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A study on role of Oxidative stress and Calcium-Phosphorous Ratio in Rheumatoid Arthritis

Pullaiah Akinepalli[1,*], Mahesh Gajula[2], Narender G[3]

[1,3]Assistant Professor, Kakatiya Medical College, Warangal, Telangana, [2]Assistant Professor, Dept. of Forensic Medicine, Govt. Medical College, Anantapuramu, Andhra pradesh

***Corresponding Author:**
Email: pullaiahakinepalli@gmail.com

Abstract

Background: Rheumatoid arthritis is one of the chronic inflammatory disease affecting joints and other organs is primarily of an unknown etiology with an incidence of 1% to 2% of total world population.one of the current school of thought implies oxidative stress related to free radical damage, auto immunity and genetic predisposition are few of the important etiological factors proposed for its causation. The current study is aimed to see whether detection of lipid peroxidation products such as Malondialdehyde**(MDA)** and estimation of serum calcium, phosphorous, Alkaline phosphatase, calcium-phosphorous ratio as biomarkers for Rheumatoid Arthritis that will help to diagnose and treat disease at an early stage.

Material and Methods: The current study is prospective study carried out at Kakatiya Medical College and Mahatma Gandhi Medical College, Warangal from January 2014 to January 2015 over 100 subjects of which 50 cases were suffering with Rheumatoid arthritis while 50 subjects were normal healthy adults. Data was recorded in a pretested proforma and was analyzed using appropriate statistical methods.

Results: During the study, it is found that there is raise in mean MDA levels in the group suffering with Rheumatoid arthritis (p value <0.001) when compared to control group. Similarly, the serum calcium levels are found decreased, serum phosphorous levels were increased in the subjects suffering with Rheumatoid Arthritis. The difference between the means of two groups in relation to Calcium-phosphorous ration was found to be statistically significant ($p < 0.001$).

Conclusion: In conclusion, the increased MDA levels in the present study may be considered as important marker but apparently not sufficient to conclude that there was an increase in oxidative stress in rheumatoid arthritis patients.

Keywords: Malondialdehyde (MDA), Rheumatoid Arthritis, Serum Calcium, Serum Phosphorous, Calcium-Phosphorous ratio

Introduction

Rheumatoid arthritis is a chronic systemic inflammatory disease affecting diarthrodial joints and frequently a variety of other organs.(1) Rheumatoid Arthritis marks the most common type of connective tissue disorder second to the commonest, osteoarthritis.(2) Its existence is reported throughout the world and affects approximately 1 to 2% of the world's population and ethnic groups. It affects 0.75% of Indian population with an annual incidence rate between 0.5% and 1% of total population every year in both developed and developing countries.(3) Although the cause of Rheumatoid arthritis remains uncertain, autoimmunity is attributed a pivotal role in its chronicity and progression.(4) Both genetic and environmental factors appear to play an important role in the etiology of Rheumatoid arthritis.(5) Family studies do indicate that genetic predisposition as an etiological factor for Rheumatoid arthritis.(6)

In recent years, the experimental & clinical data is providing compelling evidences for involvement of Free radical or Reactive oxygen species related etiology for Rheumatoid arthritis.(7) It is postulated that the development of Rheumatoid arthritis is related to excess production of reactive oxygen species and decreased ability of the body to combat oxidative stress.(8) Peroxidation of Lipids exposed to free oxygen radical species were found to be responsible for damage to tissues in vivo may cause inflammatory disease like Rheumatoid arthritis. The cytotoxic aldehydes such as Malondialdehyde**(MDA)** which are the end products of lipid peroxidation due to free radicals. Cellular proliferation of the Synoviocytes and neo-angiogenesis leads to formation of pannus which destroys the articular cartilage and bone. Increase in the intra-articular pressure above the synovial capillary perfusion cause intra-articular hypoxia.(9) Hypoxic conditions disrupt an intracellular ionic environment and alter Calcium and Phosphorus levels.(10) Hence estimating the levels of prooxidant Malondialdehyde in patients with rheumatoid arthritis and comparing with the normal healthy controls to assess the role and significance of oxygen derived free radicals in rheumatoid arthritis. Parameters such as levels of Calcium, Phosphorus their ratio and the levels of Alkaline phosphatase will connote to the evidence of disease process.

Materials and Methods

The current prospective study was carried out at Department of Biochemistry, Kakatiya Medical College and Mahatma Gandhi Hospital Warangal from January 2013 to January 2014 after approval of Institutional Ethics Committee. The study encompassed 100 subjects of which 50 people(GROUP-R) were suffering with rheumatoid arthritis and remaining 50 subjects(GROUP-C) were normal and utilized as control subjects. The

selection of subjects was done using standard diagnostic criteria postulated by American Rheumatism Association. The Control subjects were normal healthy adults who were not suffering with any diseases like Diabetes, Hypertension or renal failure.

Fasting venous blood samples were collected in all the subjects and non-hemolyzed sera were processed for all biochemical parameters. All the assays were carried out by using reagents and chemicals of analytical grade and standardized procedures. Estimation of marker of oxidative stress, Malondialdehyde**(MDA)** was done by Thiobarbituric acid assay, Calcium was determined by O-Cresolpthalien complexone method, Phosphorus by Fiske and Subbarow method and Alkaline phosphatase by the method of King and Kind.

The study data so obtained was recorded in a pretested proforma and was analyzed using appropriate standard statistical methods.

Analysis of Results

Serum MDA levels

Table 1: Mean Serum Malondialdehyde Levels

Group	No. of subjects	Mean ± S. D	p value
Group C	50	151.64 ± 21.77	<0.001
Group R	50	256.66 ± 20.56	
Group C v/s R			

The mean for serum MDA levels in group R was 256.66 ± 20.56 and the group C was 151.64 ± 21.77. The mean was found to be increased in group R when compared with group C. The difference between the means of the two groups was found to be statistically significant ($p < 0.001$) which is represented in the Table 1.

Serum Calcium levels

Table 2: Mean Serum Calcium Levels

Group	No. of subjects	Mean ± S. D	p value
Group C	50	9.31 ± 0.91	< 0.001
Group R	50	7.09 ± 1.22	
Group C v/s R			

The mean for serum calcium levels in group R was7.09 ± 1.22 and the group C was 9.31 ± 0.91. The mean was found to be decreased in group R when compared with group C. The difference between the means of the two groups was found to be statistically significant ($p < 0.001$) which is represented in the Table 2.

Serum Phosphorus levels

Table 3: Mean Serum Phosphorous levels

Group	No. of subjects	Mean ± S.D	p value
Group C	50	4.10 ± 0.45	< 0.01
Group R	50	5.32 ± 0.47	
Group C v/s R			

The mean for serum phosphorus levels in the group R was 5.32 ± 0.47 and group C was 4.10 ± 0.45. The mean was found to be increased in group R when compared with the control. The difference between the means of two groups was found to be statistically significant ($p < 0.01$) which is represented in the Table 3.

Serum Alkaline Phosphatase

Table 4: Mean Serum Alkaline Phosphatase Levels

Group	No. of subjects	Mean ± S. D	p value
Group C	50	7.51 ± 1.51	> 0.05
Group R	50	7.56± 1.39	
Group C v/s R			

The mean for serum alkaline phosphatase levels in group R was 7.56 ± 1.39 and group C was 7.51 ± 1.51. The difference between the means of two groups was found to be not significant (p value >0.05) which is represented in the Table 4.

Calcium Phosphorus ratio

Table 5: Mean Calcium Phosphorous Ratio

Group	No. of subjects	Mean ± S.D	p value
Group C	50	2.29 ± 0.32	< 0.001
Group R	50	1.35± 0.32	
Group C v/s R			

The mean for calcium phosphorus ratio in group R was 1.35 ± 0.32 and group C was 2.29 ± 0.32. The mean was found to be decreased in group R when compared with the control. The difference between the means of two groups was found to be statistically significant ($p < 0.001$) which is represented in the Table 5.

Discussion

The diseases of the Musculo-skeletal system are common, disabling and costly to economy. Rheumatoid arthritis is a chronic systemic inflammatory disease affecting diarthrodial joints and frequently a variety of other organs. Formation of reactive oxygen species and lipid peroxides because of disease activity may play an important role in rheumatoid arthritis.

The highly reactive nature of reactive oxygen species makes it difficult to directly demonstrate their presence in vivo. It is considerably more practical to measure the foot prints of reactive oxygen species and reactive nitrogen species, such as their effects on various lipids, proteins and nucleic acids. Evidence for oxidative stress in rheumatoid arthritis has in many cases generated by approach that detect oxidant induced changes to those molecules.[11] There are several indicators of the extent of oxidative damage in humans. Some of the most common include, measuring oxidative damage to lipids by measuring Thiobarbituric acid reactive substances(most prevalent substrate of which is Malondialdehyde) and others include measuring, oxidative DNA damage by detection of oxidized base products, assessment of protein damage by quantification in the term of the number of carbonyls and modified tyrosine residues, assessment of levels of antioxidant enzymes like catalase, super oxide dismutase and glutathione peroxidase, assessment of levels of low molecular weight antioxidants and vitamins like uric acid, glutathione, flavonoids, vitamin E & C and β-carotene.[12]

Lipid peroxidation generates a variety of relatively stable decomposition end products mainly α, β-unsaturated reactive aldehydes such as Malondialdehyde (MDA) which can then be measured as an indirect index of oxidative stress. Compared with free radicals the aldehydes are relatively stable and can diffuse within or even escape from the cell and attack targets far from the site of the original event. They therefore are not only end products and remnants of lipid peroxidation process but also may act on as "second cytotoxic messengers" for the primary reactions.

Malondialdehyde is an easily measured and reproducible marker of lipid peroxidation, used as a biomarker of oxidative stress. MDA is detected as a sensitive marker of inflammation in the chronic auto immune disorder like rheumatoid arthritis by the Thiobarbituric acid assay, a standard test of lipid peroxidation.

The present study was undertaken to assess the role of free radicals in the enteropathogenesis of rheumatoid arthritis. A total of 100 subjects were included in the study categorized into two groups, 50 were healthy non-rheumatoid arthritis subjects in control group and 50 were rheumatoid arthritis patients who fulfill the ARA criteria[13] in study group. The following parameters were analyzed as a measure of oxidative stress and bone involvement. Serum Malondialdehyde levels were found to be increased with a statistical significance (p value < 0.001). Similar results were noticed in studies conducted by Gulden Baskol et al,[14] Jaswinder K. Gambhir et al[15] and Karatas F. et al.[16]

Serum calcium levels were found to be decreased with a statistical significance (p value <0.001), serum phosphorus levels were found to be increased with a statistical significance (p value <0.01), decrease in Calcium phosphorus ratio with a statistical significance (p value <0.001) and with no statistical significance (value >0.05) in serum alkaline phosphatase levels in study group when compared with the control group. These findings are identical to a study conducted by S.D. Walwadkar, A.N. Suryakar et al.[10]

The observation in the present study shows that oxidative stress plays an important role in the etiopathogenesis of rheumatoid arthritis. Addition of free radical scavengers to the existing therapeutic regimens may prove to be beneficial in the treatment of this chronic disease. Based on the findings of the levels of serum calcium, phosphorus and calcium phosphorus ratio which was significantly decreased in the present study suggest that there is an altered Calcium and Phosphorus metabolism and as calcium phosphorus ratio is very important in the formation of bones, Calcium supplementation could also be beneficial in view of bone resorption.

Conclusion

In conclusion, the increased MDA levels in the present study may be considered as important marker but apparently not sufficient to conclude that there was an increase in oxidative stress in rheumatoid arthritis patients. Further studies including markers of oxidative stress like serum vitamin E and peroxidase levels and other supporting evidences based on the levels of urinary calcium levels would help in complete understanding of the etiopathogenesis and thereby prevent functional disability of this severe disabling, crippling disease with unknown etiology associated with chronic inflammation and bone resorption.

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Conflict of interest

Nil

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