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Evaluation of Osteocalcin and Pro-Collagen Type 1 Pro-Peptide as Predictors of Bone Integrity in Patients with Chronic Kidney Disease

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Abstract

Back ground: The risk of fractures progressively increased with kidney function declines and in end-stage the kidney disease regarded as the risk of fractures is more 2 to 3 times higher compared to the general population. The gold standard for assessment of bone turnover is the measurement of bone formation rate in an iliac crest bone biopsy specimen. This is limited by expense, invasiveness and availability of local expertise. Plasma bone turnover markers were developed in osteoporosis patients for fracture risk prediction and assessment of medication compliance and anti-fracture efficacy. Therefore, the present study was aimed to evaluation the chemical biomarkers of bone integrity and to assessment the efficacy of these biomarkers as diagnostic and predictors biomarkers for early detection of complications of bone diseases in patients with chronic kidney disease. Methods. A case control study, involved 129 patient and 55 participants as control group. Cobas E311, C311 automated analyzer and ELISA technique were used to analysis the parameters. Results: The current study showed significant differences in the BMI (26.8), uric acid (4.34), urea (28.18), creatinine (0.76), phosphorus (3.42), calcium (9.12), vitamin D (31.55), albumin (4.30), alkaline phosphatase (74.49), bone specific alkaline phosphatase (1.83), parathyroid hormone (33.51), osteocalcin (1.84), pro-collagen type1 propeptide and estimated GFR (88.15) in control Acknowledgments\ in comparison with BMI (24.1), uric acid (6.53), urea (139.4), creatinine (7.69), phosphorus (6.309), calcium (8.813), vitamin D (15.14), albumin (3.79), alkaline phosphatase (120.2), bone specific alkaline phosphatase (11.85), parathyroid hormone (153.7), osteocalcin (9.392), pro-collagen type1 propeptide (3.08) and estimated GFR (19.47) in patient, respectively. In the present study were no significant differences between CKD patients and healthy control, regarding to age (P =0.07) and gender (P =0.629). There were highly significant increases of urea, creatinine, uric acid, osteocalcin, pro-collagen type propeptide and estimated GFR, parathyroid hormone, Phosphorous, ALP levels; whereas showed significant decreases of vitamin D, albumin, calcium levels compared to the control group. Conclusion: There were significant decreases in each of vitamin D in patient with CKD; While, there were significant increases in each of ferritin, bone alkaline phosphatase, osteocalcin, and pro-collagen type1 propeptide. They were good noninvasive biomarkers for prediction of bone turnover, risk stratification and assessment disease severity in CKD patients

Keywords

As kidney functions decreases, the chance of fractures rises progressively. In end- stage renal disease, the danger of fractures is 2 to 3 instances better than in the preferred population (Pimentel *et al.*, 2017); also, have an improved risk of long-term hospitalization and mortality following a bone fracture (Tentori *et al.*, 2014). Renal osteodystrophy is a change in bone morphology that impacts human beings with CKD and consists of an extensive variety of bone abnormalities that are present in more than two- thirds of human beings with chronic renal disease (CKD), (Keronen *et al.*, 20 9).

The gold standard for determining bone turnover is the percentage of bone formation in an iliac crest bone biopsy specimen. This is limited by the cost, intrusiveness, and availability of local knowledge (Novel-Catin *et al.*, 2020). Plasma bone turnover markers (BTM) have been implanted in osteoporosis patients in order to anticipate fracture risk, assess medication adherence, and ascertain the efficacy of anti-fracture treatment. Mineral and bone disease in chronic renal disease, a circumstance that has a more incidence of adynamic bone disorder and in which the management of anti- resorptive medicinal drugs will increase microdamage buildup and outcomes in extra fractures, these signs have extra makes use of. Determining high from low bone turnover states and being in a position to frequently check on turnover status to adjust therapy are for this reason critical in the control of CKD patients (Dalle Carbonare *et al.*, 2021).

Additional indications that are unaffected by CKD include bone alkaline phosphatase (BALP) total alkaline phosphatase are unquestionably associated with the pace of bone growth (ALP) (Brown *et al.*, 2022).

Increased mortality and fracture rate are correlated with excessive tiers of PTH, general ALP, and BSALP. Vascular calcification caused by low bone turnover is attached to mortality (Cohen-Solal, Funck-Brentano and Torres, 2020).

Calcium, phosphate, and PTH are frequently assessed in CKD. Total ALP is a low- value enzymatic assessment of CKD, (Di Medio and Brandi, 2021).

A non-collagenous protein called osteocalcin is present in cartilage and bone matrix. It is a kidney-cleared marker of bone improvement. Osteocalcin and BALP together elevated the tremendous predictive cost for identifying adynamic bone sickness to 77%. Renal characteristic has little impact on ranges, which can be correlated with PTH and ALP (Kalia, Ansari and Regmi, 2022). Therefore, the aims of this study to assess the chemical biomarkers in chronic kidney disease, included urea, creatinine, estimated GFR (eGFR), phosphate, calcium, albumin, vitamin D, parathyroid hormone, bone-specific alkaline phosphatase, osteocalcin and amino-terminal pro-peptide of type procollagen (PNP); and evaluating the effectiveness of these biomarkers as indicators for the early diagnosis and risk stratification of bone turnover which consequences of chronic renal disease.

Materials and Methods

A case control study was included (184) participates, that include 129 patients (65 males and 64 females), who diagnosed with chronic kidney disease by specialist physicians, depend on clinical examinations and laboratory investigations in Nephrology Center of the Al-Basra Teaching hospital, Basra Governorate, Iraq; in- addition to (55) healthy individual as control group. Their age range were 17-75 years. The samples were collected throughout the period from March 2022 to December 2022. The statistical data was expressed as means ± standard deviations (SD). $P < 0.05$ was considered

statistically significant.

Results and discussion

In the present study the anthropometric data were illustrated in table (1). Which demonstrate that there were no differences in age (P =0.07) and gender (P =0.62) between CKD patients and the healthy control group.

The current study was demonstrated a significant difference in BMI (p< 0.01) between patient's (24.1) and control group (26.8). Because of the impaired capacity to digest food and the increased requirement for energy, chronic renal disease can induce weight loss and a decrease in appetite. This could result in malnutrition (Ammirati 2020). This study finding agreement with (Koppe, Fouque, and Kalantar-Zadeh 2019).

The means serum uric acid (6.539 mg/dl), urea (139.4 mg/dl), and creatinine (7.69 mg/dl) in patients were significantly higher than the means of uric acid (4.344 mg/dl), urea (28.18 mg/dl), and creatinine (0.76 mg/dl) in the control, Table, (3.1). These findings were consistent with (Nonso *et al.* 2019), (Hafez *et al.* 2021), and (Kameda *et al.* 2020). The principal organ responsible for preserving the metabolic equilibrium of uric acid (UA) is the kidney, hyperuricemia is brought on by insufficient renal excretion, and only 10% by excessive production. Hyperuricemia is caused by an imbalance between the synthesis and removal of UA, which is mostly dependent on renal excretion (James *et al.* 2021). For many years, blood urea measurements have been employed as a measure of renal function. However, it is widely acknowledged that measuring creatinine offers better data in this regard (Luft 2021). The level of creatinine in the blood may increase if there is a deficiency in the renal filtration process as a result of impaired renal function (Ali 2022).

The current study also showed that CKD patients' blood albumin levels significantly decreased (P 0.01) as compared to controls. This study supports the findings of other previous studies, including (Jiang *et al.* 2020; Tabata *et al.* 2021; Zabihi, Abbasi, and Alimoradzadeh 2021). In individuals with chronic dialysis-dependent end-stage renal disease (ESRD), low blood albumin is linked to increased mortality and the prognosis of patients undergoing peritoneal dialysis may be predicted by low albumin levels (Tsai *et al.* 2020).

Since blood calcium and phosphorous concentration are inversely related (Table 3.1), the serum calcium concentration was lowered (P< 0.05) in renal failure patients (8.81). Mohamed *et al.* 2019), who reported that the majority of patients (52%) with stage 5 chronic renal disease had hyperphosphatemia (54.6%) and hypocalcemia (mean calcium levels of 8.08 mg/dl). Because the kidneys are unable to produce the active form of vitamin D (1,25-dihydroxycholecalciferol), which is necessary for calcium absorption in the gut, the change in vitamin D synthesis caused by renal failure may be the likely reason of the drop in blood calcium levels (Janoušek *et al.* 2022). According to the current study, vitamin D3 levels are considerably lower in CKD patients than in healthy controls (15.14 against 31.55) (Kawarazaki *et al.* 2011).

The current study has also revealed that there is a high significant increase (P < 0.01) in the alkaline phosphatase (120.2) and bone ALP (11.85) in CKD patients when compared to alkaline phosphatase (74.49) and bone ALP (1.83) in control, respectively. This result was in agreement with (Wally 2016). It has been demonstrated that BALP is a sensitive and trustworthy biomarker of bone metabolism (Staykova, Bocheva, and Prodanova 2018). Since several research show a link among ALP and accelerated morbidity and mortality. The link of circulating BALP with mortality has frequently been pronounced in patients with advanced CKD. As end result, several pathways might hyperlink ALP to mortality, and strategies that relate circulating BALP to an accelerated chance of mortality could be precise to advanced CKD (Nizet *et al.*, 2020).

Osteocalcin stages in CKD patients are significantly higher (9.39) in patients when compared to control (1.84), (P < 0.01); Table (3.1). This finding was consistent with that of Chi *et al.* (2022), who determined that better blood osteocalcin degrees in CKD patients is probably used as a measure of osteoporosis or bone turnover. Osteocalcin, a hormone generated from osteoblasts, is linked to the onset of osteoporosis and arteriosclerosis. It affects the improvement of vascular calcification and osteoporosis in people with persistent renal disease. It is a nutrition K-structured hormone that influences bone mineralization and impacts both osteoblast and osteoclast (Chi *et al.*, 2022). Individuals with CKD regularly have high osteocalcin ranges; but it is unsure if those tiers are associated with vascular function in those sufferers (Thanakun et al., 2019). Marchelek- Mysliwiec *et al.*, (2018) in comparison to the control institution, the group with persistent kidney sickness (CKD) had statistically considerably higher concentrations. Additionally, there was a negative correlation between osteocalcin and bone mineral density (BMD), indicating that high osteocalcin is the maximum touchy indicator of lowering bone mass in CKD patients. Kratz *et al.,* (2021) determined that higher proportions of fragmented paperwork may contribute to the heightened stages of circulating osteocalcin visible in CKD patients. In cross-sectional studies, decreasing renal feature may bring about reduced osteocalcin excretion and, as end result, a rising degree of circulating osteocalcin. This is due to the fact some investigations showed that expanded stages of osteocalcin are as a result of a reduction in renal excretion rate (Ye *et al.*, 2022).

The amino-terminal pro-peptide of type I collagen (P1NP), as showed in Table (1), was Significant higher in CKD patients (P<0.01) than within the control group. Along with CTX-1, P1NP is maximum well-known for being a marker of osteoblastic status, that's vital for osteoporosis investigations (Choi, Lee and Lee, 2022). Although osteoblasts and fibroblasts in bone and gentle tissues, respectively, each synthesize kind I collagen, the general public of circulating PINP is comprised of activities interior bone (Tridimas, Milan and Marks, 2021). The total PINP values gathered demonstrate an exponential rise as the eGFR maintains to decline until give up-level renal failure (Jørgensen *et al.*, 2022).

The GFR measures the total quantity of plasma that is filtered via all of the lively glomeruli in a certain quantity of time. It can be calculated via searching at a substance's plasma clearance. Because it specifies renal characteristic, estimated GFR is a degree of sizable clinical importance. Chronic kidney disorder is defined as both GFR 60 mL/min/1.73 m2 or GFR 60 mL/min/1.73 m2 with evidence of kidney damage, as consistent with the global kidney disorder: (Charles and Ferris 2020).

Table 1. Comparison between the anthropometric and biochemical markers in chronic kidney diseases patients and control.

Variables		Control (n=55) Mean ± SD	CKD (n=129) Mean± SD	P. value
Age (years)		44.16 ± 13	46.89 ± 13.24	0.07
Gender	Male, n (%)	25 (45.45%	65 (50.39%)	0.629
	Female, n (%)	30 (54.55%)	64 (49.61%)	
	Total, n (%)	55 (100%)	129 (100%)	
BMI (kg/m2)		26.8 ± 3.74	24.1 ± 3.18	<0.0001
Uric Acid		4.34 ± 0.62	6.53 ± 2.05	<0.0001
Urea (mg/dl)		28.18 ± 8.95	139.4 ± 64.59	<0.0001

Creatinine (mg/dl)	0.76 ± 0.22	7.69 ± 0.75	<0.0001
Hb (g/dl)	12.78 ± 1.86	9.48 ± 1.83	<0.0001
Ferritin (ng/ml)	134.6 ± 28.38	470.3 ± 50.2	<0.0001
Phosphorus (mg/dl)	3.42 ± 0.71	6.30 ± 1.94	<0.0001
Calcium (mg/dl)	9.12 ± 0.61	8.81 ± 0.95	0.0267
Vitamin D (ng/ml)	31.55 ± 4.80	15.14 ± 3.14	<0.0001
Albumin (g/dl)	4.30 ± 0.492	3.79 ± 0.65	<0.0001
ALP (IU/L)	74.49 ± 13.72	120.2 ± 21.02	<0.0001
Bone ALP (ng/ml)	1.83 ± 0.20	11.85 ± 0.86	<0.0001
Magnesium (mmol/L)	0.93 ± 0.15	0.95 ± 0.21	0.056
PTH (pg/ml)	33.51 ± 6.78	153.7 ± 40.5	<0.0001
Osteocalcin (ng/ml)	1.84 ± 0.36	9.39 ± 1.08	<0.0001
P1NP (ng/ml)	1.10 ± 0.46	3.08 ± 0.92	<0.0001
e-GFR (mL/min/1.73m2)	88.15 ± 2.88	19.47 ± 10.04	<0.0001

SD: standard deviation; n: number of cases; CKD: chronic kidney diseases, $p < 0.05$.

3.2 Comparison of chronic kidney disease patients according to gender.

As shown in Table (2), there were no significant differences (p. value >0.05) in the means values of all paramerters.

Table 2. Comparison between pateints and control according to gender.

Variables	Males (n=65) Mean ± SD	Female (n=64) Mean± SD	P. value
Age (years)	49.75±13.26	47.98±12.68	0.128
BMI (kg/m^2)	24.01±2.82	24.12±3.53 2	0.854
Uric Acid	6.65±2.53	6.42±1.419	0.531
Urea (mg/dl)	147.5±23.15	131.2±24.90	0.153
Creatinine (mg/dl)	7.90±1.39	7.48±1.02	0.620
Albumin (g/dl)	3.69±0.62	3.88±0.67	0.093
Phosphorus (mg/dl)	6.04±2.07	6.57±1.76	0.120
Calcium (mg/dl)	8.78±0.95	8.84±0.96	0.717
Vitamin D (ng/ml)	14.66±1.78	15.63±1.50	0.372
ALP (IU/L)	123.6±17.01	116.7±16.00	0.588
Magnesium (mmol/L)	0.95±0.22	0.947±0.202	0.888
PTH (pg/ml)	133.7±21.3	158.1±51.5	0.313
Bone ALP (ng/ml)	10.14±1.12	13.58±1.61	0.300
Osteocalcin (ng/ml)	8.96±1.08	9.83±1.07	0.228
P1NP (ng/ml)	2.99±0.57	3.16±0.27	0.507
e-GFR (mL/min/1.73m2)	21.29±1.99	17.63±1.19	0.077

SD: standard deviation, significant at $p < 0.05$., n: number of cases.

3.3 Comparison of chronic kidney disease patients according to the duration of disease.

The mean values of biochemical parameters among patients with chronic renal disease according to duration of CKD were shown no significantly differences (P value >0.05), Table (3).

Table 3. Comparison of biochemical markers among patients with chronic kidney disease according to the duration.

Variables	CKD < 1 Year (n=60) Mean ± SD	CKD >1 Year (n=69) Mean ± SD	P. Value
Age (years)	48.97±8.92	45.09±7.44	0.097
BMI (kg/m2)	24.41±3.124	23.76±3.22	0.248
Uric Acid	6.78±2.45	6.32±1.626	0.210
Urea (mg/dl)	140.3±22.50	138.6±18.66	0.876
Creatinine (mg/dl)	7.08±1.13	8.23±1.35	0.169
Albumin (g/dl)	3.71±0.566	3.85±0.71	0.200
Phosphorus (mg/dl)	6.23±1.86	6.37±2.01	0.666
Calcium (mg/dl)	8.79±0.96	8.82±0.95	0.860
Vit. D (ng/ml)	15.52±1.95	14.81±1.33	0.519
ALP (IU/L)	113.3±12.22	125.3±13.27	0.351
Magnesium (mmol/L)	0.93±0.22	0.962±0.197	0.501
PTH (pg/ml)	142.1±10.5	163.8±12.1	0.417
Bone ALP (ng/ml)	11.63±1.19	12.03±1.02	0.851
Osteocalcin (ng/ml)	9.03±11.54	9.70±1.51	0.356
P1NP (ng/ml)	3.01±0.61	3.14±0.24	0.615
e-GFR (mL/min/1.73m2)	20.47±2.49	18.61±2.56	0.296
Urea (mg/dl)	140.3±22.50	138.6±18.66	0.876
Creatinine (mg/dl)	7.08±1.13	8.23±1.35	0.169

n: number of cases; SD: standard deviation; significant at $p < 0.05$.

3.4 Identification of risk of incident CKD by multivariable logistic regression analysis for males.

Multiple logistic regression was performed for male patients' group to predict risk for developed CKD, as shown in table (4). There were significant variables include: (age, uric acid, ferritin, Phosphorus, alkaline phosphatase, PTH, bone ALP, osteocalcin, P1NP, urea, and creatinine); while, the significant variables include: (BMI, Hb, albumin, calcium, vitamin D3, GFR).

Table 4. Identification of risk of incident CKD by multivariable logistic regression analysis for males.

Variables	Regression coefficient	Standard error	Odds ratio	95% CI	*P* value
Age (years)	0.039	0.017	1.041	1.006 - 1.079	0.0212
BMI (kg/m2)	-0.212	0.081	0.808	0.681 - 0.939	<0.005
Uric Acid	1.783	0.425	5.947	2.863 - 15.44	<0.0001
Albumin (g/dl)	-2.201	0.56	0.110	0.032 - 0.300	<0.012
Phosphorus (mg/dl)	1.622	0.3721	5.062	2.685 - 11.77	<0.0001
Calcium (mg/dl)	-0.601	0.306	0.547	0.287 - 0.965	0.037
Vit. D (ng/ml)	-0.887	0.318	0.4118	0.172 - 0.638	<0.001
ALP (IU/L)	0.027	0.009	1.028	1.011 - 1.049	<0.0001
Magnesium (mmol/L)	0.245	1.157	1.278	0.139 - 14.15	0.8314
PTH (pg/ml)	0.161	0.042	1.175	1.094 - 1.296	<0.0001
Bone ALP (ng/ml)	1.929	0.5013	6.884	3.015 - 22.38	<0.0001
Osteocalcin (ng/ml)	2.304	0.724	10.02	3.589 - 81.71	<0.0001
P1NP (ng/ml)	4.206	0.989	67.06	12.97 - 673.1	<0.0001
e-GFR (mL/min/1.73m2)	-0.188	0.325	0.622	0.302 - 1.101	<0.001
Urea (mg/dl)	0.206	0.647	1.132	0.892 - 1.078	<0.001
Creatinine (mg/dl)	13.31	10.1	2.18	1.9 - 2.64	<0.001

$p \leq 0.05$.

Conclusion

CKD induced bone abnormalities, proper diagnosis of the actual underlying skeletal problems helps to prevent future bone loss and fractures. There were significant decreases in each of calcium, vitamin D, albumin, estimated GFR in patient with CKD; while, there were

significant increases in each of uric acid, urea, creatinine, ferritin, phosphorus, alkaline phosphatase, bone alkaline phosphatase, parathyroid hormone, osteocalcin, and collagen type1 propeptide. These parameters were good noninvasive biomarkers for prediction of bone turnover risk stratification and assessment disease severity and beginning of the proper treatment course, which are crucial in achieving better patient outcomes in CKD patients.

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