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Original article

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ABSTRACT

Objective: Bone mineral abnormalities in post-renal transplant (RT) period can lead to mineral bone disease (MBD). We planned to analyze biochemical parameters reflecting bone mineral changes in a set of post-RT patients**.**

Design: Prospective single center study

Setting: Institute of Kidney Disease and Research Center, Institute of Transplantation sciences, Ahmedabad

Subjects: Sixty RT patients with mean age of 36.7 ± 9.4 years.

Intervention: Serum total calcium (S.Ca), phosphorus ($S.PO_4$), alkaline phosphatase (S.ALP) and intact parathyroid hormone (S.iPTH) levels pre-RT and post-RT were evaluated at 1 and 7 months respectively and correlated with serum creatinine (SCr) and calculated creatinine clearance.

Results: Mean pre-RT levels of estimated glomerular filtration rate (ml/min), SCr (mg/dL), S.Ca level (mg/dL), S.PO4 (mg/dL), S.iPTH (pg/mL) and S.ALP (IU/L) were 8.2 ± 2.1, 10.3 ± 3.2, 8 ± 1.1, 6 ± 2, 352 ± 315.2 and 147.2 ± 120 respectively. At mean follow-up of 1.08 ± 0.2 month post-RT, mean values changed to 57.3 ± 20.1, 1.6 ± 0.7, 8.9 ± 0.8, 2.8 ± 1, 135.8 ± 131.5, 123.3 ± 68; and at 7.3 ± 1.1 months, values changed to 65.3 ± 22.1, 1.3 ± 0.5, 8.9 ± 1.4, 3.5 ± 0.9, 100.5 ± 65.9 and 172.5 ± 93.4 respectively. At 1 month post-RT, all patients achieved stable graft function with hypocalcemia in 20%, hypophosphatemia in 61.7%, and high S.ALP level in 46.7%. In spite of significantly improved S. iPTH level, hyperparathyroidism was observed in 66.7% patients. At 7 months post-RT, hypercalcemia was found in 10.9%, hypophosphatemia in 26.1% and hyperparathyroidism in 47.8% patients.

Conclusion: Regular stringent monitoring of bone mineral markers like i-PTH, PO4, total Ca and ALP in serum can prevent progression of MBD in RT recipients with medical management.

Keywords: hyperparathyroidism, hypocalcemia, hypophophatemia, mineral bone diseases, renal transplant

INTRODUCTION

Renal transplantation (RT) largely restores defective exocrine and endocrine renal functions in patients with chronic kidney disease (CKD). With increasing life expectancy after RT, the prevention of long term complications has become an essential part of post-RT care. Mineral bone disease (MBD) is one of the most common complications that significantly influence the quality of life. This is possibly due to pre-existing bone damage acquired during dialysis therapy, renal insufficiency, deleterious effects of different immunosuppressive agents and post-RT mineral metabolic changes such as persistent post-RT secondary hyperparathyroidism (SHP) and post-RT hypophosphatemia (hypo-Pi)[1]. Among all these causes, the major obstacle to investigate MBD in RT recipients has been its unpredictable evolution under multiple biochemical and hormonal influences that regulate mineral metabolism and bone turnover independently.

Persistent SHP found regularly for ≥2 years post-RT is a known factor for increased bone turnover and decreased bone density[2]. Moreover, hypo-Pi observed most frequently in the early post-RT period is believed to be associated with severe alterations in bone turnover that include a decrease in osteoblast activity leading to osteomalacia[3]. We carried out a study to evaluate the changes in bone mineral metabolism markers which included serum (S.) intact parathyroid hormone (S.iPTH), S. phosphorus ($S.PO_4$), S. calcium (S.Ca) and S. alkaline phosphatase (S.ALP) at 1 and at 7 months post-RT period. Serum creatinine (SCr) was considered for evaluation of graft function status.

SUBJECTS AND METHODS

This was a prospective single center clinical study to observe bone mineral changes at 1 month and 7 months post-transplant in 60 post-RT patients with a mean age of 36.7 ± 9.4 years. Patients above 18 years of age were included in the study. We excluded patients with a history of bone disease due to causes other than CKD or any metabolic disorders affecting bone minerals.

All the patients were on the same immunosuppression regime of Tacrolimus (0.05 mg/kgBW/ day), Prednisolone (10 mg/day) and Mycofenolate (360 mg) thrice a day.

Venous blood samples were collected in a gel vacutainer and separated serum was analyzed for SCr and calculated creatinine clearance to assess graft function, and S.Ca, $S.PO_4$, S.ALP on fully automated biochemistry analyzer (Beckman Coulter AU-480, USA) and S. iPTH on fully automated immuno-analyzer (Roche Cobas-e411, Switzerland) with commercially available reagent kits. Analysis was performed before transplantation, at 1 month and 7 months post-RT. SCr was measured by isotopic dilutional mass spectrometry traceable modified Jaffe's method. S.Ca was measured by colorimetric Arsenazo-III method, S. PO_4 was measured by phosphomolybdate method, S.ALP was measured by para-Nitro PhenolPhosphate method and S.iPTH was measured by sandwich electro-chemiluminescence method. The calculated creatinine clearance was measured by using Cockcroft and gault formula[4]. Cut-off values considered to define hypercalcemia, hypophosphatemia and hyperparathyroidism were >10.5 mg/dL, <3mg/dL and >75 pg/mL, respectively.

Ethical approval

The study was approved by our institutional review board and study number was IKDRCITS-LAB-03-01-2016. Consent for publication was obtained from all the patients.

Statistical Analysis

Statistical analysis was performed using SPSS version 20. All values are expressed as mean ± SD. Comparison of pre-RT and post-RT results were made with student's paired-t test, Wilcoxon Signed Rank test and correlations were calculated with Pearson correlation coefficient. A p-value <0.05 was considered as statistically significant. Inter-patient coefficient of variance (CV%) for each analyte of interest was calculated by the formula "CV% = (SD*100) /

Mean".

RESULTS

Out of 60 patients, 48 (80%) were males and 12 (20%) were females. Mean SCr was 1.6 ± 0.7 mg/dL with calculated creatinine clearance of 57.3 ± 20.1 ml/min at 1 month post-RT, and 1.3 ± 0.5 mg/dL with calculated creatinine clearance of 65.3 ± 22.1 ml/min at 7 months post-RT. S. iPTH, S.Ca, $S.PO_4$ and S.ALP at mean 1.08 ± 0.2 and 7.3 ± 1.1 months post-RT respectively is shown in Table 1. Inter-patient CV% for SCr at pre-RT, 1 month and 6 months post-RT were 26.2%, 35.1% and 33.8% respectively.

There was a significant decrease in the mean level of S. iPTH (in pg/mL) from 352 ± 315.2 to 135.8 ± 131.5 ($p < 0.001$) and 100.5 ± 65.9 ($p < 0.01$) at mean 1 and 7 months post-RT respectively. However, 66.7% (n = 40) and 47.8% (n = 24) of the patients had persistent SHP at 1 and 7 months post-RT respectively (Fig 1). No significant changes were found between values of S.iPTH levels at 1 and 7 months ($p = 0.486$). Inter-patient CV% for S. iPTH at pre-RT, 1 month and 6 months post-RT was 89.5%, 96.9% and 65.1% respectively.

$S.PO_4$ level (in mg/dL) was decreased significantly from 6 ± 2 to 2.8 ± 1 ($p < 0.0011$) and 3.5 ± 0.9 ($p < 0.001$) at 1 and 7 months post-RT respectively. Overall, 61.7% (n = 37) patients had hypo-Pi at 1 month, and 26.1% of the patients (n = 12) had hypo-Pi at 7 months post-RT (Fig 2). There was also a significant change between level of $S.PO_4$ at 1 and 7 months post-transplant ($p < 0.001$). Inter-patient CV% for $S.PO_4$ at pre-RT, 1 month and 6 months post-RT were 33.5%, 37.1% and 25.9% respectively. Total S.Ca level (in mg/dL) increased from 8 ± 1.1 to 8.9 ± 0.8 ($p < 0.001$) at 1 month and thereafter remained steady at 8.9 ± 1.4 ($p < 0.05$) at 7 months post-RT. Inter-patient CV% for S.Ca at pre-RT, 1 month and 6 months post-RT were 13.8%, 9.4% and 15.6% respectively. Moreover, we noticed that out of 68% pre-RT hypocalcemic patients (n = 41), 23.3% (n = 14) and 32.6% (n = 15) had persistent hypocalcemia at 1 and 7 months post-RT respectively (Fig 3). In addition, 10.9% (n = 5) of the patients developed hypercalcemia at 7 months. We observed there was no significant alteration in S.ALP level at 1 month post-RT. However, significant rise in mean S.ALP level at 7 months post-RT compared to pre-RT level ($p < 0.001$) and 1 month post-RT level ($p < 0.001$) was observed. Mean levels of S.ALP (IU/L) were 147.2 ± 120.7, 123.3 ± 68, 172.5 ± 93.4 at pre-RT, at 1 month and 7 months post-RT respectively. Inter-patient CV% for S.ALP at pre-RT, 1 month and 6 months post-RT was 82%, 55.1% and 54.2% respectively.

DISCUSSION

Successful RT restores the main abnormalities responsible for SHP. In this study, we prospectively evaluated data of 60 post-RT patients with stable renal function as reflected by calculated creatinine clearance and SCr level.

Changes in S.iPTH level

Though we observed a significant decrease in mean S. iPTH level after RT, it persisted to higher than acceptable reference ranges at 1 and 7 months post-RT in spite of stable graft function. Overall, 66.7% and 47.8% of our patients had elevated S. iPTH levels at 1 month and 7 months post-RT respectively. The prevalence of SHP in our study is quite high compared with the study in European children by Marjolein *et al* who showed persistent hyperparathyroidism in 41% patients at 9 - 12 months post-transplant[5]. Our findings are fairly correlating with the findings of Heide *et al,* who showed 50% patients having higher value of S. iPTH at 1 year and 27% of patients after 2 years of post RT[2], although our follow-up period is less compared to those of Heide *et al*.

In CKD, there is a decrease in vitamin-D and S.Ca level whereas increase in fibroblast growth factor-23 (FGF-23) and $S.PO_4$ level. All these abnormalities ultimately act on the parathyroid gland through their respective receptors and lead to parathyroid cell proliferation, thereby increasing S. iPTH synthesis and its secretion[6-8]. In addition to the very high S. iPTH level before transplant, longer duration of dialysis and older age, development of tertiary hyperparathyroidism due to nodular transformation from a polyclonal hyperplasia into a monoclonal adenoma post-transplant may contribute to persistently high S. iPTH level[8,9].

Persistent SHP stimulates bone resorption by indirect effect on osteoclast. It binds with the receptor present on osteoblast, up-regulates expression of receptor activator of nuclear factor kappa-B ligand which bind with the receptor activator of nuclear factor kappa-B present on osteoclast, and thereby gives signal to bone marrow derived osteoclast precursor. Ultimately this stimulates fusion, differentiation and activation of osteoclast eventually leading to bone resorption[3,7,10].

Changes in $S.PO_4$

Hypo-Pi is a frequent problem found after RT. In our study, we found 61.7% of the patients with hypo-Pi at 1 month post-transplant and 26.1% patients with hypo-Pi at 7 months post-RT, which fairly correlated with the study of Ulrich Kunzendorf *et al*. They found hypo-Pi in 90% of RT recipients for the first 4 months and in 20 – 40% patients at 1 year post-transplant[1].

Inappropriately high S.iPTH with recovered tubular function is considered to be the most relevant hypophosphatemic factor in RT patients[11,12]. Apart from that, elevated level of one of the more recently defined phosphaturic factor FGF-23, also known as “Phosphatonin”, continues to be elevated in early post-RT period even with normal S.iPTH level[13-15,16]. Another factor contributing to post-RT hypo-Pi is steroid therapy which increases PO_4 excretion by inhibiting Na/Pi co-transporter[1,6,11]**.** Bellorin-Font *et al* showed $S.PO_4$ level correlated negatively with the number of apoptotic osteoblasts and positively with the numbers of active osteoblasts, suggesting a role of hypo-Pi in the mechanism that leads to post-RT MBD[17].

Changes in S.Ca

In this study, we observed that 68% (n = 41) of the patients had pre-RT hypocalcemia and subsequently 36.5% (n = 15) of the patients had persistent hypocalcemia at 7 months post-RT.

This indicates that about 63.5% of the patients recovered from hypocalcemia at 7 months after transplantation (Fig 3). Moreover, 10.9% (n = 5) of the patients had developed hypercalcemia at 7 months post-RT. This suggests gradual increase in S.Ca level during 7 months follow-up after transplantation, which may progress to hypercalcemia over a longer duration of follow-up. This finding supports the studies of Messa *et al* who observed hypercalcemia in 23% of the patients at 6 months and in 27% patients at 12 months post-RT follow-up[11]. The possible mechanisms for increasing S.Ca level after transplantation are persistent SHP, recovery of response of iPTH-receptor to iPTH, increased calcitriol availability and hypo-Pi[6,18-20].

Changes in S.ALP

In our study, no significant change was found in post-RT S.ALP level at 1 month, but it was significantly increased at 7 months (Fig 4). This observation is similar to the findings of Sperschneider *et al,* who showed an increase in S.ALP level at 1 month post-RT[2].

Limitations of the present study

We could not perform serum levels of vitamin-D and FGF-23, which might have thrown light on potential etiological mechanisms of post-RT MBD.

CONCLUSION

Regular stringent monitoring of bone mineral markers like intact parathyroid hormone, phosphorus, total calcium and alkaline phosphatase in serum can prevent progression of mineral bone disease in renal transplantation recipients with medical management.

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Authors' Contribution: P. A. Gandhi designed the study, carried out all the lab work and wrote the manuscript. A. V. Vanikar, R. D. Patel, K. V. Kanodia, K. S. Suthar and L. A. Nigam supervised all the lab work and approved the manuscript. H. V. Patel was the treating physician for all patients and approved the manuscript. A. V. Vanikar and U. G. Thakkar participated in the study design and finalized the manuscript.

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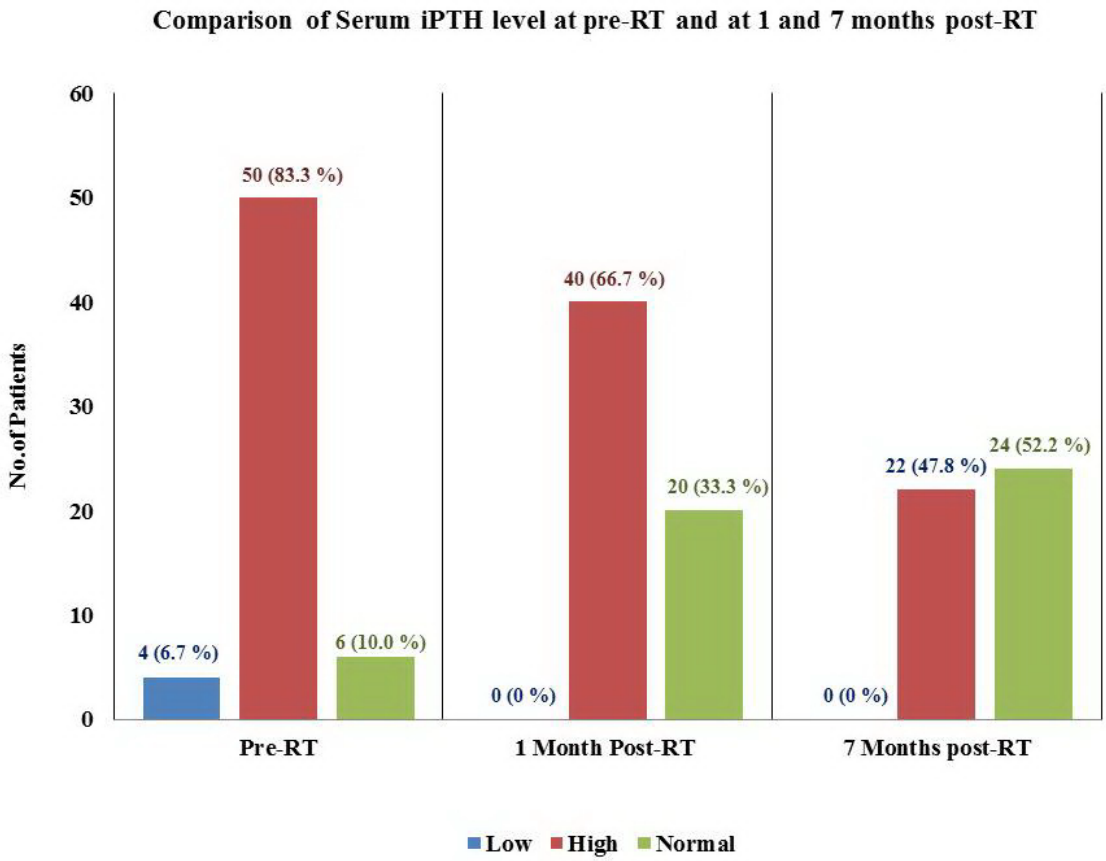

Fig 1: Comparison of Serum iPTH level at pre-renal transplant and at 1 and 7 months post renal transplant

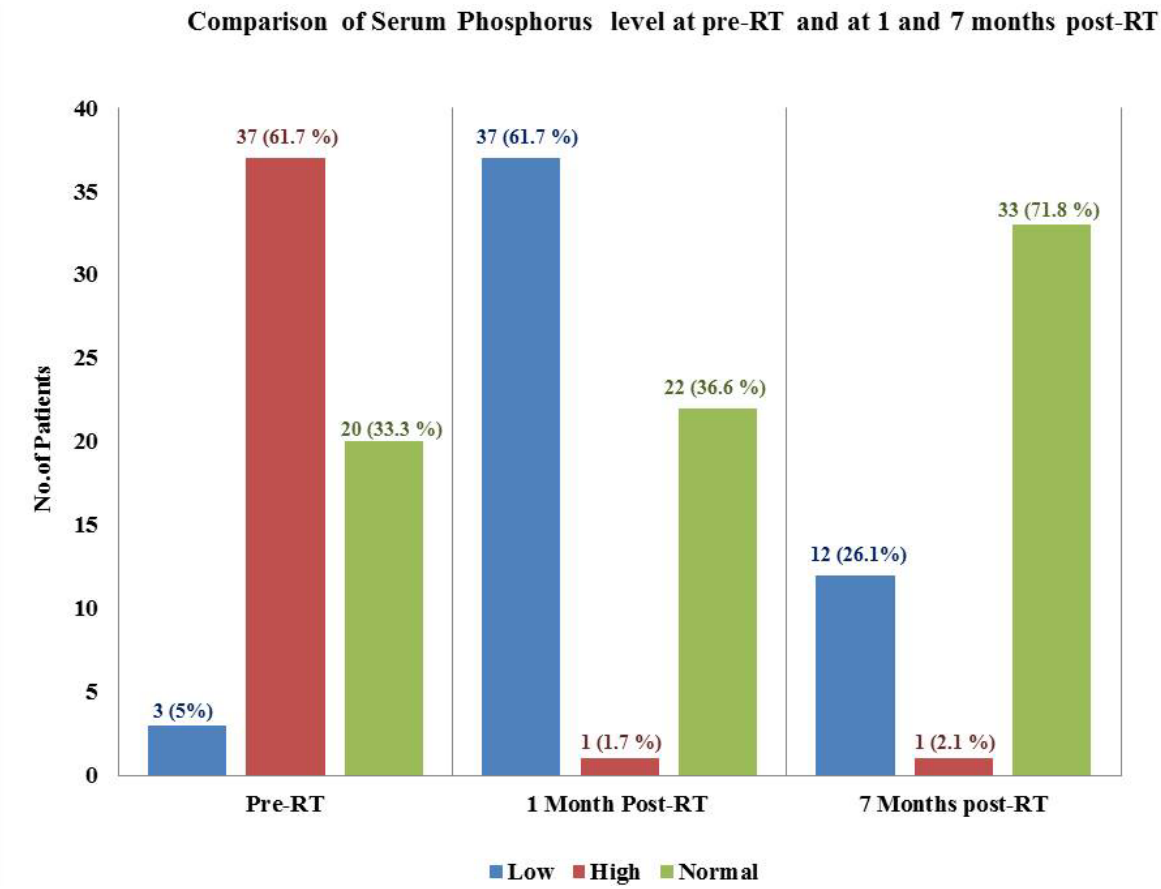

Fig 2: Comparison of serum phosphorus level at pre-renal transplant and at 1 and 7 months post renal transplant

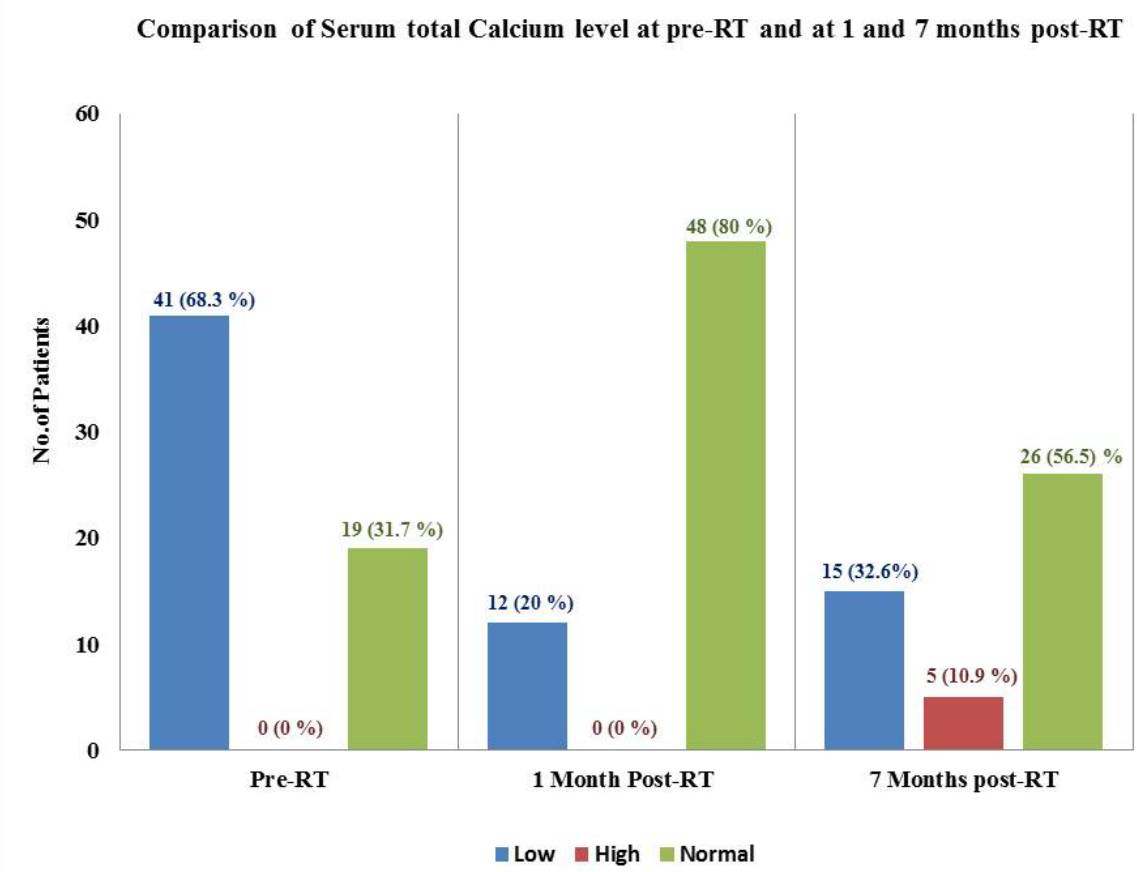

Fig 3: Comparison of serum total calcium level at pre-renal transplant and at 1 and 7 months post renal transplant

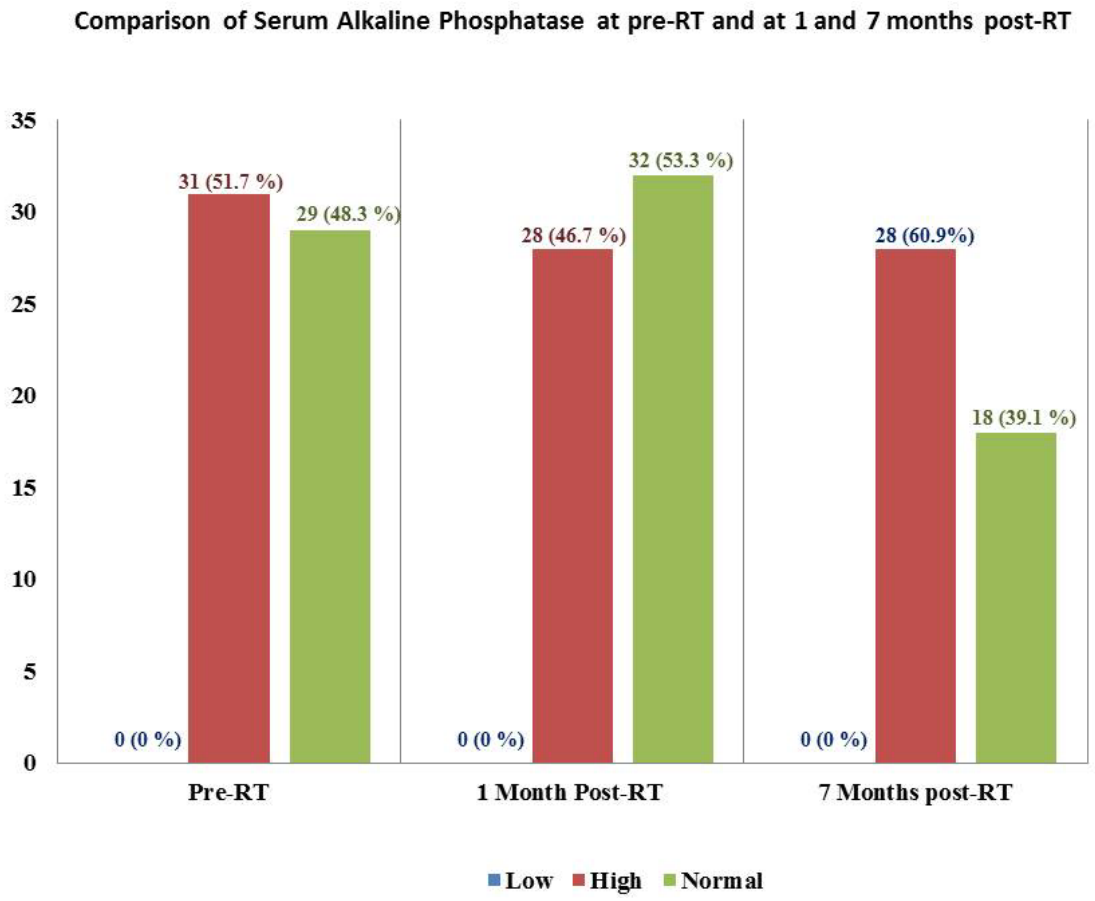

Fig 4: Comparison of serum alkaline phosphatase at pre-renal transplant and at 1 and 7 months post renal transplant

Table 1: Comparison of bone mineral markers in renal transplant patients at pre and post transplant 1 month and 7 months

Parameter	Biological Reference Range	Pre-RT (Mean ± SD) (n=60)	Post-RT 1.08 ± 0.2 months (Mean ± SD) (n=60)	Post-RT 7.3 ± 1.1 months (Mean ± SD) (n=46)	p-value		
					(Pre and post-RT 1.08 ± 0.2 Month)	(Pre and post-RT 7.3 ± 1.1 Months)	(post-RT 1.09 ± 0.21 and 7.3 ± 1.1 Months)
Calculated creatinine clearance (ml/min)	>90	8.2 ± 2.4	57.3 ± 25.5	65.3 ± 21.3	<0.01*	<0.01*	0.02*
S. Creatinine (mg/dL)	0.8 - 1.4	10.3 ± 3.0	1.6 ± 0.7	1.3 ± 0.4	<0.01*	<0.01*	0.03*
S.intact parathyroid hormone (pg/mL)	13 - 75	352.2 ± 304.6	135.8 ± 116.1	100.5 ± 56.1	<0.01*	<0.01*	0.58 (NS)
S. Calcium (mg/dL)	8.5 - 10.5	8 ± 1.1	8.9 ± 0.8	8.9 ± 1.4	<0.01*	<0.01*	0.84 (NS)
S. Phosphorus (mg/dL)	3 - 5	6 ± 2.1	2.8 ± 0.9	3.5 ± 0.8	<0.01*	<0.01*	<0.01*
S.Alkaline phosphatase (IU/L)	34 - 108	147.2 ± 134.1	123.3 ± 67.3	172.5 ± 100	0.43 (NS)	0.01*	<0.01*