Analysing the association between Serum Parathyroid Hormone, Osteocalcin, and TRACP 5b in Bone Mineral Disease among Chronic Kidney Disease Patients before and after dialysis

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Abstract:

Introduction: Changes in kidney structure and function, together with a prolonged decline in glomerular filtration rate (GFR), are symptoms of chronic kidney disease (CKD). Because chronic kidney disease (CKD) is becoming more common and widespread in India every year, it is a worldwide health concern. Males with stage five CKD have greater serum creatinine levels (over 5.0 mg/dl) than females (above 4.0 mg/dl). These result produce products of protein metabolism thus causing Hormonal imbalances and the loss of other renal functions, such as fluid and electrolyte derangement.

Objectives: We wanted to evaluate the correlation of serum iPTH, Osteocalcin and (TRACP) 5b levels with bone mineral disease in CKD patients before and after dialysis as a potential biomarker.

Methods: Out of the 300 participants in this study, 150 were healthy individuals (controls) and 150 were CKD patients (cases). Serum Glomerular Filtration Rate (eGFR), Serum Urea, Serum Creatinine, Serum Uric Acid, Serum Sodium, Serum Potassium, and Serum Chloride were measured on the Serum supernatant from 3 milliliters of peripheral venous blood using Diacetyl monoxime, Jaffe's method, Caraway's method, Cockcroft-Gault method, Colorimetric method, and Mercuric Thiocyanate method.

Results: In post-dialysis patients, serum levels of Serum Sodium (OR-5.90, $P < 0.0571$*), Serum Potassium (OR-5.80, $P < 0.0005$***), and Serum Chloride (OR-7.85, $P < 0.03$*) were lower than those in Pre-Dialysis stage. We found significantly increased levels of serum iPTH (OR-3.07, $P < 0.0001$***), Serum Osteoclacin (OR-7.12, $P < 0.001$**), (TRACP) 5b (OR-5.62, $P < 0.0001$***) pre dialysis CKD patients as compared to controls and these values were decreased significantly post dialysis in these patients

Conclusions: Patients with chronic kidney disease benefit from this study when they have bone mineral disease. By using non-invasive techniques to estimate serum iPTH, osteocalcin, and (TRACP) 5b levels, bone mineral disease in patients with chronic kidney disease can be identified early and used as a prognostic biomarker.

Keywords: Glomerular filtration rate (eGFR), Urea, Creatinine, Uric acid, Sodium, Potassium, Chloride, Pre dialysis and Post dialysis.

Introduction:

A progressive impairment of renal function is a characteristic of chronic kidney disease (CKD) [1]. Normal kidney function, particularly excretory and regulatory functions, can be lost due to infections, autoimmune diseases, diabetes, high blood pressure, cancer, and toxic chemicals [2]. Chronic renal failure is rapidly spreading over the world and is poised to become a major public health concern [3]. Nowadays, renal failure is a public health issue. Around 13–15.04 percent of Indians had chronic kidney disease. Regardless of the etiology, chronic kidney disease (CKD) is defined as kidney damage or impaired kidney function for three months or more and is a leading cause of death in India [4]. Systemic disruption of mineral and bone metabolisms are the underlying cause of the mineral bone issue and chronic renal disease, a broad clinical condition [5]. Regardless of the underlying cause of the kidney disease, chronic renal failure is defined by a gradual decline in renal function. Atherosclerosis and diabetic nephropathy have replaced glomerulonephritis and interstitial nephritis as the main causes of chronic renal failure [6]. Kidney function rapidly and permanently declines as a result of progressive glomerulopathies. In chronic renal failure, changes in renal parameters and blood minerals are brought on by a persistent decline in the glomerular filtration rate. A GFR of less than 60 ml/minute/1.73 m2 is indicative of chronic kidney disease (CKD), according to Kidney Disease Improving Global Outcomes. There are five stages of CKD. In stage five of CKD, GFR is less than 15 milliliters per minute [7]. Serum creatinine levels in stage five CKD are higher than 5.0 mg/dl in males and 4.0 mg/dl in women [8]. Biochemical and hormonal alterations that dramatically increase the risk of bone fractures, cardiovascular events, mortality, and the progression of chronic kidney disease characterize the mineral and bone issues, commonly referred to as the acronyms for chronic renal disease [9]. The gold standard for assessing and diagnosing renal osteodystrophy is bone biopsy, and in order to identify bone pathology, it is recommended to categorize patients according to bone turnover, mineralization, and volume [10]. Trabecular bone biopsy for histomorphometric analysis is the gold standard for diagnosing and monitoring mineral bone diseases and chronic renal illness [11]. In osteoporosis, a common metabolic disorder of the skeleton, the low bone mass and microarchitecture degradation of bone tissue increase the fragility and fracture susceptibility of bones [12]. The bones may degenerate to the point that they might shatter spontaneously or in reaction to minor stress. The person may have ongoing pain and a reduced ability to do everyday activities while the broken bone heals [13]. Though their clinical usefulness has not yet been shown, several additional circulating biochemical markers of bone production and resorption have been studied as clinical indications of bone turnover [14]. Imaging has been an essential component of assessing bone disease and extraskeletal calcification in CKD patients, in addition to bone histology and blood biomarkers [15]. The amount of waste in the body, the level of salts, body weight, and kidney function all affect how long dialysis takes. When kidney function is compromised, hemodialysis is essential for the extracorporeal elimination of waste products such as creatinine, urea, and free water from the blood [16]. Hemodialysis occurs on the basis of solute diffusion over a semipermeable membrane. Metabolic waste products go from the circulation into the dialysate along a concentration gradient. Larger molecules like creatinine (113 Da) are eliminated less effectively than smaller ones like urea (60 Da), which is cleaned significantly [17].

Objectives:

We wanted to evaluate the correlation of serum iPTH, Osteocalcin and (TRACP) 5b levels with bone mineral disease in CKD patients before and after dialysis as a potential biomarker.

Methods:

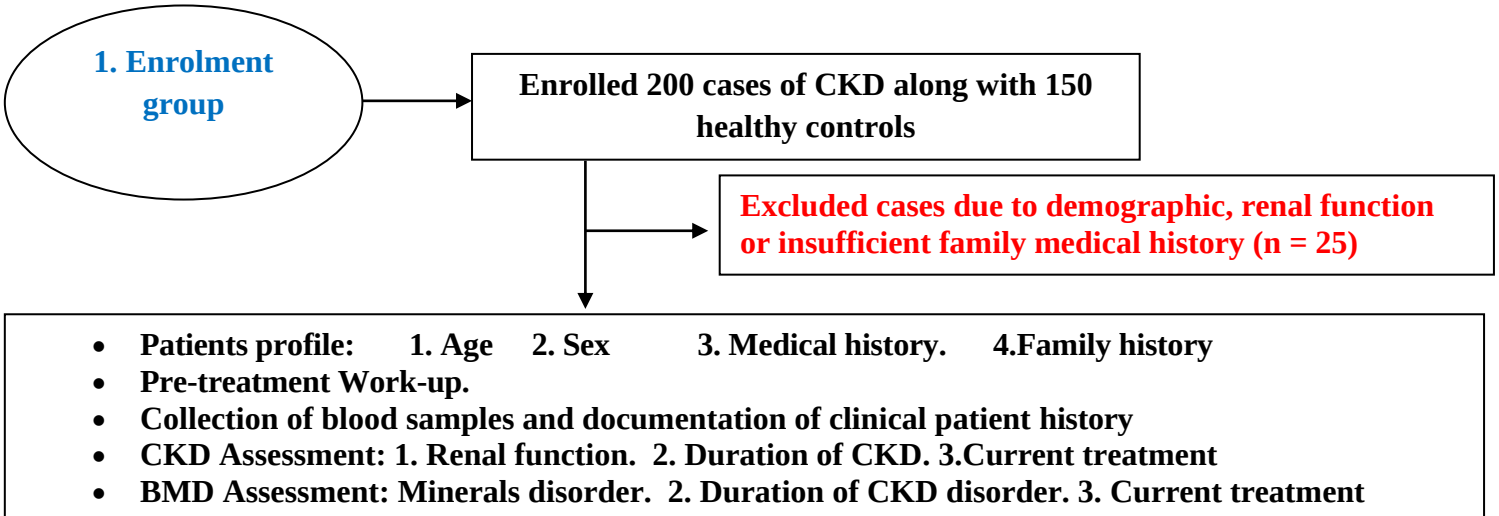
This study was a case control study conducted in Department of Biochemistry, Pacific Medical College and Hospital, Udaipur, (Rajasthan) India. Study was carried out after obtaining clearance from Pacific Medical College and Hospital, Pacific Medical University, Institutional Ethics committee, Department of Biochemistry, Pacific Medical College and Hospital, Udaipur, (Rajasthan) India. The study was approved by institutional research ethical committee of Pacific Medical University, Udaipur, (Rajasthan) India. Study involved 300 subjects, 150 healthy subjects and 150 chronic kidney disease patients attending the Dialysis Unit, Nephrology OPD/IPD and Department of Medicine in Pacific Medical College and Hospital, Udaipur, Rajasthan were recruited.

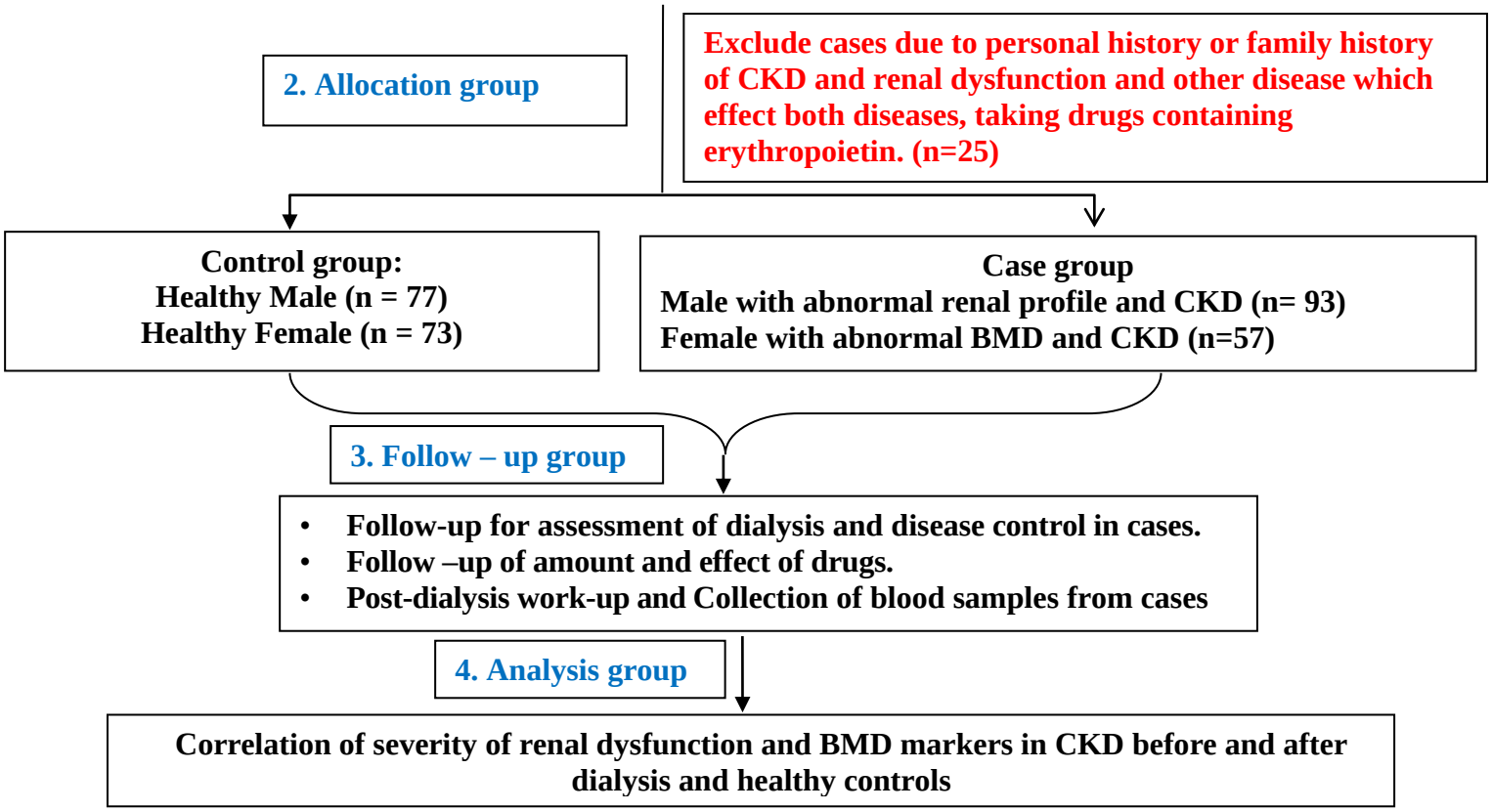
Inclusion Criteria:
Chronic Kidney Disease Patients (stage I- stage V) before starting renal replacement therapy.
Subjects between 18–65 years age group were considered.

Exclusion criteria:

- Acute infections.
- Malignancy.
- Chronic liver disease.
- Thyroid gland dysfunctions.
- Myocardial infarction.

Figure 1: Study design.





Method of Analysis:

3 ml peripheral blood samples were collected from all participants both patients and healthy controls. The blood samples were centrifuged at 4000 rpm for 15 minutes. The 50µl of centrifuge serum was used in a fully auto-analyzer Beckman Coulter AU-5800 to measure all Biochemical parameters Serum Glomerular filtration rate (eGFR), Serum Urea, Serum Creatinine, Serum Uric acid, Serum Sodium, Potassium and Serum Chloride, which is estimated by Diacetyl monoxime, Jaffe's method, Caraway's method and Cockcroft-Gault method, Colorimetric method, Colorimetric method and Mercuric Thiocyanate method using Erba kit and lot no. IX13469.. The 50µl of centrifuge serum was used in a fully auto-analyzer Roche cobas e 801 to measure the serum parathyroid hormone, which was estimated by Chemiluminescence Immunoassay (CLIA). The Serum Osteocalcin and Serum Tartrate resistant acid Phosphatase 5b (TRACP) 5b were estimated by Enzyme-linked immunosorbent assay (ELISA) semi auto-analyzer ELISA Reder Bio-Red PR 4100, Elbscience kit and lot no. E-EL-H1343. The serum is stored at -70°C until assayed. The serum is stored at -70°C until assayed.

Statistical Analysis:

All the parameter of case and control were analyzed for mean and standard deviation. The results were expressed as Mean ± standard deviation**.** The student t-test was used and a p-value < 0.05 was consider statistically significant. Pearson correlation coefficient was used to find the correlation between the level of Serum Glomerular filtration rate (eGFR), Serum Urea, Serum Creatinine, Serum Uric acid, Serum Sodium, Potassium, Serum Chloride level, Serum Parathyroid hormone, Serum Osteocalcin, and Serum Tartrate resistant acid Phosphatase 5b (TRACP) 5b in Pre dialysis and Post dialysis with Bone mineral disease level in chronic kidney disease patients as compared to controls as well as pre and post dialysis of patients. Data was analyzed using Statistical software i.e. Statistical Package for Social Sciences **(SPSS) version 21.0**. After analysis of data distribution patterns appropriate statistical tests was utilized for analyzing measures of central tendency, dispersion and odds ratio.

Results:

The study included subjects 300 participants among them 150 were chronic kidney disease patients with ages ranged from 18 to 65 years with a mean of 48.26±10.49 years and 150 were heathy controls. There were 150 Case groups (male 77% and female 73 %) and 150 Control groups (male 93% and female 53%). Older age dialysis patients with CKD were more common i.e. in the 50–60-year age group. The p-value of 0.05 was considered significant to compare the pattern of renal profile between the two groups [Table 1].

Table 1: Comparison of Bone mineral disease and Blood parameters between healthy controls and CKD cases

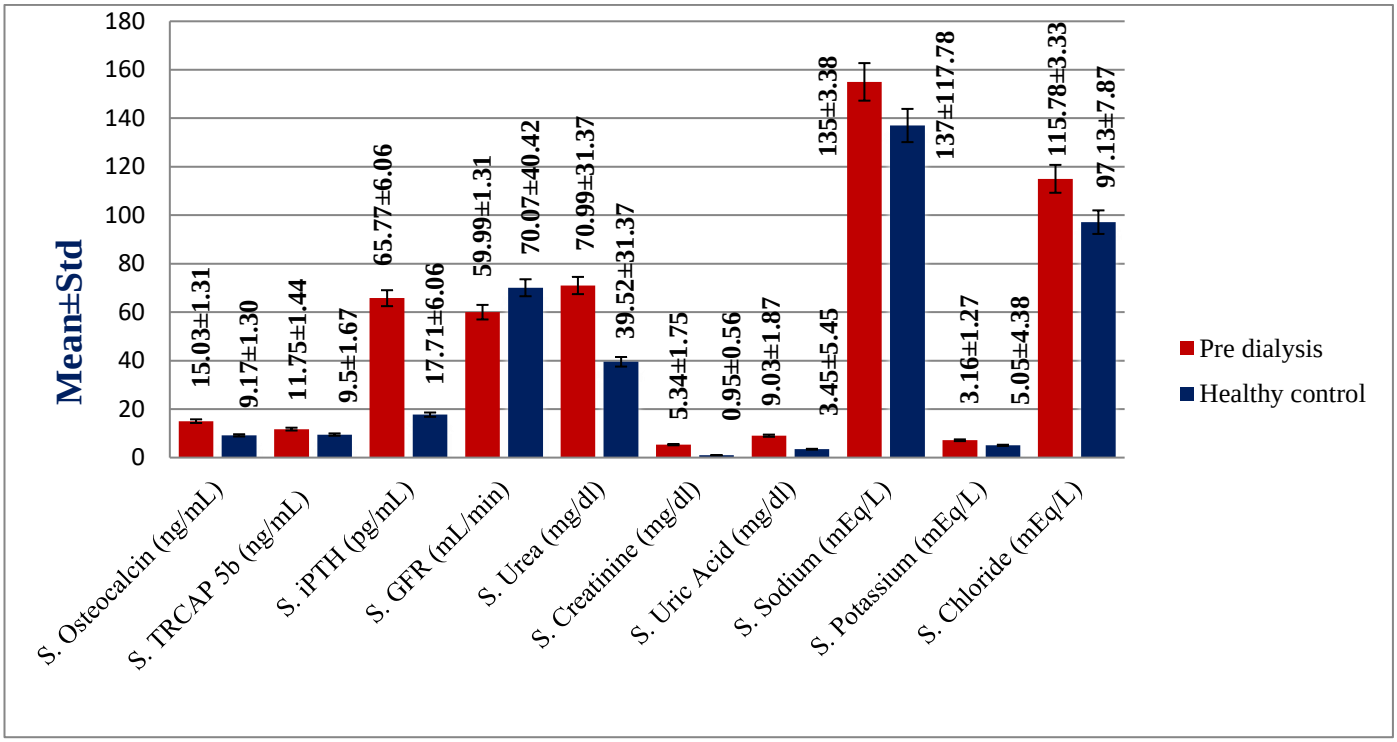
S. No.	Parameters	CKD Cases (Mean ± S.D) (150)	Healthy Controls (Mean ± S.D) (150)	OR	P Value
01.	S. Osteocalcin (ng/ml)	15.03±1.31	9.17±1.30	7.12	0.0012(**)
02.	S. TRACP 5b (ng/ml)	11.75±1.44	9.5±1.67	5.62	0.0001(***)
03.	S. iPTH (pg/ml)	65.77±6.06	17.71±5.20	3.07	0.0001(***)

04.	S. eGFR (ml/min)	70.07±40.42	59.99±1.31	5.65	0.0001(***)
06.	S. Urea (mg/dl)	70.99±6.01	39.52±31.37	3.87	0.0001(***)
07.	S. Creatinine (mg/dl)	5.34±1.75	0.95±0.56	7.85	0.0014(**)
08.	S. Uric Acid (mg/dl)	9.03±1.87	3.45±5.45	5.78	0.0001(***)
09.	S. Sodium (mEq/L)	155±3.38	137±117.78	5.90	0.0571
10.	S. Potassium (mEq/L)	7.16±1.27	5.05±4.38	5.80	0.0005(***)
11.	S. Chloride (mEq/L)	115.71±3.33	97.13±7.87	7.85	0.0354(*)

*P value<0.05, **P value <0.005, ***P value <0.0005 as considered significant

We observed significantly increased levels of S Osteocalcin (15.03±1.31), TRACP 5b (11.75±1.44) and S iPTH (65.77±6.06) in CKD patients as compared to control, Urea, Creatinine, Uric acid, eGFR, Potassium and Chloride were also significantly elevated in CKD patients as compared to controls [Table 1]. All these observations were was statistically significant ($P < 0.05$). Serum Sodium was however elevated in CKD patients but was non-significant [Figure 2].

Figure 2: Comparison of Bone mineral disease and Blood parameters between healthy controls and CKD cases



The levels of serum iPTH, Osteocalcin, (TRACP) 5b, eGFR, Uric acid, Urea, Creatinine, Sodium, Potassium, and Chloride were analysed in CKD patients both before and after dialysis. We observed mean levels of urea, creatinine, sodium, potassium, and chloride were 135.95±25.05 mg/dl, 17.95±3.50 mg/dl, and 15.29±1.51 mEq/L, respectively, before dialysis. These levels there after significantly decreased to 37.9±3.29 mg/dl, 7.02±3.29 mg/dl, and 3.50±1.75 mEq/L, respectively. $P < 0.05$ indicated that this decrease was statistically significant. Before dialysis, the mean sodium level was 155±3.38 mEq/L; after dialysis, it decreased non-significantly to 149±117.75 mEq/L [Table 2].

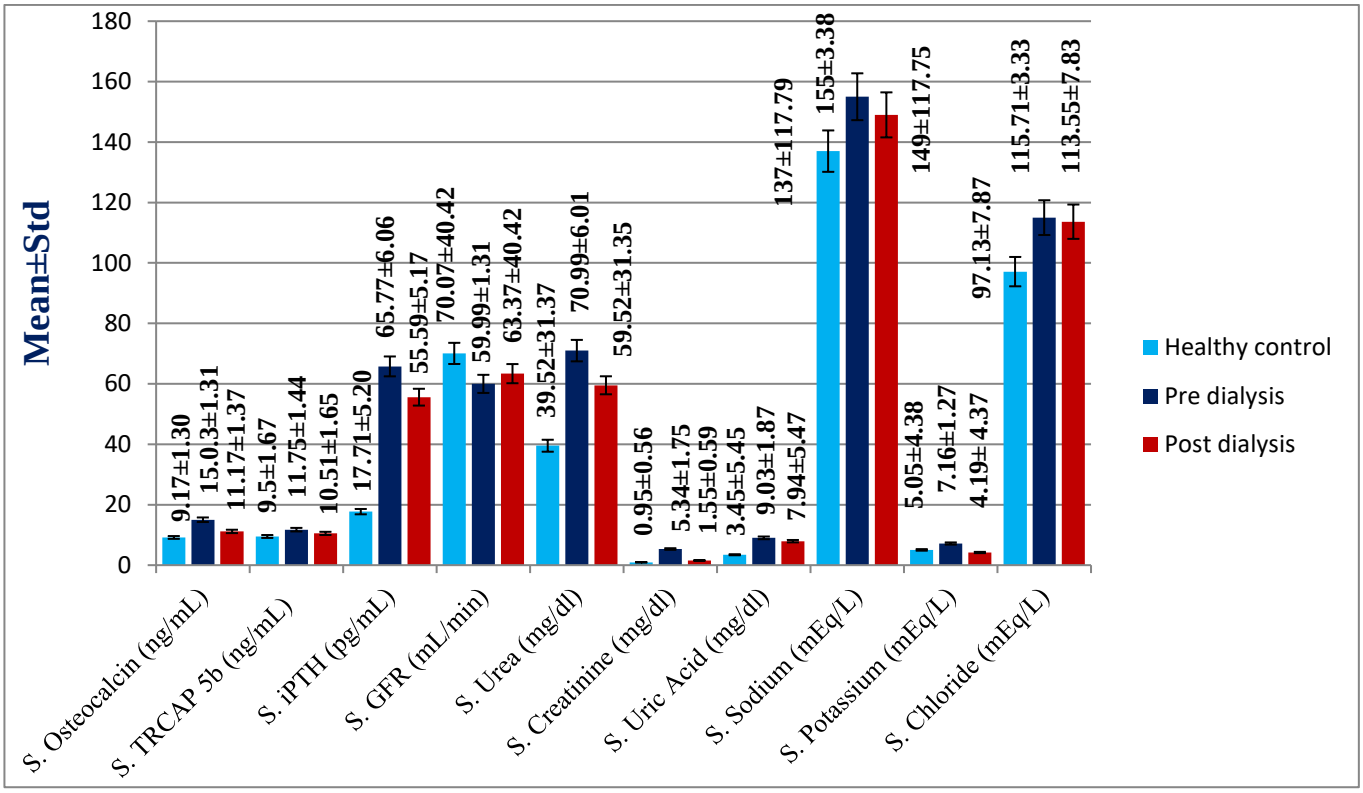
Table 2: Comparison of Bone mineral disease and Blood parameters between healthy controls and Pre dialysis values and Post dialysis values CKD patients

S. No.	Parameters	*Pre dialysis Group (Mean±S.D)* (150)	*Post dialysis Group (Mean±S.D)* (150)	*Healthy Control Group (Mean±S.D)* (150)	OR	P Value
01.	S. Osteocalcin (ng/ml)	15.03±1.31	11.17±1.35	9.17±1.30	7.12	0.0014(**)
02.	S. TRACP 5b (ng/ml)	11.75±1.44	10.51±1.65	9.5±1.67	5.62	0.0001(***)
03.	S. iPTH (pg/ml)	65.77±6.06	55.59±5.17	17.71±5.20	3.07	0.0001(***)

04.	S. eGFR (ml/min)	59.99±1.31	63.37±40.42	70.07±40.42	5.65	0.0001(***)
06.	S. Urea (mg/dl)	70.99±6.01	59.52±31.35	39.52±31.37	3.87	0.0001(***)
07.	S. Creatinine (mg/dl)	5.34±1.75	1.55±0.59	0.95±0.56	7.85	0.0017(**)
08.	S. Uric Acid (mg/dl)	9.03±1.87	7.94±5.47	3.45±5.45	5.78	0.0001(***)
09.	S. Sodium (mEq/L)	155±3.38	149±117.75	137±117.79	5.90	0.0571
10.	S. Potassium (mEq/L)	7.16±1.27	4.19±4.37	5.05±4.38	5.80	0.0005(***)
11.	S. Chloride (mEq/L)	115.71±3.33	113.65±7.83	97.13±7.87	7.85	0.0324(*)

We found significantly increased levels of serum iPTH *(OR-3.07, P < 0.0001***)*, Serum Osteoclacin *(OR-7.12, P < 0.001**)*, (TRACP) 5b *(OR-5.62, P < 0.0001***)* pre dialysis CKD patients as compared to controls and these values were decreased post dialysis in these patients [Figure 2].

Figure 2: Comparison of Bone mineral disease and Blood parameters between healthy controls and Pre dialysis values and Post dialysis values CKD patients



Discussion:

Nephrons are irretrievably destroyed in kidney failure, a very slow-moving illness. There is a significant increase prior to dialysis because the renal system's normal filtration capacity is impaired, which hinders the removal of urea and creatinine from the blood. According to our most recent research, dialysis patients of both sexes had significantly higher blood urea and blood creatinine levels. However, urea and creatinine were shown to be significantly reduced following dialysis compared to before and post-dialysis blood parameters as well as to controls. These results were consistent with Singh et al.'s earlier 2016 study [18]. Singh enlightens the concept of renal loss due to depressed renal glomerular filtration, piloting towards the accumulation of various metabolites like Urea, Creatinine, and other elements in blood and ending with permanent destruction of nephrons. Nisha et al 2017 reconfirmed that in renal failure, although the boost in serum urea levels is seen to be relative to the disease progression, the protein catabolism products in such patients cannot be ignored. Hence our findings of higher blood urea & creatinine before dialysis and their drastic reduction after dialysis were also at par with the finding of Mohammed Jumaah, in 2013 [19]. When compared to the pre-dialysis state, our results clearly show that creatinine and urea have been removed from the blood during dialysis. In contrast to control groups, which showed a significant decrease following the initiation of dialysis, phosphates were once more shown to be significantly elevated in our investigation prior to the initiation of dialysis in renal failure participants of both sexes [20]. Chronic renal failure is one of the progressive disorders that causes an irreversible decline in the glomerular filtration rate, which in turn raises blood urea nitrogen and serum creatinine levels [21]. Chronic renal failure is most often caused by autoimmune diseases, diabetes mellitus, hypertension, etc. Due to its irreversible nature, it gradually develops into a more severe form,

with high levels of uremia causing the glomerular filtration rate to drop to 5 to 10 percent [22]. The disease's signs and symptoms are reflected in these blood chemistry alterations. Overt signs of uremia decrease as a result of chronic dialysis, which lowers the frequency and intensity of these disruptions [23]. We looked into whether the serum electrolyte profile (sodium, potassium, and chloride) changed before and after dialysis. The pre-dialysis group's serum potassium levels were lower than those of the post-dialysis group (P-value 0.00005 highly significant). [24]. Patients who had undergone hemodialysis had lower serum salt levels than those who had not. P Value 0 573 not important. Serum chloride levels before and after haemodialysis were greater in the pre-dialysis group than in the post-dialysis group. Highly significant, P value: 0.2 [25]. Due to the fact that it is permanent and gradually increases, resulting in a drop in glomerular filtration rate to 5–10% and elevated uremia, [26]. The disease's signs and symptoms are reflected in these blood chemistry alterations. Serum levels of electrolytes in bodily fluids, such as sodium, potassium, etc., can be measured to determine the compounds the kidneys excrete, evaluate renal excretory functions, and use the results as a diagnostic tool for renal diseases [27]. In order to clarify the impact of dialysis on patients with CRF, we assessed the mean values of serum renal biochemical indicators before and after dialysis [28]. In the current investigation, we found that the pre-dialysis group had higher levels of creatinine and urea, which were statistically significant (p-value < 0.05) [29]. This is because people with CRF have a decline in GFR. Plasma levels of creatinine and urea increase when the GFR decreases because they are removed by tubular secretion and glomerular filtration [30]. When compared to the pre-dialysis group, the post-dialysis group's blood urea and serum creatinine levels significantly decreased. Serum sodium levels in the pre-dialysis group of the current investigation are statistically non-significant (p-value > 0.05) and lower than those in the control group [31]. A rapid mGFR drop (by CCr-U) in the pre-dialysis period is linked to almost twice as high a degree of dialysis mortality as a moderate mGFR decline, according the current study [32]. In contrast to evaluating eGFR just in patients who are in the latter stages before to starting dialysis, the significance of mGFR reduction encourages recurrent mGFR tests [32]. Numerous dialysis facilities and nephrologists have previously used the mGFR, which is based on creatinine and urea clearance in 24-hour urine collections, to assess renal function [33]. At an academic, hospital-based the Global Information Management clinic, we found that regular eGFR reporting significantly increased CKD patient comanagement. Compared to fewer than 25% of the pre-eGFR group, approximately 50% of CKD 3b-4 patients were co-managed at the end of the corresponding study periods in the post-eGFR cohort. Both CKD 3b and CKD 4 patients showed notable increases in co-management [33]. It should be mentioned that published rates of hyperkalemia in a recent non-dialysis dependent CKD 3-5 cohort were around 5-12% in pre-dialysis and post-dialysis patients, despite the fact that severe hyperkalemia and angioedema contraindications to treatment may have prevented their usage [35].

Conclusion:

Patients with renal failure were shown to have an imbalance in blood biochemical markers. In individuals with renal failure, there is a substantial correlation between serum creatinine and serum urea levels. Hemodialysis is a vital and effective technique for filtering undesirable metabolites including

urea, creatinine, and electrolytes over a broad range, which lessens the burden on the kidneys. Hemodialysis is a necessary and effective procedure to lessen the strain on the kidneys. Regular blood testing may help improve the quality of life for people with renal failure and monitor the need for dialysis due to azotemia. Patients with chronic kidney disease benefit from this study when they have bone mineral disease. By using non-invasive techniques to estimate serum iPTH, osteocalcin, and (TRACP) 5b levels, bone mineral disease in patients with chronic kidney disease can be identified early and used as a prognostic biomarker.

Additional Information:

Disclosures:

Human subjects: Consent for treatment and open access publication was obtained or waived by all participants in this study. **Ethics statement:** Institutional ethics committee was convened in Pacific Medical College and Hospital, Udaipur, (Rajasthan) India. Ethical approval for the project approved by Institutional ethics committee, Pacific Medical College and Hospital, Udaipur, Rajasthan India. Informed written patient consent form for treatment and publication in open access journal has been obtained from each study participant prior to enrolment in study and sample collection. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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