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# Study of Biochemical Parameters in Rheumatoid Arthritis and Systemic Lupus Erythematosus

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## ABSTRACT

Dynamic tissue that is remodeled constantly throughout life. The arrangement of compact and cancellous bone provides a strength and density suitable for mobility. In addition, bone provides a reservoir for calcium, phosphorus, magnesium and other ions necessary for homeostatic functions. Dead bone must be resorbed, and new bone must be formed. The purpose of this study was to evaluate the parameters like calcium, phosphorus, magnesium, alkaline phosphatase and its isoenzymes in RA and systemic SLE. A study was conducted in rheumatoid arthritis, pre and post-menopausal women with RA and systemic lupus erythematosus patients and controls. The serum calcium was decreased, while phosphorus and alkaline phosphatase were increased in rheumatoid arthritis and systemic lupus erythematosus patients as compared to control subjects. The isoenzymes of alkaline phosphatase showed one diffuse band which was originated from bone. Low serum calcium and high phosphorus and alkaline phosphatase (ALP) indicate that SLE & RA patients had sick bone. The serum ALP isoenzyme (bone) is a biochemical marker for the assessment of bone mineralization and monitoring the therapy with bisphosphonates which are bone strengthening agents.

**KEY WORDS:** bisphosphonates, isoenzymes, rheumatoid arthritis, systemic lupus erythematosus

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## INTRODUCTION:

A reduction of bone mass (or density) or the presence of a fragility fracture is prevalent among postmenopausal women but also occurs in men and women with underlying conditions or major risk factors associated with bone demineralization. It occurs with increasing age as bone tissue is progressively lost. In women, the loss of ovarian function at menopause precipitates rapid bone loss. Magaro et al have concluded that bone demineralization in rheumatoid arthritis (RA) is observed even in non-corticosteroid treated patients; articular lesions with subsequent reduction in physical activity appear to play an important role in axial bone loss in RA.<sup>[1]</sup> Stridl et al examined red cell magnesium and calcium

content in patients with disorders of bone metabolism. The results indicated the important role of magnesium in these disorders of bone metabolism.<sup>[2]</sup> Yoshikawa et al have studied the combined effects of 1- $\alpha$  (OH)D<sub>3</sub> with calcium supplement in preventing progressive decrease of bone mass in patients with osteoporosis. The combine use of calcium and 1- $\alpha$  (OH)D<sub>3</sub> had significant effect in preventing bone loss. Calcitonin has been considered to be useful in the treatment of bone demineralization due to its remarkable action on bone resorption as well as effect on bone formation.<sup>[3]</sup> Yashikawa et al have suggested that the use of calcitonin with calcium supplements had the place in the treatment of osteoporosis.<sup>[4]</sup> Eggelmeijer et al have concluded that long term treatment with an orally administered bisphosphonate overcomes bone loss and increases bone mass. This finding may have significance with regard to the treatment of patients with RA. The study of minerals, alkaline phosphatase and its isoenzymes play an important role in postmenopausal osteoporosis in RA.<sup>[5]</sup> Romagnoll et al have concluded that

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postmenopausal women had significantly higher levels of bone specific isoenzyme of alkaline phosphatase. Alkaline phosphatase levels were elevated in both serum and synovial fluid from RA patients.<sup>[6]</sup> Bone type alkaline phosphatase derived from synovial tissue may contribute to the raised activities of alkaline phosphatase in RA patients.<sup>[7]</sup> Abrahamsen and Harvey reviewed the evidence for vit. D supplementation in the management of patients with rheumatic diseases.<sup>[8]</sup>

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown aetiology predominantly affecting women in their reproductive years. Many patients with SLE have to be treated with systemic glucocorticoids for prolonged periods of time. Osteoporosis is a frequent complication of SLE.<sup>[9]</sup>

The aim of the present study was to estimate serum calcium, phosphorus, magnesium and alkaline phosphatase and separation of serum alkaline phosphatase isoenzymes by electrophoresis in RA & SLE patients. The evaluation of these parameters on specific therapy such as supplementation of calcium, vit.D, calcitonin, bisphosphonates, hormone replacement therapy and exercise.

## MATERIALS AND METHODS:

Institutional Ethics Committee approval was taken before conducting this study. Blood samples were collected from the patients admitted to the hospital suffering from rheumatoid arthritis and systemic lupus erythematosus.

RA patients with age over 50 years were included (cases n=40, control n=40). Some patients were on specific therapy like calcium, Vit D, diet advice, exercise, etc. Patients suffering from other co-morbid conditions, which are common in RA were excluded from this study.

Women suffering with RA between 40 to 50 years were considered for pre-menopausal group and women suffering with RA above 50 having menopause were considered for post-menopausal group (cases n=32, control n=32).

Female patients with known SLE were selected irrespective of their ages (Cases n=08, control n=10). Patients with any concomitant medical problems or those on any medications were excluded from the study.

40 control samples and 40 samples of the concerned patients were collected in plain bulb and allowed to clot at room temp. It was centrifuged at 3000 rpm for 10 minutes. The serum was separated

and kept in the refrigerator at -20°C till analyzed. All chemicals used were of analytical grade. Each parameter was standardized. Serum calcium was estimated by the method of Trindar.<sup>[10]</sup> Serum phosphorus was estimated by the method of Fiske and Subbarow.<sup>[11]</sup> Serum magnesium was estimated by the method of Titan yellow (Neill and Neely).<sup>[12]</sup> Alkaline phosphatase was estimated by the method of Kind and King.<sup>[13]</sup> Alkaline phosphatase were separated by the method of Smith et al (1968)<sup>[14]</sup>. 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 rheumatoid arthritis patients as compared to control subjects, while serum alkaline phosphatase levels were increased significantly ( $p \leq 0.05$ ) than control subjects. The serum phosphorus levels were increased non significantly ( $p > 0.05$ ) than controls, whereas there were a significant increase ( $p \leq 0.001$ ) in magnesium levels than controls (Table 1).

The serum calcium levels were significantly decreased ( $p \leq 0.01$ ) than control. The serum alkaline phosphatase levels were increased but non significantly ( $p > 0.05$ ) than controls. Serum phosphorus levels were decreased non significantly ( $p > 0.05$ ) than controls, whereas there were no significance seen in serum magnesium levels than controls ( $p > 0.05$ ) (Table 2).

The serum calcium levels were decreased non significantly ( $p > 0.05$ ) and magnesium levels were decreased significantly ( $p \leq 0.05$ ) as compared to control subjects in systemic lupus erythematosus. The serum alkaline phosphatase levels were increased but non significantly ( $p > 0.05$ ) while serum phosphorus levels were increased significantly ( $p \leq 0.05$ ) (Table 3).

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 photographs.

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 1).

There was appearance of only one band and that was diffuse as compared to control. The diffuse

**Table 1:** Biochemical parameters in rheumatoid arthritis (Male and Female)

Parameters	Control(n=40)	Rheumatoid Arthritis(n=40)	t-values
	Mean ± S.D	Mean ± S.D	
Calcium mg/dl	9.90 ±0.631	9.26 ±0.667	4.40***
Alkaline Phosphatase KA units	3.85 ±0.845	4.359 ±1.143	2.26*
Phosphorous mg/dl	3.68 ±0.544	4.027 ±0.673	2.53NS
Magnesium mg/dl	2.240 ±0.397	3.019 ±0.395	8.79***

\*\*\*= p ≤ 0.001

\*= p≤0.05

NS=Not Significant

**Table 2:** Biochemical parameters in pre and postmenopausal women with RA (Female).

Parameters	Control(n=32)	Pre and post -menopausal women(n=32)	t - values
	Mean ± S.D	Mean ± S.D	
Calcium mg/dl	10.06 ±0.773	9.248 ±0.635	4.59**
Alkaline Phosphatase KA units	3.70 ±0.798	4.10 ±1.17	1.59 NS
Phosphorous mg/dl	3.872 ±0.542	3.98 ±0.655	0.72 NS
Magnesium mg/dl	2.60 ±0.533	2.896 ±0.448	2.40 NS

\*\*= p ≤ 0.01

NS=Not Significant

**Table 3:** Biochemical parameters in systemic lupus erythematosus.

Parameters	Control(n=10)	Systemic Lupus Erythematosus(n=08)	t-values
	Mean ± S.D	Mean ± S.D	
Calcium mg/dl	10.15 ±0.858	9.11 ±1.59	1.78 NS
Alkaline Phosphatase KA units	3.57 ±0.759	4.471 ±1.07	2.09 NS
Phosphorous mg/dl	4.075 ±0.380	4.722 ±0.657	2.62*
Magnesium mg/dl	2.84 ±0.542	2.75 ±0.604	0.33*

\*= p ≤ 0.05

NS=Not Significant

band appeared in all patients and this band was originated from the bone. The diffuseness was most striking (Figure 2).

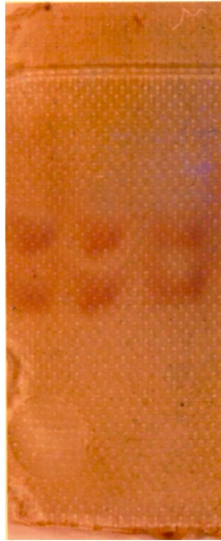
There was appearance of only one band and that was diffuse as compared to control. The diffuse band appeared in all patients and this band was originated from the bone (Figure 3).

## DISCUSSION:

In our study, calcium levels are significantly decreased in rheumatoid patients, whereas alkaline phosphatase levels are significantly increased as compared to control subjects. Serum phosphorus levels are increased non significantly than controls. Previous studies reported, the concentrations of serum calcium and phosphorus are usually reduced and serum alkaline phosphatase activity was elevated in RA patients.<sup>[15]</sup> Contrary to other studies we found

serum magnesium levels increased significantly in RA patients. Chronic inflammatory conditions are likely to alter magnesium levels and possible mechanism of decrease magnesium in RA in other studies is due to chronic inflammation and autoimmune injury.<sup>[16]</sup>

Osteoporosis is a frequent complication of rheumatoid arthritis especially in post-menopausal women.<sup>[17]</sup> Clinical laboratory tests were used to evaluate individual for osteoporosis.<sup>[18]</sup> In our study we found significantly decreased serum calcium levels in post-menopausal and pre-menopausal women suffering with RA compared to control subjects. Many factors that may cause generalized osteoporosis in inflammatory arthritis include circulating pro-inflammatory molecules, hormones alter calcium metabolism and the effects of anti-rheumatic and anti-inflammatory drugs.<sup>[19]</sup> One previous study has concluded that low dose steroid therapy is associated with increased bone loss and number of fractures in patients with rheumatoid arthritis.<sup>[20]</sup> Sambrook et al



**Figure 1:** Shows the isoenzymes of alkaline phosphatase in control subjects.



**Figure 2:** Shows the isoenzymes of alkaline phosphatase in rheumatoid arthritis patients.



**Figure 3:** Shows the isoenzymes of alkaline phosphatase in systemic lupus erythematosus.

have studied the magnitude and distribution of osteoporosis in RA. Bone mineral density (BMD) was measured by dual X-ray absorptiometry. They found that BMD was reduced at most skeletal sites with RA. Osteoporosis in RA is generalized and may be related to loss of mobility of muscle mass.<sup>[21]</sup>

Systemic lupus erythematosus results from tissue damage caused by pathogenic subsets of autoantibodies and immune complexes. In our study we get significantly higher serum phosphate levels & significantly lower serum magnesium levels in SLE patients as compared to control subjects. It is not

known why Mg levels tend to drop in patients with chronic pain problems such as SLE. It has been suggested that there may be a problem with Mg availability and/or utilization at the tissue level as opposed to a suboptimal dietary intake or an increased excretion of Mg.<sup>[22]</sup> The literature also suggests that corticosteroid treatment in SLE patients may even intensify the Mg deficiency.<sup>[23]</sup> Whatever the mechanism, Mg deficiency should not go unnoticed. To fail to consider Mg deficiency in the differential diagnosis of neuromuscular problems in SLE might expose such patients to undue risk and expense particularly if myalgias are mistakenly attributed to inflammation. Magnesium deficiency can cause complication such as osteoporosis.<sup>[24]</sup> SLE patients develop bone loss due to hypothalamo-pituitary gonadal dysfunction following corticosteroid therapy and ovarian dysfunction (SLE specific). This may be the cause of increased serum phosphorus levels in SLE patients.

In patients on steroids and calcium supplementation bone resorption must have decreased. Vitamin D is of benefit in both the treatment and prevention of steroid induced bone loss. In RA patients taking steroids, the addition of Vitamin D with elemental calcium had beneficial effects on bone density. Bisphosphonates are pyro-phosphate analogs that bind to hydroxyapatite at sites of active boneremodeling.<sup>[8]</sup> The study carried out on osteoporosis was not able to conclude that giving supplementation of folic acid, B6, B12 and other antioxidants could prevent osteoporosis due to insignificant results.<sup>[25]</sup> Some patients of RA and SLE were on calcium, phosphorus bisphosphonates which have given positive effect on bone mineral homeostasis.<sup>[26]</sup> Low calcium and high phosphorus indicates that patient had osteoporosis. For treatment of patients, attention was given to calcium, Vitamin D and bisphosphonates and also from natural sources like fruits.

The human alkaline phosphatase constitutes a system of multiple molecular forms of enzymes in which heterogeneity is partly due to genetic factors and partly to post translational modifications.<sup>[27]</sup> Raised serum alkaline phosphatase activity in RA has been reported.<sup>[28]</sup> Serum ALP isoenzymes were done by PAGE in patients with RA and SLE. The rise in serum ALP is due to the specific bone isoenzyme which is diffuse band that is responsible for the rise in serum ALP. The serum isoenzymes are biochemical markers for the assessment of osteoporosis and monitoring the therapy with bisphosphonates which are bone strengthening agents.

## CONCLUSION:

We observed low calcium, high phosphorus and increased ALP in RA and SLE patients which indicates that patients had sick bone. Separation of the serum alkaline phosphatase by electrophoresis in RA and SLE showed one diffuse band. Appearance of diffuse band is because of rise in serum alkaline phosphatase levels. The rise in serum ALP in RA and SLE is due to bonespecific ALP isoenzyme. The serum isoenzyme is a biochemical marker for the diagnosis of rheumatoid arthritis and systemic lupus erythematosus and monitoring the therapy, with bisphosphonates, which are bone strengthening agents.

Hence, it is recommended to supplement calcium bisphosphonates & vitamin D in patients with RA. Along with above, magnesium is also recommended for SLE patients.

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