filtration rate (GFR) was performed according to the Davita GFR calculator using the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation. All participants were subjected to full clinical examination and reporting including the vital data such as blood sugar, age, gender, and eGFR. Diabetic patients were excluded. The study protocol was approved by the university ethics committee (No: ASUH/NS/39/14, Date: 28/2/2018) and informed consent was obtained from all participants. **Participants (150 males)** were divided into two different groups; Group A (control group): this group included 30 of healthy male people **(average age 48.2±3)**. Group B: this group contained 120 male patients with CKD who were classified according to the eGFR into the following stages; **Stage 2; this stage included 30 CKD patients with mildly decreased GFR (eGFR 60-89 mL/min) and the average age (56.2±2.06)**. **Stage 3; this stage included 30 CKD patients with moderately decreased GFR (eGFR 30-59 mL/min) and the average age (58.2±3.1)**. **Stage 4; this stage included 30 CKD patients with severely reduced GFR (eGFR 15-29 mL/min) and the average age (58.7±3.51)**. **Stage 5; this stage included 30 CKD patients with kidney failure and on dialysis (GFR < 15 mL/min) and the average age (59.8±3.67)**.

2.2. Blood samples

Six ml blood sample was withdrawn by venipuncture and equally divided in two plain vacutainer tubes. Serum was immediately separated from the two plain tubes after centrifugation at 4500 rpm for three minutes, and then stored at -20 °C until used for biochemical analyses of creatinine, urea, calcium, albumin, phosphorus, parathyroid hormone (PTH), testosterone, alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), C-high sensitive reactive protein (CRP), tumor necrosis factor- α (TNF- α), and fibroblast growth factor-23 (FGF23).

2.3.Methods

Serum creatinine was determined on Synchrony CX-9 autoanalyser using a modified Jaffé method. Using a synchrony system, the following assays were performed. Blood urea nitrogen (BUN) was estimated by an enzymatic conductivity rate method. Albumin concentration was assessed using the bichromatic digital endpoint with bromocresol purple. Glucose concentration was measured using timed endpoint method. ALT and AST activities were measured using the kinetic rate method. Using the Cobas Integra 800* autoanalyzer system (Roche, German), the following assays were performed. Calcium and phosphorus were measured as described elsewhere [7]. Parathyroid hormone (PTH) level [8], and lactate dehydrogenase (LDH) activity were estimated using the Access Immunoassay Systems (Beckman Coulter, USA). Determination of tumor necrosis factor alpha (TNF- α) concentration was performed by ELISA kit (Alpco Diagnostics, Cat. no. 45-TNFHUU-E02) as previously described [9]. The serum level of testosterone was measured by ELISA kit provided by DRG (EIA-2924). Determination of creatine kinase (CK) activity was performed using a modified method of the IFCC. The level of C-reactive protein (CRP) was assessed by the C-reactive protein Extended Range

(RCRP) method. The fibroblast growth factor-23 (FGF-23) concentration was estimated using a sandwich ELISA kit (GLORY Science, TX, USA)

2.4. Statistical analysis

The data distribution was tested by the Kolmogorov-Smirnov test. The data obtained were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's for multiple comparison [10] for significant comparisons between the various groups using graphpad prism 5 (San Diego, CA, USA). Probability levels of less than 0.05 were considered significant ($P < 0.05$)

3. Results

There was no significant difference in the average age of the participants in this study among the different groups. Glomerular filtration rate was used as a criterion for the classification of patients of CKD. As shown in Table (1), there was a significant depression in GFR in stages 2, 3, 4 and 5 compared to control. The % of change in stages 2, 3, 4 and 5 was ~ -34%, -58%, -80% and 90% from control, respectively. On comparing different stages, significant changes were recorded between each pair of the following stages; (2 and 3), (3 and 4) and (4 and 5). Patients in stage 2 of CKD did not show any significant difference in serum creatinine level (Table 1). With the progression of CKD from stage 2 to stage 5, there was a linear increase in the serum creatinine level. The % of change in stage 3 was ~108% from control, and the change was ~303 and 704 in stages 4 and 5, respectively. There was no significant difference between stages 2 and 3, while there were significant changes between stages 3 and 4 and between stages 4 and 5. Patients in stage 2 of CKD did not show any significant difference in serum BUN level compared to control. With the progression of CKD stages from 2 to 5, there

was a linear increase in serum BUN level. The % of change in stage 2 was ~58% from control, and the change was 154%, 266% and 383% in stages 3, 4 and 5 compared to control. There was a significant difference between each pair of the following stages (2 and 3), (3 and 4) and (4 and 5). There was a significant decrease in serum albumin in all CKD stages when compared with control. The % of change was ~20%, -24.5%, -32%, -34% for stages 2, 3, 4 and 5, respectively. No significant changes were recorded between each pair of the successive stages (Table 1).

Patients in stage 5 of CKD did not show any significant difference in serum Ca^{2+} level compared to control. There was a significant decrease in serum Ca^{2+} level in stages 2, 3 and 4 compared to control group. The % of change from control was -7.8%, -15%, -12%, and -17% for stages 2, 3, 4 and 5, respectively. No significant changes were recorded between each pair of the successive stages (Table 2). Patients in stages 2, 3 and 4 of CKD did not show any significant difference in serum phosphorus level compared to the control group. Only patients in CKD stage 5 showed a significant elevation in serum phosphorus level as compared to control. The % of change in stage 4 was 21% which did not achieve a statistical significance, the change in stage 5 was 44% from control. On comparing different stages, significant changes were recorded between each pair of the following stages; (3 and 4) and (4 and 5). With the progression of CKD from stage 2 to 5, there was a linear increase in serum PTH level. However, the elevations in stages 2 and 3 did not achieve a statistical significance compared to control. The % of change in stages 2 and 3 was ~ 67% and 117% from control. The elevation in serum level of PTH in stages 4 and 5 was significant compared to control. The % of change was 710% and 1925% for stages 4 and 5, respectively. There were significant changes between stages (3 and 4) and between (4 and 5) (Table 2).

There was a significant elevation in serum CK activity in stages 2, 3, 4 and 5 of CKD when compared with control. The % of change from control was ~ 326%, 458%, 959%, and 676% for stages 2, 3, 4 and 5, respectively (Table 3). On comparing different stages, significant changes were recorded between each pair of the following stages; (3 and 4), and (4 and 5). Patients in stage 2 of CKD did not show any significant difference in serum LDH activity compared to control. There was a significant elevation in serum LDH activity in stages 3, 4 and 5 as compared to control. The % of change in stages 3, 4 and 5 was 77%, 58% and 77%, respectively. There was a significant difference between stages 2 and 3 (Table 3). There was a significant elevation in serum CRP level in stages 4 and 5 compared to control. Patients in stages 2 and 3 did not show any significant difference in CRP as compared to control. The % of change for stage 2 and 3 was ~411% and 463%, respectively. The % of change from control was ~ 2107% and 4033% for stages 4 and 5, respectively. On comparing different stages, significant changes were recorded between each pair of the following stages; (3 and 4) and (4 and 5). With the progression of CKD stages from stage 2 to 5, there was a significant linear increase in serum TNF- α level (Table 3). The change in stage 2 was ~ 340% from control and the change was 629%, 1420% and 1559% for stages 3, 4 and 5. On comparing different stages, significant changes were recorded between each pair of the following stages; (2 and 3), (3 and 4) and (4 and 5) (Table 3).

Patients in stages 2 and 3 of the CKD did not show any significant difference in serum ALT activity as compared to control. There was significant elevation in the enzyme activity in stages 4 and 5 compared to control. The % of change in stages 2 and 3 was ~ 54% and 57%, respectively from control and the change was 83% and 84% for stages 4 and 5, respectively (Table 4). Similarly, patients in stages 2 and 3

of the CKD did not show any significant difference in serum AST activity as compared to control. There were significant elevations in the enzyme activity in stages 4 and 5 compared to control. The % of change in stages 2 and 3 was ~ 21% and 23%, respectively from control and the change was 65% and 62% for stages 4 and 5, respectively. For ALT and AST, no significant changes were recorded between any stage and its precedent stage (Table 4). Patients in stages 2 and 5 of CKD did not show any significant difference in serum testosterone level. The % of change in stages 2 and 5 was ~ -11% and -34% from control. Serum testosterone level was significantly reduced in stages 3 and 4 as compared to control. The % of change was -38% and -53% for stages 3 and 4, respectively. No significant changes were recorded between each pair of the groups (Table 4).

With the progression of CKD stages from stage 2 to 5, there was a linear increase in serum FGF23 level. There was a significant elevation in serum FGF23 level in stages 3, 4 and 5 compared to control. The change in stage 2 was ~ 91% from control and the change was 313%, 549% and 1067% for stages 3, 4, and 5, respectively. On comparing different stages, significant changes were recorded between each pair of the following stages; (2 and 3), (3 and 4) and (4 and 5) (Figure 1).

4. Discussion

Chronic kidney disease (CKD) has become a major public health problem world-wide. In Egypt, the prevalence of chronic renal failure is 225/million/year, while in Europe, it accounts for 283/million/year, and in the United States, is 975/million/year [11]. CKD is defined as a gradual loss of renal function over a period of months to years. At the early stages, there are no symptoms while, later

many symptoms develop including pedal edema, fatigue, loss of appetite, anemia hypertension, bone diseases, cardiovascular complications, and confusion [12].

Staging of CKD facilitates the clinical performance, evaluation, and management of CKD [2]. Stages are established by a series of tests that calculate the estimated glomerular filtration rate (eGFR). There are five stages of CKD based on the level of kidney function. Stage 1 with eGFR ≥ 90 , stage 2 where eGFR (60-89), stage 3 where eGFR (30-59), stage 4 where eGFR (15-29), and stage 5 with eGFR < 15 . Staging and early diagnosis of CKD would be helpful in the treatment of such patients before reaching stage 5 that require kidney transplantation.

The precise and early diagnosis of CKD is very crucial in reducing the risk of progression to stage 5 and dialysis and/or kidney transplantation. Biochemical markers are used extensively in the diagnosis of many diseases. Creatinine is a metabolite of creatine phosphate in muscles. It is produced at a constant rate by the body depending on the muscle mass [13]. Elevation of creatinine is the biomarker used in the diagnosis of CKD. However, creatinine is proved to be a late marker of CKD. In the current study, it exceeds the upper normal limit only at stage 4. The major physiological function of FGF-23 is to regulate urinary phosphate excretion and maintain a constant serum phosphate level [14, 15]. The regulation of vitamin D production is an essential secondary function of FGF23. High plasma phosphate, calcitriol, and persistent hyperphosphatemia are the major triggers for enhanced FGF23 expression [16].

At the early stages of CKD, the number of normal nephrons decline resulting in high plasma phosphate level and hence elevated FGF23 is expected. Therefore, the aim of the present study was to investigate the potential of FGF23 as an early diagnostic marker of CKD and its validity in staging of CKD patients. To achieve the

aim and based on eGFR, we have selected CKD patients in stages 2-5 (n=30 for every stage), in addition to 30 healthy control volunteers. All participants were not diabetic.

The current study showed that patients in stage 2 of CKD did not show any significant difference in serum creatinine and BUN level compared to control. With the progression of CKD stages from 3 to 5, there was a linear increase in serum BUN level. Creatinine level also began to significantly increase at stage 3 (the upper normal level) and continued thereafter. Serum albumin significantly declined in all CKD stages but the decrease was not correlated to the stage.

Urea is synthesized by the liver as an end product of protein catabolism. It is filtered by the glomeruli and partially reabsorbed by the tubules [17]. In the late stages of CKD, excretion of urea and creatinine decline resulting in their elevation in the plasma [18]. However, urea and creatinine are not reliable biomarkers of CKD. They are elevated at the late stages and they are not specific. In patients with renal dysfunction, the tubular secretion of creatinine increases giving false negative value [19]. Some other disorders such as muscular dystrophy, leukemia, and hyperthyroidism can elevate the creatinine level. The reduced creatinine levels are reported with congestive heart failure, acute tubular necrosis, polycystic kidney disease, and dehydration [18]. This showed the failure of creatinine and urea to be early diagnostic markers in addition to low specificity as they are affected by many other health problems. Therefore, we need a better early diagnostic marker of CKD.

Hypoalbuminemia could indicate decreased synthesis by the liver or increased clearance by the kidneys and this could happen in many diseases other than CKD [20]. Patients with end-stage renal disease (ESRD) usually develop hypoalbuminemia due to increased albumin catabolism and reduced synthesis resulting in mortality [21].

The absence of correlation of reduction of albumin and CKD stages indicates that albumin cannot be used in staging or early diagnosis.

The current study showed that there was a significant decrease in serum Ca^{2+} level in stages 2, 3, and 5 compared to the control but there was not any significant difference in serum phosphorus compared to the control. Only patients in stage 5 showed a significant elevation in phosphorus. The results of the present study showed a linear increase in serum PTH level in CKD in all stages investigated. However, the elevations in PTH in stages 2 and 3 did not achieve a statistical significance compared to the control. The interplay between calcium, phosphorus, and calcitropic hormones (PTH, calcitonin, and vitamin D) is well understood. The damaged kidneys cannot activate calcitriol causing an imbalance of calcium in the blood. The damaged kidneys cannot clear the phosphorus from the blood either, resulting in elevated plasma phosphorus level. The elevated phosphorus level would result in calcium leakage out of the bones leading to hypercalcemia. Hypocalcemia is quite uncommon in some late stages of CKD. Hyperphosphatemia produced in CKD contributes directly to hypocalcemia through interactions of calcium ions. Hyperphosphatemia was only reported in the late stages of CKD [22], similar to the findings of the present study.

The progression of CKD is associated with consequent elevated PTH to help maintaining the homeostasis of calcium/phosphorus metabolism [23]. The elevated PTH level due to CKD is called hyperparathyroidism of renal disease (rHPT). It results in renal osteodystrophy, vascular calcification, and cardiovascular diseases. The “CKD-mineral and bone disorder” term was adopted by the National Kidney Foundation to describe this complex pathophysiology of minerals and PTH in CKD [24,25].

Patients with CKD are at increased risk for cardiovascular diseases [26]. Most mortality from CKD occurs due to heart complications. In the present study, LDH and CK were estimated to investigate the heart function. A significant elevation in serum CK in stages 2-5 was reported compared with the control with the highest elevation in stage 4. A significant elevation in serum LDH in stages 3, 4 and 5 was also reported with the most elevation in stage 5. LDH loss from the kidney is an indicator of tubular cell death [27]. High CK levels are associated with acute renal failure, stark electrolyte abnormalities, and acid base turbulences resulting in significant morbidity [28]. The elevated levels of cardiac biomarkers may reflect secondary myocardial injury caused by disturbances of electrolytes resulting primarily from CKD or could result from the CKD-associated chronic inflammation [29].

The ESRD patients have altered albumin homeostasis caused by a systemic inflammation which closely correlates with mortality [21]. It seems that inflammation is a key player in CKD and the consequent pathophysiology of different organs [30]. The results of the current study showed an elevation in the serum CRP only in stages 4 and 5 in agreement with a previous study [31] that found that CKD patients with increased CRP values are linked with the development of atherosclerosis. Elevated CRP levels are associated with an increase in the carotid intima-media area in CKD patients [32]. A linear increase of TNF- α with the progression of CKD stages (2 to 5) was reported in the current study. TNF- α was more sensitive than CRP in correlation with the CKD staging. Belinda et al. [33] showed that TNF- α is significantly elevated in patients with CKD compared to the controls normal subjects. In addition, TNF- α is significantly and positively associated with the severity of CKD. TNF- α stimulates the immune cell infiltration and cell death which severely affects the renal hemodynamics and nephron transport [34]. It was previously shown that patients with

CKD suffer various inflammations that are not caused by bacteria or viruses. These inflammations are characterized by increased expression levels of CRP and TNF- α [35].

To investigate the effect of CKD on the liver status, the ALT and AST activities were examined. The results indicated that stages 2 and 3 of CKD did not show any significant difference in the serum activities of both ALT and AST, but there were significant elevations of both enzymes in stages 4 and 5 compared to control. There are controversial data regarding the ALT and AST in CKD. Some studies have revealed that serum ALT and AST activities are lower in patients with CKD [36,37]. Elevated serum activities of AST and ALT were previously reported in CKD patients [38]. These increases in serum AST and ALT levels were attributed to the absence of renal clearance and hepatotoxicity [39].

The results of the present study did not show any significant difference in serum testosterone in stages 2 and 5, but the hormone level was significantly reduced in stages 3 and 4 as compared to control. Higher mortality among CDK male patients stages 3 and 4 who have low testosterone level, was previously reported [40]. A similar phenomenon among the super-aged Japanese men was observed [41]. This phenomenon was associated with high level of aromatase [42].

The results of the current study showed a linear increase of FGF-23 with the progression of CKD from stages 2 to 5. **There was a strong positive correlation between the median age and the level of FGF23 (R=0.73).** One of the early disturbances in the CKD patients is the perturbation in the mineral homeostasis. This is manifested by the elevation in phosphate and reduction in calcium levels and later followed by elevation in the PTH to compensate these changes [43]. Persistent phosphate retention would also elevate the level of FGF23 very early in the course of

CKD development [44]. Serum FGF23 levels gradually increased as kidney function declines and are markedly elevated once on dialysis therapy [45]. Elevated FGF23 level resulted in hypophosphatemia and low levels of vitamin D. Serum FGF23 level increases in parallel with the perturbation of renal function and the increase of serum phosphate and PTH concentrations [6]. With its elevation very early in stage 2, and continuous elevations with the progression of the CKD from stage 2 to stage 5, we think FGF2 is a reliable early and specific biomarker of CKD that could help in the therapeutic interventions to prevent the CKD deteriorations, and reduce the need for dialysis and kidney transplantation.

5. Conclusions

The progression of CKD in the current study was associated with elevations in BUN, creatinine, PTH, phosphorus, CK, LDH, CRP, TNF- α , ALT, and AST, and reductions in GFR, albumin, calcium, and testosterone. However, FGF23 was the most sensitive indicator in the diagnosis and staging of CKD. PTH and phosphorus were elevated only in late stages 4 and 5. Similarly, creatinine and urea were elevated in late stages in addition to their low specificity. Therefore, FGF23 could be used as an early indicator of CKD, staging, and prognosis of the disease in response to certain medications.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any

litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Effect of chronic kidney disease (CKD) on glomerular filtration rate (GFR) (mL/min) and kidney functions

	GFR (mL/min)	Creatinine (mg/dL)	BUN (mg/dL)	Albumin (g/dL)
Control	107.89 ± 3.31	0.77 ± 0.02	13.71 ± 0.68	4.11 ± 0.09
Stage2	71.23 ± 1.42 ^a	1.10 ± 0.02	21.68 ± 1.70	3.29 ± 0.16 ^a
Stage3	45.21 ± 1.52 ^{a,b}	1.60 ± 0.05 ^a	34.92 ± 2.58 ^{a,b}	3.10 ± 0.15 ^a
Stage4	21.21 ± 0.82 ^{a,b}	3.10 ± 0.12 ^{a,b}	50.23 ± 2.56 ^{a,b}	2.80 ± 0.10 ^a
Stage5	10.38 ± 0.54 ^{a,b}	6.19 ± 0.40 ^{a,b}	66.30 ± 3.51 ^{a,b}	2.72 ± 0.13 ^a

BUN: blood urea nitrogen. Data are expressed as mean ± SEM, n=30. ^asignificant change versus control. ^bsignificant change versus the previous stage

UNDER PEER REVIEW

Table 2: Effect of chronic kidney disease (CKD) on serum levels of calcium, phosphorus, and parathyroid hormone (PTH)

	Ca ²⁺ (mg/dL)	Phosphorus (mg/dL)	PTH (pg/mL)
Control	9.55 ± 0.20	3.87 ± 0.19	27.19 ± 1.91
Stage2	8.09 ± 0.1 ^a	3.43 ± 0.12	45.39 ± 3.33
Stage3	8.35 ± 0.17 ^a	3.73 ± 0.13	59.11 ± 4.56
Stage4	7.93 ± 0.14 ^a	4.69 ± 0.28 ^b	220.21 ± 13.57 ^{a,b}
Stage5	8.80 ± 0.40	5.58 ± 0.30 ^{a,b}	550.77 ± 78.59 ^{a,b}

Data are expressed as mean ± SEM, n=30. ^asignificant change versus control, ^bsignificant change versus the previous stage

UNDER PEER REVIEW

Table 3: Effect of chronic kidney disease (CKD) on serum activities of creatine kinase (CK) and lactate dehydrogenase (LDH), and levels of C-reactive protein (CRP) and tumor necrosis factor- α (TNF- α)

	CK (U/mL)	LDH (U/L)	CRP (mg/dL)	TNF- α (pg/mL)
Control	18.52 \pm 1.0	311.00 \pm 11.48	0.27 \pm 0.03	5.45 \pm 0.37
Stage2	78.90 \pm 7.0 ^a	365.81 \pm 14.10	1.38 \pm 0.13	24.00 \pm 1.78 ^a
Stage3	103.35 \pm 8.3 ^a	550.17 \pm 18.72 ^{a,b}	1.52 \pm 0.16	39.75 \pm 5.21 ^{a,b}
Stage4	196.15 \pm 20.4 ^{a,b}	492.07 \pm 19.84 ^a	5.96 \pm 0.60 ^{a,b}	82.86 \pm 3.16 ^{a,b}
Stage5	143.71 \pm 11.7 ^{a,b}	550.45 \pm 27.49 ^a	11.16 \pm 1.26 ^{a,b}	90.43 \pm 4.78 ^a

Data are expressed as mean \pm SEM, n=30. ^asignificant change versus control, ^bsignificant change versus the previous stage

UNDER PEER REVIEW

Table 4: Effect of chronic kidney disease (CKD) on liver functions and testosterone

	ALT (U/L)	AST (U/L)	Testosterone (ng/mL)
Control	18.09 ± 1.18	22.22 ± 1.25	3.32 ± 0.29
Stage2	27.91 ± 2.53	27.00 ± 2.54	2.95 ± 0.40
Stage3	28.33 ± 2.03	27.25 ± 1.68	2.05 ± 0.30 ^a
Stage4	33.06 ± 4.52 ^a	36.65 ± 4.66 ^a	1.51 ± 0.22 ^a
Stage5	33.31 ± 3.92 ^a	36.08 ± 3.36 ^a	2.18 ± 0.25

Data are expressed as mean ± SEM, n=30. ^asignificant change versus control

UNDER PEER REVIEW

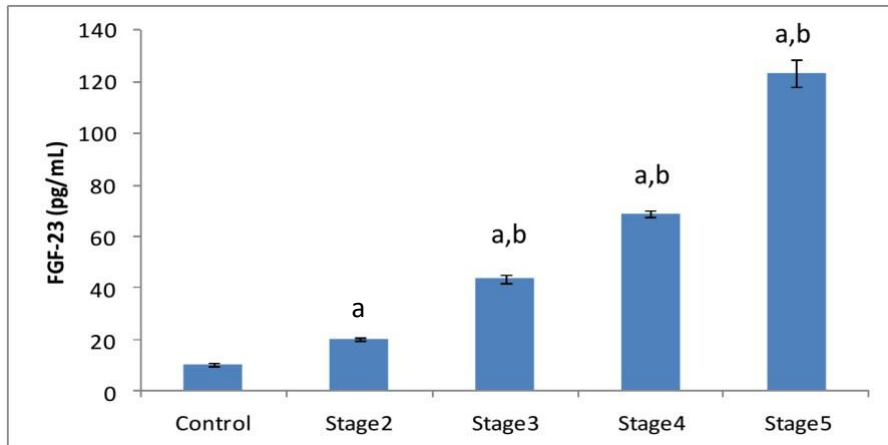


Figure 1: Effect of chronic kidney disease (CKD) on serum level of fibroblast growth factor-23 (FGF-23) (pg/mL) in human. Data are expressed as mean \pm SEM, n=30. ^a significant change versus control, ^b significant change versus the previous stage

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