CLINICAL, RADIOLOGICAL AND BIOCHEMICAL EVIDENCE OF RENAL OSTEODYSTROPHY IN PATIENTS WITH CHRONIC RENAL FAILURE AT THE KENYATTA NATIONAL HOSPITAL

BY

ANITA K. PATEL M.B,.B.S. (INDIA).

A DISSERTATION SUBMITTED IN PART FULLFILLMENT FOR THE DEGREE OF MASTER OF MEDICINE IN THE UNIVERSITY OF NAIROBI (1989)

DECLARATION

This dissertation is my original work and has not been presented for a degree in any other University.

ANITA K. PATEL, M.B.B.S

SUPERVISORS

This dissertation has been submitted for examination with our approval as University supervisors.

PROFESSOR L.S. OTIENO, MRCP(U.K), FRCP(E)
Associate Professor of Medicine,
Department of Medicine,
College of Health Sciences
University of Nairobi.

Dr. D.A.O. ORINDA, Ph.D, D.C.B, MSc
Clinical Biochemistry (London)
Senior Lecturer, Chemical Pathology
University of Nairobi.

ACKNOWLEDGMENT

I would like to express my sincere gratitude to my supervisors Prof L.S. Otieno and Dr D.A.O. Orinda for their encouragement, help and guidance throughout this study and for the constructive criticisms and suggestions they made in the final preparation of the script. I would particularly like to thank Dr Orinda for the good laboratory backup I received from him and the technicians. I would also like to thank Mr Mwai and his collegues from the department of medicine for helping me to sort out and store my samples. My gratitude goes out to Chris Hinsen of International Laboratory For Research in Animal Diseases (ILRAD) for his excellent guidance and assistance in the running of the vitamin D assay. My gratitude also goes out to my cousin Kaushik for encouraging me throughout this study and providing me with financial assistance at the time of need. I sincerely thank Avinash for word processing my draft and making it look very presentable. I would like to acknowledge the assistance of Dr. Mcligeyo who got me started with this work.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS.

1. CRF - chronic renal failure.
2. iPTH - immunoreactive parathyroid hormone.
3. Mg - magnesium.
4. Ca - Calcium.
5. inP - inorganic phosphate.
6. Po4 - phosphate.
7. PTH - Parathyroid hormone.
8. hPTH - human parathyroid hormone.
9. 1,25 - dihydroxy cholecalciferol.
10. 25,OHCC - 25,hydroxycholecalciferol.
11. GFR - glomerular filtration rate.
12. Al - aluminium.
13. HPLC - high pressure liquid chromatography.
14. CPM - counts per minute.
15. Ccr - creatinine clearance.
16. ESRD - end stage renal disease.

LIST OF TABLES

LIST OF FIGURES

SUMMARY

Between May 1988 and January 1989 40 patients with established diagnosis of chronic renal failure (CRF) on either maintenance dialysis or conservative management were studied to determine the incidence and the nature of renal osteodystrophy. Serum immunoreactive parathyroid hormone (iPTH), magnesium (Mg) and 1,25 dihydroxycholecalciferol (1,25DHCC) were assayed, as well as serum alkaline phosphatase, inorganic phosphate (inP) and calcium (Ca).

Clinical features suggestive of renal osteodystrophy were present in 80 percent of the patients. 75 percent were symptomatic. Signs suggestive of renal bone disease were seen in 62.5 percent. Radiological changes suggestive of renal osteodystrophy were found in only 7.5 percent of the patients only. 60 percent of the patients had conclusive biochemical features of renal bone disease, that is, a raised serum inP, iPTH and alkaline phosphatase levels. Serum Ca levels were normal when compared to the control group of individuals. There was no correlation between clinical features and biochemical parameters of renal osteodystrophy with the exception of pruritus which was correlated with iPTH levels. Serum 1,25DHCC levels were reduced in the patients and were positively correlated with the duration of renal failure. Serum alkaline phosphatase levels were positively correlated with the duration of renal failure. Serum biochemical changes were not significantly altered by the severity of renal failure and the duration of hemodialysis.

INTRODUCTION AND LITERATURE REVIEW

The association of renal failure with skeletal disease and hyperplasia of parathyroid glands has been known for many years (1). As early as 1962, Stanbury and Lumb (2) associated the abnormalities of divalent ion metabolism, parathyroid gland structure and function, Vitamin D metabolism and bone disease with renal insuffiency. Now that the lives of patients with chronic renal failure (CRF) can be prolonged through better conservative therapy, readily available dialysis and successful renal transplantation, the morbidity associated with renal osteodystrophy has taken formidable dimensions. The renal osteodystrophy includes several skeletal syndromes and alterations of divalent ion metabolism that occur when renal function is compromised. The manifestations of the complications range from being asymptomatic to pathological fractures and bone pain leading to severe disability.

Skeletal pathology in renal osteodystrophy includes osteitis fibrosa, osteomalacia, osteoporosis, osteosclerosis and short stature (3). The type of bone disease found depends upon the age of the patient, the rate of skeletal turnover, the duration and progression of renal insufficiency, dietary intake, the type of therapy received and the duration and adequacy of dialysis (4). The two main pathogenetic features of renal osteodystrophy are secondary hyperparathyriodism and defective mineralization of the bone.

SECONDARY HYPERPARATHYROIDISM

Parathyroid hyperplasia and high levels of immunoreactive parathyroid hormone (iPTH) are among the earliest and most consistent pathogenetic factors affecting the divalent ion metabolism in patients with chronic renal failure. Arnaud (6) in 1973 reported elevated serum levels of iPTH early in the course of renal insuffiency. Slatopolsky and coworkers (7) found an early and persistant rise in serum iPTH when the glomerular filtration rate (GFR) fell below 50-75 ml/min. Hypocalcemia is the main stimulus for iPTH release and secondary hyperparathyroidism in renal insufficiency. Hypocalcemia occurs as a result of a number of reasons :(i) phosphate (PO4) retention with a rise in the serum inorganic phosphate concentration (inP) and a resultant fall in the serum calcium (Ca) levels ;(ii) altered Vitamin D metabolism, with intestinal malabsorption of Ca ;(iii) skeletal resistance to the calcemic action of PTH ;(iv) impaired degradation of intact PTH(84 aminoacids) and iPTH(first 34 aminoacids) secondary to reduced renal function and (v) an altered feedback relationship between serum Ca level and the secretion of PTH. At any given serum Ca levels there is resultant greater secretion of PTH, i.e there is a change in the "set point" for calcium.(5)

Slatopolsky and collegues (7) suggested that a transient and mild PO4 retention occurs in early CRF and is responsible for the secondary hyperparathyroidism via generation of hypocalcemia.

PTH causes phosphaturia and increases the serum Ca via increased production of 1,25DHCC thus leading to normocalcemia but at the expense of high circulating PTH. With a GFR < 30 ml/min hyperphosphatemia is usual and hypocalcemia is related directly to the level of serum inP (8).

There is considerable evidence to suggest that patients with advanced renal failure have little or no production of 1,25 DHCC and that this accounts for several pathophysiological features of renal osteodystrophy, including :- i) decreased intestinal absorption of Ca and PO4, ii) decreased calcemic response to skeletal action of PTH, iii) altered collagen synthesis, iv) development of proximal myopathy and v) maintenance of increased secretion of PTH for any given level of serum Ca (5). Cells in the parathyroid gland have receptors for 1,25DHCC through which Vitamin D regulates the release of PTH. High doses of 1,25 DHCC suppress PTH release (9,10,11,12). On the other hand high levels of PTH in patients with mild renal insufficiency maintain near normal serum levels of 1,25DHCC, despite, a decrease in renal mass. PTH enhances the activity of 1α hydroxylase, the enzyme which converts 25,OHCC to 1,25DHCC. Many patients with advanced uremia, therefore, lack clinical features of Vitamin D deficiency (13).

Massry et al (14) have shown that skeletal resistance to the calcemic action of PTH is either due to decreased 1,25DHCC or

hyperphosphatemia and appears in both acute and chronic renal failure. However Galceron et al (15) examined parathyroid resistance in dogs with CRF and showed that a blunted calcemic response to PTH infusion occurs and that supplementation of pharmacological doses of 1,25DHCC failed to improve this calcemic action. They also showed that parathyroidectomy returned the calcemic response to normal, suggesting that, high levels of endogenous PTH desensitised the skeleton to administered exogenous PTH. Uremic toxins too have been implicated.

Higher than usual concentrations of extracellular calcium are required to suppress the release of PTH from the hyperplastic/hypertrophied parathyroid gland, that is, a shift in the so called "set point for calcium" occurs. This implies that even after normalization of Ca, PTH secretion continues unabated (16).

DEFECTIVE MINERALISATION OF BONE

Osteomalacia is the second important feature of skeletal disease in patients with advanced renal failure and occurs in association with altered Vitamin D metabolism, aluminium accumulation, altered collagen synthesis and maturation, altered bone crystal maturation and metabolic acidosis (5).

Vitamin D- 1,25DHCC is now considered to be a calciotropic hormone and causes release of Ca from the bones. This does not occur by direct-stimulation of osteoclasts, instead, 1,25DHCC

recruits undiffrentiated stem cells in the bone marrow and peripheral monocytes to become osteoclasts, thus effecting bone remodelling. Its deficiency does not cause overt osteomalacia in all patients ; the defective mineralization is attributed to deficiency of other Vitamin D sterols like 24,25DHCC and 25 OHCC, Osteomalacia is more common in uremic patients in Northern Europe than in U.S.A., probably due to decreased synthesis as a result of decreased exposure to sunlight and lack of widespread fortification of foods with Vitamin D (5).

Al accumulation is now considered the major causative agent in osteomalacia. In the 1970's an outbreak of "Dialysis Osteomalacia" along with encephalopathy was reported in certain dialysis units in the UK (17). Untreated water with high Al content (>100 ug/l) was implicated . Al is excreted by the Kidneys only and therefore accumulates in CRF. Kaehny et al (18) demonstrated hyperaluminemia in CRF patients taking aluminium hydroxide to decrease dietary PO4 absorption. Al binds to the calcification front where it appears to inhibit mineralization of osteoid by reducing the osteoblasts and osteoclasts (aplastic area) and by blocking calcium hydroxyapatite crystal formation (crystal poision). Smith et al (19) have noted that Al directly suppresses PTH release. When reverse osmosis water treatment was used as a modality of treatment of Al related osteomalacia, there was decrease in the plasma Al levels and bone AL content leading to an inprovement in the mineralization status in some patients.

Those with persistant osteomalacia after reverse osmosis water treatment remained biochemically euparathyroid and only had mild osteitis fibrosa. The responders developed biochemical and histological evidence of hyperparathyroidism consistent with the removal of AL mediated supression of PTH realease from the parathyroid gland. PTH is involved in the restoration of mineralization in paralyzed osteoid, possibly through genesis of matrix vesicles by PTH activated osteoblasts. Moderate to severe hyperparathyroidism appears to protect against osteomalacia despite the fact that the bone AL levels in hemodialysis patients with osteitis fibrosa do not differ significantly from those with osteomalacia. AL causes a crippling form of osteomalacia as compared to osteitis fibrosa. A mild reduction in dialysis fluid Ca with the aim of stimulating the parathyroid glands may offer a useful adjunct or alternative to desferroxamine in the management of distressing dialysis osteomalacia. AL related osteomalacia is refractory to treatment with 1.25DHCC .

Another cause of defective mineralization is low plasma PO4 levels. This leads to a low Ca x P product and occurs when predialysis plasma phosphate levels are below normal. Abnormal collagen synthesis and maturation occurs in uremia. Mechanism for this remains uncertain. Altered vitimin D metabolism has been implicated(5).

The deposition of mineral in the bone involves the precipitation

of amorphous calcium phosphate and its subsequent transformation to crystalline hydroxyapatite. An increase in magnesium content has been found in the bone from uremic patients and bone magnesium correlates closely with serum magnesium levels. Amorphous pyrophosphate exists as Mg salt and is less susceptible to degradation by pyrophosphatases thus contributing to the delayed bone crystal formation (20).

HISTOLOGICAL FEATURES OF RENAL BONE DISEASE

Osteites fibrosa characterised by the presence of an increased number of both osteoclasts and osteoblasts. There is increased synthesis of unmineralised osteoid matrix out of proportion to increased resorption of both bone mineral and matrix. Woven osteoid along with augmented marrow fibrosis is seen along the peritrabecular surfaces. Osteomalacia is identified by the finding of wide osteoid seams. This is due to defective mineralization of newly formed matrix and is best identified by the use of tetracycline double labelling technique. Most patients have both of these histological features occuring in varying degrees (3,5).

CLINICAL FEATURES OF RENAL OSTEODYSTROPHY:

Many symptoms are subtle and insidious in their onset. It may be difficult to seperate them from other features of uremia per se. In general clinical symptoms are present usually in far advanced renal insufficiency. Certain symptoms occur with moderate renal

failure - i.e., reduction by 15-20% of normal renal function particularly in children and in slowly progressing renal disease. Bone pain is variable, often deep-seated and aggravated by weight bearing or muscular effort, common in lower back, hips and rib cage. Physical findings are usually lacking.

Skeletal deformities develop most commonly during periods of active bone remodelling or growth, that is, in children. They also occur in adults, usually involve the wrists and the hips. Slipped epiphysis (22) is a consequence of osteitis fibrosa affecting epiphysial growth plate. Genu valgum, pigeon chest, loss of height, bowing of the tibia and femur, deformities of thoracic spine and recurrent rib fractures (23) are other features. These are more common in patients with osteomalacia. Occasionally, jaw and maxilla are involved with drifting, loosening or loss of teeth. Fractures, which are usually spontaneous, occur in the axial skeleton and involve the vertebral bodies, ribs and hips. They are most commonly due to osteomalacia (24,25,26,27,28), which is therefore a more crippling form of osteodystrophy when compared to osteitis fibrosa.

Baker et al (29) in 1974 described myopathy in patients with renal impairment. This myopathy which is usually proximal, is of varying severity and affects muscles of the shoulder and pelvic girdles in end stage renal disease. Frequently patients have

"penguin" gait. Myopathy which is attributed to both osteomalacia and secondary hyperparathyroidism, often responds dramatically to Vitamin D sterols (30,31).

Motor and sensory neuropathy as well as distal muscle weakness may occur in patients with CRF. This may be totally unrelated to the calcium metabolism. Clinically, it is difficult to distinguish between neuropathy and myopathy. Also, bone pain and myopathy coexist and it is difficult to know whether the myopathy is due to disuse of concerned part or due to uremia per se (30).

Pseudogout and periarthritis occur occasionally and are attributed to deposition of calcium pyprophosphate in and around the joints. These occur frequently with extra skeletal calcifications. Symptoms may respond to phenylbutazone or with other non steroidal anti inflammatory drugs, and may recur even if serum phosphorus levels are adequetaly controlled (20).

There is impaired growth and delayed maturation of the skeleton in uremic children whose mechanisms remain obscure. Abnormal protein metabolism, reduced caloric intake, secondary hyperparathyroidism and alteration in Vitamin D metabolism have been implicated. Substantial improvement is noted with active Vitamin D supplementation (24,32,33.)

Pruritus is a troublesome symptom in many patients with advanced renal failure. It often improves following the initiation of regular dialysis. Seperate studies done by Hampers and coworkers

(34) and Massry and Coworkers (35) have shown that uremic pruritus improves a few days after subtotal parathyroidectomy for secondary hyperparathyroidism suggesting its relation to the level of calcium and phosphate in the extra-cellular fluid.

Calciphylaxis described by Gipstein, Coburn and others (36) is an unusual yet devastating syndrome of obscure pathogenesis. It appears in patients with stable advanced renal failure, in those undergoing hemodialysis and often after successful renal transplantation. It is characterised by progressive ischemic ulcers of the extremities, extreme pain with Raynaud's phenomenon. Necrosis of digits and muscles is also seen. Extensive arterial calcification of the media of small and large arteries has been implicated and sub-periosteal resorption and secondary hyperparathyroidism are also seen in these patients. Parathyroidectomy has caused reversal of lesions in some patients (36).

Extra skeletal calcification occurs in several forms. Non visceral calcifications include tumoral calcified masses in joints, periarticular sites and vascular tissues. They are common in adults and may be seen at any age. Visceral calcification includes soft tissue calcification of the lungs with disturbances in lung function tests and cardiac calcification with conduction abnormalities or congestive cardiac failure (37).

BIOCHEMICAL FEATURES OF RENAL OSTEODYSTROPHY:

Serum phosphorus levels are generally normal in early CRF. With a decrease in GFR by 25%, the level starts rising (7,8). Despite the phosphaturic effect of PTH the serum inP levels are persistantly high in late CRF and ESRD because the main clearance of phosphate depends on the GFR.

A GFR below 10 ml/min is invariably associated with minimal tubular reabsorption of phosphate. Under such circumstances, an increase in PTH induces bone resorption and augments the release of both calcium and phosphate in the extracellular fluid with resultant hyperphosphatemia. Intestinal absorption of phosphorus is unchanged in renal failure. Dietary phosphate also contributes to hyperphosphatemia. Serum calcium levels in a patients wit advanced renal failure are lower than those seen in a control population. Patients with advanced chronic uremia often show a slight increase in the complexed fraction of plasma calcium, complexed to citrate, phosphates and other organic ions, the result being a slightly lower level of ionized calcium for any given total serum calcium concentration. Hypocalcemia also occurs due to low levels of 1,25DHCC which causes tubular and intestinal absorption of calcium to fall (38,39,40). With hemodialysis calcium levels increase toward normal and occasionally, mild hypercalcemia is seen. In ESRD hypercalcemia occurs due to secondary hyperparathyroidism (tertiary too), excess intake of

Vitamin D sterols and calcium and also dialysis - osteomalacia (41).

Serum magnesium (Mg) levels tend to increase with a decrease in GFR below 20-25% of normal. Hypermagnesemia occurs in uremic patients when there is an uncontrolled dietary Mg intake and in those ingesting Mg containing antacids or cathartics. In dialysis magnesium content of dialysate is the major factor controlling the serum Mg. Intestinal absorption of Mg is unusually normal in uremic patients but when diarrhoea occurs hypomagnesemia and hypocalcemia develop (42,43). This intensifies hyperparathyroidism. An acute elevation of serum Mg can suppress PTH secretion. However, long term hypermagnesemia does not affect PTH secretion (42). The total serum alkaline phosphatase reflects the enzyme activities of hepatic, intestinal and skeletal sources. Despite this nonspecific nature of alkaline phosphatase, a slow progressive increase in serum level of the enzyme in a patient with ESRD is characteristic of progressive renal bone disease. This includes osteitis fibrosa, osteomalacia, and mixed lesions although markedly elevated levels are most characteristic of osteitis fibrosa. The skeletal alkaline phosphatase arises largely from osteoblasts and a correlation has been observed between skeletal alkaline phosphatase and bone biopsy findings of osteitis fibrosa (44,45,46). It should be remembered that uremic patients can have significant and overt skeletal disease despite normal plasma alkaline phosphatase activity. In a study with

specific skeletal isoenzymes of alkaline phosphatase, a good correlation was observed between skeletal phosphatase and response to active vitamin D metabolites(45).

Serum iPTH levels in CRF are raised. Even in early renal failure slightly raised levels of iPTH are seen (41). The native PTH molecule is a single chain peptide of 84 aminoacids and is quickly cleaved by the liver into amino-terminal and carboxyl terminal fragments. The carboxyl terminal fragments (iPTH 53-84) are inactive but remain in the circulation with a longer "half life" than either the intact PTH molecule (PTH 1-84) or the biologically active amino terminal fragment (iPTH 1-34). The carboxyl terminal is cleared from the circulation by glomerular filtration and tubular reabsorption. Serum levels of PTH are particularly elevated when they are measured with an anti-serum directed towards the mid region or carboxyl terminal of PTH, whereas the degree of elevation is less marked when the antiserum is directed towards the amino terminus. Studies have shown that serum levels of amino terminal correlate well with acute variations in PTH secretion. carboxyl terminal, mid region, and intact assays of PTH correlate with manifestations of parathyroid activity in bone (47).

Higher serum levels have been reported in dialysis patients with radiographic evidence of bone resorption. Serum iPTH levels are sometimes normal or undetectable. This may occur in uremic

patients with severe hypomagnesemia, where, Mg depletion decreases the ability of the gland to release PTH (47,48). PTH levels are low in patients with aluminium related bone disease. The mechanisms have already been described.

Bordier et al (41) introduced a scheme of staging renal osteodystrophy using biochemical and osseous abnormalities (Appendix I). Stage I is characterised by normal serum Ca and inP concentrations despite increased serum iPTH levels. There is sufficient mass of renal parenchyma to produce 1,25 DHCC and to serve as target tissue for the phosphaturic action of PTH. Calcium mobilising action of PTH on bone is unimpaired. In this stage, increased PTH secretion can be identified as the major compensating adjustment to inP retention. The adjustment occurs in response to intermitant hypocalcemia. The compromise in maintaining the extracellular mineral homeostasis is at the cost of osseous integrity, that is, development of osteitis fibrosa.

In stage II, there is an increased in nephron loss. The rising PTH cannot decrease inP concentration by urinary excretion.At the same time production of 1,25 DHCC drops, with the result that intestinal absorption of Ca and normal osseous function are reduced. Serum iPTH is high, serum inP is high and serum Ca level falls and osteitis fibrosa develops, along with early osteomalacia. Osteitis fibrosa progresses slowly due to "Vitamin D deficiency and hyperphosphatemic blocks" to the osteolytic

action of increased circulating iPTH.

In stage III, serum Ca is normal, iPTH and inP are high and stimulation PTH is governed indirectly by the degree and duration of hyperphosphatemia. Hyperplasia of parathyroid glands occurs and PTH secretion increases. "Osteolytic block" is overcome. Serum Ca is restored towards normal.
InP is resorped from bone. There is overt osteitis fibrosa. iPTH secretion is not under control of serum Calcium.

Stage IV is characterised by high serum iPTH, high serum Ca and inP. There is massive parathyroid hyperplasia along with both florid osteitis fibrosa and worsening osteomalacia. In this stage, complications of hypercalcemia, that is, metastatic calcification occur. There is no negative feedback of hypercalcemia on PTH secretion.

RADIOGRAPHIC FEATURES OF RENAL OSTEODYSTROPHY. With the use of fine grain films, and hand lens magnification, the following changes are seen in secondary hyperparathyroidism. 1) Increased bone resorption which may be on the sub-periosteal, intracortical or endosteal surfaces of cortical bones. Also seen are periosteal neostosis, osteosclerosis and osteopenia. Erosion in conjunction with formation of new bone appear as cysts or osteoclastomas (Browns tumour). The skull may show mottled radiolucent appearance with areas of osteosclerosis. In osteomalacia widening

action of increased circulating iPTH.

In stage III, serum Ca is normal, iPTH and inP are high and stimulation PTH is governed indirectly by the degree and duration of hyperphosphatemia. Hyperplasia of parathyroid glands occurs and PTH secretion increases. "Osteolytic block" is overcome. Serum Ca is restored towards normal.
InP is resorped from bone. There is overt osteitis fibrosa. iPTH secretion is not under control of serum Calcium.

Stage IV is characterised by high serum iPTH, high serum Ca and inP. There is massive parathyroid hyperplasia along with both florid osteitis fibrosa and worsening osteomalacia. In this stage, complications of hypercalcemia, that is, metastatic calcification occur. There is no negative feedback of hypercalcemia on PTH secretion.

RADIOGRAPHIC FEATURES OF RENAL OSTEODYSTROPHY. With the use of fine grain films, and hand lens magnification, the following changes are seen in secondary hyperparathyroidism. 1) Increased bone resorption which may be on the sub-periosteal, intracortical or endosteal surfaces of cortical bones. Also seen are periosteal neostosis, osteosclerosis and osteopenia. Erosion in conjunction with formation of new bone appear as cysts or osteoclastomas (Browns tumour). The skull may show mottled radiolucent appearance with areas of osteosclerosis. In osteomalacia widening

of the epiphysial growth plate and other deformities are seen. Loosers zones or pseudofractures may be the only pathognomonic finding of osteomalacia in an adult. Protusio acetabuli, skeletal demineralization and increased haziness of the trabeculi are the other findings. Osteopenia, osteosclerosis and metastatic calcification may also be seen (48,49,50).

STUDY OBJECTIVES

The objectives of the study were :-

1) To look at the clinical, radiological and biochemical evidence of renal osteodystrophy in patients with CRF on or not on hemodialysis.

2) To correlate the above with the severity of CRF.

METHODS

Study population :- Patients with established diagnosis of CRF seen in the medical and pediatric wards, the renal unit and the renal clinic were recruited. The following categories of patients were excluded from the study, that is, those with multiple myeloma, other bone or soft tissue neoplasms with secondaries to the bone, other metabolic and non-metabolic bone disease, liver disease, congestive cardiac failure and malabsorption syndrome. Als excluded were patients on drugs like phenobarbitone, diphenylhydantoin, acetazolamide and steroids.

Control population:- The control group of individuals was obtained from healthy members of the hospital staff as well as the patients from the ENT clinic with minor medical problems. They were matched with the patients for age and sex. Presence of hypertension, diabetes mellitus, liver disease, kidney problems or bone disorders were ruled out on history and physical examination.

Clinical Methods :- A history was taken in all patients, especially , details concerning the duration of renal failure, symptoms of renal osteodystrophy and the kind of drug taken. Physical examination included in particular looking for bone and joint tenderness, fractures, skeletal deformities, myopathy, growth retardation, pruritus and signs of dystrophic calcification of organ systems (table 3-7).

Investigations:-

Radiology :-All the patients had the following xrays:- Hand anteroposterior view, skull lateral view and thorocolumbar spine lateral view. In addition to these, x-rays of other clinically involved bones and joints were taken, whenever necessary. The x-rays were taken using standard x-ray films, standard energy of exposure and standard exposure time. Two radiologist viewed them independently. 24 hour urine was taken for estimation of creatinine clearance.

Serum Biochemistry:- Venous blood obtained from both the patients and controls by venepuncture with minimal hemostasis was collected in heparinised tubes, centrifuged and the serum stored at -30 degrees centigrade. It was used for the following analysis:-

1) Serum calcium by O cresolphalein complexone method(51).
2) Serum magnesium by colorimetry(52).
3) Serum inorganic phosphates and albumin by photometric estimation(53,54).

4) Serum alkaline phosphatase by method of Bowers et al(55).

5) Serum iPTH & 1,25DHCC by radioimmunoassay (Appendix II,III) (56,57).

6) Serum and urinary creatinine were done by colorimetric method on patients only(58).

STATISTICAL ANALYSIS.

All the variables were expressed in terms of mean ± standard deviation (SD). Difference between the patient and control means were statistically analysed using the student t-test. A p value of less than .05 was taken as significant. Pearson's correlation coefficient of association (r) was used to correlate variables, the t-test was used to check the strength of the association.

Diagnostic criteria for biochemical renal osteodystrophy.

The scheme presented by Bordier et al (41) for staging the renal osteodystrophy was utilised to define the biochemical criteria for the diagnosis of renal osteodystrophy.

Stage I	Normal Serum calcium Normal Serum inP Sightly High serum iPTH
Stage II	Low Serum calcium High Serum inP High Serum iPTH
Stage III	Normal Serum calcium High Serum inP High Serum iPTH

	High Serum calcium
Stage IV	High Serum inP
	High Serum iPTH

Patients were grouped into the above four categories.

RESULTS

40 patients with an established diagnosis of CRF were recruited and taken into the study. Their ages ranged from 10 - 70 years (mean age 28.1 ± 14.17 years). Figure 1 illustrates the age distribution of the patients. 33 (82.5%) patients were below the age of 40 years. The male to female ratio was 1:1. The mean duration of renal failure ranged from 2 to 68 months with a mean of 26.18 ± 40.97 months. The underlying causes of CRF were glomerulonephritis (10/40), hypertension (12/40), renal artery stenosis (1/40), adult polycystic kidney disease (1/40) and unknown etiology (16/40).

Table 1, gives the modalities of treatment in the patients. Majority (52.5%) were on chronic intermittant hemodialysis. Drug treatment included a number of antihypertensive drugs (captopril, enalpril, nifedipine, alphamethlydopa, hydrallazine and frusemide) taken in various combinations and doses by almost all the patients. Antacid/phosphate binding agents were taken by 33 (82.5%) patients and contained Al and Mg compounds. Calcium lactate and vitamin D (calciferol) were taken by 33 (82.5%) patients. All patients were on ferrous sulphate 200mg three times a day and folate 5 mg twice daily.

Table 2, shows the catagories of patients based on the severity of renal failure. Majority (72.5%) were in end stage renal disease.

Table 3-5, shows the break down of the symptoms that are associated with renal osteodystrophy. 30 (75%) patients had

Figure 1: Age distribution of patients.

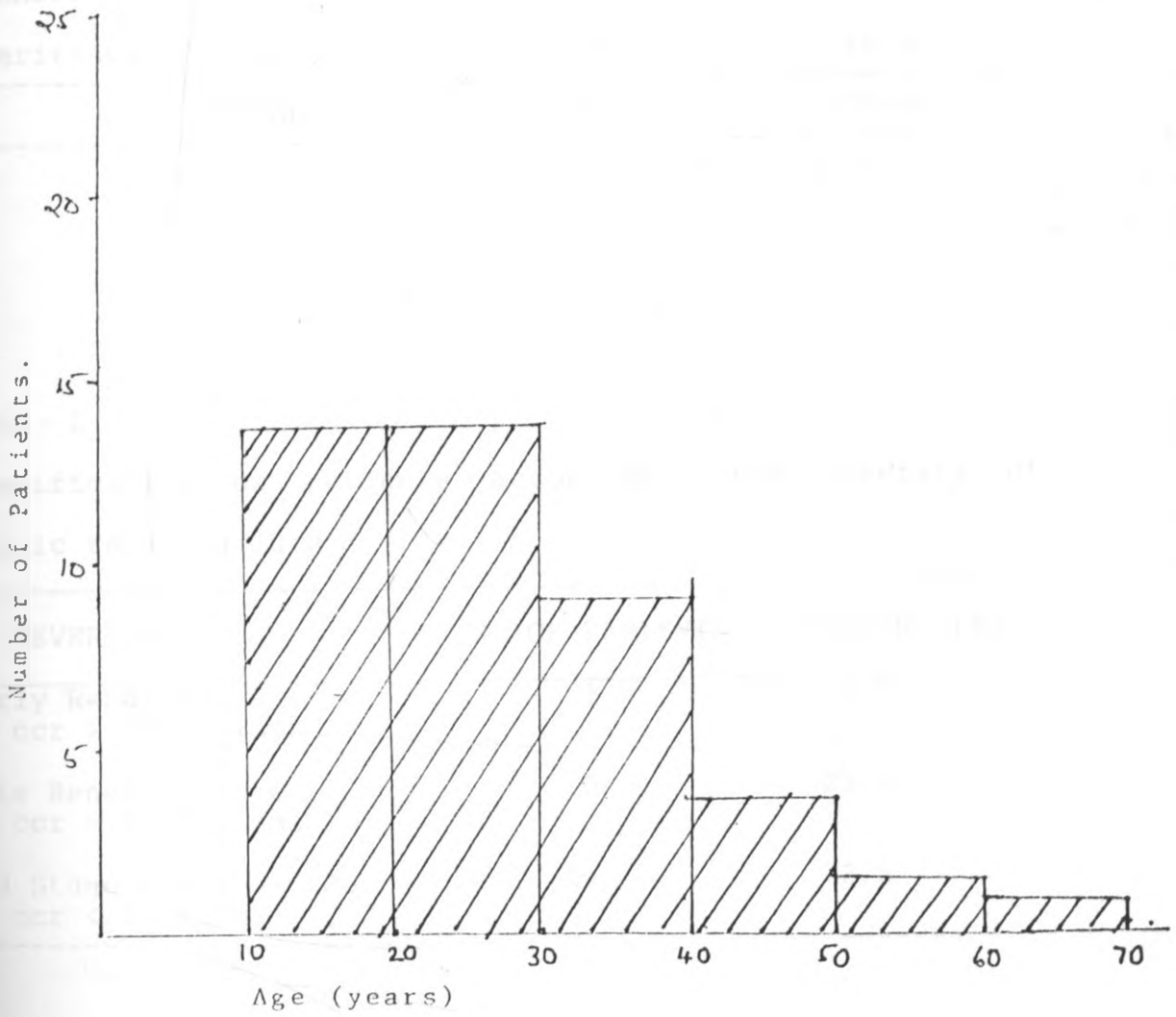


TABLE 1

Modalities of treatment in patients with chronic renal failure.

TREATMENT	NO OF PATIENTS	PERCENT (%)
Hemodialysis	21	52.5
Conservative	15	37.5
Peritoneal Dialysis	4	10.0
TOTAL	40	100.00

TABLE 2

Classification of patients according to the severity of chronic renal failure.

SEVERITY	NO OF PATIENTS	PERCENT (%)
Early Renal failure ccr > 30 ml/min	1	2.5
Late Renal failure ccr = 5-30 ml/min	10	25.0
End Stage Renal Disease ccr < 5 ml/min	29	72.5

TABLE 3

Symptoms suggestive of renal osteodystrophy.

	SYMPTOM	NO OF CASES	PERCENT (%)
*	Pain in the bones	27	67.5
*	Pain in the joints	25	62.5
	Proximal muscle weakness	24	60.0
	Pruritus	21	52.5
	Parasthesias	18	45.0
	Growth retardation	6	15.0
	Tetany	1	2.5
	Raynauds phenomenon	1	2.5

* Aggravated by muscular effort and Wt bearing.

TABLE 4

Distribution of pain in the bones.

SYMPTOM	NO OF PATIENTS	PERCENT (%)
Pain in the thoracolumbar Spine	16	40.0
Pain in the bones of the lower limbs	8	20.0
Generalised bone pain	3	7.5
Pain in the bones of upper limbs.	2	5.0
No bone pain	5	12.5

TABLE 5

Distribution of joint pains.

SYMPTOM	NO OF PATIENTS	PERCENT (%)
Pain in the large joints or lower limbs	17	42.5
Pain in the large joints of upper limbs	8	20.0
Pain in the small joints of lower limbs	5	12.5

symptoms suggestive of renal osteodystrophy. Pain in the bones and joints and proximal muscle weakness were the most frequent symptoms. Pruritus present in 21 (52.5%) patients was generalised and was neither disturbing nor disabling (Table 6). The most common site of bone pain was the thoracolumbar spine seen in 16 (40%) patients. Regarding joint pain, it was found that large joints of the lower limbs i.e. knees, ankles and hips were most commonly effected (42.5%) followed by pain in large joints of the upper limbs i.e. shoulders and elbows, seen in 8 (20%) of the patients. Small joints of the lower limbs were painful in 5 (12.5%) patients.

Table 6 and 7, respectively give the breakdown and distribution of the signs found on examination of the patients. Joint tenderness was the commonest sign and was seen in 17 (42.5%) patients. This involved the ankles, knees and shoulders. Tenderness along the thoracolumbar spine was noted in 15 (37.5%) patients. Proximal muscle weakness involving the shoulder and pelvic girdles occured in 14 (35%) patients. Bony tenderness was noted in 11 (27.5%) patients and predominantly affected the femur, ribs, tibia and humerus. 8 (20%) patients had muscle tenderness and 4 (10%) had skeletal deformity with genuvalgum, pigeon chest in two, and kyphosis of spine in one patient. None of the patients had distal muscle weakness, spontaneous fractures, tendon rupture, joint effusion or masses around the joints.

TABLE 6

Signs suggestive of renal osteodystrophy.

SIGN	NO OF PATIENTS	PERCENT (%)
Joint tenderness	17	42.5
Tenderness along the spine	15	37.5
Proximal muscle weakness	14	35.0
Bony tenderness	11	27.5
Muscle tenderness	8	20.0
Skeletal deformity	4	10.0
Restriction of joint movement	1	2.5

TABLE 7

Breakdown of the signs in renal osteodystrophy.

SIGN	NO OF PATIENTS	PERCENT (%)
Tenderness along the thoracolumbar spine	15	37.5
Proximal muscle weakness	14	35.0
Tenderness in the large joints of the lower limbs	10	25.0
Bony tenderness in lower limbs	10	25.0
Tenderness in large joints of upper limbs	9	22.5
Genuvalgum	4	10.0
Costochondral tenderness	3	7.5
Tenderness in ribs	3	7.5
Tenderness in small joints of lower limbs	3	7.5
Pigeon chest	2	5.0
Kyphosis of spine	1	2.5

Table 8, describes certain aspects of pruritus in chronic renal failure. It was present in 21 (52.5%). There was relief following dialysis in 11 (52.3%) patients. No relief was noted in 7 (33.3%) patients. It was aggravated on exposure to sunlight in two patients and one patient had pruritus after dialysis was initiated.

Radiological features suggestive of renal osteodystrophy were seen in only 3 out of 40 (7.5%) patients.

Table 9, indicates the breakdown of changes, all 3 had subperiosteal erosion in the phalanges, 2 had ground glass appearance of the skull with sclerotic foci, 2 had the rugger jersey spine and cystic changes in the bone, while one showed loss of lamina dura in the teeth as well as delayed epiphyseal fusion and coarse trabeculation in the bones (Figures 2-7). Loosers zones/spontaneous fractures, periosteal neostosis or prutrusio acetabuli were not seen.

The normal serum levels of Ca, Mg and inP, alkaline phosphatase and iPTH used in this study were obtained by studying the sera of 37 controls and the results are shown in table 10.

Figure 8, is a histogram showing the number of patients in each of the stages of renal osteodystrophy. 16 (40%) patients were in stage I, 5 (12.5%) patients were in stage II, another 16 (40%) were in stage III and 3 (7.5%) patients were in stage IV. Table 10 reviews the various biochemical parameters of the patients in stage I and compares them with controls. It is noted that serum inP, calcium, magnesium and alkaline phosphatase were not significantly different from control values ($p > 0.05$ in all).

TABLE 8

Pruritus in chronic renal failure.

FEATURES	NO OF PATIENTS	PERCENT (%)
Relief following hemodialysis	11	52.3
No relief following dialysis	7	33.3
Aggravated by sunlight	2	9.6
Onset after dialysis	1	4.8
TOTAL	21	100.0

TABLE 9

Radiographic features suggestive of renal osteodystrophy.

FEATURES	NO OF PATIENTS	PERCENT (%)
Subperiosteal erosion in the phalanges	3	7.5
Ground glass appearance of skull with osteosclerosis	2	5.0
Rugger Jersey appearance of the spine	2	5.0
Cystic changes in the bone(Browns tumor)	2	5.0
Loss of lamina dura (teeth)	1	2.5
Coarse trabeculation with loss of corticomedullary definition	1	2.5
Delayed epiphysial fusion	1	2.5

FIGURE 2

Abnormalities in the skull radiograph of a 19 year old patient with renal osteodystrophy. Note the focal areas of sclerosis and widening of the diploe with loss of cortical definition.

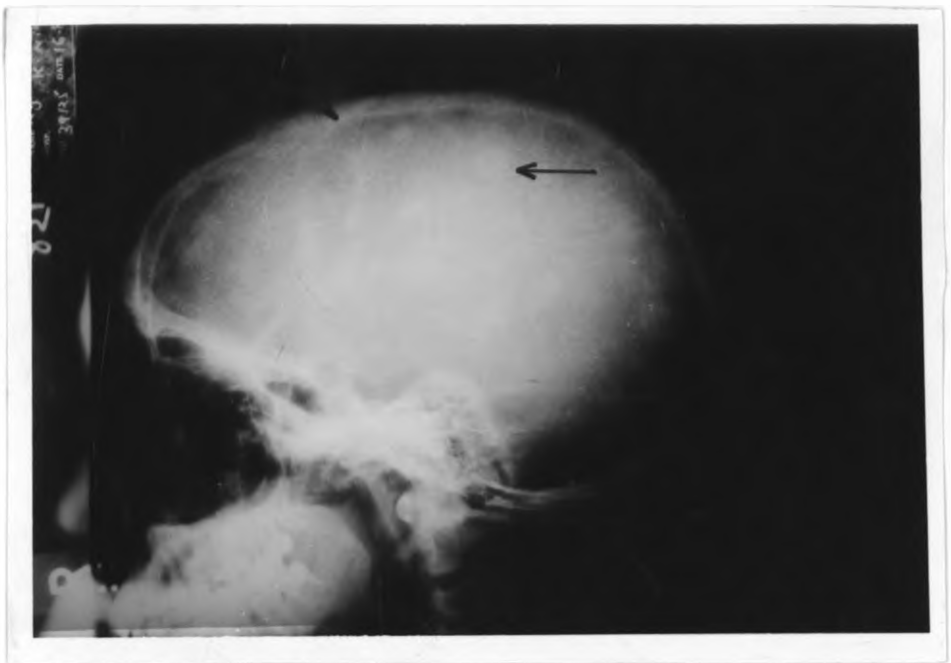


FIGURE 3:

Radiograph of the hand of the 19 year old patient showing subperiosteal bone resorption. There is diffuse coarse trabeculation with delayed skeletal maturation. Bone age is 13 years.

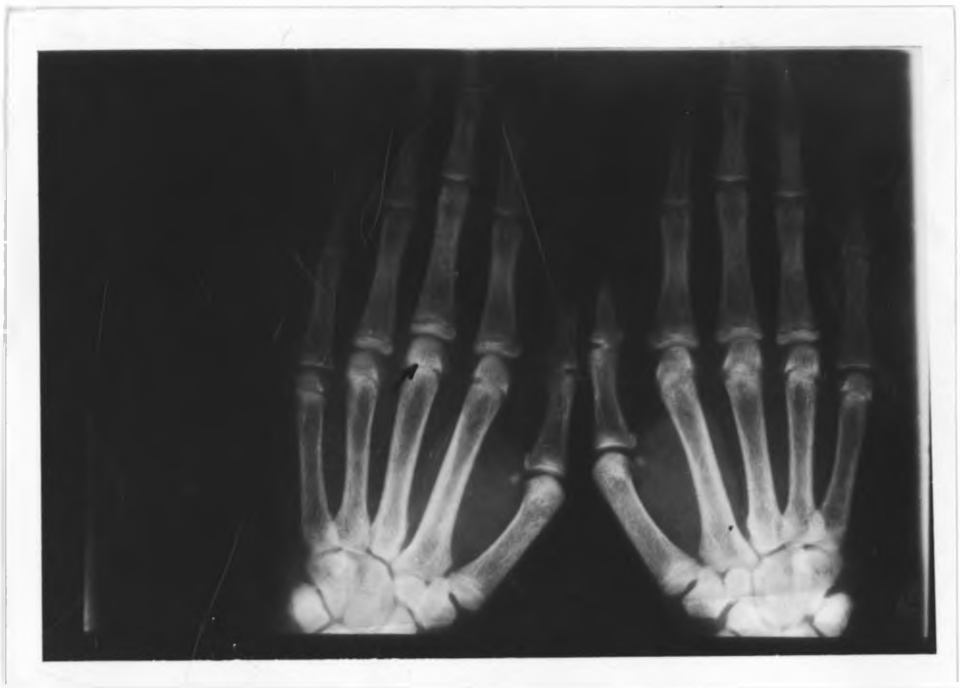


FIGURE 4:

Radiograph of the pelvis of a 19 years old patient showing delayed union of the proximal femoral epiphysis with associated coxa vara. Coarse trabeculation is seen in the femoral neck and the proximal end of femur. Subperiosteal reaction is seen on lateral aspect of the right femur.

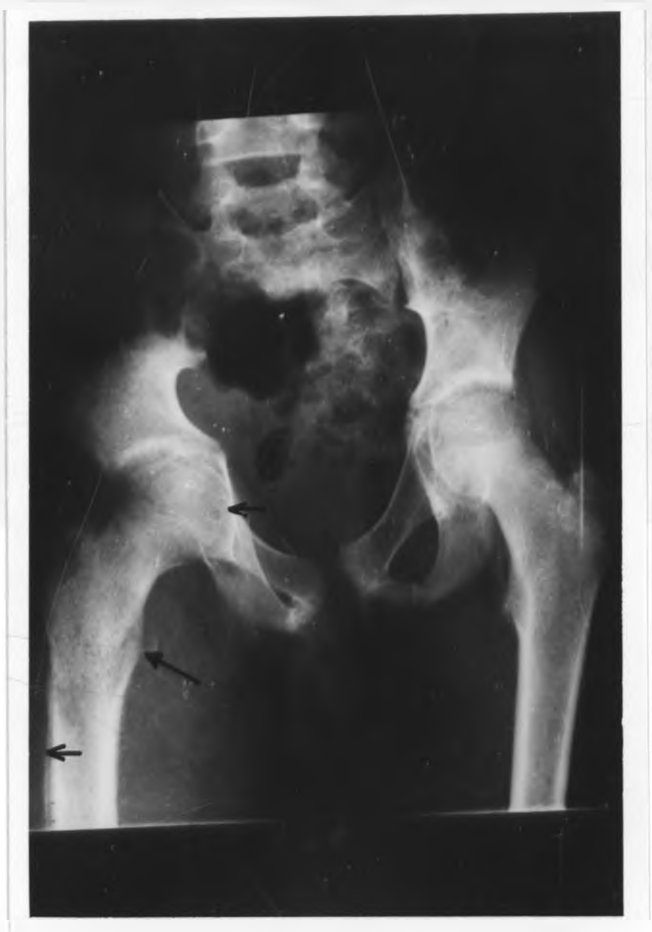


FIGURE 5:

Radiograph of the knees of the same patient showing delayed epiphyseal fusion of the femoral, tibial and fibular ends. Sub periosteal erosion is also seen .

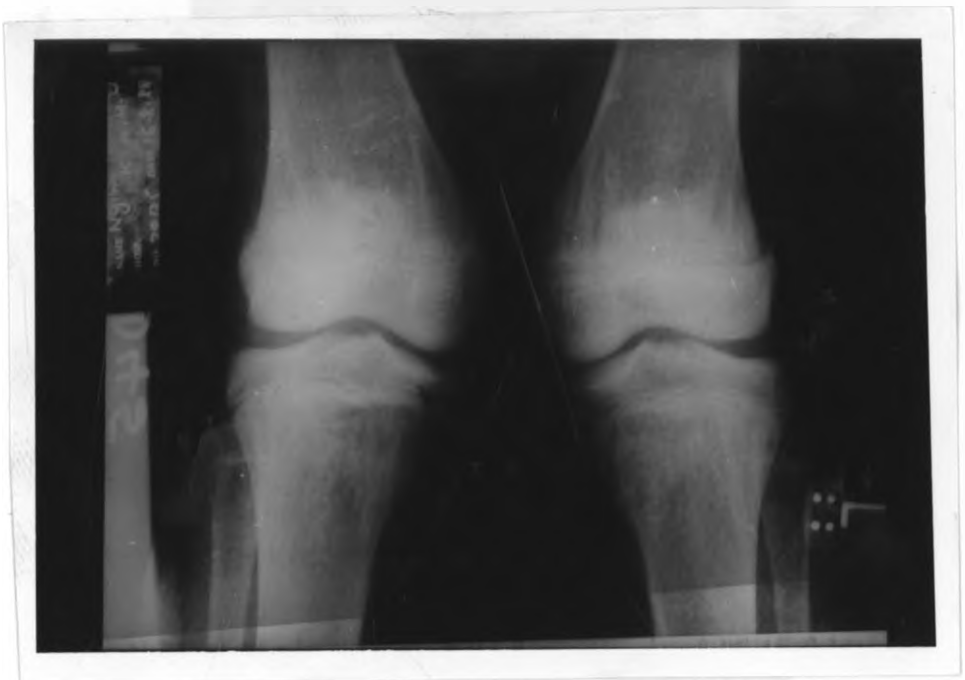


FIGURE 6:

Radiograph of the hand of a 14 year old patient showing juxta articular osteoporosis.

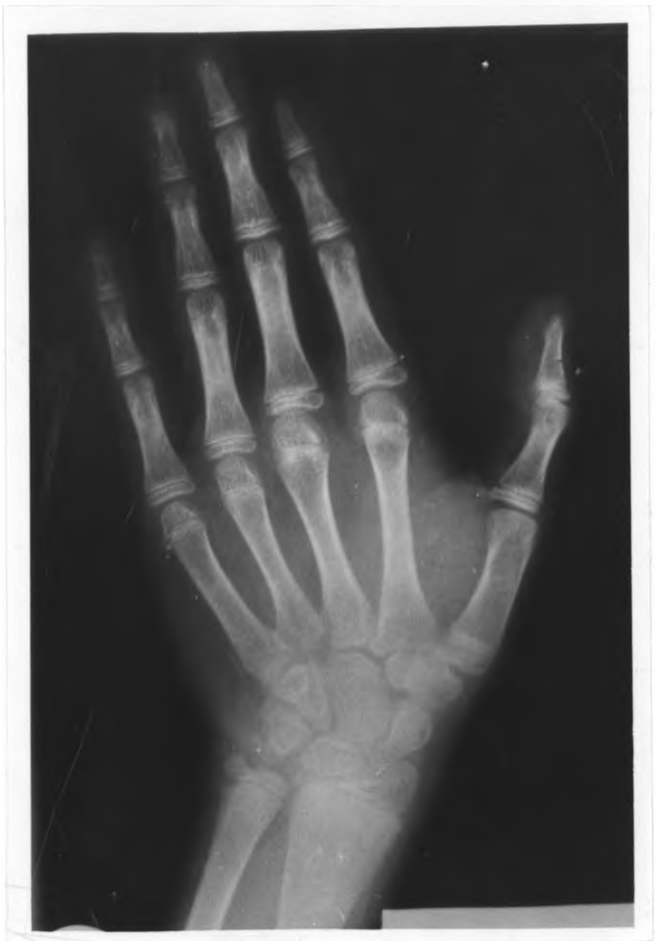


FIGURE 7:

Radiograph of the spine showing "Rugger Jersey" appearance.

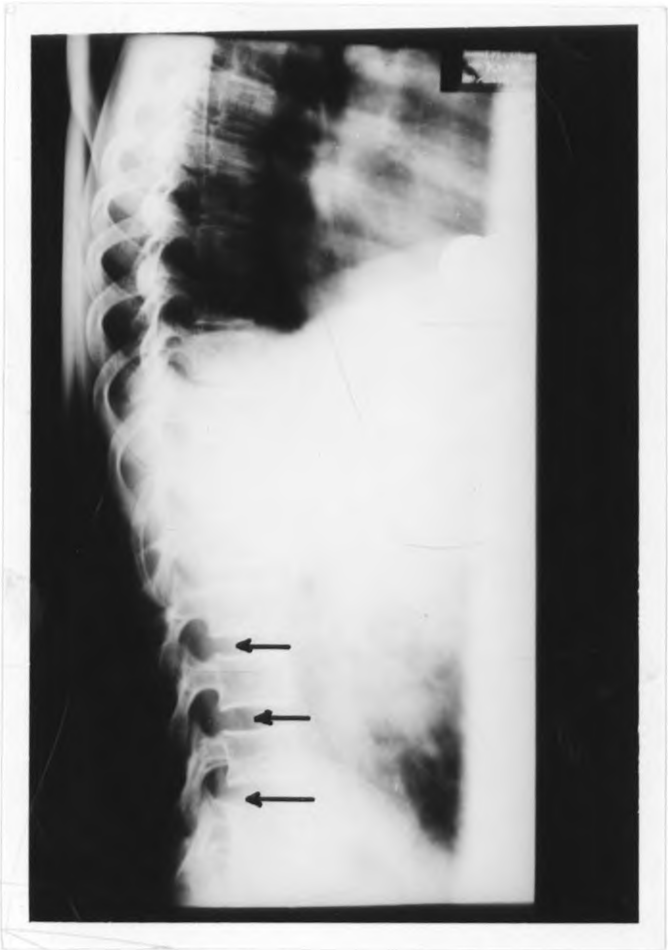
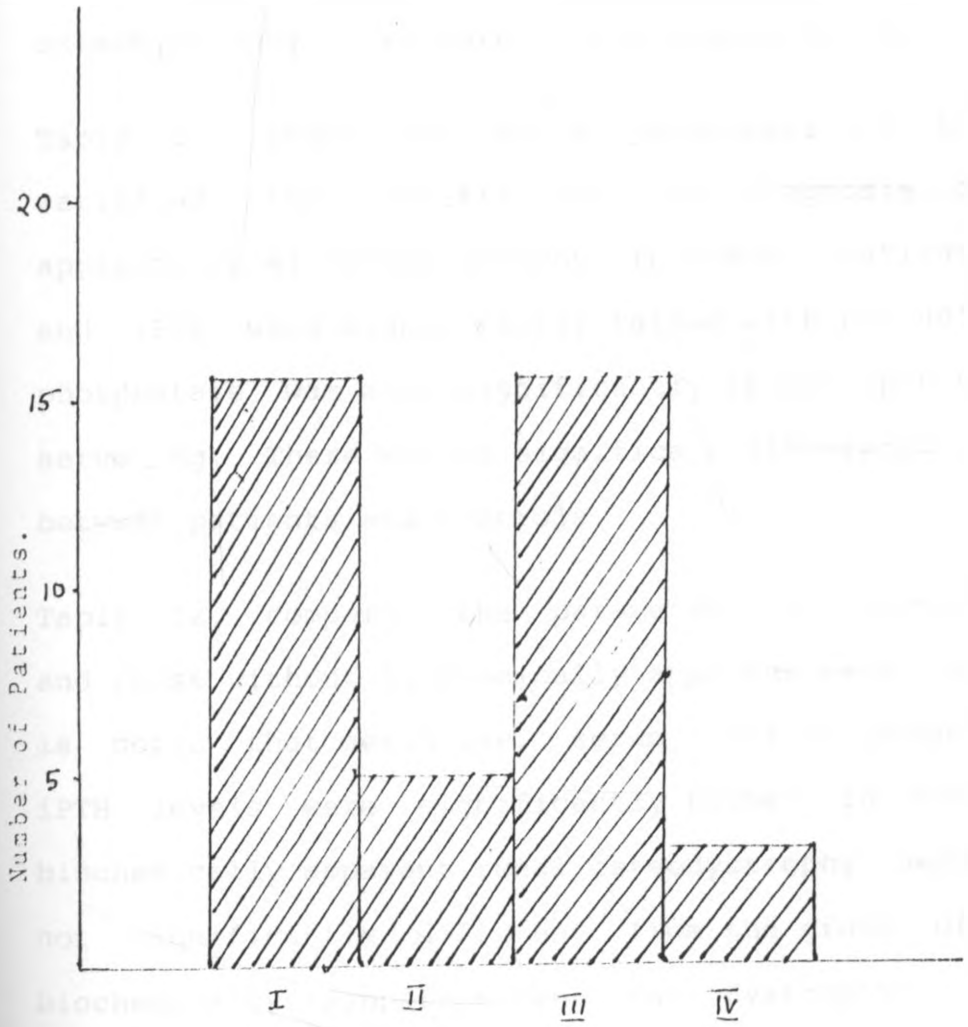


TABLE 10

Serum values of uremic patients with biochemically inapparent renal osteodystrophy. (n=16).

Serum parameters	Uremic patients with biochemically inapparent osteodystrophy n=16	Controls n=37	p value
Serum inP (mmol/l)	1.912 ± .663	1.605 ± .369	> 0.05
Serum calcium (mmol/l)	1.975 ± .336	2.013 ± .303	> 0.05
Serum iPTH (pg/ml)	2.34.687 ± 166.648	113.054 ± 60.52	< 0.011
Serum Alkaline Phosphataes (Iu/l)	202.375 ± 62.218	184.604 ± 98.013	> 0.05
Serum magnesium (mmol/l)	1.131 ± .285	.913 ± .249	> 0.05

Figure 8: Histogram of number of patients in various stages of renal osteodystrophy.



Stages of remal osteodystrophy.

Serum iPTH was slightly high 234.687 ± 166.648 pg/ml ($p < 0.011$) as defined in stage I. This group of patients did not have the biochemically apparent changes of osteodystrophy that have been described previously in uremic patients (2,53,54). They were therefore classified as biochemically inapparent osteodystrophy. 24 (60%) patients had biochemically apparent renal osteodystrophy. They were in the stages II, III and IV.

Table 11, shows the serum parameters of the patients who satisfied the criteria for the diagnosis of biochemically apparent renal osteodystrophy. In these, patients, the serum inP and iPTH were significantly raised with $p<0.001$. Serum alkaline phosphatase was also significantly raised ($p<0.001$), as well as serum Mg. There was no significant difference in the serum Ca between patients and controls.

Table 12, compares the parameters of uremic patients with and those without biochemically apparent renal osteodystrophy. It is noted that serum inP, serum alkaline phosphatase and serum iPTH levels were significantly higher in the patients with biochemically apparent renal osteodystrophy. Serum Ca and Mg were not significantly different from the group of patients with biochemically inapparent renal osteodystrophy.

Table 13, shows the serum 1,25DHCC levels in 20 patients on the hemodialysis the duration of the which ranged from 1 to 15 months and the duration of renal failure ranged from 3 to 35 months. The levels were also estimated in 16 controls matched for age and sex with the patients. The mean 1,25DHCC levels in controls were

TABLE 11

Serum values of uremic patients with biochemically apparent renal osteodystrophy.

Serum Parameters	Uremic patients with biochemically apparent osteodystrophy n=24	controls n=37	p value
Serum inP (mmol/l)	2.341 ± .546	1.605 ± .369	< 0.001
Serum calcium (mmol/l)	1.95 ± .392	2.013 ± .303	> 0.05
Serum iPTH (pg/ml)	547.916 ± 250.829	113.054 ± 60.62	< 0.001
Serum alkaline Phosphatase (Iu/l)	372.125 ± 222.202	184.604 ± 98.013	< 0.001
Serum magnesium (mmol/l)	1.304 ± .29	.913 ± .249	< 0.001

TABLE 12

Comparison between uremic patients with and those without biochemically apparent renal osteodystrophy.

Serum Parameter	Uremic patients with biochemically apparent osteodystrophy n=24	Uremic patients without biochemically apparent renal osteodystrophy	p value
Serum inP (mmol/l)	2.341 ± .546	1.912 ± .663	< 0.041
Serum calcium (mmol/l)	1.95 ± .392	1.975 ± .336	> 0.05
Serum magnesium (mmol/l)	1.304 ± .29	1.131 ± .336	> 0.05
Serum Alkaline Phosphatase (Iu/l)	372.125 ± 222.202	202.375 ± 62.218	< 0.001
Serum iPTH (pg/ml)	547.916 ± 250.829	234.687 ± 166.648	< 0.001

TABLE 13

1,25DHCC levels in 20 patients with CRF on hemodislysis

SAMPLE POPULATION	MEAN LEVELS OF 1,25DHCC (pg/ml)	p value
Patients (N=20)	14.885 ± 16.166	< 0.035
Controls (N=16)	24.89 ± 11.007	

24.89 ± 11.007 pg/ml. The mean serum 1,25DHCC levels in the patients (14.885 ± 16.166 pg/ml) were significantly reduced when compared with the control population ($p < 0.035$).

Variables were compared between the patients with and those without pruritus (Table 14). Serum iPTH alone was significantly raised ($p<0.030$), in patients with pruritus.

TABLE 15, is a summary of the significant correlations found with the parameters of renal osteodystrophy. These correlations are also shown in figures 9-12. In all the patients taken together, significant positive correlations were realised between iPTH and serum Mg ($r=.5155, p<0.001$). In the 24 patients with biochemically apparent renal osteodystrophy, significant positive correlations were found between serum iPTH and serum Ca ($r=.4949$, $p<0.01$); between serum iPTH and serum Mg ($r=.4926, p<0.01$); serum alkaline phosphatase and the duration of renal failure ($r=.4734, p<0.01$), and between the symptoms and signs ($r=.5078$, $p<0.01$). In the 20 patients in whom vitamin 1,25DHCC levels were estimated, a positive correlation was realised between serum 1,25DHCC and the duration of renal failure ($r=.7458, p<0.001$).

Table 16, cross tabulates the patients according to the clinical features and biochemical evidence of renal osteodystrophy described above. It was found that 20 patients (50%) had both clinical and biochemical features of renal osteodystrophy while 12 patients (30%) showed only the clinical features. 4 patients without clinical features showed biochemical changes of renal osteodystrophy and 4 others (10%) had neither features.

TABLE 14
Comparision of variables in patients with and without Pruritus.

VARIABLES	PATIENTS WITH PRURITUS N=21	PATIENTS WITHOUT PRURITUS N=19	p value
Serum inP (mmol/l)	2.085 ± .613	2.263 ± .64	.378
Serum Ca (mmol/l)	1.938 ± .372	1.984 ± .369	.696
Serum iPTH (pg/ml)	508.095 ± 286.123	328.157 ± 216.694	< 0.030
Serum Alkaline Phosphatase (mmol/l)	306.523 ± 132.537	301.684 ± 249.302	.940
Serum Mg (mmol/l)	1.29 ± .362	1.173 ± .194	.207
Duration of Renal failure (month)	13.904 ± 10.421	39.736 ± 56.098	.063
BUN (mmol/l)	36.13 ± 20.055	30.179 ± 21.199	.368
AGE (years)	27.714 ± 17.135	28.526 ± 10.416	.856

TABLE 15

Summary of significant correlations.

CORRELATION OF VARIABLES			CORRELATION COEFFICIENT r	p value
Serum iPTH (n=40)	vs	Serum Mg (n=40)	.5155	< 0.001
Serum iPTH (n=24)	vs	Serum Ca (n=24)	.4949	< 0.01
	vs	Serum Mg (n=24)	.4926	< 0.01
Serum Alkaline Phosphatase (n=24)	vs	Duration of Renal failure (n=24)	.4734	< 0.01
Symptoms (n=24)	vs	Signs (n=24)	.5078	< 0.01
Serum 1,25DHCC (n=20)	vs	Duration of Renal failure (n=20)	.7458	< .001

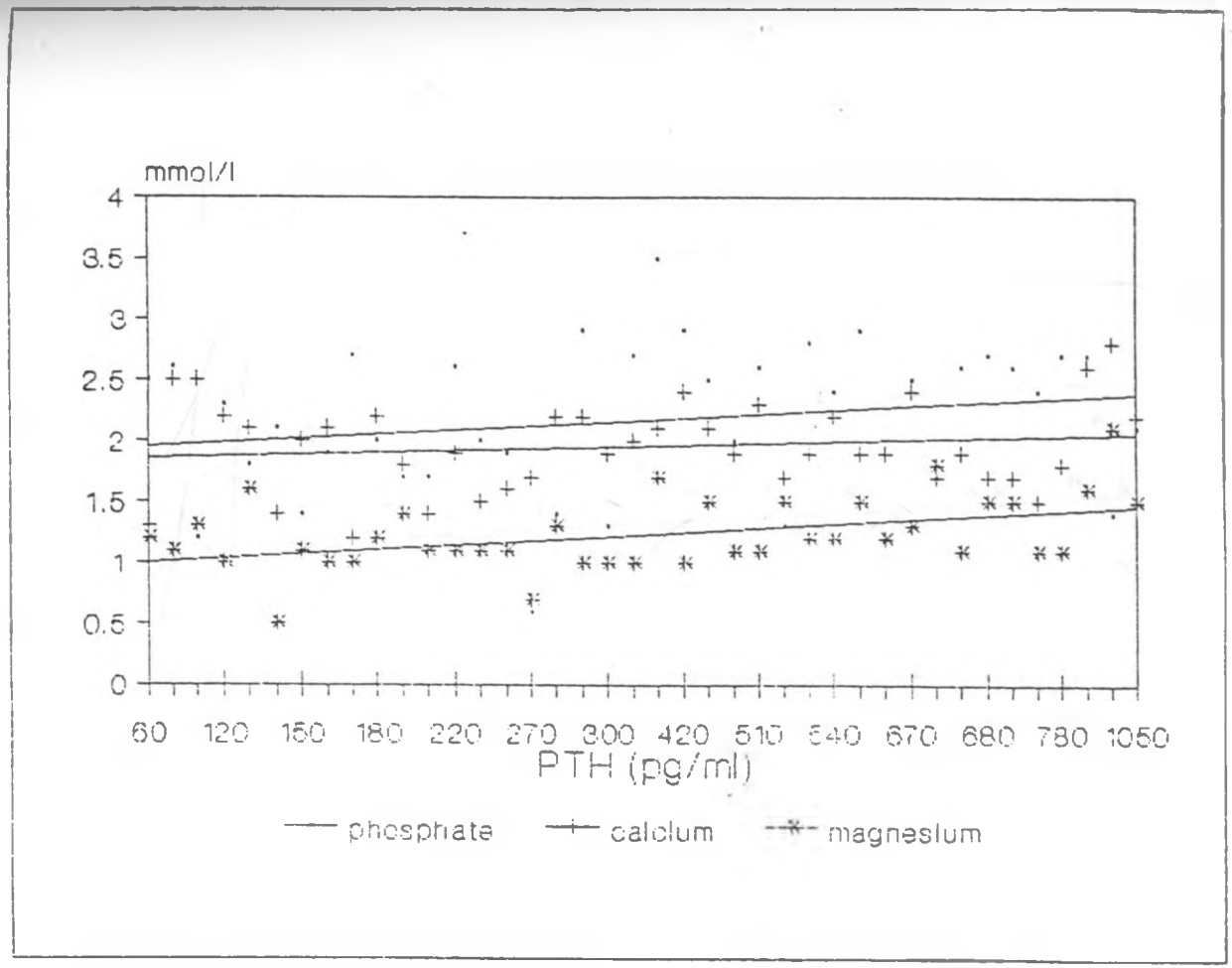


Figure 9: Graph showing the trend in serum Ca, Mg and inP in relation to increasing serum iPTH.

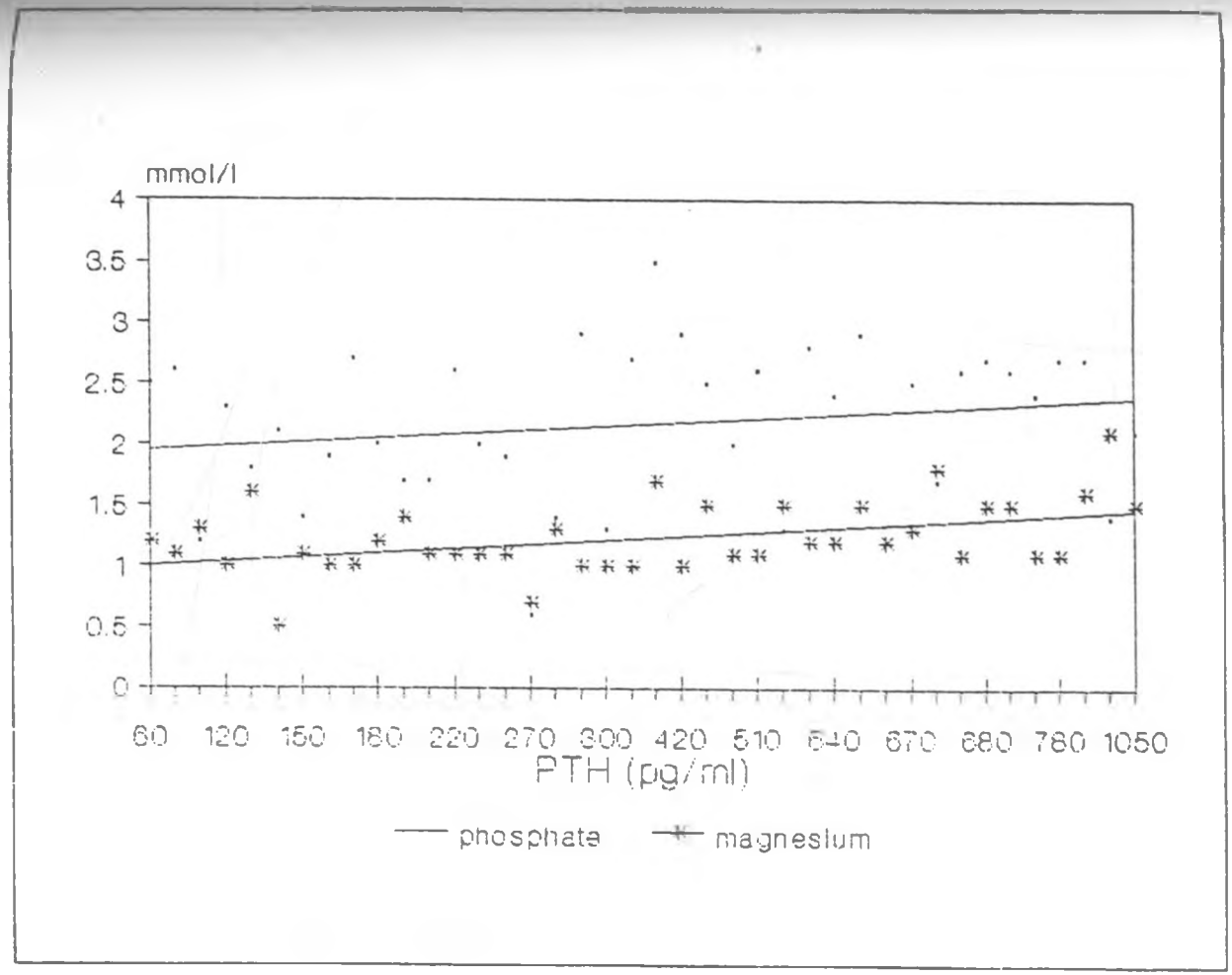


Figure 10. Graph showing the trend in serum Mg and inP in relation to increasing serum iPTH.

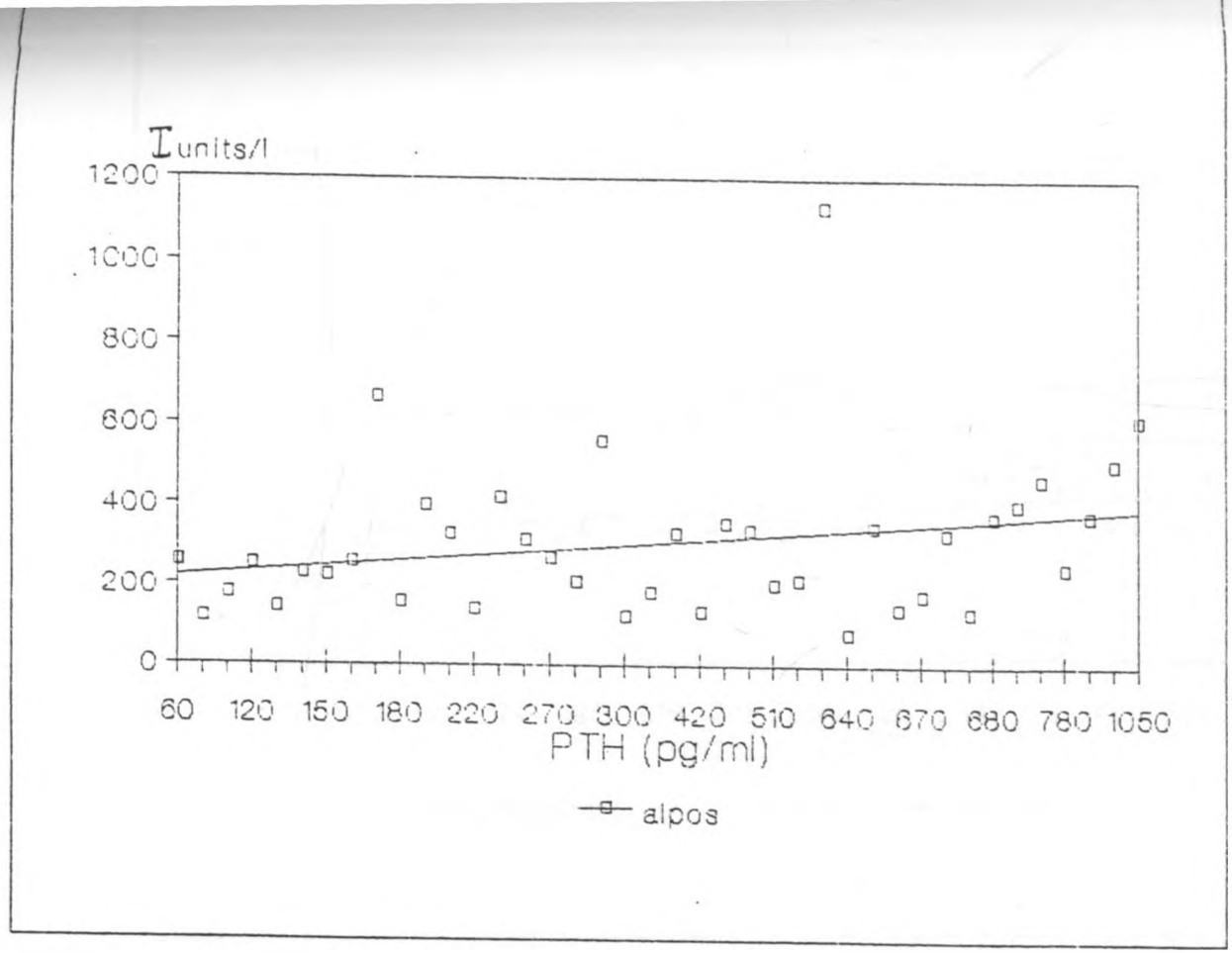


Figure 11: Graph showing change in alkaline phosphatase with increasing concentration of serum iPTH.

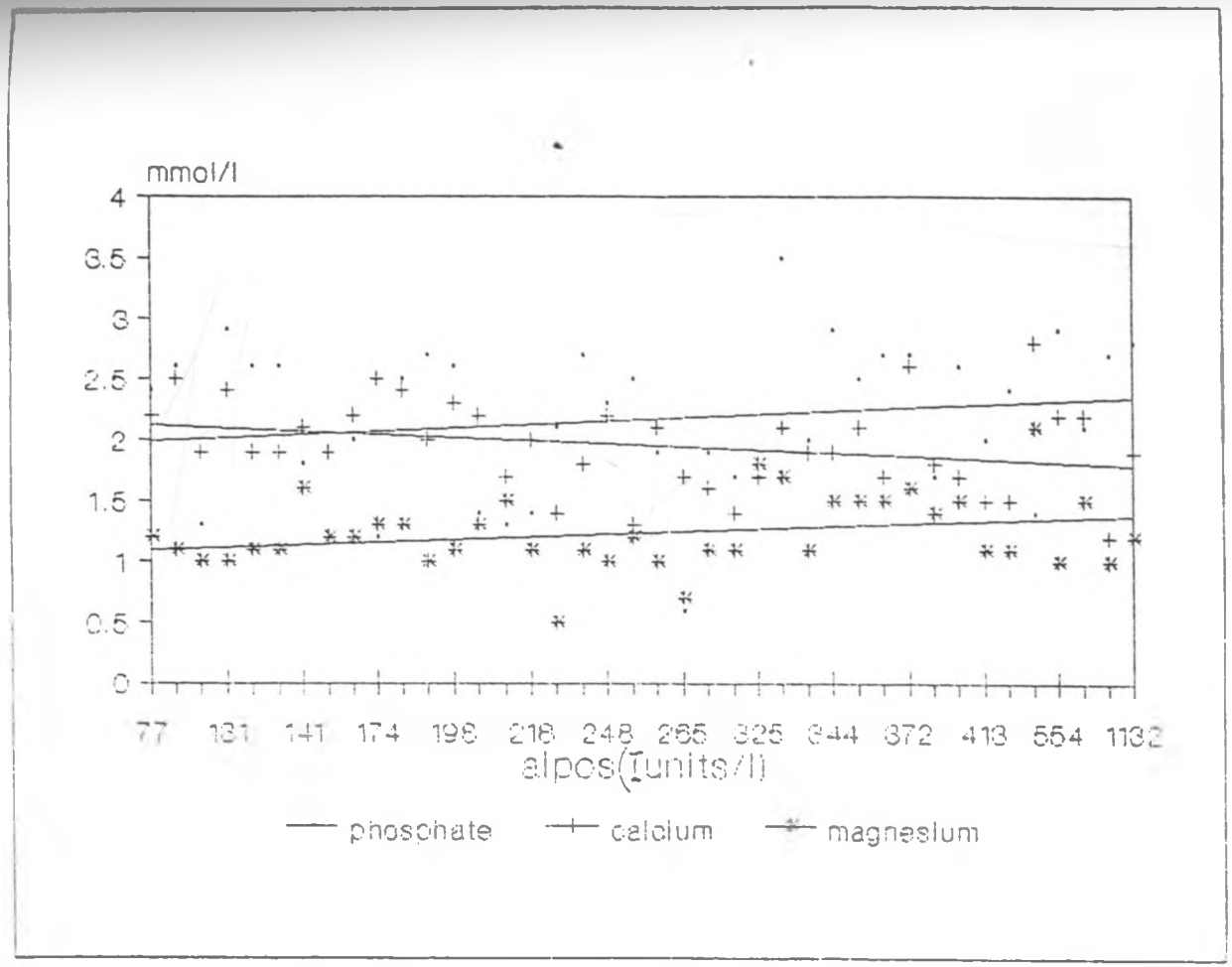


Figure 12. Graph showing the trend in serum. Ca, Mg and inP in relation to alkaline phosphatese.

TABLE 16

Cross tabulation of patients according to the clinical features and biochemical evidence of Renal osteodystrophy.

	CLINICAL FEATURES PRESENT	CLINICAL FEATURES ABSENT
BIOCHEMICAL EVIDENCE PRESENT	20	4
BIOCHEMICAL EVIDENCE ABSENT	12	4

DISCUSSION

Renal osteodystrophy as a consequence of CRF is seen with increasing frequency with the advent of replacment therapy. This is because the longer duration of life and prolonged dialysis exposes the bones to worsening alterations of the divalent metabolism. Dialysis does the excretion work of the kidney but not the endocrine funtion.

The age of onset of renal osteodystrophy depends on the age of the onset of renal failure, the duration and progression of renal failure,the duration and efficacy of dialysis and the control of the divalent ion abnormality (5). In the present study, most patients were young (figure 1). This is probably due to the early age of onset of renal failure and delay in the institution of therapy for various reasons. In the West, renal bone disease is seen in an older age group (mean age 49.2 + 11.2 years) (63). This is due to the fact that prompt treatment and adequate dialysis has prolonged the life expectancy of patients.

No sex difference was seen in our population unlike the figures quoted in the Western literature, where the ratio is varying (male to female 3:1 to 2:1) (61). This is also dependent on the etiology of CRF.

Symptomatic bone disease was seen over 75% of the patients . The commonest symptom was a vague and deep seated pain in the mid and lower back and legs, and proximal muscle weakness. The pain in the back and the legs seemed to be aggravated by sudden movement,

pressure or weight bearing. These findings were similar to those reported by Swao (59) who studied a similar population in which dialysis was not used as a modality of treatment. He described clinical renal osteodystrophy in 67.7% of the patients. This was defined as bony tenderness in the long bones, lumbar spine and pelvic bones with restriction of joint movements. Proximal myopathy which mainly involved the pelvic girdle was not disabling in majority of our patients. One patient had "penguin gait". This is different from the observation made in the Western population where crippling bone disease makes patients bedridden, and , very often this is due to osteomalacia and pathological fractures (29,30).

Pruritus was seen in over 50% of the patients in the present study. Relief following initiation of regular dialysis and the positive correlation between pruritus and iPTH are confirmed in this study (34,35). Several studies have reported relief from pruritus in 85 to 92% of the patients following parathyroidectomy (34,35). The reason for aggravation of pruritus on exposure to sunlight remains obscure.

Tenderness of large joints of both the upper and lower limbs, along with tenderness along the thoracolumbar spine, the ribs and long bones was found in many of our patients. Most earlier reports have shown similar involvment of the joints (27).
Growth retardation reported by some of the young patients could not be objectively demonstrated as most of the patients had no documented growth kinetics before and after they became uremic.It

has been reported that 30 to 50% of the children with "preterminal" renal failure have markedly reduced height and that the growth velocity remains subnormal in 65% of the children treated with dialysis (61,62). Appearance of puberty as well as closure of epiphysis may be delayed. This was evident in one patient in the present study (Figure 3,5). "Catch-up" growth has been reported in some children on treatment with 1,25DHCC (62). Caloric supplements have been shown to improve growth in some azotemic patients (32).

Parasthesias noted at some time during the course of renal failure may have been due to hypocalcemia, although some uremic toxins have been implicated (30,40). Motor and sensory neuropathy were not seen in our patients. The explanation for this is not obvious.

Skeletal deformities were observed in only 4 patients. One patient had signs of frank rickets. This patient was a 19 year old female on conservative treatment for CRF. Her height was 140cm (normal = 190cm). Her bone age was estimated to be 13 years(Figure 3). Studies reviewed in the Western literature have reported higher incidence of skeletal deformities with severe stunting of growth (33). It has been observed that adults on hemodialysis for 1-2 years may develop lumbar scoliosis, thoracic kyphosis and deformities of the thoracic cage, particularly in association with aluminium associated osteomalacia (19,67). The Western population seems to have a higher incidence of pathological fractures. Severe osteomalacia and osteitis fibrosa

are seen in the patients and are the main causes of crippling bone disease. The shorter life expectancy and therefore the short period of hemodialysis probably explain why the patients in this study have not developed severe osteodystrophy.

Radiological features of renal osteodystrophy were seen in only 7.5% of the patients. This low incidence of radiographic features could be attributed to two reasons: 1) use of standard radiograhic techniques, that is, use of standard films and standard screens; energy of x-rays in the range of 42-77 volts and current strength and time of exposure in the range of 3-200 mas (milliamperes x seconds), depending on the bones radiographed, and, 2) The patients do not survive long enough or progress too rapidly to show the changes. Swao (59) reported no radiographic findings in the patients with CRF. On the contrary, literature from the West shows that 65-70% of the patients about to start maintenance hemodialysis have radiographic features of osteitis fibrosa, osteomalacia or vascular calcifications (63). Meema et al routinely used fine grain films (Kodak, type M) for radiographs of the hand along with a six fold magnification with a hand lens. This was highly sensitive and subperiosteal resorption was picked up in 8% of the patients without magnification, improving to 29% with magnification (25,48). Mamography films used by Carr et al showed periosteal resorption in 6% of the patients and on magnification the resorption could be detected in 56% of the patients with bone disease. The 3 patients who showed radiographic changes were in the stage IV of osteodystrophy (Figures 2-7), thereby proving the highly

significant association between radiographic features and high serum iPTH and alkaline phosphatase levels. This observation shows that the radiographic changes appearing on standard x-ray films are associated with severe hyperparathyroidism and vice versa. Changes suggestive of osteomalacia like Loosers zone, periosteal neostosis and protrusio acetabuli were not seen in the present study. Rugger jersey spine, which is a manifestation of osteoporosis and osteosclerosis was seen in 2 patients. Histology and scintography are better in picking up early bone disease (63,64).

In this study using serum iPTH, Ca and inP the patients were grouped into various stages of renal osteodystrophy. The profile of serum Mg and serum alkaline phosphatase were studied after staging the patients. The biochemical parameters in stage I patients, that is, normal serum inP and Ca and high serum iPTH are also seen in early CRF, here retention of carboxyl terminal of iPTH, is spuriously suggestive of hyperparathyroidism which may or may not be present as the carboxyl terminal of iPTH is cleared by the glomeruli (47). Occasionally serum iPTH levels may be normal or low, in which case it is important to measure the serum Mg levels. Hypomagnesemia may cause hypoparathyroidism. In such circumstances histology is the most specific parameter for the diagnosis of the renal osteodystrophy, as even a slight fall in the GFR is known to produce histological changes. Woven osteoid is seen when GFR is more than 80 ml/min (68). The renal osteodystrophy in this stage is not biochemically apparent or conclusive. 40% of the patients in the present study had

biochemically inapparent renal osteodystrophy. Serum alkaline phosphatase and serum Mg levels were not significantly different from the control values, a finding well in keeping with earlier studies (44,45,46).

Stage II,III and IV have two serum parameters in common, that is, high serum iPTH and inP levels. These two parameters are therefore defined as the biochemically apparent changes in renal osteodystrophy. 60% of the patients in the present study showed biochemically apparent renal osteodystrophy. 40% of the patients were in stage III of the renal bone disease while 7.5% were in stage IV, an observation suggesting that many patients do not survive beyond stage III bone disease. This is unlike the Western studies where majority of the patients are in stage IV renal disease (17,20,60).

Apart from serum iPTH and inP, both serum alkaline phosphatase and serum Mg levels were raised in the patients with biochemically apparent renal osteodystrophy (table 11). There was no significant difference in the serum Ca levels between the patients and their controls, a finding, which reflects the mean of Ca levels seen in patients of stage II, III and IV renal bone disease. This finding is in contrast to the study by Swao who reported hypocalcemia in majority of the patients (59).

The significance of raised alkaline phosphatase as a diagnostic criterion is brought out in table 12, where, the biochemical parameters of the two groups of patients with renal bone disease are compared. The significantly raised serum alkaline phosphatase

in patients with biochemically apparent renal osteodystrophy may be important in the diagnosis of renal bone disease but only in the presence of raised inP and iPTH levels. It is known that uremic patients have significant renal bone disease despite normal alkaline phosphatase. The levels should be interpreted with caution as they may be raised in patients with liver disease (45). Studies in the West have shown that some patients with Al related osteomalacia have normal alkaline phosphatase levels (19).

In patients on dialysis, the levels have been noted to correlate with the area of osteoid surface covered with osteoblasts, the osteoclastic resorption surface, the number of osteoclasts and the percentage of osteoid seams (45,68). The present study has shown a positive correlation between serum alkaline phosphatase and the duration of the renal failure. This observation was noted in other studies where serial measurements of alkaline phosphatase over months proved to be useful in detecting the slow progression of skeletal disease in uremic patients (44,45). A slow rise in alkaline phosphatase with values that may be within the normal range is also characteristic of progressive bone disease (5).

The serum inP levels in this study were comparable with the controls in the early stage of renal bone disease. Patients with biochemically apparent osteodystrophy had significantly raised serum inP. Studies in the West have shown that serum inP are significantly influenced by diet, 1,25DHCC therapy, phosphate binders, catabolic state of the body and the degree of hyperparathyroidism. Levels may be high even in early renal

failure (7,38). On the other hand, serum inP levels have been known to be low or normal in some patients on dialysis despite the ingestion of a normal diet and without the use of phosphate binding gels (7,8,38). It is, therefore, important to measure the serum iPTH levels in conjunction with serum inP when the biochemical diagnosis of renal osteodystrophy is sought. The study done on a similar population (59), used raised inP with raised serum alkaline phosphatase to diagnose biochemical osteodystrophy. Renal bone disease was seen in 51.8% of the patients in that study. Although figure 10 shows, a positive correlation between inP and iPTH, the correlation was not statistically significant.

Serum Ca levels in the present study were comparable with the control population in the patients with and those without biochemically evident bone disease. The reason for this normocalcemia in the first group has already been discussed. Marked hypercalcemia was seen in the three patients who were in stage IV of renal osteodystrophy (Fig 8, Appendix I). Although they had severe hyperphosphatemia and very high iPTH levels they did not show any manifestations of extra skeletal calcification. There was a significant positive correlation between serum calcium and iPTH levels in the patients with biochemically apparent osteodystrophy. This could be explained on the basis of the transient hypercalcemia which is known to occur in some patients who ingest large amount of calcium lactate and vitamin D; many of our patients were on both of these drugs (5).It is also possible that dialysis in some of these patients rendered

failure (7,38). On the other hand, serum inP levels have been known to be low or normal in some patients on dialysis despite the ingestion of a normal diet and without the use of phosphate binding gels (7,8,38). It is, therefore, important to measure the serum iPTH levels in conjunction with serum inP when the biochemical diagnosis of renal osteodystrophy is sought. The study done on a similar population (59), used raised inP with raised serum alkaline phosphatase to diagnose biochemical osteodystrophy. Renal bone disease was seen in 51.8% of the patients in that study. Although figure 10 shows, a positive correlation between inP and iPTH, the correlation was not statistically significant.

Serum Ca levels in the present study were comparable with the control population in the patients with and those without biochemically evident bone disease. The reason for this normocalcemia in the first group has already been discussed. Marked hypercalcemia was seen in the three patients who were in stage IV of renal osteodystrophy (Fig 8, Appendix I). Although they had severe hyperphosphatemia and very high iPTH levels they did not show any manifestations of extra skeletal calcification. There was a significant positive correlation between serum calcium and iPTH levels in the patients with biochemically apparent osteodystrophy. This could be explained on the basis of the transient hypercalcemia which is known to occur in some patients who ingest large amount of calcium lactate and vitamin D; many of our patients were on both of these drugs (5).It is also possible that dialysis in some of these patients rendered

them susceptible to the calcemic action of PTH on bone. Serum Ca levels are highly variable in renal bone disease, being influenced by PTH profile, dietary Ca, Al accumulation, Ca in dialysate, mobility of the patients, use of thiazide diuretics and Ca cycle ion exchange resins and lastly renal transplantation (5,16,40,41,68). Hypocalcemia was reported in the study by Swao. It is quite possible that most of the patients in that study were in stage II renal osteodystrophy. In the West, majority of the patients show hypercalcemia. This is due to the fact that they are on long term dialysis and also taking therapautic doses of calcitriol and calcium carbonate (12,44,63).

Serum iPTH has been evaluated for the first time in our population. The normal levels in the controls were comparable with the Western population (56). All patients had raised iPTH levels. Patients in stages II, III nd IV had fairly high levels (table 11). The 3 patients who showed radiographic changes of osteodystrophy had mean serum iPTH levels of 890 $\pm$ 161 pg/ml. Serum Ca levels were 2.6 $\pm$ .9 mmols/l. Earlier studies have reported similar findings although, a large number of patients have been reported to have markedly raised iPTH in the range of "autonomous" secretion. Massive parathyroid hyperplasia was reported in such patients (16,41). In some patients, the high PTH levels have been known to fall when blood Ca is raised by a Ca infusion or during dialysis with a high Ca dialysate. Patients with non suppressible "autonomous" hyperparathyriodism may need parathyroidectemy to avoid the devastating complications of extra skeletal calcification (20,41). Pruritus, seen in 52.5% of the

patients in this study was associated with raised iPTH levels (table 14). Massry et al (35) speculate that increased Ca content of the skin is responsible for the pruritus. Hypercalcemia, per se, also has been implicated as well as uric acid (5) .

Serum Mg levels were comparable with the controls in patients with biochemically in apparent osteodystrophy. The levels were significantly raised in patients with biochemically apparent renal osteodystrophy. However, the levels were not significantly different between the two groups of patients. These findings show that Mg is retained in patients with CRF, but the raised serum Mg levels are not diagnostic of renal osteodystrophy. Most of the patients were ingesting phosphate binders which contained magnesium trisilicate. This could also explain the hypermagnesemia seen in the present setup. A highly significant positive correlation was realised between serum iPTH and serum Mg in the patients with biochemically apparent osteodystrophy (39,72). High Mg levels in the serum have been shown to increase the bone Mg content, which, in turn contribute to the abnormal mineralisation seen in renal osteodystrophy (20). Hanerer et al (72) have shown supression of PTH secretion following acute hypermagnesemia. There is no convincing data that long standing hypermagnesemia can effect PTH secretion. It has been speculated that the suppressive effect of hypermagnesemia on PTH secretion is offset by the stimulating effect of mild hypocalcemia (5). This probably explains the positive correlation between iPTH and Mg in the present study.

1,25DHCC is a calciotrophic hormone produced by functional renal tissue. The control population in this series showed serum 1,25DHCC levels comparable with the West, with no variation with age and sex (71). Most patients had reduced serum levels of 1,25DHCC. In some patients the levels were undetectable. Brickman et al (71) reported similar findings. Most patients, in my study, showed no radiological signs of osteomalacia. This observation has been reported in the Western literature where many patients lacked features of vitamin D deficiency (13,70). This has been attributed to the persistence of normal or elevated levels of serum inP and high levels of serum iPTH (9,10,11,12). In uremic patients osteomalacia has been shown to correlate best with serum 25,OHCC (73). There was a positive correation between 1,25DHCC and the duration of renal failure in the patients in this study This could be due to the fact that most of these patients who were also undergoing hemodialysis were on vitamin D supplementation (calciferol). It can also be attributed to the increasing levels of PTH which enhanced the activity of 1αhydroxylase (13).

There was a significant correlation between the signs and symptoms in the patients with biochemically apparent renal osteodystrophy. While 30% of the patients had clinical features suggestive of renal bone disease without any biochemically apparent changes, 10% of the patients with biochemically evident osteodystrophy were found to be symptomatic. This goes on to show that absence of clinical features does not rule out osteodystrophy. The presence of clinical features does not mean

that biochemical osteodystrophy is always present. The absence of both clinical features and biochemical evidence does not mean that osteodystrophy is not present. Very often histological changes are seen in the bones.

CONCLUSIONS AND RECOMMENDATIONS

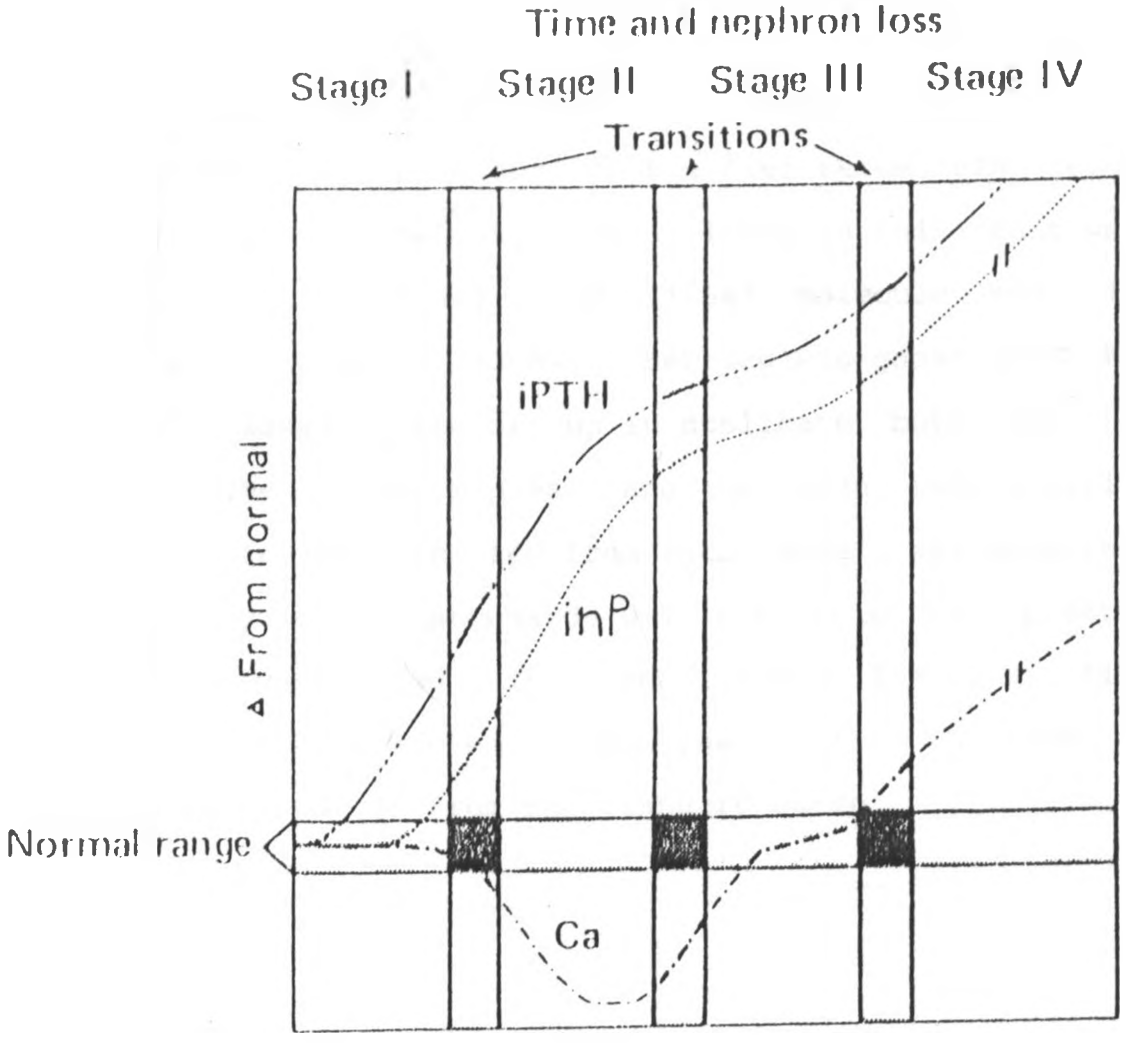
From the present study it becomes clear that renal osteodystrophy does occur with varying degrees of severity in patients with chronic renal failure. Because of hyperphosphatemia, iPTH secretion is stimulated to start with and becomes autonomous at a later date. In view of the fact that patients have not been exposed to long periods of chronic intermitant hemodialysis at the present moment, overt osteomalacia has not as yet been seen. Nevertheless, the problem should be anticipated. It is, therefore very important to control the divalent ion abnormalities early in renal failure, so that the complications of renal osteodystrophy can be prevented or controlled. Use of phosphate binders which do not contain Al or Mg is essential. Control of dialysate Ca and Mg concentrations should be implemented.

Assessment of nutritional status including measurement of height should be carried out at intervals when patients are on treatment, so that the growth kinetics can be monitored. The xray techniques need to be revised so that one is able to pick up the changes of renal bone disease by screening the patients regularly. It is important to do bone histomorphometric studies along with estimation of serum Al, iPTH using antiserum directed against N-aminoterminal, 25,OHCC and 1,25DHCC to enable one to know the etiopathogenesis of the type of bone lesion seen in our setup. It would be of great help in the treatment of patients with renal osteodystrophy where one can decide whether parathyroidectomy and/or supplementation with 1,25DHCC is

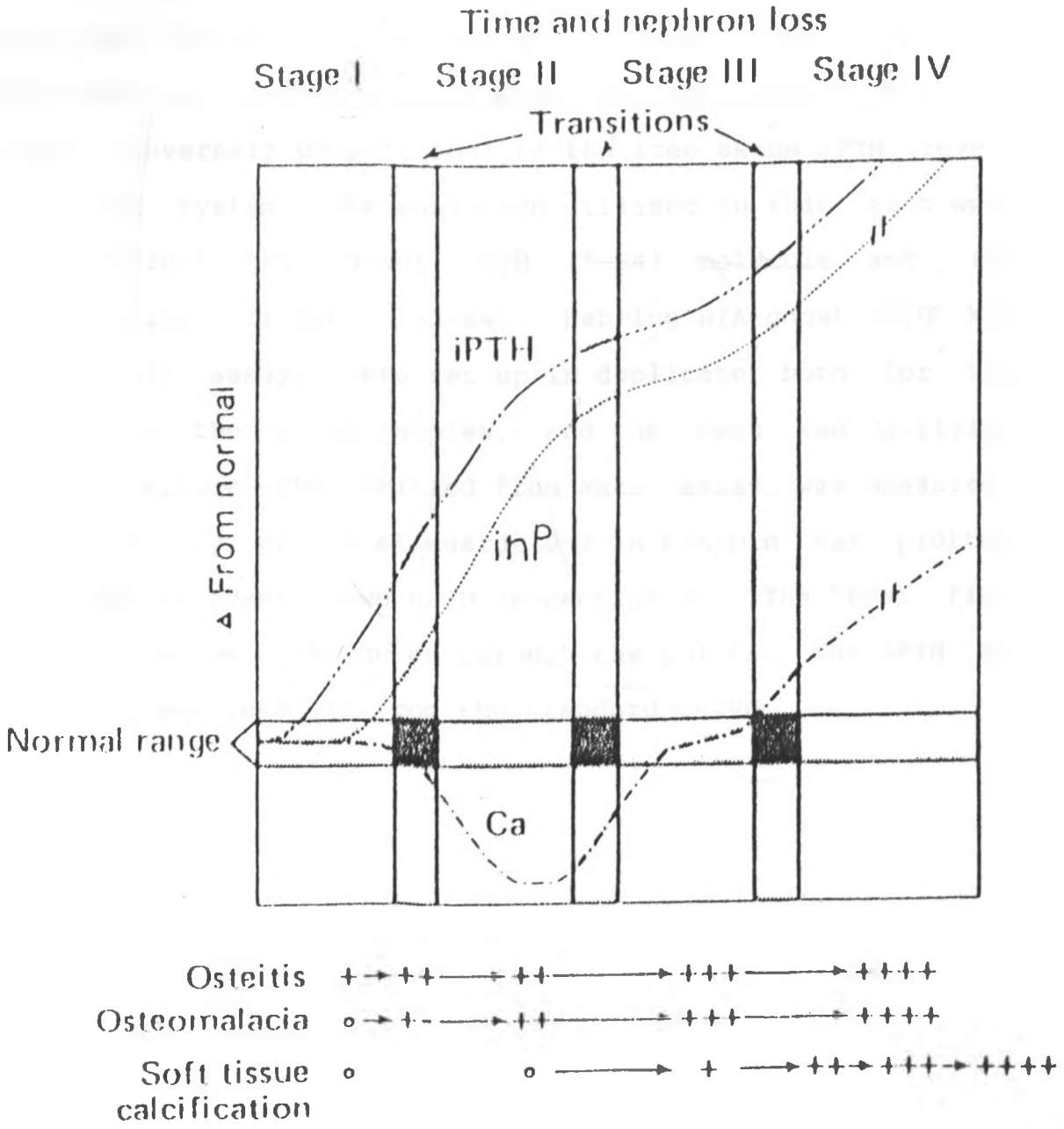
indicated, in addition to other modalities of treatment. The final goal is to correct the divalent ion abnormalities and give the patients a painless and ambulatory lifestyle.

indicated, in addition to other modalities of treatment. The final goal is to correct the divalent ion abnormalities and give the patients a painless and ambulatory lifestyle.

Appendix I: Scheme of staging renal osteodystrophy using biochemical and osseous abnormalities (adapted from Kidney Int., 7 - S102, 1975, Ref. 41).

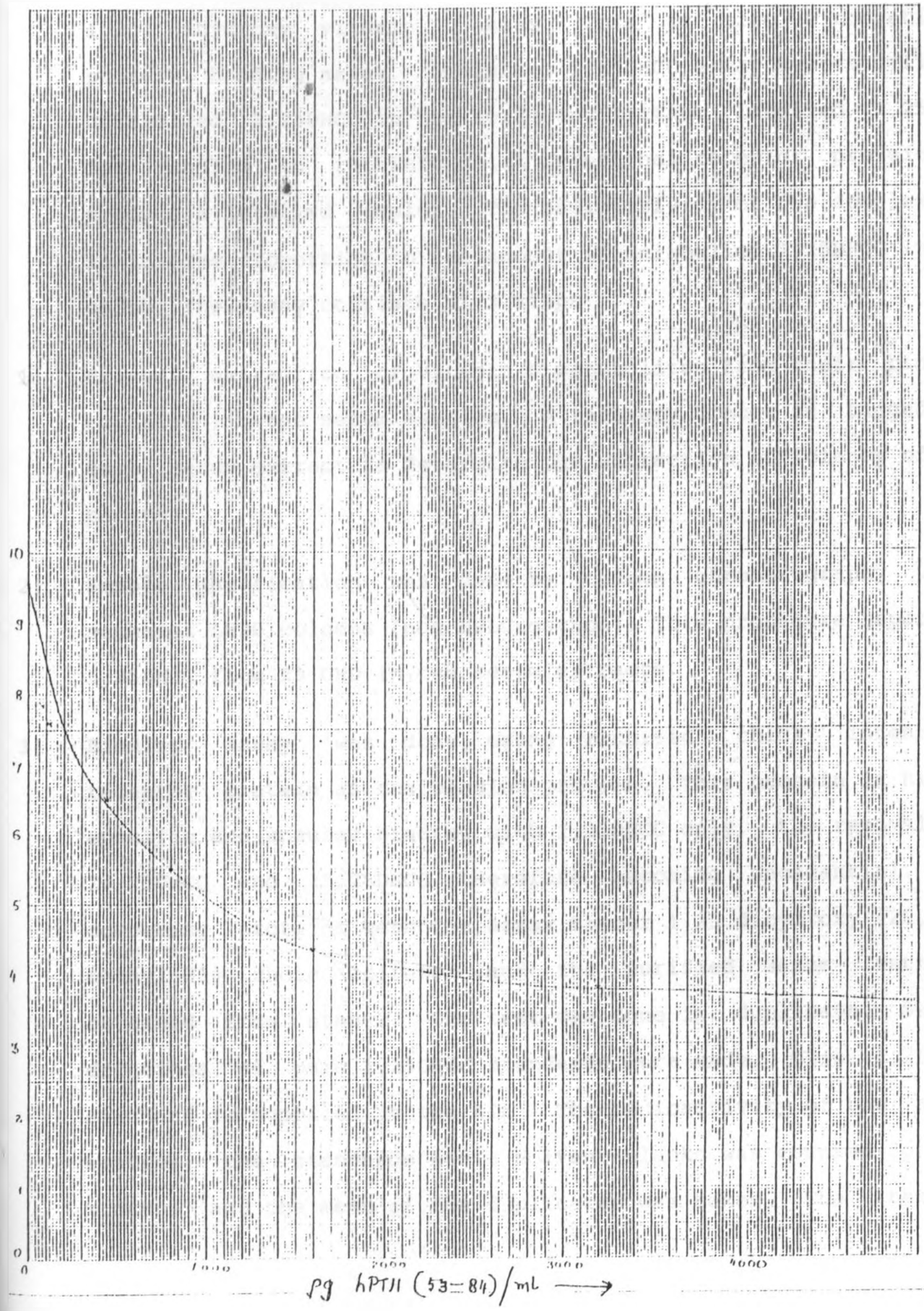


Appendix I: Scheme of staging renal osteodystrophy using biochemical and osseous abnormalities (adapted from Kidney Int., 7 - S102, 1975, Ref. 41).



APPENDIX II

Radioimmunoassay for human parathyroid hormone (RIA for hPTH). This method utilizes the principle of competitive protein binding (56). The serum iPTH and labelled hPTH , that is, 125 I-hPTH compete with antihPTH antibodies for the combining sites. The radioactivity of the antigen antibody complex formed is measured and this is inversely proportional to the free serum iPTH levels in the test system. The antiserum utilised in this test was directed against the intact hPTH (1-84) molecule and its carboxyl-terminal fragment (53-84). Behring RIA-gnost hPTH kit was used. All assays were set up in duplicate both for the standards and the serum samples, and the mean radioactivity (counts per minute- CPM) emitted from each assay was measured. The radioactivity of the standards 0-7 in CPM/min was plotted against the relevant known hPTH concentration. The "best fit" standard curve was constructed through the points. The iPTH in serum samples was read off from the standard curve.



Appendix II

Standard Curve for iPTH assay.

APPENDIX III

1,25 Dihydroxy vitamin D assay - This is also a competitive protein binding assay. Amershams 1,25DHCC assay system utilizes unlabelled 1,25DHCC in the serum and tritium labelled 1,25DHCC for binding to rachitic chick intestinal receptor protein to produce a labelled complex. The radioactivity of the complex is in inverse proportion to the amount of unlabelled 1,25DHCC in the serum. The assay consists of 3 steps.

1) Solvent extraction of Vitamin D metabolites from their binding proteins in the serum samples. A recovery tracer is tagged to each serum extract to determine the extraction efficiency of the process.

2) Sample purification was done by column chromatography using sephadex LH20 columns (non HPLC method). This procedure seperates 1,25DHCC from 25 OHD in the serum extracts.

3) Binding assay was done using rachitic chick intestinal receptor protein as the binding protein. This protein is highly specific and ensures binding with 1,25DHCC only, the removal of 24,25DHCC and 25,26DHCC therefore becoming unessential. The competing proteins were the assay tracer 1,25 dihydroxy (26,27 - methyl-H3) cholecalciferol and the purified serum extracts obtained from step 2. The test was done in duplicate. Serial dilutions of pure 1,25DHCC standards were made. This 1,25DHCC standard is supplied in the kit. The radioactivity of the assay systems, the standards and 2 solvent blanks was counted for 10 minutes and the mean of the duplicates was taken. The standard

curve was plotted using CPM bound on the y-axis and standard serial dilutions as pg/tube on the x-axis. The following formulae were used :-

1) $$\% \text{ recovery} = \frac{(\text{CPM} - \text{instrument blank}) \times 4 \times 100}{(\text{Mean total recovery} - \text{instrument blank})}$$

where

- % recovery is the radioactivity of tagged 1,25DHCC extracted from the serum sample, Instrument blank is the background radioactivity of the scintilation counter,
- Mean total recovery is the total activity of the recovery tracer. The radioactivity of each assay when read off from the standard curve gave the pg of 1,25DHCC per assay tube.

2) Corrected pg/tube = pg/tube - solvent blank

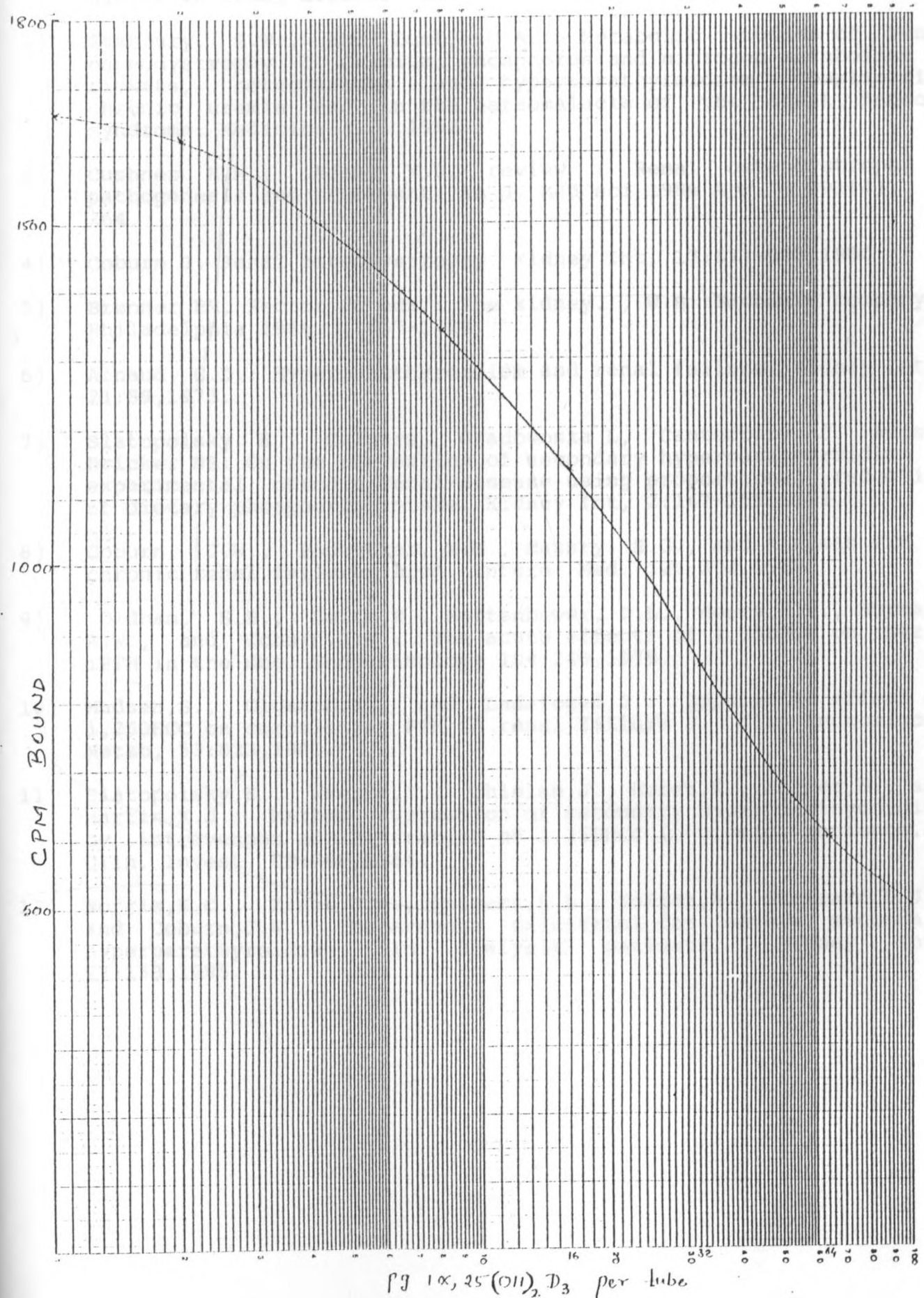
Where

- The corrected pg/tube is the actual concentration of 1,25DHCC per assay tube.
- The solvent blank is the radioactivity of the solvents used in the extraction, purification and assay systems and which gives a false rise in the actual 1,25DHCC levels.

3) $$\text{pg/ml of 1,25DHCC} = \frac{\text{Corrected pg/tube} \times 200}{\% \text{ recovery}}$$

Appendix III:

Standard curve for assay of 1, 25DIHCC.



REFERENCES

1) Pappenheimer, A.M., and Wilen S,S.l.: Enlargement of parathyroid glands in renal disease. Am. J. Pathol. 11:73,1935.

2) Stanbury, S.W., and Lumb, G.A.: Metabolic studies of renal osteodystrophy. I. Calcium, phosphorus and nitrogen metabolism in rickets, osteomalacia and hyperparathyroidism complicating chronic uremia and in the osteomalacia of the adult fanconi syndrome. Medicine 41:1,1962.

3) Cushner H.M., Adams N.D. review : Renal osteodystrophy - pathogenesis and treatment. Am J. Med sci 1986 Apr. : 291(4), 264.

4) Coburn J. Renal osteodystrophy. Kidney Int. 17:677-693.1980.

5) Brenner BM, Rector JC eds : The kidney. , W.B. Saunders company, Philadelphia 1986. ,1657-1729.

6) Arnaud, C.D.: Hyperparathyroidism and renal failure. Kidney Int 21:89,1973.

7) Slatopolsky E , Calgars, Gradowoska L, Canterbury J. Reiss, Bricker NS: On the prevention of secondary hyperparathyroidism in experimental chronic renal disease using proportional reduction of dietary phosphorus intake. Kidney Int, 2:147-151,1972.

8) Coburn, J.W., Popovtzer, M.M., Massry, S.G., and Kleeman PTH in chronic renal failure. Arch. Intern. Med. 124:302,1969.

9) Oldham, S.B., Smith,R., Hartenbower, D.L., Henry,H.L., Norman, A.W., and Coburn,J.W.: The acute effects of 1,25DHCC on serum iPTH in the dog. Endocrinology 104:248,1979.

10) Madsen,S., Olgaard,K., and Ladefoged,J.: Suppresive effect of 1,25DHCC on circulating PTH in renal failure J. Clin Endocrinol. Metab, 53:823,1981.

11) Slatopolsky,E., Weerts,C., Thielan,J., Horst,R., Harter,H., and Martin,K.J.: Marked supression of secondary hyperparathydoidism by intravenous administration of 1,25DHCC in uremic patients.J. Clin. Invest. 00:000,1984.

12) Norris,K.C., Kraut,J.A., Andress,D.L., Koffer,A., Sherrard,D.J., and Coburn,J.W.: Intravenous calcitriol for severe secondary hyperparathyroidism in dialysis patients. Kidney Int. 27:158,1985.

13) Bordier,P.J., Tun-chot,S., Eastwood,J.B., Fournier,A., and DeWardner,H.E.: Lack of histological evidence of vitamin D abnormality in the bones of anephric patients. Clin Sci 44:33,1973.

14) Massry,S.G., Coburn,J., Lee,D,B., Jowsey,J., Kleeman,C,R.: Skeletal resistance to PTH in CRF. Ann Int Med 78:357,1973.

15) Galceron,T., Martin,K.J., Morrissey,J.J, and Slatopolsky,E.: Role of 1,25-dihyroxy vitamin D on Skeletal resistance to PTH. Kidney Int. 32 ,801,1987.

16) Brown.M., Wilson,R.E., Eastman,R.C., Pallota,J., and Marynick, S.P.: Abnormal regulation of PTH release by calcium in secondary hyperparathyroidism due to CRF. J.Clin. Encocrinol. Metab 54:172,1982.

17) Parkinson,I.S., Feest,T.G., Ward,M.K., Fawcett,R.W., Kerr,D.N.: Fracturing dialysis osteodystrophy and dialysis encephalopathy: An epidemiological survey. Lancet 1: 506,1979.

18) Kaehny,W.D., Hegg,A.P., Alfrey,A.C.: Gastrointestinal absorption of Al from Al containing antacids N Engl J Med 296:1389,1977.

19) George D. Smith, Robin J. Winney, Alexander Mclean & James S Robson: Aluminium- related osteomalacia: Response to reverse osmosis water treatment. Kidney International 32: 96,(1987)

20) Alfrey ,A.C., Solmon,C.C., Circillo,J., & Miller.N,L.: Extraosseus calcification. Evidence for abnormal pyrophosphate metabolism in uremia. J clin Invest, 57: 692,1976.

21) Broyer,M.: Growth in children with renal insufficiency. Pediatr clin. North Am 29(4) 991,(1982).

22) Mehls O, Ritz E, Burkhard K., Gill G., Link W., Willich E., Scharer K.: Slipped epiphysis in renal osteodystrophy. Archives of disease of children 50:545-554. 1975.

23) Muspratt S. : Thoracic deformity and flail chest in renal osteodystrophy. J Am Med Assoc 243: 1458, 1980.

24) Chesney RW., Moorthy AV., Eisman JA., Jax DK, Mazess RB., De Luca HF.: Increased growth after long-term 1,25DHCC in childhood renal osteodystrophy. New Engl J. Med 298: 238-242. 1978.

25) Meema H E., Rabinovch S., Meema S., Llyord GJ., Oreopoulos DG.: Improved radiological diagnosis of azotemic renal osteodystrophy Radiology. 102:1, 1972.

26) Ritz E, Prager P, Krempien B, Bommer J, Malluche HH, Schmidtgayk H.: Skeletal X-ray findings and bone histology in patients on hemodialysis. Kidney Int. 13:316, 1978.

27) Simpson W, Ellis HA, Kerr Dn, McELROY M, McNARY RA, Peart K.: Bone disease in long term hemodialysis: the association of radiological with histological abnormalities. British J radiol. 49:105, 1976.

28) Sundaram M, Joyce PF, Shields JV, Riaz MA, Sagar S.: Terminal phalangeal tufts; earliest site of renal osteodystrophy findings in hemodialysis patients. Am J Roent 133: 25, 1979.

29) Baker L, Ackrill P, Cattell W, Stamp T, Watson I.: Iatrogenic osteomalacia and myopathy due to phosphate depletion.Brit. Med J 3:150, 1974.

30) Mallette L, Pattern BM and Engel WK.: Neuromuscular disease in secondary hyperparathyroidism. Ann of Intern Med 82:474, 1975.

31) Matthews C, Heimberg K, Ritz E, Agostine S, Fritzsche J, Hasselback W.: Effect of 1,25DHCC on impaired calcium transport by the sarcoplasmic reticulum in experimental uremia. Kidney Int 11: 227, 1977.

32) Simmons J. Wilson CJ, Potter DE, Holliday MA.: Relation of calorie deficiency to growth failure in children on hemodialysis and growth response to calorie supplementation. N Engl J Med 285:653, 1971.

33) Johannsen A, Nielsen HE, Hansen HE.: Bone maturation in children with chronic renal failure. Effects of 1 hydroxyvitamin D3 and renal transplantation. Acta radiol diag 20:193, 1979.

34) Hampers CL, Katz AJ, Wilson RE, Merrill JP.: Disappearance of uremic itching after subtotal parathyroidectomy. New Engl J Med 279:695, 1968.

35) Massry SG, Popvtzer MM, Cobur JW, Makoff Dl, Maxwell MH, Kleeman CR.: Intractable pruritus as a manifestation of secondary hyperthyroidism in uremia, disapearance of itching following subtotal parathyroidectomy. New Engl J Med 279:697, 1968.

36) Gipstein Rh, Coburn JW, Adam DA, Lee DBN, parsa KP, Sellers A, Suki W, Massry SG.: Calciphylaxis in man; a syndrome of tissue necrosis and vascular calcification in 11 patients with chronic renal disease. Arch Inter Med. 136:1273, 1976.

37) Schwatrz KV.: Heart block in renal failure and hypercalcemia. J Am Med Assoc letters, 235:1550. 1976.

38) Slatopolsky E and Bricker N.: Role of phosphorus restriction in prevention of secondary hyperparathyroidism in chronic renal disease. Kidney Int. 4:141, 1973.

39) Coburn J, Popovtzer M. Massry SG, Kleeman CR.: The physiochemical state and renal handling of diavalent ions in chronic renal failure. Arch Intern Med 124:302, 1969.

40) Malluche HH, Werner E, Ritz E.: Intestinal absorption of calcium and whole body calcium retention in incipient and advanced renal failure. Mineral and electrolyte metabolism 1:263, 1978.

41) Bordier, P,J; Marie ,P.J; and Claude D. Arnaud: Evolution of renal osteodystrophy: Correlation of bone histomorphometry and serum mineral and immunoreactive parathyroid hormone values before and after treatment with calcium carbonate or 25,OHCC. Kidney Int : 7:s102, 1975

42) Anast OA, Mohs JM, Kaplan SL, Burns TW.: Effect of parathyroid failure in magnesium deficiency. Science 177: 606-608. 1972.

43) Estep J, Shaw W, Watlington CO, Hobe C, Holland W, Tucker H.: Hypocalcemia due to reversible hypomagnesemia and parathyroid hormone unresponsiveness. J of clin Endocrinol. 29:842, 1979.

44) Alvarez UDE F, Feest RG, Ward MK, Pierides AM, Ellise HA, Peart Km, Simpson W, Weightman D and Her D.S.: Hemodialysis and bone disease correlation between clinical histological and other findings. Kidney Int 14:68, 1978.

45) Pierides AM, Skillen AW, Ellis HA.: Serum alkaline phosphatase in isotemic and hemodialysis patients with renal osteodystrophy : a study of isoenzyme patterns , their correlation with bone histology, and their changes in response to treatment with 1,hydroxy D3 and 1,25DHCC. J of Lab Clin Med 93:899, 1979.

46) Hodsman AB, Sherrard DJ, Wong EG, Brickman AS, Lee DBM Alfrey A, Singer FR, Norman AW, Coburn JW.: Vitamin D resistant osteomalacia in hemodialysis patients lacking secondary hyperparathyroidism. Ann Intern Med 94:629, 1981.

47) Hawker D, DE Bella FP.: Parathyroid hormone in chronic renal failure; studies with two different parathyroid hormone immunoassays. Contributions to Nephrol 20:2137. 1980.

48) Meema HE and Meema S.: Micro radioscopic quantitation of subperiosteal resorption in secondary hyperparathyriodism of chronic renal failure. Clin Ortho. 130:297, 1987. Comparision of microradioscopic and morphometric findings in the hand bones with densitometric findings in the proximal radius in thyrotoxicosis and in renal osteodystrophy. Invest radiol 7:88,1972.

49) Ellis KJ and Hochstin RJ.: The skull in hyperparathyroid bone disease. AM J Roent. 83:732,1960.

50) Meema HE & Meema S.: Improved radiological diagnosis of osteomalacia by microradioscopy of hands and bones. AM J Roent. 125:925,1975.

51) Baginsky E.S et al: Manual determination of calcium by O-Cresolphthalein complexone method. Clin chem. Acta 1973 (46) 49.

52) Alfrey, A.C; Miller, N.L and Butkus, D: Evaluation of body Mg stones. J. Lab clin Med : 84:153,1974

53) Varley H., Gowenlock, A.H, and Bell M.: Determination of inorganic phosphorous. Practical chemical biochemistry Vol 1 PP 88, Wilham Heinmann Medical Books London, 1980.

54) Doumas, B.T., Watson, W.A, and Biggs, H.G: Albumin standards and the measurments of serum albumin by Bromocresol Green. Clin Chem. Acta 31: 87, 1971.

55) Bowers, J.N. and McComb, R.B.: Determination of alkaline phosphatase activity. Clin. Chem 75, 21;1988.

56) Reiss E, Canterbury J, Egdahl R.: Experience with radioimmuno assay of parathyroid hormone in human sera. Trans Assoc Am Physic. 81:104, 1968.

57) Eisman JA, Hamstra AJ, Kream BE and De Luca HF.: 1,25DHCC in biological fliuds: a simplified and sensitive assay. Sci 193:1021, 1976.

58) Tausky, H.H.: Manual determination of creatinine. J. Biol Chem 208;853, 1954.

59) Swao JH.: Renal osteodystrophy as seen at KNH. M. Med Dissertation 1984.

60) Dawborn JK, Brown DJ and Douglas MC, Eddy HH, Heale WF, Thomas DP and Xipell JM : Parathyroidectomy in chronic renal failure. Nephron 33,100,1983.

61) Scharer K.: Growth in children with CRF. Kidney Int 13 : S,68,1978.

62) Chessney RW, Deluca HF, Hamstra A.: Influence of long term 1,25DHCC in children with renal osteodystrophy. N Engl J Med ,298:238,1978

63) Memos DE, Eastwood JB, Talner LV, Gower PE, Curtis JR, Alaghband Zadeh J, DeWardner HC.: Double blind trial of oral 1,25DHCC versus placebo in asymptomatic hyperparathyroid patients receiving maintenance hemodialysis. Brit.Med J . 282, 1919, 1981.

64) Olgaard K, Hearfort J, Madson S.: Scintographic skeletal changes in uremic patients on regular hemodialysis. Nephron 17,329,1976.

65) Hruska KA, Koelman R, Rutherford W, Klaur S, Slatopolsky E.: Metabolism of immunoreactive parathyroid hormone in the dog. The role of kidney and the effects of chronic renal disease. J Clin Invest 56,39, 1975.

66) Ingham JP, Kleerekoper N, Stewart SH.: Symptomatic skeletal disease in non terminal renal failure. Med J Aust 1:873,1974.

67) Hodsman Ab, Sherrard Dj, Wong EGC, Brickman As, Lee DBN, Alrey AC, Singer FR, Norman AW, Coburn JW.: Vitamin D resistant osteomalacia in hemodialysis patients lacking secondary hyperparathyroidism. Ann Intern Med. 94:629 1981.

68) Malluche HH, Ritz E, Lange PH, Kutschera J, Hodgsen M, Seiffert U, and Schoeppe W.: Bone histology in incipient and advanced renal failure. Kidney Int, 9:355,1976

69) Teitelbaum SL: Renal osteodystrophy. Human Pathology 15:4,306,1984.

70) Slatopolsky E, Gray R, Adams ND, Lewis J, Hruska K, Matrin K, Klaur S, De Luca H, Lemann J.: Pathogenesis of secondary hyperparathyroidism in early renal failure in Morman AW, Schaeffer K, Hewath DR, Gringoleit HG, Coburn JW, De Luca HF.: Vitamin D. Basic research and its clinical application. Berlin Watter De Grufter :32:1209,1979.

71) Brickman AS, Coburn JW, Massry SG.: 1,25DHCC in normal man and patients with chronic renal failure. Ann Intern Med 80:161, 1974.

72) Habener JF, and Potts J.T Jr.: Relative effectiveness of Mg and Ca on secretion and biosynthesis of PTH in vitro: Endocrinology 98: 197,1976.

73) Eastwood JB, Harris E, Stamp TCB, Dewardner, Bordier PJ, and Arnaud CD.: The effect of 25 hydroxy vitamin D3 in osteomalacia of chronic renal failure. Sci. Mol. Med. 52:499,1977.

****