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Evaluation of Bone Resorption Markers in Leprosy

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Abstract

Background: Leprosy is among the world's oldest and most dreaded diseases and it has been synonymous with stigma and discrimination due to the hideous deformities it produced. Bone involvement in leprosy is one of the causes of deformity and disability. There are conflicting results on association of bone resorption (BP) in various forms of leprosy. Hence the aim of the study was to evaluate bone resorption markers in leprosy patients and to compare with healthy controls and to find out whether these findings were related to various forms of leprosy, its duration and bacterial load.

Methods: Seventy newly diagnosed, untreated leprosy patients were selected as cases and compared with sixty age and sex matched healthy controls. Bone resorption was studied by measuring fasting urinary calcium / creatinine level. Bone resorption may be associated with new bone formation. So, Serum Alkaline phosphatase (ALP) activity was estimated as a marker of bone formation.

Results: There was a significant increase in the serum ALP activity in cases when compared to controls and it was associated with increased duration, bacterial load and multibacillary type of leprosy. There was a significant increase in the level of Urinary calcium/creatinine in cases when compared to controls and it was not associated with disease parameters.

Conclusion: Our data supports that there is an increased bone resorption in leprosy. Therapy aimed at reducing bone loss may benefit leprosy patients with high bone turnover.

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Keywords: Bone resorption (BR), Alkaline phosphatase, Urinary calcium/creatinine(Ca/Cr), Multibacillary (MB), Paucibacillary (PB).

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1. INTRODUCTION

Leprosy is a chronic granulomatous disease caused by Mycobacterium leprae (M. leprae). M. leprae was discovered by Hansen in 1873, the first bacterium to be identified as causing disease in humans[1]. It is believed to be transmitted via droplets from the nose and mouth, through close contact with a person affected by the disease who has not received treatment. Leprosy primarily affects the skin, the nasal tissues, the bones and the peripheral nerves, and can damage the eyes and testes. The response to this disease is highly variable, and the immune status of each infected person determines the type and severity of pathological change[2].

Although leprosy has been eliminated from many areas of the world, its prevalence is still high in endemic areas. New cases continue to occur in almost all endemic countries and high-burden pockets exist against a low-burden background. India records the highest number of new leprosy cases in the world. The number of new cases detected during 2011, as reported by 105 countries to WHO was 219,075. India contributes to a maximum of 127,295 new cases registered worldwide. New cases with grade 2 deformities have still not changed significantly over the years. Among the 127,295 new cases in India, 63,562 are MB leprosy and about 3,834 people have suffered from grade-2 disabilities at the time of detection[3].

Leprosy continues to be a health problem due to its ability to cause disability and permanent deformities. Approximately three million people are disabled by the disease worldwide.[4] It has been estimated that in 2015, there will be nearly 5,00,000 people living in India with grade2 disability due to leprosy[5]. Bone disease is one of the principal prognostic factors in leprosy .The frequency of the bone changes which has been recorded in different studies has varied from 15% to 95% [4]. Leprous bone lesions mainly affect the hands and feet. The disease also involves bones of the face and in more advanced cases, the bones of the cranium or axial skeleton. The leprosy bacilli invade the nerves supplying arterioles of the bone, leading to an enlargement of the nutrient canals and formation of small bone cysts.

Bone changes in leprosy can be specific, resulting from direct invasion of bones by M. leprae and include formation of bone cysts, honeycombing, enlarged nutrient foramina, subarticular erosion, concentric cortical erosions, and primary periosteitis or nonspecific, secondary to damage to the nerve supply with superadded factors including vascular changes, trauma, and secondary infection[6]. Specific bone lesions are relatively rare, occur in the lepromatous forms and are confined to the small bones of hands, feet and nose. Non-specific lesions are common and are of four general types: distal absorption of the digits, osteoarthritis, osteomyelitis and osteoporosis[7]. Histologically, these lytic bone lesions present foamy macrophages with numerous bacilli, few lymphocytes, epithelioid cells and Langhans giant cells[8].

Bone resorption is one of the disease processes in leprosy and occurs in the early stage of the disease [7]. It leads to permanent deformities and is one of the major causes for morbidity. About 25% of the leprosy patients who are not treated at the early stages of the disease develop deformities of the hands and

feet4. Osteoporosis is the second most common sign in patients with leprosy[2]. Localised osteoporotic changes result from immobilisation, most frequently because of disuse associated with fixed contractures of the fingers. Motor denervation is sometimes associated with absorption of the cancellous bone and the development of a concentric type of bone atrophy. It affects both the length and the width of bone. Osteoporosis mainly causes vertebral fractures, intertrochanteric fractures and Colles fractures[4].

Bone resorption in leprosy has been well established by radiographic studies[9,10,11]. Radiological studies in leprosy patients found bone resorption involving face [12], alveolar bone[12,13] and limbs [14,15,16]. A clinical study has analysed facial deformities in leprosy and foundnasal deformities andseptal perforation along with eye deformities and facial nerve palsy[17]. Archaeological studies have found bone resorption in skeleton of leprosy patients [18, 19, 20]. Very few biochemical studies have been done to evaluate bone resorption in leprosy [21, 22, 23, 24] with conflicting results.

Bone is a dynamic tissue characterized by continuous formation and resorption. Bone formation predominates in growing bone, whereas resorption often predominates in aging bone, leading to osteopenia and osteoporosis. Many disease states may also lead to bone fragility[25]. Bone turnover can be studied by many biochemical markers[26]. The level of fasting urinary calcium corrected for creatinine levels serves as a marker of bone resorption [26, 27]. The resorption of bone may be associated with new bone formation. The measurement of Serum Alkaline Phosphatase is considered as an index of osteoblastic activity[26].

Patients with leprosy are treated with Multi Drug Therapy (MDT) consisting of Rifampicin, Dapsone and Clofazimine. MDT, although can disinfect leprosy patients, it has limited impact on various pathologic processes that occur in leprosy. Leprous bone disease can progress even several years after having completed specific treatment for the disease[11, 15,16]. So, there is a need to improve the treatment modalities and decrease the burden of the disease. Therefore, the present study was undertaken to evaluate bone resorption by measuring serum ALP activity and urinary Calcium / Creatinine in various forms of leprosy and compared to healthy controls.

2. MATERIALS AND METHODS

In the present study, seventy newly diagnosed patients of leprosy attending Osmania General Hospital, Department of Dermatology were included as cases before starting Multidrug therapy. The diagnosis is based on clinical grounds into Paucibacillary (PB) (2 to 5 skin patches) and Multibacillary (MB) (≥ 6 skin patches) cases as per the WHO Operational classification[2].The patients who were already undergoing treatment for leprosy or taking any form of antioxidant / multivitamin supplementation and leprosy patients suffering from reactions, ulceration, co-infection andhistory of smoking, infectious diseases and other major illnesses were excluded from the study.

This study was approved by institutional ethical committee. After prior consent from the subjects, slit skin smear was done to grade Bacteriological Index (B.I) according to Ridley`s logarithmic scale [28]. Sixty age

and sex matched healthy individuals without any past history of leprosy disease were taken as controls after fulfilling the inclusion and exclusion criteria.

Under aseptic precautions, 5 ml of fasting venous blood samples were collected from the study subjects. Sample is centrifuged at 3000 r.p.m for 10 minutes and serum was separated and used for the estimation of Alkaline phosphatase (ALP) by - pNPP-AMP (IFCC) Kinetic Assay29 using semi-autoanalyser. 5 ml of Random urine sample was collected in the fasting state in a clean, sterile glass bottle. 1-2 drops of 6M HCL is added as a preservative as calcium salts like calcium oxalate precipitate during and after collection , and is used for the estimation of urinary Calcium by - O-Cresolphthalein Complexone(OCPC) 30 Method and urinary Creatinine by Alkaline Picrate Method[31].

The data was analysed by using SPSS 15.0 version. The results were expressed as Mean ± Standard Error (SE). Independent sample 't' test was used to assess the significance of difference of means between the cases and controls. P<0.05 is considered as significant.

3. RESULTS

The mean values of serum ALP and Urinary Ca/Cr. were significantly increased in cases when compared to controls as shown in Table 1.

Table-1: Comparison of Analysed Parameters in Controls and Cases

S.No.	Parameter	MEAN±S.E of Controls	MEAN±S.E of Cases	't ' Value	'p' Value
1.	Serum Alkaline Phosphatase (ALP) IU/L	79.72 ± 2.56	110.87 ±6.2	4.65	0.000*
2.	Urinary Calcium / Creatinine (U.Ca/Cr)	0.09 ± 0.01	0.13 ±0.02	2.17	0.03*

* Variations in Superscripts indicate significance of mean differences among groups

The cases (n = 70) were further divided into sub-groups according to the duration of the disease into Group 1 with duration <1 year, n=41 and Group 2 with duration > 1 year, n=29. The mean value of serum ALP was significantly increased in group 2 when compared to group 1. The mean value of Urinary Ca/Cr was increased in Group 2 when compared to Group 1 and it was not statistically significant. The values are given in Table 2.

Table-2: Comparison of Analysed Parameters in Group1 and Group 2 of cases

S.NO.	Parameter	MEAN ± S.E of Group 1	MEAN±S.E of Group 2	't ' Value	'p' Value
1.	ALP	98.93 ± 7.62	127.76 ±9.69	2.37	0.021*
2.	U.Ca/Cr	0.12± 0.01	0.14±0.03	0.86	0.395

* Variations in Superscripts indicate significance of mean differences among groups

The cases (n = 70) were categorized on the basis of Bacteriological Index (BI) into Group 3 consisting of B.I negative (n=43) and Group 4 with B.I positive (n=27)

cases. The mean value of serum ALP was significantly increased in Group 4 when compared to Group 3. The mean value of Urine Ca/Cr was higher in Group 4 when compared to Group 3 and it was not statistically significant. The values are shown in Table 3.

Table-3: Comparison of Analysed Parameters in Group 3 and Group 4 of cases

S.NO.	Parameter	Mean ± S.E of Group 3	Mean ± S.E of Group 4	't' Value	'p' Value
1.	ALP	100.88 ± 7.43	126.78 ± 10.32	2.08	0.041*
2.	Urine Ca/Cr	0.12± 0.01	0.14± 0.03	0.46	0.649

* Variations in Superscripts indicate significance of mean differences among groups

The various parameters analyzed were compared between Multibacillary (MB) n=40 and Paucibacillary(P.B) n=30 leprosy cases and controls. Anova (Analysis of Variance) test was used to assess the significance of difference of means between the 3 groups. The values are shown in Table 4.

Table-4: Comparison of Analysed Parameters in Multibacillary Cases , Paucibacillary Cases and Controls

Parameter	Groups	Mean ± S.E	' F' Value	' p ' Value
ALP	Multibacillary	124.55 ± 8.21 [a]	16.21	0.000
	Controls	79.72 ± 2.56 [b]		
	Paucibacillary	92.63 ± 8.51 [c]		
U.Ca / Cr.	Multibacillary	0.13 ± 0.02 [a]		
	Controls	0.09 ± 0.01 [b]	2.06	0.13
	Paucibacillary	0.13 ± 0.02 [a]		

* Variations in Superscripts indicate significance of mean differences among groups

The serum ALP activity was significantly increased in MB cases in comparison to PB and Controls. The level of Urinary Ca/Cr. was increased in MB Cases in comparison to PB and Controls but it was not statistically significant. The serum ALP activity was significantly increased in PB cases in comparison to controls. The Urinary Ca/Cr. was increased in PB cases in comparison to controls and it was not statistically significant.

4. DISCUSSION

Leprosy has a multisystem involvement that leads to wide range of biochemical changes in the body. Bone deformities are hallmarks of leprosy, which are characterized by bone loss of the nasal spine, maxillary structures, hands and feet. Bone changes occur in untreated disease and when started cannot be arrested even on treatment [14, 15, 16]. Bone resorption is an early event in leprosy and is frequently already present at diagnosis[23]. The amount of bone resorption may vary depending on the severity of the disease. Bone resorption in leprosy was found to be associated with male sex, grade of disability at diagnosis with

other deformities and with the occurrence of lepra reactions[9, 16, 32].

In the present study, Urinary calcium excretion corrected for creatinine was used as a marker of bone resorption. Serum ALP activity was used as a bone formation marker. From the present study, it was observed that there was a statistically significant increase in mean value of urinary calcium/creatinine (0.13 Vs 0.09) and serum ALP activity (110.87 Vs 79.72)in Cases as compared to Controls. These results are in concordance with previous studies such as Mautalen et al[21]., Asako ishikawa et al[22].,and contrary to studies done by Ribeiro et al[23]., and Vidal et al[24]., .

The increased calcium excretion in urine of leprosy patients, as observed in the present study, may be due to underlying increased bone resorption. The amount of calcium excreted into the urine reflects intestinal absorption, skeletal resorption, renal tubular filtration and reabsorption. Under fasting conditions, the intestinal and renal components are fixed and calcium excretion is used to assess the skeletal component. The amount of creatinine excreted in the urine is usually constant and is little influenced by the diet. In the present study, the increase in urinary calcium/creatinine in leprosy cases was not associated with duration, bacterial load and type of the disease.

There was a statistically significant increase of serum ALP activity in MB cases and in Cases with increased duration of the disease and higher bacterial load. Serum ALP activity is raised in hepato-biliary and bone diseases. In this study, none of the patients have shown elevated liver function tests and other bone diseases leading to an increase in ALP activity. The raised serum ALP activity in cases is attributed to compensatory new bone formation coupled with bone resorption.

The present study suggests an increase in bone turnover in leprosy. The aetiology of increased bone resorption is multifactorial. The development of bone loss in leprosy is a complex process and probably involves circulatory alteration on which is superimposed infection, inflammation, trauma or disuse [7]. It was proposed that bone loss in patients with leprosy is an acceleration of a normal cellular process and could be due to local release of products from M. leprae or host cells [7] .The nerve damage in leprosy may affect the nutrition of bones leading to osteomalacia and bone atrophy [6]. The increased bone turnover in leprosy may result from sympathetic neuropathy and alteration of peripheral vascular bed dynamics selectively stimulating extracortical osteoclastic and endosteal osteoblastic activity[33]. Cytokines released from immune cells of host in response to infection may play a role in bone resorption[34]. M. leprae is directly involved in the downregulation of phosphate-regulating gene with homologies to endopeptidase on the X chromosome(PHEX) expression in Schwann cells and osteoblasts, probably leading to an imbalance in bone turnover[35]. Bone resorption in leprosy in males may be due to primary hypogonadism secondary to leprosy [36]. The oxidative damage may also play a role in bone resorption [37].

As MDT in leprosy has limited impact on bone loss, specific therapeutic treatment aimed at BR have been studied by Kanji et al[32]., and they have found the need

for anti-resorptive drugs along with MDT in leprosy. Honeji et al[38]., have found that in leprosy, there is a definite absorption of bone salts irrespective of the host nutritional status and suggested that in leprosy, administration of calcium may be of benefit as an additional therapeutic measure to retard or arrest the progress of the bone changes characteristic of the disease. In the present study, the BR in leprosy was found in both PB and MB type of leprosy, irrespective of duration and bacterial load. This emphasizes the need for specific treatment aimed at BR in the early stages of the disease.

CONCLUSION

The present study suggests an increase in bone resorption in leprosy patients. Therapy aimed at reducing bone loss may benefit leprosy patients with high bone turnover and decrease the deformities and disease burden due to leprosy.

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