Research Article

Study of biochemical parameters in metabolic bone disease with chronic renal failure

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ABSTRACT

Background: Renal osteodystrophy and metabolic bone disease are terms that encompass a number of skeletal abnormalities, including osteomalasia, osteitis fibrosa cystica and impaired bone growth in children. The proposed work aimed to evaluate the status of bone health by estimating levels of elements like calcium, phosphorus, magnesium, alkaline phosphatase and alkaline phosphatase isoenzymes in serum of patients suffering from metabolic bone disease with chronic renal failure.
Methods: The routine biochemical investigations were estimated by known standard methods and alkaline phosphatase isoenzymes were done by polyacrylamide slab gel electrophoresis. The assay values were statistically validated.
Results: Serum calcium levels were significantly decreased (p<0.001) in chronic renal failure cases than control whereas, phosphorus and serum alkaline phosphatase levels were significantly increased. Electrophoresis on polyacrylamide gel separated the serum alkaline phosphatase isoenzymes. In control group, two bands of isoenzymes appeared. Whereas, in patients with metabolic bone disease with chronic renal failure, only one diffused band appeared.
Conclusions: Decreased calcium levels and increased phosphorus levels in serum indicate metabolic bone disease with chronic renal failure. Elevation of serum alkaline phosphatase is a marker of sick bone. Increased levels of serum alkaline phosphatase are responsible for appearance of diffused band of isoenzyme which is marker for metabolic bone disease with chronic renal failure.

Keywords: Metabolic bone disease, Chronic renal failure, Isoenzymes, Alkaline phosphatase

INTRODUCTION

Renal osteodystrophy and metabolic bone disease are terms that encompass a number of skeletal abnormalities, including osteomalasia, osteitis fibrosa cystica and in children impaired bone growth. Osteodystrophy is more frequent in younger patients and in those with the lower plasma levels of calcium and phosphorus. In addition, there may be a primary defect in intestinal calcium absorption. Renal bone disease which develops in patients with chronic renal failure (CRF) is not a uniform metabolic disorder.[1] Numerous biochemical markers have been developed to measure bone formation and resorption.

Serum or plasma levels of calcium, phosphorus, alkaline phosphatase, parathyroid hormone, calcitonin and tartrate resistant acid phosphatase are measured to evaluate hyperparathyroidism and metabolic bone disease.[2] Plasma bone specific alkaline phosphatase (bAP) has been demonstrated to be more reliable than total alkaline phophatase (tAP) in providing information about bone

turnover in patients with metabolic bone diseases. Because of the suggestion that alkaline phosphatase was elevated in the serum of patients with chronic renal failure, Alpers DH et al found that the kidney is the likely source of the observed increase in serum intestinal type phosphatase activity noted in patients with chronic renal failure.[3]

An elevation in the intestinal isoenzyme rather than the presence of early metabolic bone disease or hepatic disease should be considered in renal failure patients with mildly elevated total serum alkaline phosphatase.

Bone diseases develop relatively early in the development of chronic renal failure. Alkaline phosphatase (Alps) originating from different organs frequently detected in the serum and urine of patients with renal failure.[4]

Separating ALP into its isoenzymes adds considerable value necessary for total ALP activity.[5] The determination of alkaline phosphatase bone isoenzyme activity in serum is sensitive and reliable diagnostic tool in assessing the beginning and degree of metabolic bone disease.[6]

The aim of this study was to evaluate the status of bone health and to establish the relevance of bone specific alkaline phosphatase isoenzymes by carrying out routine biochemical investigations like serum calcium, phosphorus, magnesium, alkaline phosphatase in patients with MBD-CRF.

METHODS

Institutional ethics committee approval was taken before conducting this study. Written informed consent was taken from all participants. Blood samples were collected from the patients admitted to the hospital suffering from metabolic bone disease with chronic renal failure. The patients had no history of steroid exposure or any agents that suppress parathyroid hormone production.

30 blood samples each of control group and patients suffering from metabolic bone disease with chronic renal failure were collected in plain bulb belonging to age groups 21-60 years.

Blood samples were allowed to clot at room temperature and centrifuged at 3000rpm for 10 minutes. The serum was separated and kept at -20°C till analysed. All chemicals used were of analytical grade. Each parameter was standardized. Serum calcium was estimated by the method of Trinder.[7]

Serum phosphorus was estimated by the method of Fiske and Subbarow.[8] Serum magnesium was estimated by the method of Titan Yellow (Neill and Neely).[9] Alkaline phosphatase was estimated by the method of Kind PRN and King EJ.[10] Alkaline phosphatase isoenzymes were separated by the method of Smith et al modified. The statistical analysis was done by measuring mean standard deviation and 't' test.

RESULTS

Serum calcium levels were significantly decreased ($P \leq 0.001$) in metabolic bone disease with CRF as compared to control subjects, while phosphorus and alkaline phosphatase levels were increased significantly ($P \leq 0.001$) than control subjects. The serum magnesium levels were decreased non-significantly ($P \geq 0.05$) than control subjects.

Table 1: Biochemical parameters in metabolic bone disease in chronic renal failure (age group: 21-60).

Parameters	Control (Mean±S.D) n=30 (Mean:S.E)	Metabolic bone disease with CRF (Mean±S.D), n=30 Mean 1 SE	't' Value
Calcium (mg/dl)	9.84 ±0.574	6.86±0.473	20.41 ***
Phosphorus (mg/dl)	3.70±0.384	6.573 ±0.422	25.65 ***
Magnesium (mg/dl)	2.145 ±0.271	2.108±0.232	0.524 NS
Alkaline Phosphatase (K.A.Units)	7.98±2.72	11.82±2.37	5.95 ***

*** (P< 0.001); NS = Not significant.

Various methods are available to identify the isoenzymes of alkaline phosphatase. Polyacrylamide slab gel electrophoresis is the necessary means to separate various isoenzymes of alkaline phosphatase. The mobilities of the isoenzymes are as shown in figures for control subjects and patients.

Figure 1: shows the isoenzymes of alkaline phosphatase in control subjects. There were two bands present in the control group. Bone and liver isoenzymes are always present, one was originated from bone and the other was originated from liver.

Figure 2 shows the isoenzymes of alkaline phosphatase in metabolic bone disease in chronic renal failure. There was appearance of only one band and that was diffuse as compared to control. The diffuse band appeared in all patients and this band was originated from the bone.
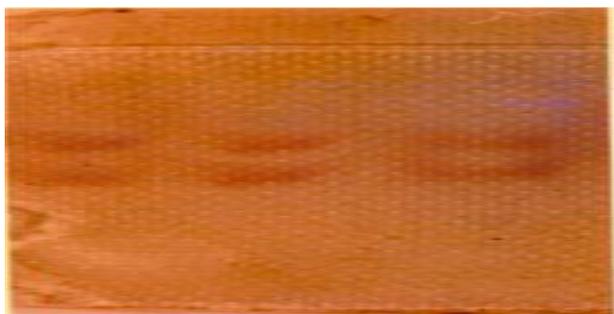

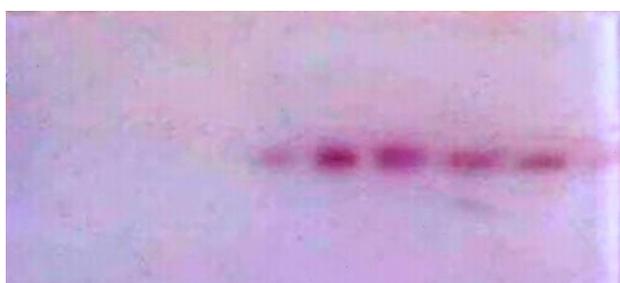**Figure 1: Isoenzymes of alkaline phosphatase in control subjects.**

Figure 2: Isoenzymes of alkaline phosphatase in metabolic bone disease in chronic renal failure.

DISCUSSION

In present study calcium levels were significantly decreased in MBD-CRF as compared to control subjects, whereas alkaline phosphatase and phosphorus levels were increased significantly than control subjects. There were no significant changes in magnesium levels in CRF as compared to control. In CRF, the serum calcium may decrease in part because of increased losses into the urine.

Decreased renal excretion of phosphorus is the most common cause of hyperphosphatemia. Osteomalacia is common in patients with CRF; it tends to be the predominant type of renal osteodystrophy in younger patients and is more frequent in those with the lower plasma levels of calcium and phosphorus. In addition, there may be a primary defect in intestinal calcium absorption.

Previous study had reported the measurement of PTH, phosphate, calcium, alkaline phosphatase, osteocalcin and creatinine in serum[1]. Several experiments were conducted that support many mechanisms. Retention of phosphates and declining renal function suppress the synthesis of 1, 25 dihydroxy cholecalcipherol. Experimental evidence in patients with moderate renal failure demonstrates that serum 1, 25 DHCC levels decline before serum calcium decreases and this decrease correlates inversely with the increase in PTH levels.

The demonstration that intravenous calcitriol suppresses PTH independent of its effect on serum calcium level, appears to provide the proof that calcitriol is a direct inhibitor of PTH secretion.[12]

For many years it was thought Barker's "Trade off Hypothesis", adequately explained the PTH changes in renal failure. According to this theory as renal failure advances the level of serum phosphate increases, this lowers serum calcium levels, which stimulates PTH secretion. The latter normalizes serum calcium levels by acting on the bone and brings serum phosphorus levels towards normal by promoting phosphate excretion.[13]

Renal excretion is the major root of magnesium elimination from the body and a positive magnesium balance would be expected under condition of renal insufficiency.[14]

While magnesium intoxication is a real hazard when magnesium containing drugs are given, magnesium balance may be normal or even decreased in uremic patients. This is usually due to decreased dietary intake combined with an impaired intestinal magnesium absorption which characterizes CRF. Our results (Table I) are in agreement with above findings.

In our study we found significantly increased serum ALP levels as compared to control subjects. The increase in serum ALP activity may be derived from the injury to the brush border membrane of the renal tubular cells. Serum ALP may be a marker for involvement of the kidneys in pathological process.[15]

Elevation of ALP activity in the serum may be the first sign of disease involving skeletal systems. The isoenzymes could be separated and the isoenzymes responsible for serum elevation could be identified. The most prominent band was identified as the major isoenzyme. Band was diffused and appeared most frequently in patients with MBD-CRF. ALP is a marker of sick bone. To monitor ALP measurements in patients with MBD-CRF and bring it to normal, vit D metabolites or analogous to calcitonin and bisphosphonates were given as bone strengthening agents which are useful for management of renal bone disease and prevention of bone resorption.

CONCLUSION

Decreased calcium levels and increased phosphorus levels in serum indicate MBD-CRF. Elevation of serum ALP is a marker of sick bone. Appearance of diffused band of serum ALP isoenzymes, which is bone originated is indicative of MBD-CRF.

REFERENCES

1. Franke-S, Lehmann-G, Abendroth-K, Hein-G, Stein-G. PICP as bone formation and NTx as bone resorption marker in patients with chronic renal failure. Eur J Med Res. 1998;3(1-2):81-8.
2. Morion-K, Koide-K. Secondary hyperparathyroidism and tertiary hyperparathyroidism in chronic renal failure, uremia. Nippon- Rinsho. 1995;53(4):958-64.
3. Alper S-DH, DeSchryver- Kecskemeti-K, Goodwin-CL, Tindira-CA, Harter-H, Slatopolsky-E. Intestinal alkaline phosphatase in patients with chronic renal failure. Gastroenterol. 1988;94(1):62-7.
4. Tsumura M, Ueno Y, Kinouchi T, Koyama I, Komoda T. Atypical alkaline phosphatase isoenzymes in serum and urine of patients with renal failure. Clin Chim Acta. 2001;312(1-2):169-78.
5. Meema HE, Oreopoulos DG, Rapoport A. Serum magnesium level and arterial calcification in end stage renal disease. Kidney Int. 1987;32(3):388-94.
6. Lindeman RD. Chronic renal failure and magnesium metabolism. Magnesium. 1986;5(5-6):293-300.
7. Trinder P. Colorimetric micro-determination of calcium in serum. Analyst. 1960;85:889-94.
8. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem. 1925;66:375.
9. Neill DW, Neely RA. The estimation of magnesium in serum by titan yellow. Clin Path. 1956;(9):162.
10. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol. 1954;7(4):322-6.
11. Smith I, Lightstone PJ, Perry JD. Separation of human tissues alkaline phosphatases by electrophoresis on acrylamide disc gels. Clin Chim Acta. 1968;(19):499-505.
12. Slatopolsky E, Weerts C, Thielan J, Horst R, Harter H, Martin KJ. Marked suppression of secondary hyperparathyroidism by intravenous administration of 1,25 DHCC in uremic patients. J Clin Invest. 1984;74:2136-43.
13. Bricker NS, Slatopolsky E, Reiss E, Avioli LV. Calcium, phosphorus and bone in renal disease and transplantation. Arch Intern Med. 1969;123:543-53.
14. Mountokalokis TD. Magnesium metabolism in chronic renal failure. Magnes Res. 1990;3(2):121-7.
15. Leibovitch I, Ben-Chaim J, Ramon J, Goldwasser B. Increased serum alkaline phosphatase activity: a possible indicator of renal damage. J Clin Lab Anal. 1991;5(6):406-9.

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