ABSTRACT: Background/Aims- Osteoarthritis is an inflammatory & degenerative disorder of joints. The exact pro-oxidant & antioxidant status is not clear in osteoarthritis. The aim of this study was to estimate levels of Malondialdehyde (the marker of lipid Peroxidation) & Reduced Glutathione (Non-enzymatic antioxidant marker) in serum of osteoarthritis patients & compare them with the levels in normal healthy controls. MATERIAL & METHODS: A study was performed at the Department of biochemistry at Pd. Dr. D.Y. Patil medical college, Pimpri, Pune-18(M.S.) In 30 patients of osteoarthritis serum levels of Non enzymatic Antioxidant & lipid Peroxidation marker were estimated by spectrophotometry. Thirty healthy controls were also included in the study & serum levels of same parameter also measured in them also. STATISTICAL ANALYSIS: It was performed by using the student unpaired t- test & correlation between variables was studied by using the Pearson’s correlation coefficient tests RESULT: Serum level of Reduced Glutathione was significantly lower in the patients than in the controls. (P<0.05). The serum Lipid Peroxidation marker (MDA) level was significantly increased in the patients than in the controls (P<0.05) CONCLUSION: The increased oxidative stress in terms of lipid Peroxidation marker (MDA) in the osteoarthritis patients is evidenced by decreased serum levels of non-enzymatic (GSH) antioxidant. KEYWORDS: Osteoarthritis (OA), Reduced Glutathione (GSH), Malondialdehyde (MDA)

INTRODUCTION: Osteoarthritis is called degenerative & inflammatory joint disease is most common type of joint disease. It is characterized by progressive erosion of articular cartilage. Osteoarthritis joins heart disease & cancer as one of the dividends of growing older. The age related changes in cartilage include alterations in proteoglycan& collagen .Chondrocyte plays a important role in the process & Plays a cellular basis of the disease.(1)

At the sites of inflammation of joint increased free radical activity is associated with activation of neutrophils, phagocytosis by macrophages, which involve respiratory bursts phenomenon& uncoupling of variety of cellular redox systems (2, 3). These process lead to ultimately increased peroxidation of unsaturated lipids of the membrane.

Lipid Peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction & cell damage. (4) Antioxidants are compounds that dispose, scavenge, & suppress the formation of free radicals or oppose their actions. (5)

This study evaluates the association between lipid Peroxidation & non-enzymatic antioxidant marker in osteoarthritis Patients.

To the best of our knowledge, only very few studies have been performed with respect to the estimation of the serum non-enzymatic antioxidant levels in patient with osteoarthritis & their role in prevention & treatment of osteoarthritis. In the light of this explanation, the present study was
undertaken to determine the levels of the non-enzymatic antioxidant (GSH) in the serum of osteoarthritis patients.

**MATERIAL & METHOD:** This study was conducted in the Department of Biochemistry, Pd. Dr. D.Y. Patil medical college, Pimpri. Pune-18

The present study consists of thirty clinically diagnosed cases of Osteoarthritis ranging in the age from 30 to 60 years. The control group consists of age & sex matched thirty healthy volunteer.

A thorough physical examination was carried out on all the patients. Routine hematological & radiological investigation was also done. Thirty cases selected from orthopedics OPD diagnosed by orthopedician. The presence of osteoarthritis in patients was diagnosed by carrying out X- ray analysis of joint destruction as well as C-reactive protein & antinuclear antibody test.

This study was also approved by the institutional ethical committee.

**Inclusion Criteria:** Subjects with normal nutritional habits without supplementing with any vitamins during the last six months included in the study.

**Exclusion criteria:** None of these subjects were alcoholic or chronic smoker, & none of them suffered from any systemic diseases like hypertension, diabetes, not having any history of trauma to joints, & also subject’s history of receiving any anti-inflammatory drugs in the last six months were excluded from the study.

3 ml of fasting venous blood samples were collected in Plain vials for estimation of MDA. & 3ml of whole blood in anticoagulated with 1ml ACD (acid citrate dextrose) collected in vial for estimation of reduced glutathione (GSH).

Serum for MDA estimation was separated by centrifuging the blood at 3000 rpm for 10 minute.

- Serum MDA was estimated by Thiobarbituric acid method (6)
- Reduced glutathione was estimated by using Beauter et al (7)

All estimation was done within 24-48 hrs after specimen collection.

The result were present as Mean ± SD. Statistical analysis was performed by using the student unpaired t- test & correlation ( r value) between variables was studied by using Pearson's correlation coefficient tests. P value < 0.05 was considered as significant.

**RESULT:** In present study, serum Malondialdehyde, reduced glutathione levels were estimated

Table 1: Levels of serum MDA, reduced glutathione, in patients with osteoarthritis and controls.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Sample size (n)</th>
<th>MDA (nmol/ml) Mean + SD</th>
<th>Reduced glutathione μmol / gm Hb Mean + SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>30</td>
<td>9.37±5.33**</td>
<td>3.90±0.99**</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>3.98±1.98</td>
<td>5.60±1.19</td>
</tr>
</tbody>
</table>

**P < 0.05 compared to controls – highly significant**

The serum MDA levels in the osteoarthritis patient was 9.37±5.33 nmol/ml which was significantly higher than that of controls (3.98±1.98 nmol/l, p<0.05)
Reduced Glutathione (non-enzymatic antioxidant) levels were also significantly decreased in osteoarthritis patients than controls (3.90±0.99 vs. 5.60±1.19 μmol/gm Hb, P<0.05)

Table 2: Statistical correlation between serum MDA & reduced glutathione levels in patient with osteoarthritis.

<table>
<thead>
<tr>
<th>Correlation variable</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA with reduced glutathione</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

MDA & reduced glutathione are negatively correlated & its value is -0.05.

Graph-1: Serum MDA level in osteoarthritis patients & controls (MDA conc. in nmol/ml)

Graph-2: Reduced Glutathione levels in osteoarthritis patients & controls (reduced glutathione conc. in μmol/gm Hb)
DISCUSSION: Osteoarthritis is characterized by increased markers of oxidative stress. Recent studies have suggested that human articular Chondrocyte can actively produce reactive oxygen species (ROS) (8).

ROS are released during inflammation of the Synovial membrane of synoviocyte. These radical oxygen species with oxidative activity play an important role in the Chondrocyte catabolic program: being the mediators and effecters of cartilage damage. The damaging effect of the process is initiated by a chain reaction that provides continue supply of free radicals which initiates further Peroxidation. This involves the mechanism of oxidative decomposition of n-3 and n-6 PUFA membrane phospholipids leading to formation of complex mixtures of lipid hydroperoxide aldehydic end products such as MDA. (9)

Antioxidant status of the body is again a subject of intrigue to medical researchers. The present study showed significant increased in lipid Peroxidation product MDA & significant decrease in non-enzymatic antioxidant marker reduced glutathione.

Since MDA is an index of lipid Peroxidation, its level was estimated in patients with osteoarthritis to estimate the extent of lipid Peroxidation. MDA levels were found to be significantly increased in osteoarthritis patients than in healthy individuals, indicating an increase in the process of lipid Peroxidation in osteoarthritic patients. Results are in agreement with Ruby K B. I et al (1998) (10) and K. K. Mane et al (1999) (11) and Tiku et al (2000 & 2003) (12,13).
Reduced glutathione a non-enzymatic antioxidant marker estimated in OA and healthy subjects (control). Reduced glutathione levels were found to be significantly decreased in patients with osteoarthritis than in healthy subjects indicating inadequate antioxidant mechanism in patients suffering from osteoarthritis. Negative correlation was established between the product of lipid Peroxidation (MDA) and non-enzymatic antioxidant (reduced glutathione). Suggesting that inadequacy in antioxidant mechanism may result in increased lipid Peroxidation which contributes to more progression and severity of disease. Results are in agreement with M Maneesh et al (2005) (14) and suprapaneni Krishnamohan (2007) (15)

From the above discussion it is presumed that oxidative stress involved in pathogenesis of OA which results due to increased free radical production. This leads to alteration in the antioxidant status which varies with the individual antioxidant depending upon their biochemical action. This is evident by noting significant decreased in GSH.

Further research required in this area to know the status of other antioxidant marker & about their beneficial therapeutic effect in the management of osteoarthritis.

REFERENCES:
8. Loeser R F, Shakoor N Aging or osteoarthritis which is the problem Rheum Dis. Clin North Am; 29[4]: 653-673
10. Rubyk BI, Fil chagin NM, Sabadyshin RA Change in lipid peroxidation in patient with primary osteoarthritis deformans. Ter Arkh 1988; 60 (9) : 110 – 113

Authors:
1. Manoj Narayan Paliwal
2. A.N. Sontakke
3. Prachi Paliwal

Particulars of Contributors:
1. Assistant Professor, Department of Biochemistry, R.D. Gardi Medical College, Ujjain.
2. Professor and HOD, Department of Biochemistry, MIMER, Medical College, Pune.
3. Demonstrator, Department of Medical Biochemistry, R.D. Gardi Medical College, Ujjain.

Name Address Email ID of the Corresponding Author:
Dr. Manoj Narayan Paliwal,
C/O Sonu Saluja,
35, Golden Palace Colony,
Near Basantpuri, AB Road,
Indore, M.P. 452012.
Email – dr.manojpaliwal@yahoo.co.in

Date of Submission: 22/07/2013.
Date of Peer Review: 23/07/2013.
Date of Acceptance: 24/07/2013.
Date of Publishing: 26/07/2013.