

Manuscript Title :

STUDY OF LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS.

First page file

- Title :** STUDY OF LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS.
- Category:** Original research paper
- Author:** Mrs.Nutan Yogesh Chaudhari¹
- Degree:** M.Sc.(Medical Biochemistry)
- Department:** Biochemistry
- Post:** Assistant Professor
- Institute:** M.I.M.E.R. Medical College, Talegaon Dabhade.
- Co-author:** Dr. A.N. Sontakke
- Department :** Biochemistry
- Degree:** M.D.(Biochemistry)
- Post :** Principal
- Institute:** M.I.M.E.R. Medical College, Talegaon Dabhade.
- Number of tables:** 1
- Number of graphs :** 3
- Author :** Mrs. Nutan Yogesh Chaudhari
Department of Biochemistry
M.I.M.E.R. Medical College ,Talegaon Dabhade, Pune.
Pincode: 411044
Contact : 9923009330

Emailaddress: chaudhari.nutan@gmail.com

Title : STUDY OF LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS.

ABSTRACT:

BACKGROUND AND AIMS:

Rheumatoid arthritis (RA) is characterized by local and systemic effects of inflammation and osteoarthritis (OA) is an inflammatory degenerative disorder of joint. A wide range of biochemical markers are implicated directly or indirectly to pathogenesis of rheumatoid arthritis (RA) and osteoarthritis (OA). The exact oxidant and antioxidant status is not clear. Our aims were to estimate serum levels of lipid peroxidation in terms of malondialdehyde (MDA) and serum levels of antioxidants (in terms of vitamin E and reduced glutathione) rheumatoid arthritis and osteoarthritis patients and compare them with the levels in normal healthy controls.

MATERIALS AND METHODS: A study was performed in the department of biochemistry at M.I.M.E.R. Medical College, Talegaon (D), Pune. Serum levels of lipid peroxidation status (MDA) and antioxidants (vitamin E and reduced glutathione) were estimated by spectrophotometric methods in thirty patients of RA and OA each. Age and sex matched thirty healthy controls were also included in the study and serum levels of same parameters were also measured. Statistical analysis was performed by using the student unpaired t- test.

RESULTS: serum levels of MDA was significantly increased ($p < 0.001$) while serum levels of vitamin E and reduced glutathione were significantly decreased ($p < 0.001$) in RA and OA as compared to healthy controls and in that especially MDA was more elevated ($p < 0.001$) in RA than OA. Negative correlation was established between MDA and vitamin E ($r = -0.42$) and reduced glutathione ($r = -0.41$) in RA.

Conclusion: Lipid peroxidation end product MDA and antioxidants like vitamin E and reduced glutathione showed statistically significant differences between controls and RA and OA patients which may have clinical utility in the management and administration of specific treatment to rheumatoid arthritis and osteoarthritis .

ABBREVIATIONS: RA - Rheumatoid arthritis, OA - Osteoarthritis, MDA - Malondialdihyde

KEYWORDS: Rheumatoid arthritis, osteoarthritis, vitamin E, reduced glutathione, malondialdihyde

doi: 10.15713/ins.mmj.6

❑ **TITLE:** STUDY OF LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS.

❑ **INTRODUCTION:**

Rheumatoid arthritis is a chronic systemic inflammatory disorder of joints characterized by synovitis that progresses to destruction of articular cartilage and have important role in the pathogenesis of disease. Osteoarthritis is degenerative , inflammatory and age related joint disease characterized by the progressive erosion of articular cartilage¹

It is established that reactive oxygen species plays an important in inflammation in RA and OA. Control mechanisms like oxidative modification of low density lipoprotein, DNA damage, lipid peroxidation and heat shock protein with the activation of neutrophil, NADPH oxidase and endothelial cell xanthine dehydrogenase contribute significantly to the inflammatory process².

Hence, the attempt of this study is to evaluate the association between lipid peroxidation & antioxidant status by measuring MDA as an index of lipid peroxidation , vitamin E and reduced glutathione as non-enzymatic antioxidants in rheumatoid arthritis and osteoarthritis patients.

❑ **MATERIALS AND METHODS:**

This study was conducted in the department of biochemistry, M.I.M.E.R. Medical college, Talegaon (D), Pune-18. The present study includes thirty clinically diagnosed patients of rheumatoid arthritis and osteoarthritis each in the age group of 30-55 years. The control includes age and sex matched thirty healthy volunteer.

- ❑ Subjects were selected from the departments of Medicine and Orthopaedics of Dr. Bhausaheb Sardesai Talegaon Rural Hospital ,Talegaon (D), Pune-18. Rheumatoid arthritis patients fulfilled the American Rheumatism Association criteria³ and osteoarthritis patients were diagnosed by carrying out x-ray analysis of joint destruction. Informed consent was obtained from all the study subjects.
- ❑ **INCLUSION CRITERIA:** Clinically diagnosed subjects with normal with normal nutritional habits were included in the study.
- ❑ **EXCLUSION CRITERIA:** Subjects having clinical history of diabetes mellitus, hypertension, cardiovascular disease, inflammatory disease, infectious disease, other type of arthritis and pregnancy were excluded from study. This study was also approved by the institutional ethical committee.
- ❑ 3ml of fasting venous blood samples were collected under aseptic condition in plain vials for estimation of MDA and vitamin E and also 2ml of fasting venous samples were collected in vials anticoagulated with 1ml acid citrate dextrose for estimation of reduced glutathione under aseptic precautions.
- ❑ Serum for MDA and vitamin E estimation was separated by centrifuging the blood samples collected in plain vials at 3000 rpm for 10 minutes.
- ❑ Serum MDA was estimated by Thiobarbituric acid method⁴. Serum vitamin E was estimated by Baker and Frank method⁵. Serum reduced glutathione was estimated by Beutlers method⁶. All specimens were processed within 72 hours after specimen collection.
- ❑ Results were presented as mean \pm SD. Statistical analysis was performed by using the student unpaired t-test and p value < 0.001 was considered as significant.

❑ **RESULTS:**

□ **TABLE 1.**

Study Group	Sample size	MDA (nmol/ml) Mean ± SD	Vitamin E (mg%) Mean ± SD	Reduced glutathione (μmol/gm Hb) Mean ± SD
Rheumatoid arthritis Patients	30	9.18±4.09**	0.57±0.21**	4.01±0.58**
Osteoarthritis patients	30	4.99±1.22**	0.64±0.23**	4.88±1.33**
Controls	30	2.76±0.75	1.18±0.28	5.60±1.16

**P<0.001 indicate significant.

In present study, serum Malondialdehyde, vitamin E and reduced glutathione levels were estimated.

P < 0.001 compared to controls – considered as a statistically significant.

There was a significant increase in serum MDA levels (p<0.001) and a significant decrease in levels of non enzymatic antioxidants like vitamin E and reduced glutathione levels p<0.001) in RA and OA as compared to controls. Serum MDA level was elevated especially in rheumatoid arthritis than osteoarthritis.

Negative correlation was established between lipid peroxidation end product MDA and antioxidants such as reduced glutathione (r = – 0.42) and vitamin E (r = –0.41) in RA.

❑ DISCUSSION:

Rheumatoid arthritis and osteoarthritis are characterized by increased biochemical markers of oxidative stress.

Recent studies have suggested that reactive oxygen species can be produced by human articular chondrocytes in osteoarthritis and also in rheumatoid arthritis^{7,8}.

Increased free radical activity is associated with activation of neutrophils, phagocytosis by macrophages at the site of inflammation. These processes increase lipid peroxidation of membrane which suggests the increased oxidative stress in joint disorders⁹.

The present study showed increased level of lipid peroxidation end product MDA while non-enzymatic antioxidant vitamin E and reduced glutathione were decreased.

MDA, the product of lipid peroxidation reacts with lysine residues in protein to produce immunogenic molecules which enhance inflammation. The long chain polyunsaturated fatty acids are more potent at increasing lipid peroxidation and causing cell damage by oxidative stress¹⁰.

The serum level of MDA, as an index of lipid peroxidation was estimated in patients with rheumatoid arthritis and osteoarthritis which was significantly increased in rheumatoid arthritis and osteoarthritis as compared to normal healthy controls, indicating an increase in the process of lipid peroxidation. In that serum MDA levels were more in rheumatoid arthritis than osteoarthritis. Results are in agreement of Rubyk BI et al(1998)¹¹ and Chaturvedi et al (1999)¹² and Vasanthi P et al(2009)¹³.

Vitamin E is a major chain breaking antioxidant, which is crucial for proper antioxidant protection. A significant decrease in vitamin E was found in patients with rheumatoid arthritis and osteoarthritis as compared to healthy controls in present study. The results are comparable with previous studies of low alpha tocopherol level in the synovial fluid of patients with inflammatory joint disease^{14,15}.

Serum Reduced glutathione (GSH) level is lowered as it is used for scavenging free radicals and peroxides by being oxidized to either GSSG or to a mixed disulphide, which prevents lipid peroxidation of cell membrane¹⁶.

From the above discussion it is presumed that increased free radical formation leads to oxidative stress which is involved in pathogenesis of rheumatoid arthritis and osteoarthritis. This leads to alterations in antioxidant status.

Further research is needed in this area to identify biochemical markers for differentiating between rheumatoid arthritis and osteoarthritis and hence in administration of appropriate treatment.

❑ CONCLUSION:

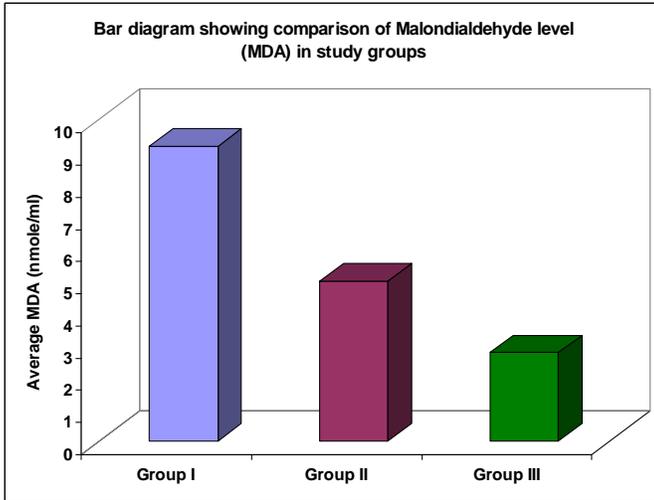
The present study has demonstrated that the serum biochemical markers (MDA, vitamin E and reduced glutathione) play an important role in the pathogenesis of rheumatoid arthritis and osteoarthritis. Further, these findings may help in finding biochemical marker for differentiating between rheumatoid arthritis and osteoarthritis and in instituting the treatment to patients of rheumatoid arthritis and osteoarthritis.

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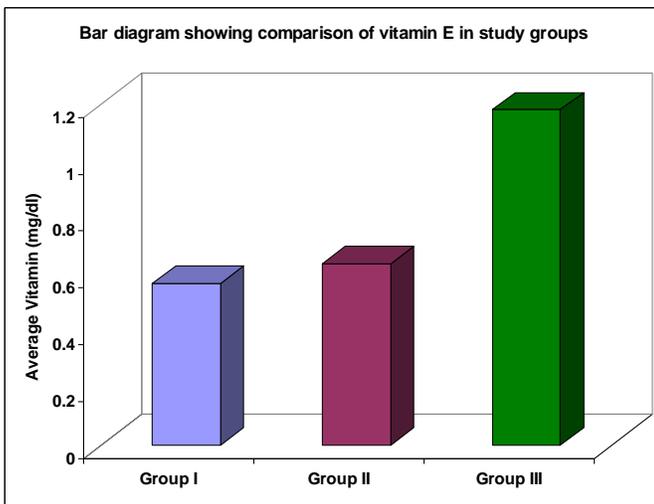
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Graph No.1:

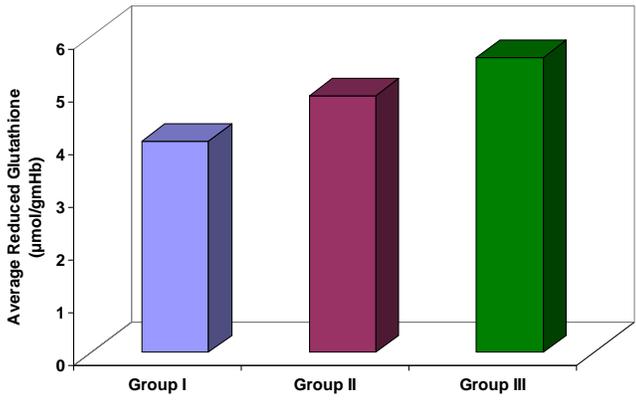


GRAPH No.2.



Graph No.3.

Bar diagram showing comparison of Reduced Glutathione in study groups



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