

Clinical Significance of Osteoprotegerin, Vitamin D, Obestatin and some Biochemical Variables in Kidney Failure Patients

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Abstract

Chronic kidney disease (CKD) is described as an abnormality of renal function existing for a long period of time. Because the initial stages of CKD can be asymptomatic, its early diagnosis is difficult. The earlier stage of CKD can lead to several complications, such as anemia and bone mineral metabolism disorders. The study was conducted to assess the effect of the chronic renal disease stage on osteoprotegerin, 1,25 dihydroxyvitamin D, obestatin levels and some biochemical parameters in patients who have not undertaken dialysis therapy. In this case-control study, fifty-five patients with kidney failure and forty healthy people were examined. Circulating concentrations of osteoprotegerin, 1,25 dihydroxyvitamin D and obestatin were estimated by ELISA technique, serum urea, uric acid, total protein and total Calcium were estimated using spectrophotometry technique. The serum concentration of osteoprotegerin, obestatin, and renal function markers (Urea, Creatinine and Uric Acid) in patient groups were higher (328.3±41.68 pg/mL; 15.52±4.28 pg/mL; 183.2±10.35 mg/dL; 8.88±0.54 mg/dL; 6.57±0.22 mg/dL) respectively in comparison with the control group (172.6±55.48 pg/mL; 11.64±3.26 pg/mL; 33.45±1.08 mg/dL; 0.95±0.03 mg/dL; 4.35±0.22 mg/dL) respectively. Vitamin D, total Calcium and total Protein level in the patient group were lower (24.49±2.53 ng/mL; 8.06±0.18 mg/dL; 6.49±0.12 g/dL), respectively, as compared to controls (57.11±12.39 ng/mL; 10.15±0.23 mg/dL; 6.82±0.10 g/dL) respectively. The current study reveals that circulating levels of osteoprotegerin and obestatin are remarkably linked with renal failure; the current data identify a high prevalence deficiency and insufficiency of 1,25 dihydroxyvitamin D in patients with moderate and severe chronic nephropathy.

Keywords: Osteoprotegerin, 1,25 dihydroxyvitamin D, Obestatin, Kidney failure.

Introduction

Chronic Kidney Disease (CKD) is one of the leading causes of death and disability. CKD is associated with a wide range of life-threatening diseases⁽¹⁾. It is a disease condition associated with premature mortality, increased healthcare expenditures, and decreased quality of life. The terminal stage of the disease, End-Stage Renal Disease (ESRD), necessitates dialysis or kidney transplantation⁽²⁾. Osteoprotegerin (OPG) is a glycoprotein that plays an important regulatory role in the skeletal, vascular, and immune systems. It has been shown that OPG predicts chronic kidney disease (CKD). PG is increased in patients with non-diabetic and diabetic chronic kidney disease (CKD), where it predicts deterioration of kidney function, vascular events, and cardiovascular and all-cause mortality. Consistent with it, it has been recently reported that elevated OPG is associated with increased 5- and 10-year risk of rapid renal decline, renal disease hospitalization, and/or deaths in elderly women⁽³⁾.

Vitamin D deficiency is very frequent in CKD, affecting more than 80% of patients in pre-dialysis. Vitamin D insufficiency arises at an early stage of the disease and tends to worsen with the progressive loss of renal function⁽⁴⁾. Moreover, decline in glomerular filtration in CKD patients

causes vitamin D deficiency, derangements in calcium and phosphate homeostasis, and secondary hyperparathyroidism resulting in bone destruction and vascular calcification⁽⁵⁾. However, the role of native vitamin D supplementation (ergocalciferol, cholecalciferol or calcifediol) remains unclear in chronic kidney disease (CKD), particularly in the pre-dialytic phase⁽⁴⁾. Obestatin, a peptide hormone, might be participated in the loss of appetite in end-stage renal disease patients. The obestatin hormone is encoded by the same gene as ghrelin and has the opposite effects compared with ghrelin; it blocks nourishment and causes weight gain, whereas ghrelin promotes nourishment. The balance between concentration of obestatin and ghrelin in final-stage nephropathic patients influences balance of energy and appetite, which involves malnourishment in final-stage nephropathic patients. Thus, obestatin is a nourishment parameter identifying the fattening of the body and insulin resistance⁽⁶⁾.

Urea is the primary metabolite derived from dietary protein and tissue protein turnover. It is freely filtered at the glomerulus but not secreted, and it is reabsorbed by the renal tubules. In addition, as urine flow rates decrease, more urea is reabsorbed. Blood urea nitrogen (BUN) measures the nitrogen component of serum urea levels are inversely

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correlated with the decline of kidney function and are also affected by extrarenal factors such as protein intake, gastrointestinal bleeding, catabolic states, malnutrition, heart failure, dehydration, use of glucocorticoids, and hepatic urea synthesis⁽⁷⁾. The role of uric acid in chronic kidney disease (CKD) has been a controversial topic for decades. Studies supporting the role of uric acid as an important mediator of CKD are extensive. Experimentally raising uric acid could cause low-grade kidney damage and accelerate established CKD⁽⁸⁾. Creatinine is the most commonly used marker to measure GFR. GFR is estimated from measurements of creatinine concentrations in blood, using various prediction equations. In adults, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) study equations are the most widely used⁽⁹⁾.

Several pieces of research have demonstrated that nourishment factors are essential in averting the development of persistent nephropathy. According to prior research, a very low protein food source as a section of nourishment treatment has serviceable impacts in decelerating the development of chronic kidney disease⁽¹⁰⁾. Nevertheless, restricted research has investigated the effect of continuous protein consumption and its crucial sources on the beginning of chronic kidney disease, comprising the protein of both plant and animal sources. In an investigation, it was recorded that a higher intake of animal protein (red meat) contributes to elevated chronic kidney disease health risk, and replacing animal protein with plant protein diminished the health risk of chronic kidney disease⁽¹¹⁾.

Methods

Study population and design

Ninety-five participants with age range (29-67) years, have been subjected to the present study, and these selected participants were divided into two groups. Group (1) includes subjects with chronic renal failure (n=55). Group (2) consisted of apparently healthy individuals (n=40). Patients were clinically diagnosed with kidney failure. The samples were collected from Ashty Hospital in Soran City. Patients were assessed by full medical

history to exclude any existing systemic disease. The two groups were matched in age and gender.

Collection of blood samples

Five to six mL of venous blood were taken from each participant, and collected in gold-top serum separator tubes (SST), to get the serum, the samples were centrifuged at (3000 rpm) for fifteen minutes. The obtained serum samples were transferred immediately to pre-labelled and coded Eppendorf tubes. These samples were frozen at -20°C for subsequent analysis.

Biochemical assays

Circulating concentrations of osteoprotegerin (OPG), 1,25-dihydroxycholecalciferol and obestatin in serum samples were estimated by sandwich enzyme-linked immunosorbent assay (ELISA) technique using BioVision (USA) research-purpose kits. The concentration of urea, creatinine, uric acid, total protein and total Calcium were estimated using the enzymatic colorimetric method using BIOLABO kits (France).

Statistical analysis

Statistical data analysis was performed using SPSS version 21 and GraphPad Prism version 8 computer programs. Comparing the study parameter means between patients and control groups was performed by using Unpaired T-test (Man-Whitney U) test. Bar graphs and Statistical test results were expressed as Mean±SE.

Results and Discussion

As shown in Table 1, osteoprotegerin, urea, creatinine, and uric acid levels were significantly higher in renal failure patients (328.3±41.68pg/mL; 183.2±10.35 mg/dL; 8.88±0.54 mg/dL; 6.57±0.22 mg/dL) respectively. Similarly, the levels of Obestatin were higher in Kidney failure patients (15.52±4.28 pg/mL), but the variation was not statistically remarkable. The levels of vitamin D, total proteins and total Calcium were significantly lower in kidney failure patients (24.49±2.53 ng/mL; 6.49±0.12; g/dL 8.06±0.18 mg/dL) respectively than that of the control group (57.11±12.39 ng/mL; 6.82±0.10 g/dL; 10.15±0.23 mg/dL) respectively.

Table 1. Comparison between biochemical variable levels in KF and control group

Parameters	Controls	Patients	P-Value
Osteoprotegerin (pg/mL)	172.6±55.48	328.3±41.68*	0.0001
Obestatin (pg/mL)	11.64±3.26	15.52±4.28	0.241
Vitamin D (ng/mL)	57.11±12.39	24.49±2.53*	0.030
Urea (mg/dL)	33.45±1.08	183.2±10.35**	<0.0001
Creatinine (mg/dL)	0.95±0.03	8.88±0.54**	<0.0001
Uric Acid (mg/dL)	4.35±0.22	6.57±0.22**	<0.0001
Total Proteins (g/dL)	6.82±0.10	6.49±0.12*	0.0503
Total Calcium (mg/dL)	10.15±0.23	8.06±0.18**	<0.0001

Value of * indicates significant, ** indicates highly significant

Serum level of OPG

Table (1) and Figure (1) showed significant elevation ($P=0.0001$) in circulating concentration of OPG in patients (328.3 ± 41.68 pg/mL) as compared to controls (172.6 ± 55.48 pg/mL).

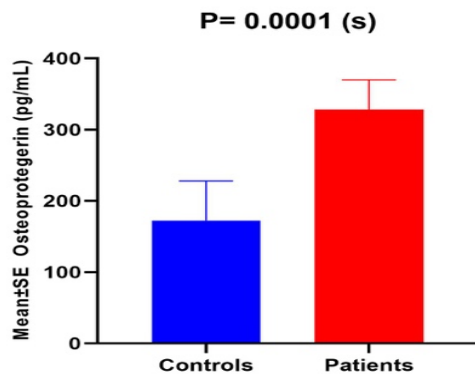


Figure 1. Serum level of OPG

Possible explanations for increased serum concentration of osteoprotegerin (OPG) with CKD may include decreased clearance of OPG or increased inflammatory activity. Inflammatory mediators are known to promote the production of OPG. As renal failure is associated with increased inflammatory activity, likely, inflammatory responses may partly explain increased serum OPG in CKD. Several mechanisms underlying OPG increase in CKD might include low-grade inflammation, fibroblast growth factor 23 (FGF-23) elevation, and kidney function. Inflammation might elevate OPG levels as several proinflammatory cytokines, such as TNF- α , regulate OPG production in vascular smooth muscle cells⁽¹²⁾. Secondly, increased concentrations of Fibroblast growth factor 23 found in persistent nephropathic patients might also promote osteoprotegerin expression⁽¹³⁾. Thirdly, given that renal secretion is assumed to control the clearance of osteoprotegerin, the reservation of osteoprotegerin attributed to kidney destruction might supply another section to explain the mechanisms whereby osteoprotegerin levels are elevated in chronic kidney disease⁽¹⁴⁾. As for inflammation, it has been revealed that osteoprotegerin promoted the expression of endothelial cells of cell surface proteins mediating the interaction between cells. It elevated white blood cell adhesion to the single layer of squamous endothelial cells, as for scarring of the tissue. It has been revealed that osteoprotegerin could begin Transforming growth factor beta 1-dependent alters in stromal cells of the vascular wall, promoting multiplication, fibrosis and inflammation. Furthermore, osteoprotegerin expression is elevated in response to transforming growth factor beta 1, which could contribute to a vicious cycle that causes the self-elicitation of both Osteoprotegerin and Transforming growth factor beta 1⁽³⁾.

Osteoprotegerin transportation remarkably elevated the gene expression of interleukin six and transforming growth factor beta 1, as well as the quantity of protein cysteine nitrosylation, which all participated in the progression of renal damage⁽¹⁵⁾. In this process, not only Transforming growth factor beta 1 but also various cytokines and growth factors stimulate the abnormal aggregation of collagen protein, fibronectin glycoprotein, and other constituents accountable for kidney scarring⁽¹⁶⁾. Consequently, our finding supposes that osteoprotegerin has the possibility to directly instigate renal damage⁽¹⁷⁾.

Serum levels of obestatin

Table (1) and Figure (2) showed a non-significant increase (0.241) in the circulating level of obestatin in patients (15.52 ± 4.28 ng/mL) as compared to controls (11.64 ± 3.26 ng/mL).

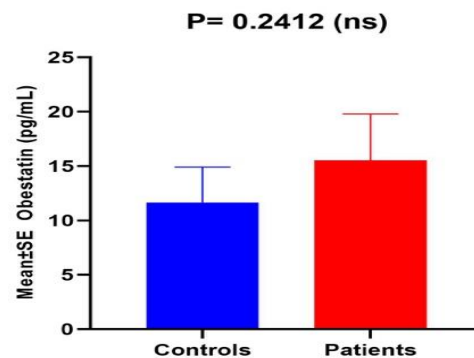


Figure 2. Serum level of obestatin

Obestatin, originally identified as an anorectic peptide, plays a vital role in several pathological activities besides physiological activities, such as substance and generation of energy from the metabolic pathway, motility of gastrointestinal tract, pancreatic secretion, cell multiplication, memory and sleep, etc.⁽¹⁸⁾. Obestatin-arginine vasopressin (AVP) contributes in the regulation of metabolic pathway of body water. The secretion of arginine vasopressin (AVP) in basic status is elevated after the management of obestatin antiserum; the secretion of arginine vasopressin is additionally elevated after water impoverishment. Earlier investigations have examined that the release of obestatin is abnormally elevated in patients with persistent heart failure and nephropathy. Compared to other groups, The current study found that patients with complicated renal failure have remarkably increased concentrations of obestatin^(19,20).

Circulating level of 1,25-dihydroxycholecalciferol

The results in Table (1) and Figure (3) showed that there was a remarkable decline ($P=0.030$) in circulating concentration of vitamin D in patients (24.49 ± 2.53 ng/mL) as compared to controls (57.11 ± 12.39 ng/mL).

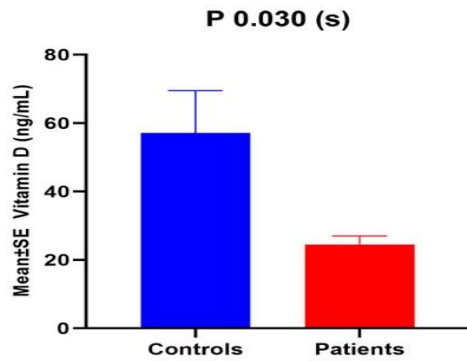


Figure 3. Serum level of Vitamin D.

Our results accompany previous findings proposing that 1,25 dihydroxyvitamin D insufficiency is vigorously linked with significant advancement in chronic kidney diseases among adults. Among 14,679 united states adult participants, the mean circulating concentration of 1,25-dihydroxycholecalciferol was lower in patients with stage four-five persistent nephropathy in comparison with normal renal function (21). Likewise, another research estimated the circulating concentration of 1,25-dihydroxycholecalciferol in patients with persistent nephropathy. The mean serum concentration of 1,25-dihydroxycholecalciferol was lower in patients with stages three-four (22). Furthermore, participants with a serum concentration of 1,25-dihydroxycholecalciferol less than 15 ng/mL had a 2fold greater occurrence of final stage nephropathy than those with more than 15 ng/mL during a long-lasting, Follow-up (23). Additionally, few researches revealed that low 1,25 dihydroxyvitamin D concentrations were independently linked with albumin-urea in chronic kidney diseases and T1DM (24,25). The present result illustrates that 1,25 dihydroxyvitamin D mild decrease / greater decrease is a persistent state in patients with chronic

kidney diseases, mainly those with an estimated glomerular filtration rate of less than 15 mL/min/1.73 m². Various mechanisms might describe the deficiency of 1,25-dihydroxycholecalciferol in persistent nephropathic patients. First, most patients with chronic kidney diseases have limited protein sources besides caloric consumption, so 1,25 dihydroxyvitamin D is fairly few. Second, many chronic kidney disease patients have restricted environmental activities with declined sunlight exposure (26). Finally, a considerable lack of urinary 1,25 dihydroxyvitamin D metabolites takes place in patients with urinary protein-creatinine ratio ≥1.0 g protein/g creatinine. Investigators reported an interrelationship between parathyroid hormone, 1,25-dihydroxycholecalciferol and persistent nephropathy (27). In chronic kidney diseases, the kidney 1 α -hydroxylase expression is blocked to remunerate for phosphate reservation, which results in an elevated expression of 24-hydroxylase for the breakdown of 1,25-dihydroxycholecalciferol. The concentration of 1,25 dihydroxyvitamin D in dialysis patients is lower than in healthy individuals (28). Renal malfunctioning, usually found among patients with renal diseases, is involved in the generation of 1,25-dihydroxycholecalciferol insufficiency that contributes to lowering serum calcium levels and secondary hyperparathyroidism, which results in secondary osteoporosis (29). It has been recorded that 1,25 dihydroxyvitamin D insufficiency in renal disorders is elevated in severe stages of chronic kidney disease (28).

Serum level of kidney function markers

Table (1) and Figure (4) revealed a significant ($P < 0.0001$) increase in serum level of urea, creatinine and uric acid in patients (183.2 ± 10.35 mg/dL), (8.88 ± 0.54 mg/dL) and (6.57 ± 0.22 mg/dL) respectively as compared to controls (33.45 ± 1.08 mg/dL), (0.95 ± 0.03 mg/dL) and (04.35 ± 0.22 mg/dL), respectively.

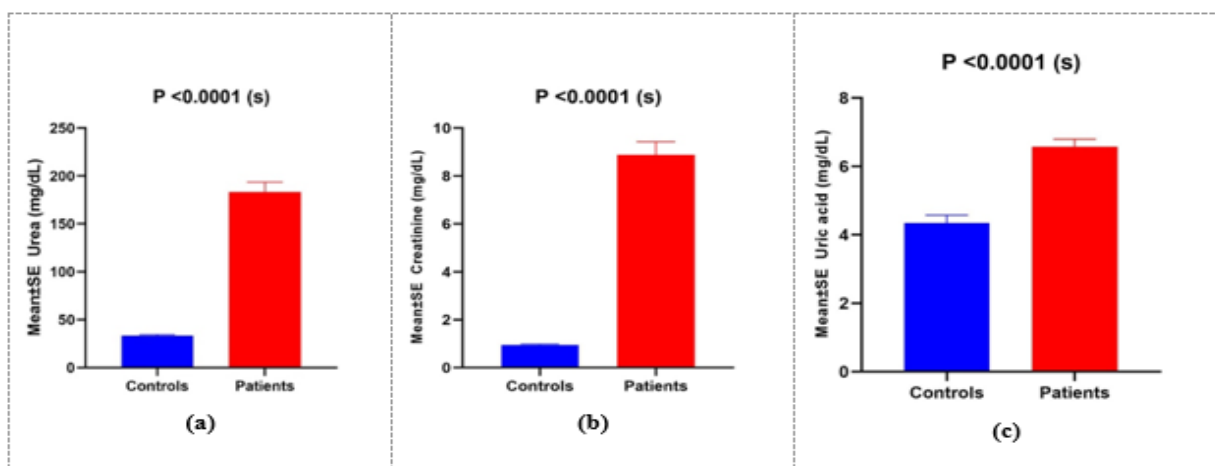


Figure 4. Serum levels of (a) urea, (b) creatinine (c) uric acid.

The current research revealed that higher blood urea nitrogen concentrations were linked with negative kidney consequences unconstrained of estimated glomerular filtration rate in patients with moderate to severe kidney damage. Previous cohort study recorded that both blood urea nitrogen and serum osmolality were unconstrained health risks for persistent nephropathy in patients with conserved renal action⁽³⁰⁾.

Urea formation directly corresponds to dietary protein consumption, and curtailment of daily protein consumption contributes to decrease urea formation. Various research concerning the potency of daily protein curtailment on renal disease development have been performed; however, findings have been incompatible. The wide-ranging irregular trial, the improvement of diet in kidney disorder study, revealed that a diet with very low-protein provided with oxoacids contributes to the reduction of the degree of development of chronic kidney disease compared with a diet that contains a limiting amount of high protein foods, but this was not statistically remarkable⁽⁷⁾. Serum creatinine descriptive of glomerular filtration rate has acquired its restriction since a decline in glomerular filtration only contributes to an imperceptible elevate in serum creatinine since its tubular release elevated; so, an imperceptible elevate in serum creatinine does not definitely means that glomerular filtration rate is normal, but a serum creatinine elevate more than 2 mg/dL result in saturation of excretion begins to consider glomerular filtration rate⁽³¹⁾.

The current study supports that hyperuricemia is associated with a greater decline in renal function and a higher risk of progressing to kidney failure. The influences of hyperuricemia on renal function decline and the risk of kidney failure are more significant in patients without proteinuria than those with proteinuria⁽³²⁾.

Uric acid is the final oxidation product of purine metabolism and is renally excreted. Therefore, elevated serum uric acid levels are seen in patients with reduced glomerular filtration rate (GFR). However, it has been proposed that uric acid itself plays a causal role in the pathophysiology of chronic kidney disease and possibly in acute kidney injury. A review of the literature demonstrates uric acid-related cellular changes that contribute to renal disease. Studies performed on rats have demonstrated that there are fundamental changes in the renal vasculature in the presence of hyperuricemia. It was found that uric acid decreased the expression of E-cadherin in epithelial cells resulting in a loss of cell-to-cell contact in the renal tubular cells of rats. Without cell-to-cell contact, epithelial cells are unable to coordinate efforts to secrete substances needed to increase renal blood flow, such as nitric oxide⁽³³⁾. In addition, a recent study utilizing immortalized proximal tubular epithelial cells from normal adult human male

kidneys has demonstrated that increased uric acid levels cause NAPDH-dependent oxidative changes that promote apoptosis⁽³⁴⁾. This finding shed light on the connection between hyperuricemia and tubulointerstitial renal damage.

Serum level of total protein

Table (1) & Figure (5) revealed a non-significant decrease ($P=0.0503$) in serum level of total protein in patients (6.49 ± 0.12 mg/dL) as compared to controls (6.82 ± 0.10 mg/dL).

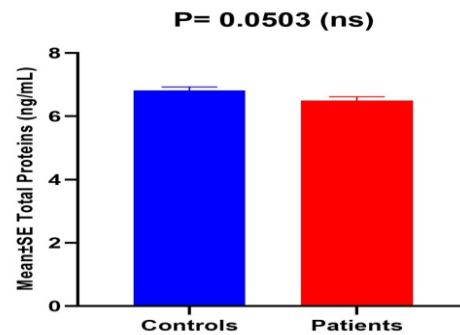


Figure 5. Serum level of total protein

It has been reported that serum total protein concentrations are strongly associated with estimated glomerular filtration rate decline, rapid eGFR decline and incident CKD. These findings were independent of albuminuria, self-reported health, biomarkers of inflammation, as well as demographic and clinical risk factors for kidney disease progression⁽³⁵⁾.

Serum total protein concentration is a well-known predictor of mortality in patients with CKD. Only a few studies have examined serum albumin as a risk factor for the development of ESRD^(36,37). Few studies have examined the associations of serum albumin with kidney function decline. However, in one study of eight inflammatory markers, including serum albumin, serum albumin was the only laboratory measure associated with kidney function decline. Few studies have examined the associations of serum albumin with kidney function decline. However, in one study of eight inflammatory markers, including serum albumin, serum albumin was the only laboratory measure associated with kidney function decline⁽³⁸⁾.

The underlying mechanisms for these associations are unclear. To our knowledge, there is no direct physiological basis for low serum albumin to cause the development of CKD. Serum albumin levels may be reduced for several reasons: liver damage and disease may decrease production; they may decrease as a negative acute phase reactant or in response to inflammation; and substantial and sustained albuminuria can lead to lower serum concentrations. Uncommonly low serum albumin can also reflect malnutrition, but the only malnourished state that consistently produces lower

serum albumin is kwashiorkor, a rare disease in the developing world⁽³⁹⁾.

Previous studies hypothesized the strong relationship between lower serum protein and kidney function decline in their research must be multi-factorial, representing general ill health. Lower protein concentrations may reflect much broader abnormalities in inflammation, thrombosis and microvascular function processes than can be captured by the markers available in this cohort. Lower serum protein and kidney function decline may also be related to multiple factors that are currently poorly understood. These findings are consistent with the results from our previous study of HIV-infected women and other studies of serum protein in ESRD⁽³⁵⁾. In a 2011 retrospective cohort analysis of 1800 men and women from the UK, Wagner et al. found that serum albumin levels were an essential component of a multivariable risk model for mortality in patients with ESRD⁽³⁶⁾. With some types of kidney disease, protein may be lost in the urine (proteinuria). With peritoneal dialysis, some protein crosses the peritoneal membrane and exits the body in the effluent dialysate (the solution drained from the peritoneal cavity). This loss increases in a person with peritonitis, an infection of the peritoneum.

Serum level of total calcium

Table (1) and Figure (6) revealed a remarkable reduction ($P < 0.0001$) in circulating concentration of total calcium in patients (8.06 ± 0.18 mg/dL) as compared to controls (10.15 ± 0.23 mg/dL).

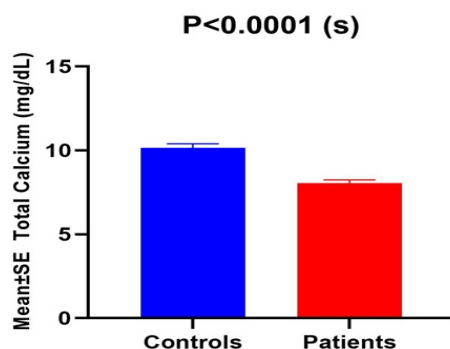


Figure 6. Serum level of total Calcium

Throughout nephropathy, the renal may no more filter out additional phosphorus and eliminate it from the body. With time, phosphorus may elevate in the blood. Ca and PO_4^{3-} in the body interact in opposite directions: as blood Ca levels rise, PO_4^{3-} levels fall. With renal disorder development, elevated phosphorus concentrations may cause low circulating calcium concentration by accumulating onto the osseous matter and other tissues. Disturbances in the metabolic pathway of calcium metabolism disorders are characteristic features of chronic kidney disease⁽⁴⁰⁾. In various investigations, the circulating concentration of phosphorus was

confirmed as a principal health risk for heart failure and all-cause death in kidney failure patients. In an American group of 35114 patients undertaken hemodialysis with an average follow-up of one year, among the patients with excessive remaining kidney urea authorization, there was an increased mortality health risk in patients with an elevated circulating concentration of phosphorus and decreased serum calcium⁽⁴¹⁾.

Conclusion

The study showed disturbances in the circulating concentration of osteoprotegerin, obestatin, and 1,25 dihydroxyvitamin D in patients with kidney failure. The serum osteoprotegerin level and obestatin were considered contributors to Kidney failure. These findings indicated that OPG, a bone-modulating protein, might have a role in developing KF patients. Decreased concentrations levels of 1,25 dihydroxyvitamin D are frequently observed in patients suffering from chronic kidney failure; these serve as a principal element in the progression of KF. More investigations are needed to explain the importance of these findings in the etiopathogenesis of Kidney failure.

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Conflict of Interest

The authors declare no conflict of interest during this study.

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