



## MDA, CALCIUM, PHOSPHORUS AND ITS RATIO IN RHEUMATOID ARTHRITIS PATIENTS

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### ABSTRACT

Rheumatoid arthritis (RA) is an inflammatory polyarthritis, leading to joint destruction, deformity and loss of function. Rheumatoid arthritis (RA) is an autoimmune disorder, occurs when there are attacks of the immune system on body's tissues especially the joint, causing a painful, swelling, that finally results in bone deformity, increased free radical level in defect joint and reduce the level of the antioxidant system can cause tissue damage. Serum levels of lipid peroxides, calcium, phosphorus and ratio of calcium/phosphorus in RA patients were determined and compared with normal healthy controls. Significant increases in lipid peroxides ( $p < 0.001$ ) phosphorus ( $p < 0.001$ ) levels were found in patients presenting with RA as compared to controls. Whereas significant decrease in calcium ( $P < 0.001$ ) and calcium/phosphorus ratio ( $p < 0.001$ ) were found in Rheumatoid arthritis patients as compared to controls. Negative correlation was observed between MDA and Calcium/Phosphorus ratio in patients with rheumatoid arthritis.

**KEYWORDS :** Rheumatoid Arthritis (ra), malondialdehyde, Calcium, phosphorus

### INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and some times shows multisystem involvement (1). While lowered concentrations of antioxidants in the blood considerably increase the probability of the occurrence of RA (2). Many investigators have focussed on oxidative stress since last few years and suggest that RA patients are more prone to lipid peroxidation (3). The calcium role is not clear in RA, however, the relationship between calcium, vitamin D, and parathyroid hormone suggests the possible role of calcium in RA and there is a change in mineral within bone which are calcium, and phosphorus (3). Changes in lipid profile have been observed in different inflammatory disease such as RA (4). RA patients are in increased risks of atherosclerosis and cardiovascular diseases (CVD) than the overall population (5). Bone contains both organic and inorganic material. The organic matter is mainly protein; the inorganic or mineral component is mainly crystalline hydroxyapatite  $\{Ca_{10}(PO_4)_6(OH)_2\}$ . Approximately of body's 99% Calcium and 85% Phosphorus are present in bone. The exact reason behind bone erosion and joint deformities is not fully understood.

This study was aimed to study MDA level as a marker of oxidative stress in patients with RA as well as the changes in Ca, and P level in recently diagnosed RA patients compared to healthy subjects and there is continuing interest in the metabolic changes occurring in RA and to estimate the serum Calcium /Phosphorus ratio in Ra patients.

### MATERIAL AND METHODS:

The sample was taken from a group of 100 patients with RA (50 male and 50 female), age (45-70 years). The control was diagnosed if they having any diseases such as diabetes, infectious diseases. RA was diagnosis based on clinical histories such as ESR and rheumatoid factor.

### STATISTICAL ANALYSIS:

Statistical analysis was done by using student t-test to compare between the two groups and statistically significance set at  $p < 0.05$ . The data of this study were given as mean  $\pm$  standard deviation. Results were diagnosed with rheumatoid arthritis at SLIMS hospital with 100 healthy people who represent controls. In this study patient with RA

belonged to the age group of 45-70 years. Among patients of RA 50 patients were males and 50 were females as shown in table 1. The mean  $\pm$  SD of age in males was  $53.21 \pm 8.12$  and the mean  $\pm$  SD of the female was  $55 \pm 11.1$ .

### RESULTS:

**Table. 1:** Serum levels of total lipid peroxide (MDA), calcium, phosphorus and calcium/phosphorus in control subjects and rheumatoid arthritis patients.

**Table. 2 :** Pairwise correlation analysis in RA patients

Our results shows comparison of MDA, Calcium and Phosphorus levels in RA with control subjects. Statistically significant increase in levels of lipid peroxide ( $P < 0.001$ ) and phosphorus ( $P < 0.001$ ) whereas statistically significant decrease in Calcium ( $P < 0.001$ ) was found in patients of RA as compared to control subjects. whereas phosphorus level was increased significantly ( $P < 0.001$ ) in RA patients as compared to control subjects. On the contrary, calcium/phosphorus ratio was decreased significantly ( $P < 0.001$ ) in RA patients as compared to control subjects. On the contrary, MDA was negatively correlated with calcium/phosphorus ratio ( $r = -0.74$ ).

### DISCUSSIONS :

Serum calcium levels decreased significantly, phosphorus levels increased significantly and Calcium / Phosphorus ratio decreased significantly in RA patients compared to the controls. Rheumatoid arthritis (RA) is an inflammatory polyarthritis, leading to joint destruction, deformity and loss of function. Extra-articular features and systemic symptoms usually occur and may accompany the onset of joint symptoms. Serum calcium levels decreased significantly, phosphorus levels increased significantly and Calcium / Phosphorus ratio decreased significantly in RA patients compared to the controls. Lower calcium level may be due to insufficient calcium intake and quicken osteoporosis, more sodium intake in our food may cause calcium depletion (6). Free radical that produced by ROS that cause chronic inflammatory cells nearby bone with subsequent bone destruction (7). RA is usually associated with localized or generalized osteoporosis, erosions and hand and generalized bone mineral density (BMD) loss resulting in functional disability and increased risk of clinical fractures.

Many drugs have an affected-on calcium metabolism and cause a low level of calcium in RA. It was assumed that high level of phosphorous was related to tissue hypoxia with an elevated in ATP breakdown resulting in the release of inorganic phosphorous from cells. However, hypoxia create by hypertrophy and hyperplasia within synovial joints [(8). It was observed that Calcium in RA a low level in serum whereas Phosphorous has shown a high level in contrast to control, that can be concluded to be a danger factor for RA cases. These results recommend to increase Mg, Ca, P in the diet with a supplement that might be helpful for a patient with RA. Further studying is necessary to estimate various biochemical markers that influences patient with RA. Statistical analysis by unpaired t test shows that the levels of serum calcium were decreased and phosphorus levels were increased statistically, which were highly significant ( $p < 0.0001$ ). Low calcium may be due to prolonged inadequate calcium intake and accelerated osteoporosis. In RA patients as compared to healthy controls. These results are in accordance with several other studies (9-13).

RA patients are vulnerable to steroid induced and disease associated osteoporosis. The high salt intake in our population may exacerbate calcium deficiency. At the proximal tubule, where sodium and calcium absorption are linked. Although not specifically studied, many disabled people rely heavily on pre-packed food and meals, much of which is high in sodium (14). RA is associated with collection of chronic inflammatory cells occurring adjacent to bone with subsequent bone destruction. It is possible that generated oxygen derived free radicals may be important in bone resorption (15). Calcium/phosphorus levels were disturbed in RA patients and more disturbed in anemic RA patients. So, supplements of calcium, iron and vitamin D are needed for RA patients. In the formation of bones, calcium/phosphorus ratio is very important. It is possible that, generation of reactive oxygen species may be particularly important factor for bone resumption in inflammatory process (16). Hypoxic conditions also disrupt an intracellular ionic environment and alter calcium and phosphorus level (17).

These results are also in accordance with A significantly decreased calcium/phosphorus ratio in RA patients as compared to controls in our study clearly indicates that, there is an altered calcium and phosphorous metabolism in RA. As calcium and phosphorous are important constituent of bone, ultimately bone metabolism is altered in RA, the event observed by many workers (18-19).

Negative correlation between calcium/phosphorus ratio and lipid peroxide ( $r = -0.89$ ) suggests that the generation of reactive oxygen species in excess may be particularly important in the bone resorption that occurs in association with inflammatory diseases (20). The earlier studies (21). Generation of reactive oxygen species is an important factor in the development and maintenance of rheumatoid arthritis (RA) in humans. MDA, the product of lipid peroxidation reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. The longer chain polyunsaturated fatty acids are especially potent at increasing lipid peroxidation and causing cell damage by oxidative stress (22). RA is associated with collection of chronic inflammatory cells occurring adjacent to bone with subsequent bone destruction. It is possible that generated oxygen derived free radicals may be important in bone resorption.

There is a contradiction with present data on the calcium-phosphorus ratio with another study of RA (rheumatoid arthritis) patients that a decreasing level of calcium-phosphorus ratio [23-24] and researchers have documented alterations of calcium and phosphorous metabolism [23]. It is

postulated that the elevation of phosphorous was related to tissue hypoxia with an increase in ATP degradation resulting in the release of inorganic phosphorous from cells. In RA, hypoxic environment triggers oxygen free radicals generation and alters oxidative metabolism within the cell. It leads to disruption in intracellular ionic environment and altered calcium and phosphorus levels. Some authors suggest that hypocalcemia results due to drugs used in RA, but decreased mean total body calcium levels in RA patients who did not receive that corticosteroid drugs strongly suggest that, this is an integral feature of RA (25).

**CONCLUSION:**

Calcium and Phosphorus level can be studied for the preferable therapeutic management of RA. The decrease in serum calcium / phosphorus ratio indicated that, calcium and phosphorus metabolism is altered in RA. In patients serum levels of calcium and calcium/phosphorus decreased and further studies on dietary management of calcium and phosphorus are needed. Increased oxidative threat in rheumatoid arthritis is evidenced by raised lipid peroxides. With a better understanding of the pathophysiology of RA, new therapeutic approaches are emerging to provide precision medicine for individuals.

**Table. 1: Serum levels of Malondialdehyde (MDA), calcium, phosphorus and calcium/phosphorus in control subjects and rheumatoid arthritis patients.**

Sr. no	Parameters	No. of Subjects	Control Subjects	Rheumatoid Arthritis patients
1	Serum Malon di aldehyde (MDA) $\mu\text{mol/L}$	100	1.98 $\pm$ 0.67	4.88 $\pm$ 1.52*
2	Serum Calcium (mg/dl)	100	10.65 $\pm$ 1.81	7.98 $\pm$ 1.48*
3	Serum inorganic phosphorus (mg/dl)	100	2.95 $\pm$ 0.40	4.95 $\pm$ 0.92*
4	Calcium/ phosphorus ratio	100	3.73 $\pm$ 0.63	41.96 $\pm$ 0.673*

Values are expressed as mean  $\pm$  SD; \*P < 0.001

**Table. 2 : Pairwise correlation analysis in RA patients**

Sr. no	Pairwise correlation of serum parameters	r' value
1	Negative correlation between MDA and calcium/phosphorus ratio	- 0.89

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