COMPARATIVE STUDY OF INTACT PARATHYROID HORMONE, CALCIUM, PHOSPHATE AND RADIOLOGICAL CHANGES IN PATIENTS WITH END STAGE RENAL DISEASE ON MAINTENANCE HAEMODIALYSIS AND NEWLY DIAGNOSED END STAGE RENAL DISEASE.

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1. SUMMARY

Secondary hyperparathyroidism and renal bone disease are invariable consequences of chronic kidney disease. Calcitriol deficiency, hypocalcemia and hyperphosphatemia are responsible for raised parathyroid hormone and renal bone disease in both pre-dialysis and maintenance dialysis patients. Calcitriol therapy and control of serum phosphate with protein restricted diet and phosphate binders result in correction of hyperparathyroidism and reversion of the renal bone histology.

Since the initiation of nephrology service in Nepal, these therapies are practiced in all chronic kidney disease patients without any studies on the severity of hyperparathyroidism, renal bone disease and the beneficial and adverse effects of these drugs. So this study was carried out to initiate the study on renal bone disease and to see the degree of hyperparathyroidism, hypocalcemia, hyperphophatemia and the radiological changes in Nepalese patients with end stage renal disease on maintenance haemodialysis (ESRD on MHD) and newly diagnosed end stage renal disease (NESRD) and to evaluate the beneficial effect of haemodialysis on secondary hyperparathyroidism.

Twenty three (16 male, 7 female) ESRD on MHD patients with twice a week dialysis for 6 to 78 (22 $\pm$ 3, mean $\pm$ SEM) months with protein restricted diet and calcium acetate as a phosphate binder but without calcitriol therapy and twenty three (16 male, 7 female) NERSD patients without protein restricted diet, phosphate binder and calcitriol therapy were included in this study and fasting blood samples were collected for estimation of serum intact parathyroid hormone (PTH), calcium, phosphate and alkaline phosphatase and all were subjected to X-ray hands A/P view and X-ray lumbosacral spine lateral view.

Serum PTH was found to be significantly lower ($z = -4.251$, $p < 0.0001$) in ESRD on MHD (mean $\pm$ SD) 118.7 $\pm$ 195.8 pg/ml than in NESRD (mean $\pm$ SD) 335.0 $\pm$ 214.3 pg/ml. On grouping of study population according to K/DOQI guide line with serum PTH 150-300 pg/ml as the optimal level, sub optimal PTH level was found in 82.6% MHD (<100 pg/ml in 65.2%) and 30.4% NESRD patients, optimal PTH in 4.3% MHD and 26.1% NESRD patients and hyperparathyroidism in 13% of MHD and 43.5% of NESRD patients. In NESRD patients PTH level was found to be >100 pg/ml in all but one patient.

On grouping of ESRD on MHD patients according to dialysate calcium concentration, mean PTH level was found to be significantly lower ($F=7.984$, $p < 0.05$) in high dialysate calcium (1.75 mmol/l) group [45.3 $\pm$ 40.8 pg/ml (mean $\pm$ SD)] than in low dialysate calcium (1.25 mmol/l) group [256.4 $\pm$ 289.9 pg/ml (mean $\pm$ SD)].

Serum calcium was found to be significantly higher ($t = 6.86$, $p<0.00001$) in MHD (mean $\pm$ SD) 9.6 $\pm$ 1.2 mg/dl than in NESRD (mean $\pm$ SD) 6.9 $\pm$ 1.4 mg/dl. On grouping of study population according to serum calcium level, ESRD on MHD patients showed hypocalcemia in 17.4%, normocalcemia in 56.5% and hypercalcemia in 26.1% patients and NESRD showed severe hypocalcemia with serum calcium (mg/dl) of 6.6 $\pm$ 1.1 (mean $\pm$ SD) in 91.3% patients.

Serum phosphate was found to be significantly lower ($t = -2.43$, $p<0.05$) in ESRD on MHD (mean $\pm$ SD) 7.9 $\pm$ 2.3 mg/dl than in NESRD (mean $\pm$ SD) 10.3 $\pm$ 4.1 mg/dl. On grouping according to K/DOQI, normal serum phosphate of 3.5 – 5.5 mg/dl was found in

8.7% of ESRD on MHD and 13% of NESRD. Hyperposphatemia was observed in 91.3% ESRD on MHD patients with serum phosphate (mg/dl) of 8.2 $\pm$ 2.3 (mean $\pm$ SD) and in 87% of NESRD patients with serum phosphate (mg/dl) 11.2 $\pm$ 3.6 (mean $\pm$ SD).
PTH showed negative correlation with serum calcium and positive correlation with serum phosphate in both groups but statistically significant positive correlation of PTH and phosphate was observed only in NESRD patients.
Osteopenia and osteoarthritis were the dominant radiological findings along with tuft erosion, radius bone erosion, rugger jersey spine and vascular calcification. None of these findings showed any relation with PTH level in both groups.

The result suggests that hypocalcemia, hyperphosphatemia and hyperparathyroidism are the invariable consequences of chronic kidney disease and it becomes severe in advanced renal failure if not treated earlier, maintenance haemodialysis and calcium containing phosphate binder therapy can control the hypocalcemia, hyperphosphatemia and secondary hyperparathyroidism even without calcitriol therapy although hyperphosphatemia still remains a significant problem in maintenance haemodialysis patients, undue suppression of PTH has occurred in maintenance haemodialysis and probably it is related to high dialysate calcium with calcium containing phosphate binder therapy and plain X-rays have not been found helpful to diagnose renal bone disease.

Therefore, more effort should be exercised for meticulous control of phosphate in dialysis patients, bone biopsy should be done to evaluate the renal bone disease and further elaborate research should be done in Nepalese haemodialysis patients to identify the etiology of excessive parathyroid hormone suppression and to allocate the desired dialysate calcium concentration.

2. INTRODUCTION

Secondary hyperparathyroidism and renal bone disease are invariable consequences of chronic kidney disease (CKD). Raised serum parathyroid hormone (PTH) is documented in early CKD with glomerular filtration rate of more than 60 ml/min (Reichel *et al* 1991). Reduced calcitriol biosynthesis by the failing kidney is responsible for elevated serum PTH in early CKD (Lucas *et al* 1988, Ritz *et al* 1991) and the treatment with calcitriol in these patients corrects the hyperparathyroidism (Ritz *et al* 1995).

Baker et al 1989 had also documented the histologically proven renal bone disease and raised PTH in moderate renal failure with glomerular filtration rate of 20-59 ml/min and improvement of bone histology with calcitiol therapy.

In advanced CKD, besides calcitriol deficiency, hypocalcemia and hyperphosphatemia aggravates the secondary hyperparathyroidism and renal bone disease in both pre-dialysis and maintenance dialysis patients (Slatopoisky and Deimez 1994). Serum phosphate correction with protein restricted diet and phosphate binders (Combe and Aparicio 1994, Teruel *et al* 1999), calcitriol therapy (Tsukamoto *et al* 1991) and maintenance dialysis with positive calcium and negative phosphate balance ameliorates the hyperphosphatemia, hypocalcemia and hyperparathyroidism and heals the renal bone disease (Massry 1995). But development of hypercalcemia (Fischer and Harris 1993) and adynamic bone disease with vigorous control of parathyroid hormone (Goodman *et al* 1994) by calcitriol therapy limits its irrational use.

Since the initiation of nephrology service in Nepal by starting intermittent peritoneal dialysis in 1973 and haemodialysis in 1987 in Bir Hospital (Khakurel *et al* 2000), all CKD patients are managed with phosphate binders and calcitriol therapy for renal bone disease. CKD has remained the major nephrological problem in Nepal with many patients landing up at the stage of end stage renal disease (ESRD) itself and to cope with them nephrology service including haemodialysis is expanded to other centers after 1995 (Hada 2003). But till date no study is carried out on renal bone disease, secondary hyperparathyroidism and beneficial and adverse effects of calcitriol and phosphate binder therapy in Nepalese CKD patients. So the purpose of this study is to initiate the study on renal bone disease in Nepalese CKD patients and to evaluate and compare the incidence of hyperparathyroidism, hypocalcemia, hyperphosphatemia and the radiological changes in end stage renal disease on maintenance haemodialysis (ESRD on MHD) patients with more than 6 months of dialysis but without calcitriol therapy for at least 12 weeks and newly diagnosed end stage renal disease (NESRD) patients not on phosphate binder, calcitriol therapy and protein restricted diet.

3. AIMS AND OBJECTIVES

The present study was aimed to:

1. initiate the study on renal bone disease in Nepalese CKD patients.
2. estimate and compare serum parathyroid hormone in end stage renal disease on maintenance haemodialysis patients with phosphate binders and newly diagnosed ESRD patients not on phosphate binders, protein restricted diet & calcitriol therapy and to establish the beneficial effect of maintenance haemodialysis with phosphate binder in secondary hyperparathyroidism.
3. estimate and compare serum calcium, phosphate and alkaline phosphatase in study population and see their relation with parathyroid hormone.
4. see the radiological changes in study population.

4. PATIENTS AND METHODS

Twenty three end stage renal disease patients (16 male and 7 female) on maintenance haemodialysis for more than 6 months (19 in Bir hospital and 4 in National Kidney Center) were included initially for the study. Their median age was 51 (range 25 to 71) years and the etiology of their renal failure was chronic glomerulonephritis 14, diabetic nephropathy 3, hypertensive nephrosclerosis 2, autosomal dominant polycystic kidney disease 2 and chronic tubulointerstitial nephropathy 2. Patients were explained about the purpose of study and written consents were obtained. Six Patients were taking calcitriol therapy and it was stopped for washout period of 12 weeks. All were receiving calcium acetate as the phosphate binder in different dosage and twice weekly haemodialysis with total duration of 8 hours/week. Monthly serum calcium and serum phosphate were estimated and the dosage of phosphate binder (calcium acetate) was adjusted accordingly. At the time of sample collection 5 (10.9%) patients were receiving 2 tablet of calcium acetate with total elemental calcium of 338 mg/day and 18 (39.1%) patients were receiving 3 tablets of calcium acetate with total elemental calcium of 507 mg/day. 8 (17.4%) patients were having acetate dialysis with dialysate calcium concentration of 1.25 mmol/l and 15 (32.6%) patients were on bicarbonate dialysis with dialysate calcium concentration of 1.75 mmol/l. All were on protein restricted diet of 1gm/kg as prescribed at the time of initiation of dialysis but diet analysis and re-prescription of diet was not done for the study. The duration of haemodialysis of these patients by the time of sample collection ranged from 6 to 78 months (22 $\pm$ 3 months, mean $\pm$ SEM, median 22 months). All patients were subjected to X-ray hands anterior posterior view and X-ray lumbosacral spine lateral view. Pre-dialysis fasting blood samples were collected after overnight fast on the first dialysis day of the week for estimation of serum PTH, calcium, phosphate and alkaline phosphatase.

Later twenty three newly diagnosed end stage renal disease patients (16 male and 7 female) attending Bir hospital emergency and nephrology out-patient department needing urgent dialysis but not on previous therapy with phosphate binder and calcitriol, not on protein restricted diet and not on follow up for kidney disease were included. Patients with acute on chronic kidney disease and chronic kidney disease on regular follow up and reaching recently in ESRD stage were excluded from the study. Their median age was 41 (range 13 to 68) years and the etiology of end stage renal disease was chronic glomerulonephritis 16, diabetic nephropathy 2, hypertensive nephropathy 4 and reflux nephropathy 1. Their serum creatinine ranged from 7.9 to 21.5 mg/dl with mean $\pm$ SD (12.8 $\pm$ 4.4 mg/dl) and the calculated creatinine clearance by Cockroft- Gault method ranged from 3.16 to 9.7 ml/min with mean $\pm$ SD (5.18 $\pm$ 1.7 ml/min).

Written consent was taken from all patients. Fasting blood samples were taken after one session of life saving intermittent peritoneal dialysis in 10 patients and without single dialysis in 13 patients. All patients had X-Ray hands anterior posterior view and X-Ray lumbosacral spine lateral view.

After collection of the fasting blood, the serum was separated and preserved in the freezer at -4^{o}C for estimation of intact parathyroid hormone once a week. But serum calcium, phosphate and alkaline phosphatase were estimated on the same day.
The level of intact parathyroid hormone was determined by immunoenzymatic assay using the Biosource hPTH-EASIA (Enzyme Amplified Sensitivity Immunoassay) and the normal value was 16-46 pg/ml. Serum calcium was detected by o-Cresolphthalein Direct Colorimetric Method and the normal value was 8.8-10.2 mg/dl. Serum phosphate was estimated by Photometric UV test and the normal value was 2.6-4.5 mg/dl. Serum alkaline phosphatase was detected by Kinetic Photometric test with normal value of 53-128 unit/L and it was not the bone-specific isoenzyme. Serum albumin was not estimated and corrected calcium value could not be calculated.

The result of this study was analyzed by using the SPSS package. The results are expressed as mean $\pm$ SD. The difference between the groups is analyzed by student's t test for continuous variables (calcium and phosphate) and Mann-Whitney U test for nonparametric variables (PTH and alkaline phosphatase). Correlation is described with the Pearson product moment coefficient.

5. RESULTS

Biochemistry

Serum parathyroid Hormone (PTH)

PTH of ESRD on MHD were (mean ± SD) 118.7 __ 195.8 pg/ml and PTH of NESRD were (mean ± SD) 335.0 ± 214.3 pg/ml as shown in Table - I.
Comparison of PTH of ESRD on MHD with NESRD showed significantly lower PTH in ESRD on MHD with ($p < 0.0001$) (Mann-Whitney U test).

On sub grouping of study population according to serum PTH level (K/DOQI guideline, National Kidney Foundation) as shown in Table II, ESRD on MHD showed hyperparathyroidism of >300 pg/ml in 3 (13%) patients, optimal parathyroid hormone of 150 – 300 pg/ml in 1 (4.3%) patient and sub optimal parathyroid hormone of <150 pg/ml in 19 (82.6%) patients. Surprisingly among the patients with sub optimal parathyroid hormone, 15 (65.2%) patients have PTH <100 pg/ml with 7 (30.4%) patients having PTH <60 pg/ml.
NESRD revealed hyperparathyroidism in 10 (43.5%) patients, optimal parathyroid hormone in 6 (26.1%) and sub optimal parathyroid hormone in 7 (30.4%) patients. None of the NESRD patients had parathyroid hormone of <60 pg/ml and out of 7 patients with sub optimal parathyroid hormone, only one was below 100 pg/ml i.e. 92.9 pg/ml.
Comparison of these sub groups of the study population according to PTH level showed significant difference with $p < 0.01$ (chi- square).

In MHD patients, 15 patients were receiving dialysis with dialysate calcium concentration of 1.75 mmol/l and 8 patients were receiving dialysis with dialysate calcium concentration of 1.25 mmol/l. Mean PTH (pg/ml) was significantly lower (mean ± SD) (45.3 ± 40.8 vs 256.4 ± 289.9) in 1.75 mmol/l dialysate calcium group than 1.25 mmol/l group ($p<0.05$, Anova).

Serum calcium

Serum calcium of ESRD on MHD were (mean ± SD) of 9.6 ± 1.2 mg/dl and the serum calcium of NESRD were (mean ± SD) of 6.9 ± 1.4 mg/dl as shown in Table I.
Comparison of serum calcium of ESRD on MHD with NESRD showed significantly lower serum calcium in NESRD patients with $p<0.00001$ (unpaired t test).

On sub grouping of study population according to serum calcium level without correcting for serum albumin value as it was not estimated initially, ESRD on MHD showed hypocalcemia with serum calcium <8.8 mg/dl in 4 (17.4%) patients, normocalcemia with serum calcium 8.8 – 10.2 mg/dl in 13 (56.4%) patients and hypercalcemia with serum calcium >10.2 mg/dl in 6 (26.1%) patients. NESRD patients revealed hypocalcemia in 21

(91.3%), normocalcemia in 1 (4.3%) and hypercalcemia in 1 (4.3%) patients as shown in Table – III.
Comparison of these sub-groups according to serum calcium level revealed significant difference with $p < 0.00001$ (Chi-Square).

Serum phosphate

Serum phosphate of ESRD on MHD were (mean $\pm$ SD) of 7.9 $\pm$ 2.3 mg/dl and the serum phosphate of NESRD were (mean $\pm$ SD) of 10.3 $\pm$ 4.1 mg/dl as shown in Table I.
Comparison of serum phosphate of ESRD on MHD and NESRD showed significantly lower serum phosphate in ESRD on MHD with $p < 0.05$ (unpaired t test).

On sub grouping of study population according to serum phosphate level (K/DOQI) as shown in Table-IV, we have not found hypophosphatemia in both groups, normal phosphate level of 3.5 – 5.5 mg/dl in 2 (8.7%) of ESRD on MHD and 3 (13%) of NESRD patients and hyperphosphatemia of >5.5 mg/dl in 21 (91.3%) of ESRD on MHD and 20 (87%) of NESRD patients. Comparison of these subgroups according to serum phosphate level did not show any statistical difference. But among the patients with hyperphosphatemia, serum phosphate (mean $\pm$ SD) of ESRD on MHD were 8.2 $\pm$ 2.3 mg/dl and that of NESRD were 11.2 $\pm$ 3.6 mg/dl.

Serum alkaline phosphatase

Serum alkaline phosphatase of ESRD on MHD were (mean $\pm$ SD) 116.4 $\pm$ 120.5 U/L and serum alkaline phosphatase of NESRD were (mean $\pm$ SD) 131.7 $\pm$ 87.8 U/L as shown in Table- I.
Comparison of serum alkaline phosphatase of ESRD on MHD and NESRD revealed significant difference with ($p< 0.05$) (Mann-Whitney U test). Alkaline phosphatase did not show any relation with PTH value in both study population.

Serum PTH showed negative correlation with serum calcium in both ESRD on MHD and NESRD but there was no statistical significance. Serum PTH showed positive correlation with phosphate in both ESRD on MHD and NESRD and it was statistically significant only in NESRD with ($r = 0.503$ and $p<0.05$).

Serum PTH, calcium and phosphate level was not different in MHD patients with different dosage of calcium acetate {2 tab (n=5) vs 3 tab (n=18)}.

Table – I

Serum parathyroid hormone, serum calcium, serum phosphate And serum alkaline phosphatase of the study population

Groups	Serum PTH[a] (pg/ml)	Serum calcium[b] (mg/dl)	Serum Phosphate[b] (mg/dl)	Alkaline phosphatase[a] (U/L)
ESRD on MHD (n=23)	118.7 ± 195.8	9.6 ± 1.2	7.9 ± 2.3	116.4 ± 120.5
NESRD (n=23)	335.0 ± 214.3	6.9 ± 1.4	10.3 ± 4.1	131.7 ± 87.8
P value	**< 0.0001**	**< 0.00001**	**< 0.05**	**< 0.05**

[a] Results expressed as mean ± SD and Mann Whitney U test was performed as the test of significance.

[b] Results are expressed as mean ± SD and unpaired t test was performed as the test of significance

Table – II

Sub – grouping the study population according to PTH level (K/DOQI)

PTH value (pg/ml)	ESRD on MHD (n=23)	NESRD (n=23)
<150.000	19 (82.6%)	7 (30.4%)
150.000 – 300.000	1 (4.3%)	6 (26.1%)
>300.000	3 (13%	10 (43.5%)
p < 0.01 (Chi -Square test)		

Table – III

Sub grouping the study population according to serum calcium level

Serum calcium (mg/dl)	ESRD on MHD (n=23)	NESRD (n=23)
<8.8	4 (17.4%)	21 (91.3%)
8.8 – 10.2	13 (56.5%)	1 (4.3%)
>10.2	6 (26.1%)	1 (4.3%)
P < 0.00001 (Chi - Square Test)		

Table - IV

Sub grouping of the study population according to serum phosphate level

Serum phosphate (mg/dl)	ESRD on MHD (n=23)	NESRD (n=23)
<3.5	0 (0%)	0 (0%)
3.5 - 5.5	2 (8.7%)	3 (13%)
>5.5	21 (91.3%)	20 (87%)
P = NS (not significant**)**		

Table- V

Radiological findings of the study population

	ESRD on MHD	NESRD	P value
Osteopenia	17 (73.9%)	15 (65.2%)	**NS**
Osteoarthrosis	11(47.8%)	10 (43.5%)	**NS**
Vascular calcification	6 (26%)	2 (4.3%)	**NS**
Radius erosion	3 (13%)	4 (17.4%)	**NS**
Tuft erosion	2 (8.7%)	4 (17.4%)	**NS**
Rugger jersey spine	1(4.3%)	2 (8.7%)	**NS**

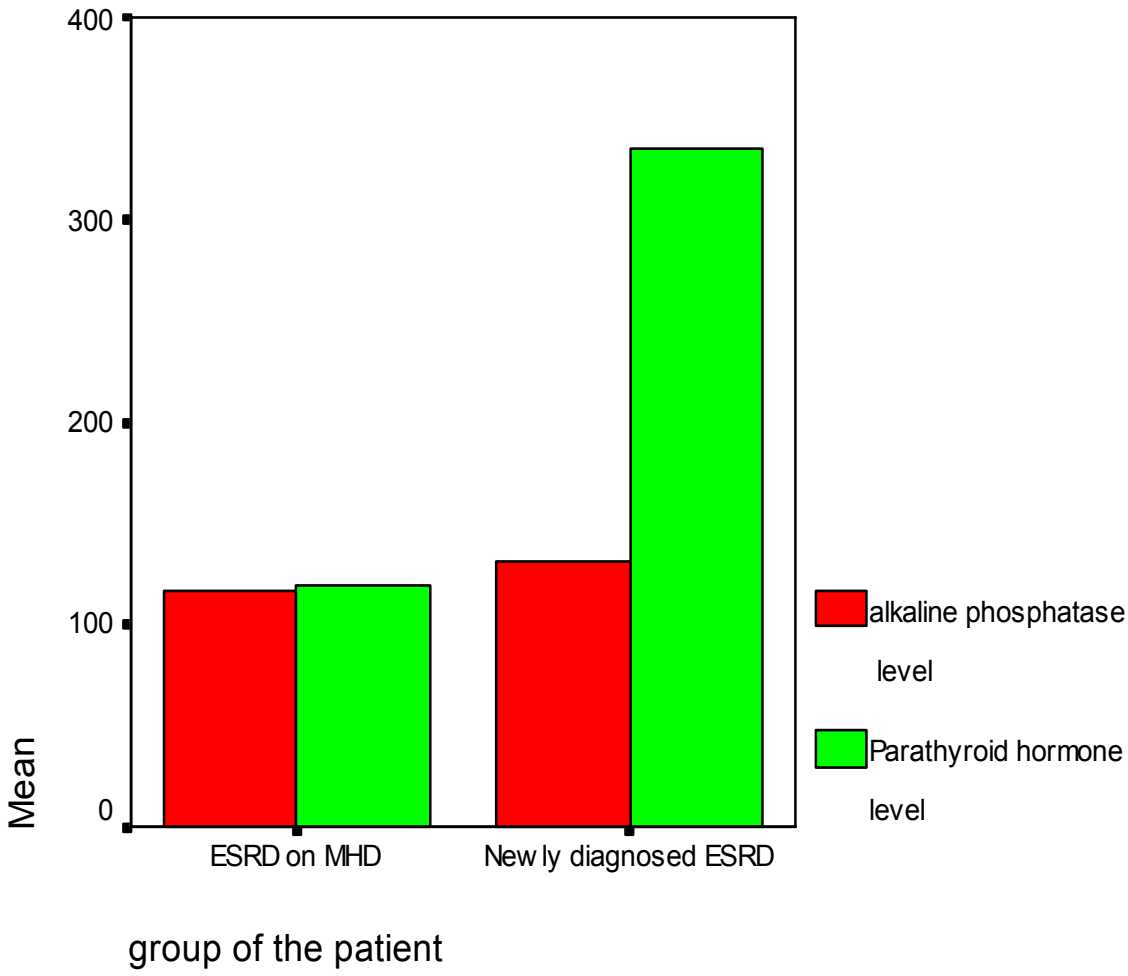

Fig. 1. Serum Parathyroid hormone (pg/ml) and serum alkaline phosphatase (U/L) of study population

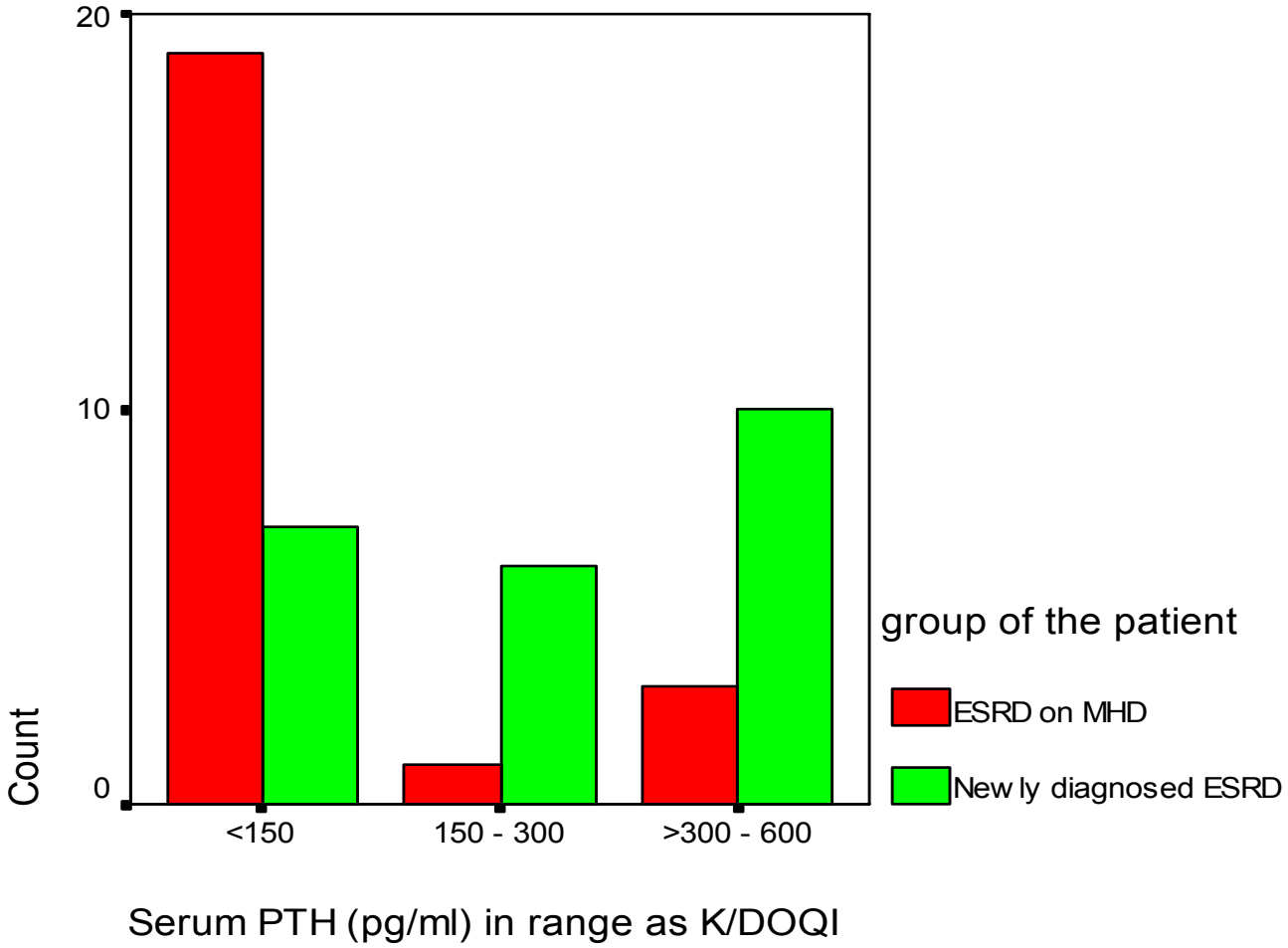

Fig. 2. Sub grouping of study population according to PTH level (K/DOQI)

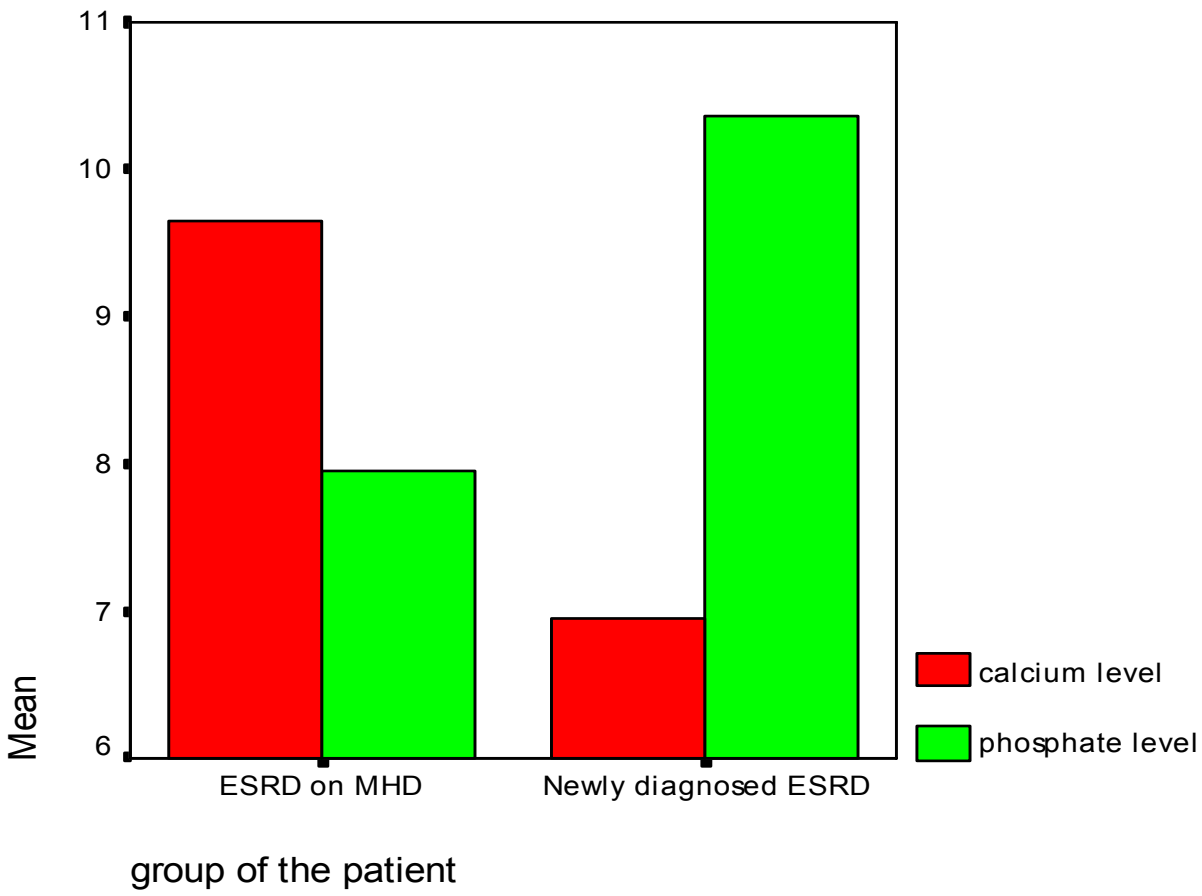

Fig. 3. Serum calcium (mg/dl) and serum phosphate (mg/dl) study population

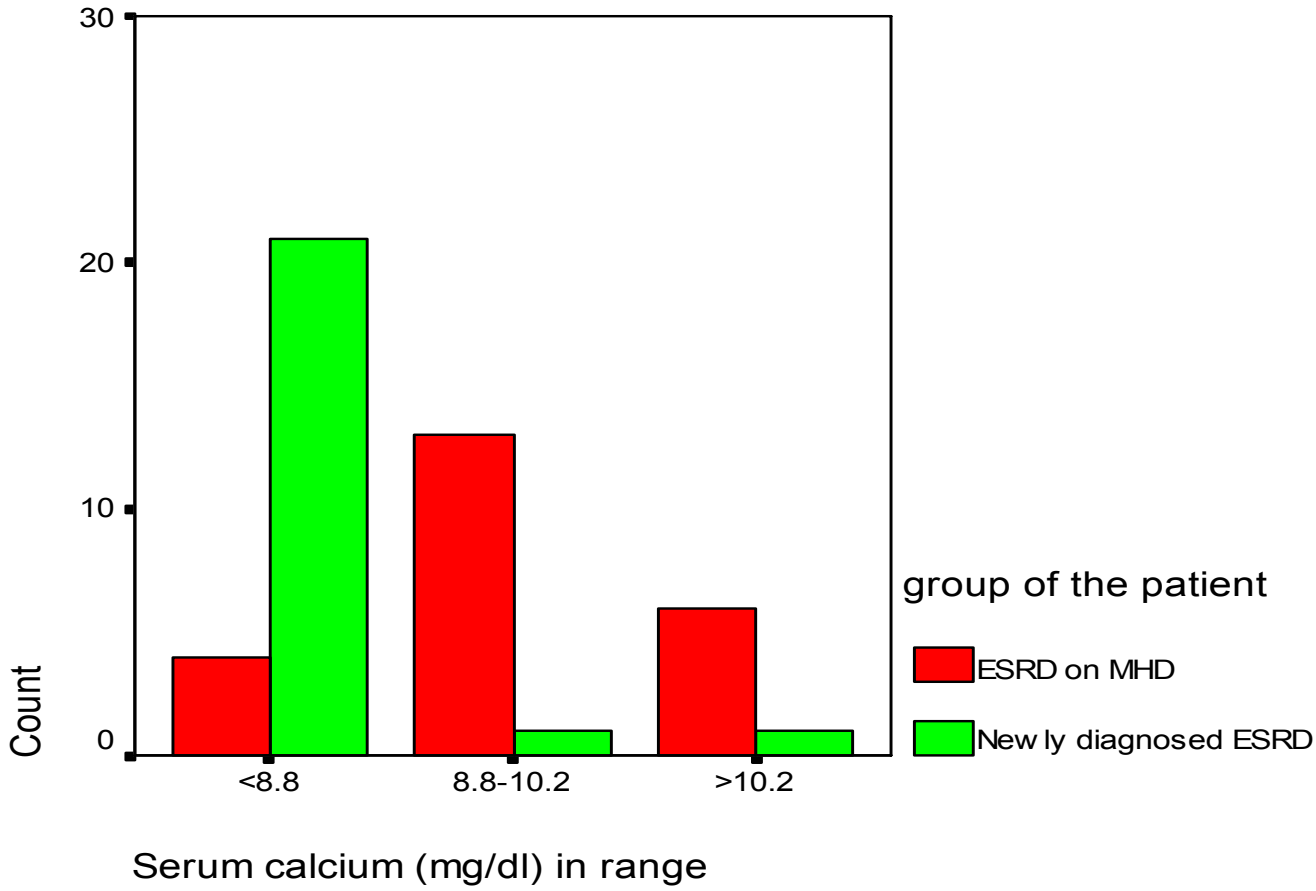

Fig. 4. Sub grouping of study population according to calcium level

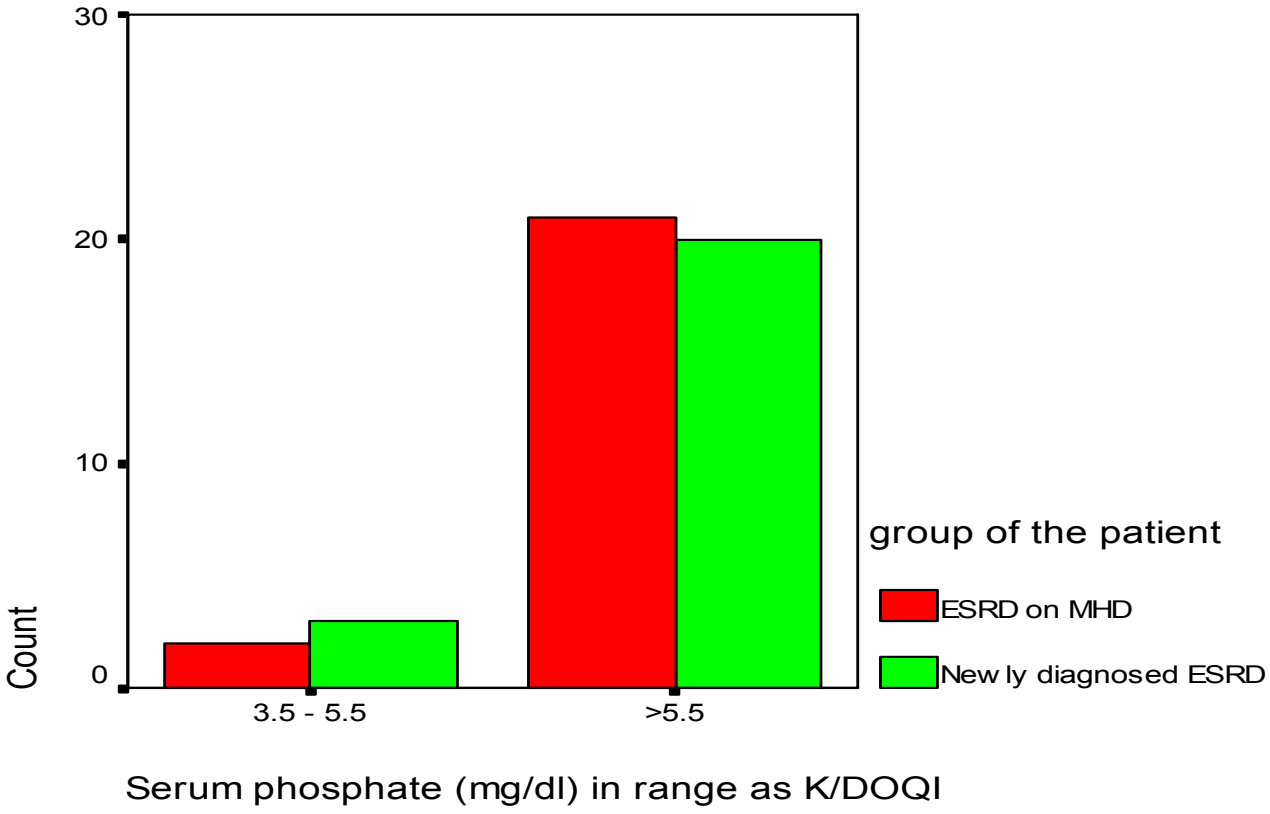

Fig. 5. Sub grouping of study population according to phosphate level K/DOQI)

Radiological findings

Osteopenia

Osteopenia was the commonest radiological finding affecting 17 (73.9%) of ESRD on MHD and 15 (65.2%) of NESRD patients and there was no statistical difference between these 2 groups. Out of 17 patients of ESRD on MHD, 13 (56.5%) patients had mild osteopenia and 4 (17.4%) patients had moderate osteopenia and out of 15 NESRD patients 12 (52.2%) patients had mild osteopenia and 3 (13.0%) patients had moderate osteopenia. Statistical analysis did not reveal any association of osteopenia with parathyroid hormone level, phosphate level and calcium level in both groups.

Osteoarthrosis (OA)

Osteoarthrosis was the second commonest radiological finding affecting 11 (47.8%) of ESRD on MHD and 10 (43.5%) of NESRD patients and there was no statistical difference. OA did not show any association with PTH level, phosphate level and calcium level in both groups. Both in ESRD on MHD and NESRD group, OA were present only in patients with 30 years and above age and totally absent in patients below 30 years of age. But statistically significant association of OA with age was found only in NESRD group ($p<0.01$, chi-square).

Tuft erosion

Renal osteodystrophy with tuft erosion of distal phalynx in X-ray hand was found in 2 (8.7%) patients of ESRD on MHD and 4 (17.4%) patients with NESRD and statistically there was no difference. There was no association of tuft erosion with PTH, calcium and phosphate level in MHD and NESRD patients.

Rugger jersey spine

Rugger jersey spine was found in 1 (4.3%) of MHD patient and 2 (8.7%) of NESRD patients. It did not show any association with PTH and phosphate level in both groups.

Vascular calcification

Vascular calcification was found in 6 (26%) of ESRD on MHD and 1 (4.3%) of NESRD patient. ESRD on MHD had calcification only in descending aorta in 3 patients, only in wrist vessel in 1 patient and calcification in both vessel in 2 patients and NESRD had calcification only in wrist vessel in 1 patient.

Vascular calcification could not show any significant relation with calcium level, phosphate level and PTH level in both groups.

The product of serum calcium and serum phosphate both in mg/dl was found to be <55 in 6 (26%), 55 and more in 17 (74%) patients of ESRD on MHD and < 5 in 8 (34.8%), 55 and more in (65.2%) of NESRD and there was difference between the groups.

Vascular calcification also did not show association with calcium and phosphate product.

Erosion of Radius bone

Radius erosion was found in 3 (13%) of ESRD on MHD and 4 (17.4%) of NESRD patients and it did not show any association with any parameter in both groups.

6. DISCUSSION

The present study has compared the parathyroid hormone level, phosphate level, calcium level and radiological changes of newly diagnosed and maintenance haemodialysis end stage renal disease patients. In this study, as the newly diagnosed ESRD patients are without prior treatment with protein restricted diet, phosphate binders and calcitriol therapy, any finding in them are purely due to the effect of severe chronic kidney disease and such findings may or may not be comparable with other studies on pre-dialysis ESRD patients, who were on regular treatment for renal bone disease prior to reaching ESRD. Moreover, these NESRD patients are the perfect control to study the beneficial effect of maintenance haemodialysis in secondary hyperparathyroidism and renal bone disease.

In this study, as the ESRD on MHD patients were receiving calcium acetate as a phosphate binder in different dosage according to phosphate level, any finding in them is due to the additive effect of maintenance dialysis and the phosphate binders.

In this study we have estimated intact parathyroid hormone and grouped them mainly according to K/DOQI guide line. So comparison of this study findings with other studies may not be homogenous as previously PTH level >200 pg/ml were considered significant hyperparathyroidism (Owda *et al* 2003).

In the present study parathyroid hormone of maintenance haemodialysis patients are significantly lower than NESRD patients indicating the beneficial effect of maintenance haemodialysis in the control of hyperparathyroidism. Reduction of serum PTH level was observed by Petrauskiene *et al* 2005 after 1 year of MHD in 47.8% patients.

The normal range of PTH in our laboratory is between 16-46 pg/ml. K/DOQI guide line of National Kidney Foundation (Eknoym *et al* 2003) has recommended the optimal PTH value in ESRD patients to be 150-300 pg/ml to prevent adynamic bone disease related to low or normal PTH (Hercz *et al* 1993, Goodman *et al* 1994). Even on analysis of the study population after grouping them according to PTH level, we have found significant difference between two groups with 82.6% of ESRD on MHD having sub optimal parathyroid hormone and 43.5% of NESRD having hyperparathyroidism.

Although PTH level in MHD patients could be high (50-60%), normal (30-40%) and low or undetectable (Reichel *et al* 1998), the sub optimal parathyroid hormone level with PTH <150 pg/ml in 82.6% patients with majority 65.2% having <100 pg/ml and hyperparathyroidism with >300 pg/ml in 8.7% patients contradicts study of Sun *et al* 2005 in Chinese MHD patients with intact PTH >300 pg/ml in 46% patients and <100 pg/ml in 18% patients and many other studies on MHD patients where hyperparathyroidism is the common finding (Owda *et al* 2003, Torres *et al* 1995).

Hyperparathyroidism in MHD patients are reverted by calcitriol (Tsukamoto *et al* 1991, Fisher and Harris 1993, Frazao *et al* 1997) and phosphate binder (Teruel *et al* 1999) therapy. In the present study the low level of PTH could be due to treatment with calcium acetate as a phosphate binder as none of them were on calcitriol for more than 12 weeks although there was no difference of PTH between patients with different dosage of calcium acetate.

Moreover, low dialysate calcium concentration (1.25mmol/l) worsens the hyperparathyroidism and high dialysate calcium (1.75mmol/l) can cause undesirable low PTH particularly if they are taking calcium containing phosphate binder and / or calcitriol (Argiles *et al* 1993, Argiles and Mourad 1998). So the dialysate calcium concentration of 1.5mmol/l is preferred in MHD patients on calcium containing phosphate binder and / or calcitriol therapy (Malberti and Ravani 2003). We have found significantly low PTH (pg/ml) [45.3 $\pm$ 40.8 vs 256.4 $\pm$ 289.9] in MHD patients with high dialysate calcium of (1.75 mmol/l) than with low dialysate calcium (1.25mmol/l).
So in this study the unexpectedly lower PTH value could be related to high dialysate calcium with calcium acetate therapy.
PTH value in MHD patient is also influenced by the duration of dialysis with worsening of hyperparathyroidism with prolonged dialysis (Chertow *et al* 2000). But in this study we have not found any association of PTH with the duration of dialysis. Moreover serum PTH in MHD patient is also related with serum magnesium level (Navarro-Gonzalez 1998) and in this study as we have not estimated serum magnesium we can not exclude its role in it.

Raised parathyroid hormone level observed in 100% of NESRD patients with minimum of 92.9pg/ml supports that hyperparathyroidism is the invariable consequence of chronic kidney disease if they are not intervened earlier. Though hypocalcemia and hyperphosphatemia have major role in the pathogenesis of hyperparathyroidism in ESRD patients (Slatopoisky and Deimez 1994) we have found significant impact of hyperphosphatemia in secondary hyperparathyroidism of NESRD patients only.

Significantly lower serum phosphate level observed in ESRD on MHD than in NESRD in this study could be due to additive effect of protein restricted diet (Combe and Aparicio 1994), calcium containing phosphate binder therapy (Almirall *et al* 1994) and maintenance dialysis with phosphate removal during dialysis (Schaefer 1994). But still the mean phosphate level (8.2 $\pm$ 2.3 mg/dl) of MHD group is more than the expected value of 3.5-5.5 mg/dl in these patients with 91.3% of them having hyperphosphatemia. This result is much higher as described by Sun et al 2005 in 81% Chinese MHD patients indicating the need of much effort to control the phosphate level in these patients.
We have found hyperphosphatemia with mean phosphate level (11.2 $\pm$ 3.6 mg/ dl) in 87% of NESRD patients indicating hyperphosphatemia is the invariable consequence of CKD and becomes very severe in advanced renal failure if not intervened earlier.

In MHD patients, calcium acetate in two different dosages was prescribed during study period according to their serum phosphate level with interest to control it adequately but we have not found any difference of phosphate level between them.

In present study as the corrected calcium could not be calculated for not estimating the serum albumin initially, the normal laboratory value of 8.8-10.2 mg/dl is used as a land mark to diagnose hypo, normal and hypercalcemia in the study population.

Serum calcium becomes normal in ESRD patients when maintenance dialysis is started even without calcitriol therapy and probably it is related to use of large oral dose of calcium containing phosphate binder (Goodman 2001). Similarly in this study we have found significantly higher serum calcium of ESRD on MHD than that of NESRD and probably it is also related to regular dialysis and calcium acetate therapy.
Hypercalcemia can occur in MHD patients with large dose of calcium containing phosphate binder or dialysis with high dialysate calcium (Mactier *et al* 1987, Oettinger *et al* 1992). In MHD patients though we have observed hypocalcemia in 17.4% patients, normocalcemia in 56.4% patients and hypercalcemia in 26.1% patients, calcium level was not influenced by dose of phosphate binder, dialysate calcium concentration and duration of dialysis.
Hypocalcemia developed in CKD patients due to calcitriol deficiency and phosphate retention (Coburn et al 1969) can be corrected with calcitriol therapy (Frazao *et al* 1997) calcium containing phosphate binder therapy (Recker *et al* 1988), and maintenance dialysis. In the present study as the NESRD patients were not treated previously, 91.3% of them have hypocalcemia with mean serum calcium of 6.6 $\pm$ 1.1 mg/dl indicating severe hypocalcemia is the invariable consequence of advanced renal failure if they are not treated earlier.
Serum alkaline phosphatase is significantly lower in ESRD on MHD than in NESRD but we could not establish any relation of alkaline phosphatase with PTH in this study.

Histologically evident renal bone disease is described in moderate CKD patients even prior to radiological changes (Baker *et al* 1989), in predialysis ESRD patients, in MHD patients and in CAPD (continuous ambulatory peritoneal dialysis) patients ((Shin *et al* 1999, Torres *et al* 1995, Sherrard *et al* 1993). Radiology is designated as the useful tool for diagnosing renal bone disease by soma authors (Piraino *et al* 1986, Ritz *et al* 1978) and questionable by others (Hutchison *et al* 1994, Hutchison *et al* 1993). Moreover X-ray hand is evaluated to be the most diagnostic radiological measure (Hutchison *et al* 1993).
In this study, the predominant radiological changes observed in plain X-ray hand and lumbosacral spine are the osteopenia and the osteoarthrosis in both groups. Other findings included tuft erosion, radius bone erosion, rugger jersey spine and vascular calcification. None of these x-ray finding showed any difference between the study populations. Although NESRD group has significantly higher PTH level, the bony changes did not show any relation with it.
Osteopenia with decreased bone density is the common X-Ray finding and it can be due to secondary hyperparathyroidism, osteomalacia, osteoporosis and adynamic bone disease (Massry 1995). In the present study osteopenia is evident in patients with suboptimal, optimal and high PTH level in both ESRD on MHD and NESRD and we can not assume the osteitis fibrosa related to high PTH and adynamic bone disease related to normal / low PTH in these patients with osteopenia. So, radiology is not useful to diagnose renal bone disease and histology should be the gold standard (Balon and Bren 2000).

7. CONCLUSION AND FUTURE RECOMMENDATIONS

1. Hypocalcemia, hyperphosphatemia and hyperparathyroidism are the invariable consequences of chronic kidney disease and it becomes severe in advanced renal failure if they are not treated earlier.
2. Maintenance haemodialysis and calcium containing phosphate binder therapy can control the hypocalcemia, hyperphosphatemia and hyperparathyroidism even without calcitriol therapy.
3. Hyperphosphatemia still remains a significant problem in maintenance haemodialysis patients and more effort should be exercised for its meticulous control.
4. Undue suppression of parathyroid hormone has occurred in maintenance haemodialysis patients and it is probably due to high dialysate calcium with calcium containing phosphate binder therapy.
5. Plain X-ray is not very helpful to diagnose renal bone disease and bone biopsy should be done to evaluate it.
6. Further elaborate research should be done in Nepalese haemodialysis patients to identify the etiology of excessive parathyroid hormone suppression and to allocate the desired dialysate calcium concentration.

8. REFERENCES

1. Almirall J, Veciana L and Llibre J. (1994). Calcium acetate versus calcium carbonaten for the control of serum phosphorus in haemodialysis patients. Am J Nephrol, 14, 192-196.
2. Argiles A, Kerr PG, Canaud B, Flavier JL and Mion C. (1993). Calcium kinetics and the long term effects of lowering dialysate calcium concentration. Kidney Int, 43, 630 – 640.
3. Argiles A and Mourad G (1998). How do we have to use calcium in the dialysate to optimize the management of secondary hyperparathyroidism? Nephrol Dial Transplant suppl (3) S62-S64.
4. Baker LRI, Abrams LSM, Roe CJ, Faugere MC, Fanti P, subayti Y and Malluche HH. (1989). 1, 25 $(OH)_2$ D_3 administration in moderate renal failure: a prospective double blind trial. Kidney Int, 35, 661-669.
5. Balon BP and Bren A. (2000). Bone histomorphometry is still the golden standard for diagnosing renal osteodystrophy. Clinical Nephrology, 54, 463-469.
6. Chertow GM, Plone M, Dillon MA, Burke SK and Slatopolsky E. (2000). Hyperparathyroidism and dialysis vintage. Clinical Nephrology, 54, 295-300.
7. Coburn JW, Popovitzer MM, Massry SG and Kleeman CR. (1969). The phisicochemical state and renal handling and divalent ions in chronic renal failure, arch Intern Med, 124, 302-311.
8. Combe C and Aparicio M. (1994). Phosphorus and protein restriction and parathyroid function in chronic renal failure. Kidney Int, 46, 1381-1386.
9. Eknoyan G, Levin A and Levin NW. (2003). Bone metabolism and disease in chronic kidney disease. Am J Kidney disease, 42 (suppl 3): S1-S201.
10. Fischer ER and Harris DCH. (1993). Comparison of intermittent oral and intravenous calcitriol in haemodialysis patients with secondary hyperparathyroidism. Clinical Nephrology, 40, 216-220.
11. Frazao JM, Levine BS, Tan AU, Mazess RB, Kyllo DM, Knutson JC, Bishop CW and Coburn JW. (1997). Efficacy and safety of intermittent oral 1 α (OH)-vitamin D_2 in suppressing 2^o hyperparathyroidism in haemodialysis patients. Dialysis and Transplantation, 26, 583-595.
12. Goodman WG, Ramirez JA, Belin TR, Chon Y, Gales B, Segre G and Salusky IB. (1994). Development of adynamic bone disease in patients with secondary hyperparathyroidism after intermittent calcitriol therapy. Kidney Int, 46, 1160-1166.
13. Goodman WG. (2001). Recent development in management of secondary hyperparathyroidism. Kidney Int, 59, 1187-1201.
14. Hada R. (2003). Kidney Transplantation in Nepal. Post-Graduate Medical Journal of Nepal, 4, 51-54.
15. Hercz G, Pei Y, Manuel A, Saiphoo C, Goodman W, SegreGV, Fenton S and Sherrard DJ (1993). Aplastic osteodystrophy without aluminium: the role of suppressed parathyroid function. Kidney Int, 44, 860-866.

16. Hutchison AJ, whitehouse RW, Boulton HF, Adams JE, Mawer EB, Freemont TJ and Gokal R. (1993). Correlation of bone histology with parathyroid hormone, vitamin D_3, and radiology in end-stage renal disease. Kidney Int, 44, 1071-1077.
17. Hutchison AJ, Whitehouse RW, Freemont AJ, Adams JE, Mawer EB and Gokal R. (1994). Histological, radiological and biochemical features of the adynamic bone lesion in continuous ambulatory peritoneal dialysis patients. Am J Nephrol, 14, 19-29.
18. Khakurel S, Agrawal RK and Hada R. Nephrology Service at Bir Hospital. Souvenir, Bir Hospital-111th Anniversary, 6-8.
19. Lucas PA, Brown RC and Woodhead JS. (1988). Vitamin D_3 metabolites in chronic renal failure and after renal transplantation. Nephrology Dialysis Transplantation, 3, 70-76.
20. Mactier RA, Van Stone J, Cox A et al (1987). Calcium carbonate is an effective phosphate binder when dialysate calcium concentration is adjusted to control hypercalcemia. Clinical Nephrology, 28, 222-226.
21. Malberti F and Ravani P. (2003). The choice of the dialysate calcium concentration in the management of patients on haemodialysis and haemodiafiltration. Nephrol dial Tranplant, 18 suppl 7: :vii 37 – 40.
22. Massry SG. (1995). Divalent ion metabolism and renal osteodystrophy. In: Massry SG, Glassok JR, eds. Textbook of Nephrology Vol. II, 3rd ed. Baltimore: William and Wilkins, 1441 – 1473.
23. Navarro-Gonzalez. (1998). Magnesium in dialysis patients: serum levels and clinical implications. Clinical Nephrology, 49, 373-378.
24. Oettinger CW, Oliver JC and Macon EJ. (1992). The effects of calcium carbonate as the sole phosphate binder in combination with low calcium dialysate and calcitriol therapy in chronic haemodialysis patients. J Am Soc Nephrol, 3, 995-1001.
25. Owda A, Elhwaris H, Narra S, Towery H and Osama S. (2003). Secondary hyperparathyroidism in haemodialysis patients: prevalence and race. Ren fail, 25, 595 – 602.
26. Petrauskiene V, Bumblyte IA, Kuzminskis V and Sepetauskiene E. (2005). Early risk factor for secondary hyperparathyroidism in haemodialysis patients. Medicina (Kaunas), 41, suppl. 1: 44-49.
27. Piraino BM, Rault R, Dominiguez JH and Puschett JB. (1986). Renal osteodystrophy in patients on haemodialysis for more than 10 years. Miner Electrolyte Metab, 12, 390-396.
28. Recker RR, Bammi A, Barger-Lux MJ and Heaney RP. (1988). Calcium absorbability from milk products, an imitation milk and calcium carbonate. American Journal of Clinical Nutrition, 47, 93-95.
29. Reichel H, Deibert B, Schmidt-Gayk H and Ritz E. (1991). Calcium metabolism in early chronic renal failure. Implications for the pathogenesis of hyperparathyroidism. Nephrology Dialysis Transplantation, 6, 162-169.
30. Reichel H, Drucke TB and Ritz E. (1998). Skeletal disorders. In: Davison AM, Cameron JS, Grunfeld JP, Kerr DNS, Ritz E and Winearls CG, eds. Oxford Textbook of Clinical Nephrology VOL lll, 2nd ed. Oxford: Oxford University Press, 1954 – 1981.

31. Ritz E, Prager P, Krepien B, Bommer J, Malluche HH and Schmidt-Gayak H. (1978). Skeletal X-ray findings and bone histology in patients on haemodialysis. Kidney Int, 13, 316-323.
32. Ritz E, Seidel A, Ramisch H, Szabo A and Bouillon R. (1991). Attenuated rise of 1, 25 $(OH)_2$ vitamin D_3 in response to parathyroid hormone in patients with incipient renal failure. Nephron, 57, 314-318.
33. Ritz E, Kuster S, Schmidt-Gayk H, Stein G, Scholz C, Kraatz G and Heidland A. (1995). Low dose calcitriol prevents the rise in 1, 84 iPTH without affecting serum calcium and phosphate in patients with moderate renal failure (prospective placebo-controlled multicenter trial). Nephrology dialysis Transplantation, 10, 2228-2234.
34. Schaefer K. (1994). Unsatisfactory control of serum phosphate: why is it so common and what can be done? Nephrology Dialysis Transplantation, 9, 1366-1367.
35. Sherrard DJ, hercz G, Pei Y, Maloney NA, Green wood C, Manuel A, Saiphoo C, fenton SS and Segre GV. (1993). The spectrum of bone disease in end-stage renal failure – an evolving disorder. Kidney Int, 43, 436-442.
36. Shin SK, Kim DH, Kim HS, Shin KT, Ma KA, Kim SJ, Kwak YS, Ha SK and Sherrard DJ. (1999). Renal osteodystrophy in pre-dialysis patients: ethnic difference? Perit dial Int, 19 suppl 2: S402-7.
37. Slatopoisky E and Deimez JA. (1994). Pathogenesis of secondary hyperparathyroidism. Am J Kidney Disease, 23, 229-36.
38. Sun LY, Wang M and Yang L. (2005). Study of calcium–phosphorus metabolism and intact parathyroid hormone levels in end stage renal disease patients. Beijing Da Xue Xue Bao, 37 (2), 147 – 150.
39. Teruel JL, Tenorio MT, Rodriguez JR, Marcen R, Orofino L, Rivera M and Ortuno J. (1999). Treatment of secondary hyperparathyroidism in haemodialyzed patients with high-dose calcium carbonate without vitamin D_3 supplements. Am J Nephrol, 19, 428-432.
40. Torres A, Lorenzo V, Hernandez D, Rodriguez JC, Concepcion MT, Rodriguez AP, Hernandz A, De Bonis E, Darias E, Gonzalez-Posadas JM, Losada M, Rufino M, Felsenfeld AJ and Rodriguez M. (1995). Bone disease in predialysis, haemodialysis and CAPD patients: Evidence of a better response to PTH. Kidney Int, 47, 1434-1442.
41. Tsukamoto Y, Nomura M, Takahashi Y, Tagaki Y, Yoshida A, Nagaoka T, Togashi K, Kikawada R and Marumo F. (1991). The oral 1, 25-dihydroxyvitamin D_3 pulse therapy in haemodialysis patients with severe secondary hyperparathyroidism. Nephron, 57, 23-28.