

## Study of mineral status & alkaline phosphatase activity in rheumatoid arthritis patients in Shivamogga district

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### Abstract

**Introduction:** Rheumatoid arthritis (RA) is a prototypical inflammatory joint disease of unknown etiology affecting various joints of the body leading to stiffness, swelling, pain, and finally functional inability. Elevated serum alkaline phosphatase (ALP) is a common feature in rheumatoid arthritis (RA) and its origin remains unclear. Inflammatory conditions are more likely to alter mineral status.

**Aim of the Study:** The aim of this study was to investigate the level of serum ALP, Calcium, Phosphorus & Magnesium levels in Rheumatoid arthritis patients and compare it with healthy subjects.

**Materials and Methods:** Fifty four RA patients and fifty four age matched healthy controls between 35-65 years were included in the study. Serum Calcium, Phosphorus, Magnesium & ALP activity were measured in all the subjects and correlated.

**Results:** Serum ALP (145.17±15.87 versus 83.19±17.55 U/L) and phosphorus levels (4.84±0.24 versus 3.84±0.44 mg/dl) were found significantly higher; serum Calcium (7.66±0.34 versus 9.27±0.49) and serum Magnesium levels (1.72±0.24 versus 1.95±0.29) were found to be lower in RA cases as compared to controls and were statistically significant (p<0.001).

**Conclusion:** Increase in ALP in RA patients when compared to healthy controls suggests the role of serum ALP as a marker of disease activity in RA. The altered mineral status in rheumatoid arthritis, observed in this study reflects on the pathogenesis of RA. Therefore we conclude that serum ALP & mineral estimation can also be used as markers of disease activity in rheumatoid Arthritis.

**Keywords:** Alkaline phosphatase, Serum calcium, Phosphorus, Magnesium, Rheumatoid arthritis.

### Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune disorder of multi factorial etiology in which both genetic and non-genetic factors contribute to disease susceptibility. Symmetric polyarticular inflammation of the joints is a significant finding in RA.<sup>1</sup>

About 0.8% of the total population of the world are affected. An annual incidence of 0.5 - 1% is seen in both developed and developing countries.<sup>2,3</sup> In Indian adult population it is the most common inflammatory disease affecting approximately 0.75%.<sup>4</sup>

Microbiological and pathological investigations are mainly used in the diagnosis and prognosis of RA, but the utility of biochemical parameters is limited. Many recent studies are exploring various biochemical parameters in RA.<sup>5-8</sup> Inflammation, is capable of inducing marked systemic alterations in trace metal distribution and metabolism.<sup>9</sup>

Alkaline Phosphatase is a biochemical marker of bone turnover. It provides useful clinical evidence of both pathologic and normal process that reflect bone cell activity on the skeleton. Of the variety of biochemical markers that reflect the activity of osteoclasts and osteoblasts, alkaline phosphatase in clinical practice was the first biochemical marker of bone turnover. It is still the most widely used clinical marker in managing patients with a variety of skeletal disorders.<sup>10</sup>

Calcium is the fifth most common element in the body and the most prevalent cation. The skeleton contains 99% of the body's calcium. It is present predominantly as extracellular

crystals of unknown structure with a composition approaching that of hydroxyapatite.<sup>11</sup>

Phosphorus in the form of organic and inorganic phosphate is important and widely distributed element in the human body. Inorganic Phosphate is the fraction measured in serum and plasma by clinical laboratories. The major component of hydroxyapatite of bone is organic phosphate.<sup>11</sup>

For the formation of bone, calcium / phosphorus ratio is very important. RA is associated with localized or generalized osteoporosis. One of the earliest radiological signs of RA is periarticular osteoporosis. It represents an important criterion for the diagnosis of RA.<sup>12</sup>

Magnesium (Mg) is an essential nutrient and fourth most abundant mineral found in the body. Mg levels are altered in chronic inflammation. A decreased level of Mg has been suggested to be reasonable marker of RA.<sup>5</sup>

Therefore the present study was undertaken to estimate the Alkaline phosphatase activity & mineral status in Rheumatoid Arthritis in Shivamogga district.

### Materials and Methods

This study was conducted in Subbaiah Institute of Medical Sciences between January 1<sup>st</sup> 2017 and July 31<sup>st</sup> 2017 for a period of 6 months. A total of fifty four patients diagnosed with Rheumatoid Arthritis without any medication and fifty four healthy controls aged between 35-65 years were included in the study. Informed consent was obtained from

each subject. Ethical approval for the study was obtained from the Institutional research ethical committee.

### Inclusion Criteria

All the patients included in this study fulfilled the criteria of American Rheumatism Association.<sup>13</sup> Presence of synovitis in at least one joint was considered. Patients with synovitis more than 6 weeks duration were included. In all the patients Rheumatoid Factor (RF) was positive.

### Exclusion Criteria

RA patients with Tuberculosis, Diabetes Mellitus, Cardiovascular diseases, HIV/AIDS and patients with other types of musculoskeletal disorders, osteoarthritis, osteoporosis, spinal disorders, severe limb trauma and gouty arthritis were excluded from the study.

### Laboratory Analysis

Taking aseptic precautions, after an overnight fast of 10-12 hours, 5 ml of venous blood was collected in plain vacutainer [BD Biosciences] from antecubital vein from each patient. Serum separated from clotted blood was analyzed for ALP activity, Calcium, Phosphorus & Magnesium levels in the central laboratory of Subbaiah Institute of Medical Sciences.

Serum magnesium was measured by Calmagite method,<sup>14</sup> Calcium by Arsenazo III End point Method,<sup>15</sup> Phosphorus by Ammonium Molybdate end point assay<sup>16</sup> & ALP was estimated by IFCC kinetic method<sup>17</sup> by using Erba Mannheim reagent kits obtained from Transasia Bio-Medicals. Analysis was done on Erba Mannheim Chem -5 analyser.

### Statistical Analysis

Data analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago). The data was carefully evaluated to obtain the mean values and SD and compared as student's unpaired 't'-test between subjects and control. A p-value < 0.001 was considered as statistically significant.

**Table 1:** Demographic characteristics

Groups	Males	Females	Mean Age
Non-rheumatoid arthritis controls (NRA)	27	27	49.87
Rheumatoid arthritis patients (RA)	14	40	48.31

### Results

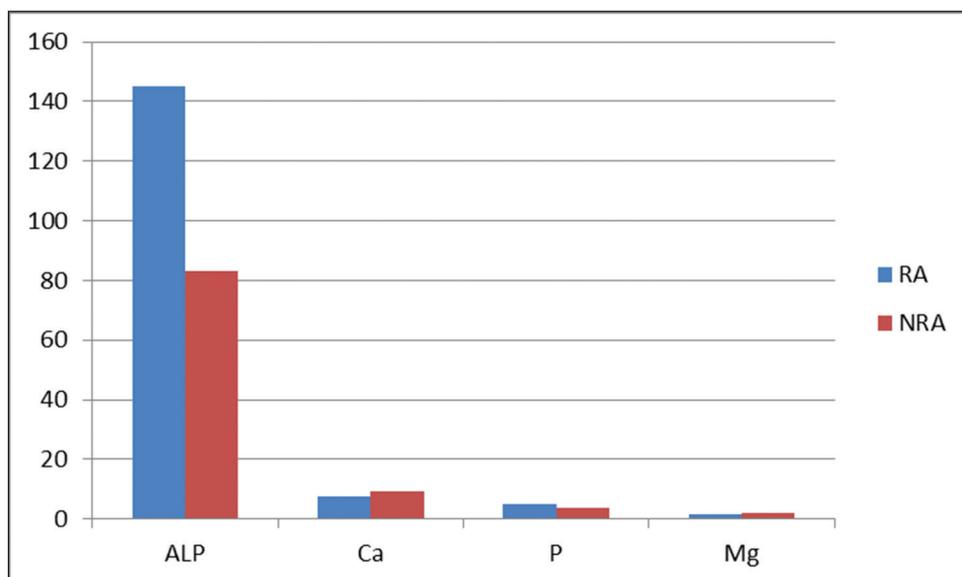
In the present study, patients with rheumatoid arthritis and non-rheumatoid arthritis controls were in the age group of 35-65 years. Among the 54 patients with RA, 14 (25.9%) were males and 40 (74.1%) were females as shown in Table 1. The mean age of males and females in cases was 49.87 years and in controls was 48.31 years.

Table 2 and Fig.1 shows the Mean±SD of ALP, serum Calcium, Phosphorus and Magnesium levels in serum of RA patients and controls. As compared to the controls, serum ALP & phosphorus levels were elevated & serum calcium and Magnesium levels were decreased in cases and significant difference (p<0.001) was found between the two groups.

### Discussion

RA is likely to affect women approximately twice to that of men.<sup>10,18</sup> Between 35 & 50 years of age, 80% of people with RA develop signs and symptoms of the disease.<sup>19</sup> Our study also showed similar findings.

In this study, serum ALP was found to be raised in patients of rheumatoid arthritis when compared to the healthy controls. Similar results observed by other studies<sup>20-22</sup> support our study. Elevated ALP in rheumatoid arthritis has been attributed to osteoblastic activity indicating an increased bone turnover. Promotion of disease activity is known to induce more active bone resorption. Activated



**Fig. 1:** Mean serum ALP, Calcium, Phosphorus and Magnesium levels in cases & controls

**Table 2:** Serum ALP, Calcium, Phosphorus and Magnesium levels in cases & controls, Values are expressed in Mean  $\pm$  SD

Parameters	NRA meanSD	RA meanSD	P-value
ALP (IU/L)	83.19 $\pm$ 17.55	145.17 $\pm$ 15.87	P<0.001, HS
Calcium (mg/dl)	9.27 $\pm$ 0.49	7.66 $\pm$ 0.34	P<0.001, HS
Phosphorus (mg/dl)	3.84 $\pm$ 0.44	4.84 $\pm$ 0.24	P<0.001, HS
Magnesium (mg/dl)	1.95 $\pm$ 0.29	1.72 $\pm$ 0.24	P<0.001, HS

bone resorption is accompanied by rise in serum ALP and concomitant bone formation.<sup>23</sup> Increased activity of ALP may be due to its leakage from injured or killed cells. One more explanation for increased ALP may be due to selective induction of ALP by inflammatory mediators such as Interleukin -1 which is known to circulate in active RA.<sup>24</sup> Liver involvement in rheumatoid arthritis has been reported. The hepatic changes may be a response to chronic inflammatory disease.

Magnesium levels are likely to be altered by chronic inflammatory conditions. Decrease in magnesium levels in RA may be due to chronic inflammation and autoimmune injury.<sup>5, 21, 25</sup> In the present study, we found decreased level of serum magnesium in RA subjects as compared to controls. The results of our study are correlating with the study by Manole et al.,<sup>5</sup> Amin et al.,<sup>21</sup> and Cortes et al.,<sup>25</sup> suggested that the RA, an autoimmune disease is associated with serum magnesium disturbances.

Serum mineral disturbances and oxidative stress are known to be associated with RA.<sup>21</sup> Decreased levels of serum magnesium and calcium may be due to various reasons. Calcium and magnesium in drinking water may be decreased because of water softeners and purifiers used for purification of water.<sup>12</sup>

This investigation revealed that serum calcium levels decreased significantly, phosphorus levels increased significantly and Calcium / Phosphorus ratio decreased significantly in RA patients compared to the controls. Several studies revealed altered calcium and phosphorus levels in RA patients.<sup>26-28</sup> RA is usually associated with generalized bone mineral density (BMD) loss, erosions and localized or generalized osteoporosis resulting in increased risk of clinical fractures and functional disability.

#### Limitations of the study & Recommendations

Limitations of our study are small sample size and non measurement of other markers of inflammation & Cardiovascular risk factors in patients with Rheumatoid Arthritis. We have not estimated serum uric acid levels in RA patients. Interleukin-6 (IL-6) a pleiotropic cytokine which plays a pivotal role in the pathophysiology of RA was not estimated. Moreover, a large cross-sectional study needs to be done to conclude the fact.

#### Conclusion

Our study concludes that newly diagnosed RA patients had significantly lower serum magnesium, calcium levels and increased levels of alkaline phosphatase and phosphorus levels as compared to controls. Lowered serum Magnesium is associated with cardiovascular risk. The biochemical alterations in rheumatoid arthritis observed in this study reflect on the pathogenesis of RA. The understanding of pathophysiology of rheumatoid arthritis may help open new therapeutic approaches in the management of rheumatoid arthritis. Therefore we conclude that serum ALP & mineral estimation can also be used as markers of disease activity in rheumatoid Arthritis.

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#### Conflict of Interest: None

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