

# A Study of Oxidative Stress and Antioxidant Levels in Osteoarthritis

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## ABSTRACT

*Osteoarthritis (OA), a joint disease, is progressive and also degenerative. This has been one of the primary causes of morbidity in elderly population, affecting their social functioning and mental health due to the pain associated with degeneration. An increase in the levels of oxidative stress is associated with the degeneration of articular cartilage and promotes the progression of the disease.*

**Objective:** Our study was designed to evaluate the amount of oxidative stress in osteoarthritis, by quantifying the levels of Malondialdehyde (MDA) and antioxidant (enzymatic – Superoxide dismutase (SOD) and non-enzymatic – Vitamin C and Vitamin E) levels. Correlation between lipid peroxidation levels and antioxidants was also done along with the above said.

**Materials and Methods:** A total of 100 subjects were included in the study and were divided into two groups. The first group comprised of 50 patients who had been confirmed with osteoarthritis after clinical and radiological examinations. Another 50 subjects who were age and sex matched controls, formed the other group. Statistical analysis was done using Student's *t* test and Pearson's correlation coefficient was used for correlations.

**Results:** A significant ( $p < 0.001$ ) increase in the serum MDA levels and a significant ( $p < 0.001$ ) decrease in the enzymatic and non-enzymatic antioxidants was observed when compared with the normal controls.

**Conclusion:** There is an increased level of oxidative stress observed in patients with osteoarthritis, which is indicated by the increased MDA levels and the decreased antioxidant levels. Thus, these findings cite the biochemical rationale for performing clinical trials of antioxidants, in an attempt prevent and treat osteoarthritis.

**Key Words:** Osteoarthritis, Oxidative stress, Malondialdehyde, Superoxide dismutase, vitamin C.

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## INTRODUCTION

Osteoarthritis (OA) is a chronic degenerative disease. In this condition, the loss of cartilage due to destruction is a key feature, which results in chronic pain leading to compromised mobility and reduced quality of life<sup>(1)</sup>. The estimates around the world indicate that, of the men and women aged more than 60 years, 9.6% and 18% of them have symptomatic OA respectively<sup>(2)</sup>. Osteoarthritis related associated with hip as well as knee were ranked to be the 11<sup>th</sup> major contributors to world-wide disability, resulting in non-fatal conditions as burdens. This also adds up to 25% of all the visits to a physician and 50% of NSAIDs (non-steroidal anti-inflammatory drugs) prescribed<sup>(3)</sup>. This is expected to rise to a level of becoming an important public health issue in due time, owing to the increase in obesity levels and sedentary life-styles. This could add to the burden when considered from the perspective of developments in developing

nations, where life expectancy is expected, which could be hindered by unavailability of arthroplasty techniques and joint replacement facilities.

Oxidative stress is implicated in the pathogenesis of OA. Due to their high reactivity and non-specific nature, reactive oxygen species (ROS) can attack almost all the biomolecules. Chondrocytes play a key role in cartilage degradation by releasing metalloproteinase (MMP) and ROS. Nitric oxide (NO) and the superoxide anion ( $O_2^{\bullet-}$ ) are the main ROS produced by chondrocytes and these are also secondary radical generators. The factors such as interleukin (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) act as stimulants in such productions<sup>(4)</sup>. Articular cartilage is unique because it is non-vascular and depends on the movement of the joint for its nourishment. Normally, the movement at the joint, results in motion of the ligaments and tendons which is essential for dilation of the vessels around and passing the nutrients to the cartilage. Whereas in OA patients, the movement at the joint is restricted, resulting in increased synovial pressure than the related blood vessels around, which could lead to the collapse of blood vessels causing hypoxic reperfusion injury<sup>(5)</sup>.

The iron mediated Fenton reaction in this state, results in the conversion of superoxide and hydrogen peroxide from their less oxidant form to their strong oxidant

hydroxyl radical form. Such free radicals cause lipid peroxidation and oxidative damage of the cartilage leading to increase in lipid peroxidation products like malondialdehyde MDA and 4 hydroxynonenal (HNE). Such mechanisms in the body act as defense mechanisms against the oxidative damage caused by ROS. Based on this information, the present study was designed to correlate the levels of oxidative stress and antioxidant levels in OA by assessing MDA (product of lipid peroxidation), along with SOD, vitamin C and vitamin E that are part of the antioxidant defense mechanisms. The participating population in the study belonged to the regions of northern part of Karnataka state which is close to the borders of another state, Andhra Pradesh. Here, the dietary consumption is mainly sorghum and are also located in the endemic fluorosis belt. These factors make them more prone for bone and joint disorders and our study was designed considering these factors. However, we did not come across such studies done in these regions, which make this study a need from these populations.

#### MATERIALS AND METHODS

The study comprised of 100 participants with their age ranging between 50 to 70 years. Of them, 50 were patients suffering from osteoarthritis, visiting the orthopedics OPD and 50 of them were healthy persons (age and sex matched control group). The study was conducted from September 2010 to June 2011 at Navodaya Medical College Hospital and Research center, Raichur. Ethical clearance was obtained for the study from the institutional ethical committee and an informed consent was obtained from all the participants of the study.

#### Inclusion Criteria:

1. OA patient group: Confirmed (Clinical and Radiological) cases of Osteoarthritis.
2. Control group: Healthy, age and sex matched individuals.

#### Exclusion Criteria:

Malnourished individuals, smokers, alcoholics and patients with Diabetes Mellitus or cardiovascular diseases or any inflammatory diseases were excluded from the study group.

#### Collection and storage of blood samples:

About six ml of blood was collected under aseptic conditions using precautionary measures as sterile disposable syringe. The blood was collected in a plain bulb and serum was separated after centrifugation. SOD was estimated immediately after separating the serum. Serum was stored at 4°C until analysis of:

- Serum Malondialdehyde (MDA) by thiobarbituric acid Method<sup>(6)</sup>.
- Serum Vitamin C by 2, 4 dinitrophenylhydrazine Method<sup>(7)</sup>.
- Serum Vitamin E (tocopherols) by baker and frank Method<sup>(8)</sup>.
- Serum Super Oxide Dismutase by Marklund and Marklund Method<sup>(9)</sup>.
- The chemicals and reagents which were used for the assay were of Analytical grade.

#### Statistical Analysis

The data are expressed as mean and standard deviation. Student's t test was performed and p value less than 0.05 ( $p < 0.05$ ) was considered significant. The relationship between variables was analyzed by performing the Pearson's correlation coefficient.

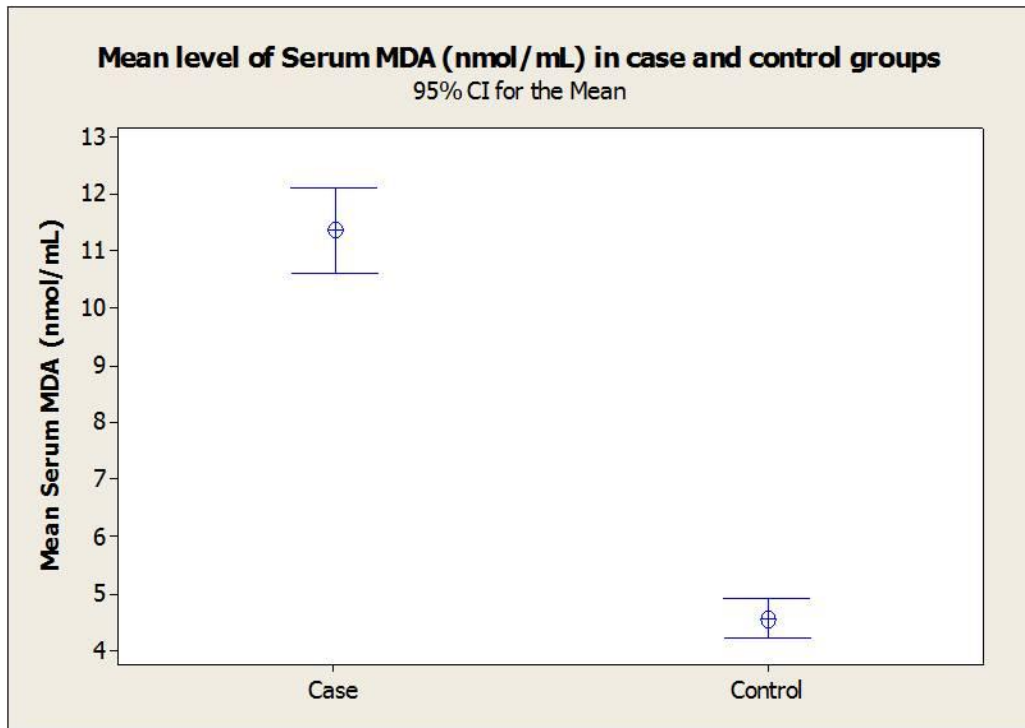
#### RESULTS

In the present study the mean level of serum MDA (nmol/mL) in controls was  $4.58 \pm 1.18$  and in cases was  $11.36 \pm 2.64$ . The value of enzymatic antioxidant SOD (units/ml) in controls was  $8.09 \pm 1.93$  and  $5.53 \pm 2.02$  in cases. The level of non-enzymatic antioxidant vitamin C (mg/dL) in controls was  $1.02 \pm 0.22$  and  $0.51 \pm 0.17$  in cases. The mean vitamin E (mg/L) level in controls was  $11.62 \pm 2.10$  and  $5.733 \pm 1.22$  in cases.

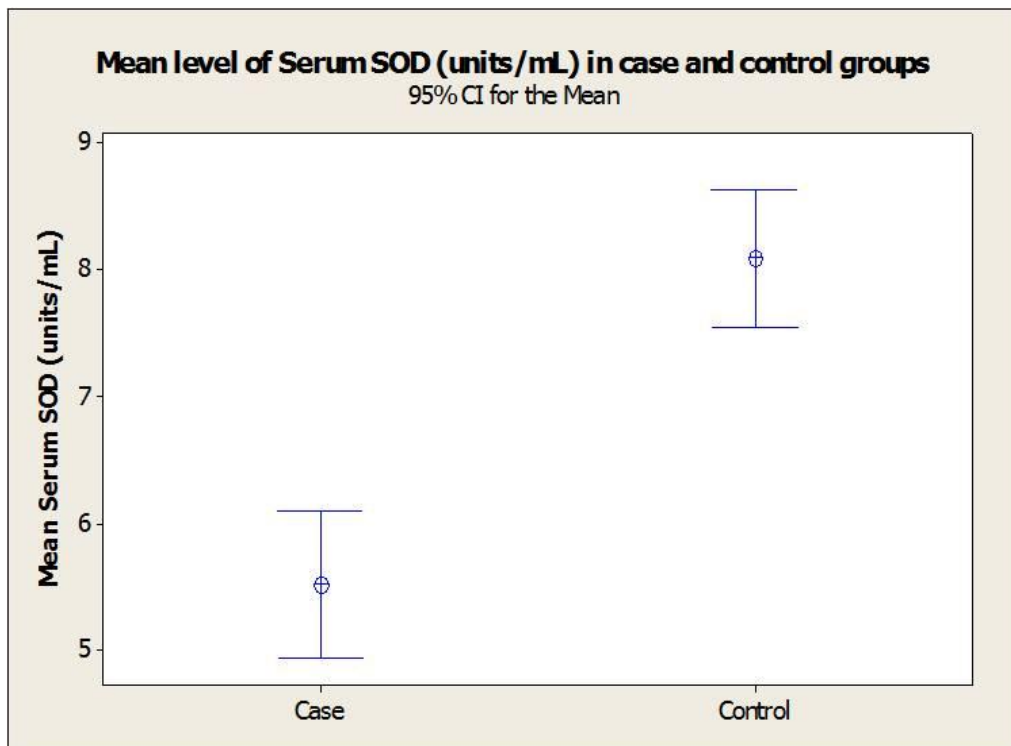
Table 1: Values of Serum MDA, SOD, Vitamin C & Vitamin E in control and OA groups

Parameters (measuring units)	Control	OA group
Serum MDA (nmol/ml)	$4.58 \pm 1.18$	$11.36 \pm 2.64^{***}$
Serum SOD (units/ml)	$8.09 \pm 1.93$	$5.53 \pm 2.02^{***}$
Serum Vitamin C (mg/dl)	$1.02 \pm 0.22$	$0.51 \pm 0.17^{***}$
Serum Vitamin E (mg/l)	$11.62 \pm 2.10$	$5.733 \pm 1.22^{***}$

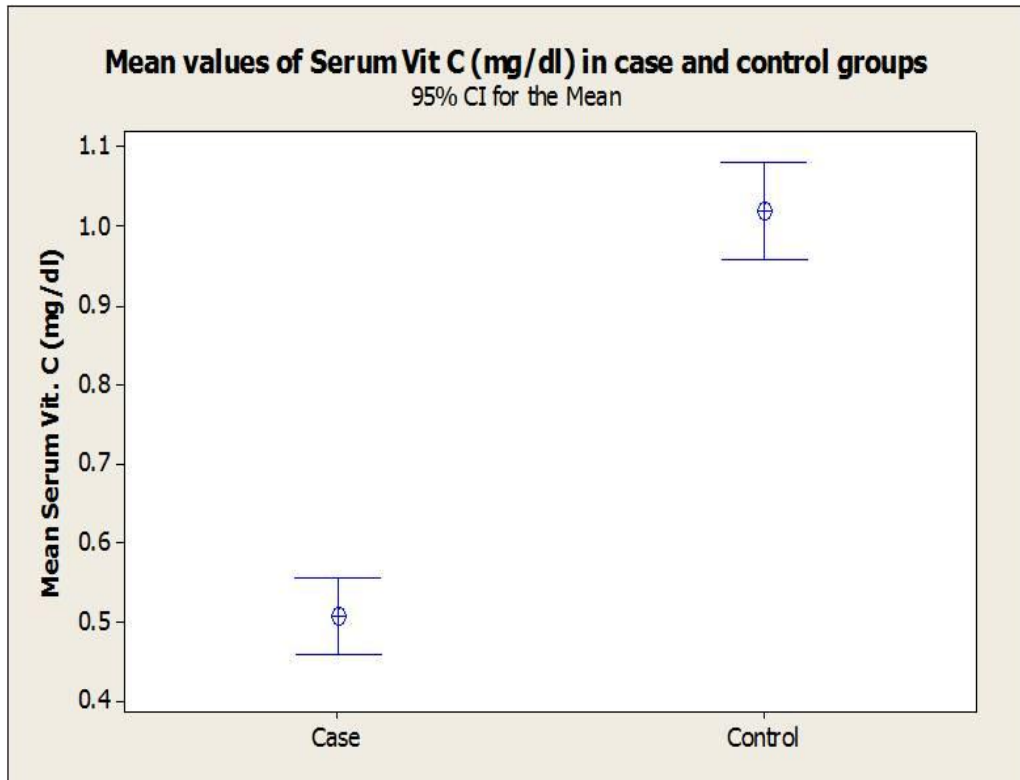
[\*\*\* =  $p < 0.001$ ]



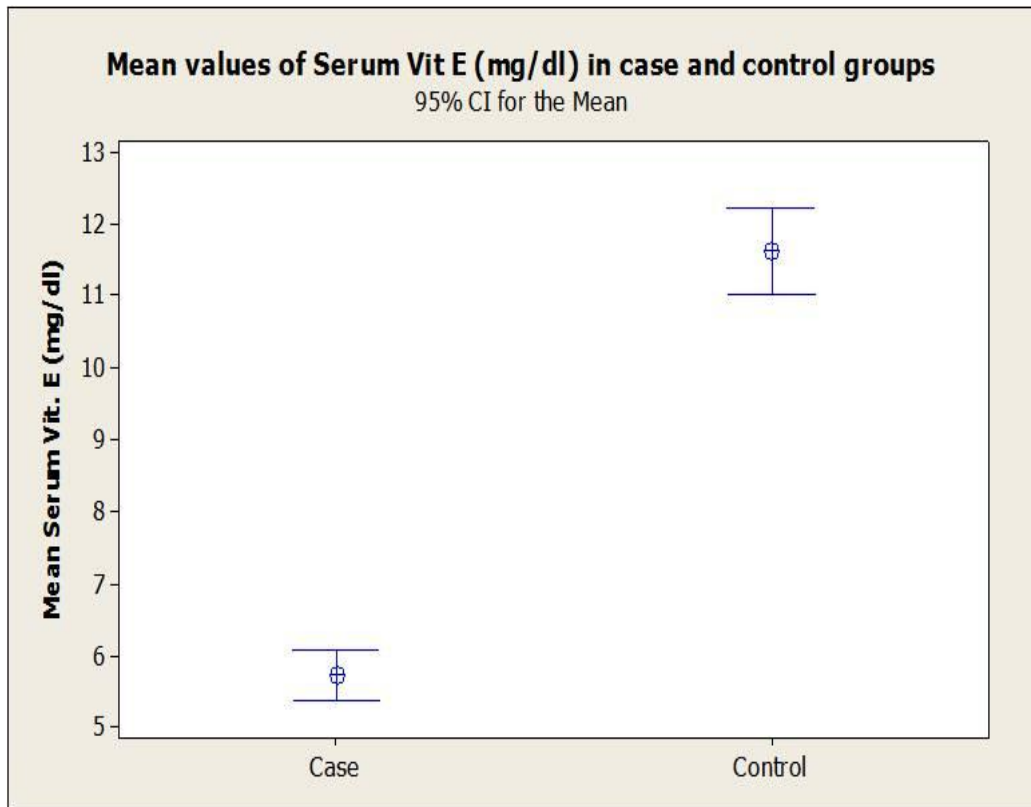
Graph 1: Graph showing significant (\*\*\*) -  $p < 0.001$ ) increase in serum MDA levels in cases when compared to control group.



Graph 2: Graph showing significant (\*\*\*) -  $p < 0.001$ ) decrease in serum SOD levels in cases when compared to control group.



Graph 3: Graph showing significant (\*\*\*) -  $p < 0.001$ ) decrease in vitamin C levels in OA group when compared with the control group.



Graph 4: Graph showing significant (\*\*\*) -  $p < 0.001$ ) decrease in the levels of vitamin E in OA group when compared with the control group.

**Table 2: Pearson's correlation coefficient between lipid peroxidation and enzymatic antioxidant (SOD) and non-enzymatic antioxidant vitamins (Vitamin C and Vitamin E)**

Parameter (measurement units)		r value
Antioxidant	Product of lipid peroxidation	
Serum SOD (units/ml)	Serum MDA (nmol/ml)	-0.052
Serum Vitamin C (mg/dl)		-0.122
Serum Vitamin E (mg/l)		-0.104

It was observed that there was negative correlation between serum MDA and both enzymatic and non-enzymatic antioxidants. This indicates that as the serum MDA levels increase, the levels of antioxidants (SOD, vitamin C and vitamin E) decrease, however this negative correlation was not statistically significant.

## DISCUSSION

The correlation of antioxidants in the body and osteoarthritis has a role to play with the healing process and treatment process of the latter. The presence of oxidative stress in osteoarthritis has been reported earlier<sup>(10)</sup> along with the relative reduction in the levels of antioxidants (vitamin C, GSH and catalase). In another report, a reduction in the levels of antioxidants in both osteoarthritis and rheumatoid arthritis<sup>(11)</sup> has been shown. This presence of oxidative stress could lead to the increased levels of free radicals, which in turn can hinder with the treatment in osteoarthritis. Oxidative stress and inflammation in OA has been reported to be prone in people consuming diets with antioxidant deficiency<sup>(12,13)</sup>. Hence, in cases of OA, there has been a continuous effort by researchers to find the antioxidant supplements which can be provided along with the treatment for OA. Some of the antioxidants reported were Resveratrol<sup>(14)</sup>, vitamin A, E and C<sup>(15)</sup> all combined and these vitamins have been studied individually too. There are also reports of homeopathic treatments found to be beneficial in OA. Another study had shown changes with respect to levels of lipid peroxidation in the chondrocytes and synovial fluid, whose levels have been found to reflect in the erythrocytes in OA<sup>(16)</sup>. However, there are various other factors too which can alter the effects on the level of antioxidant and the supplements which could be provided during treatment of OA. One of the aspects involving population studies and dietary supplements could be linked to the variations in the diet of the population itself. Choosing the mode of administration of antioxidant supplements and the time and stage of the condition being treated could be considered along with the geographical location (such as endemic fluorosis belt in this study) for better results. Endemic fluorosis has also been suggested to be a possible causative factor for knee OA<sup>(17)</sup>. Fluoride tends to have a cumulative poisonous effect which results in increased levels of formation of bones. It is said to stimulate proliferation of bone by boosting the signals of growth factors promoting bone formation<sup>(18,19)</sup>. In our study, the major staple food being sorghum (known for

very minimal vitamin content) and the population located in areas with high content of fluoride in water<sup>(20)</sup> were the reasons for the design and selection in the study. The contents of food cannot be ignored in such cases, which would alter the way the antioxidant supplements act on the condition and the healing process. This makes it essential for such studies to be conducted in varying populations from varying geographic locations.

In our study, we found a significant increase in serum MDA levels in patients with osteoarthritis as compared to the controls. The levels of SOD, vitamin C and vitamin E in cases were significantly decreased when compared to the controls. There was also a negative correlation observed between MDA and the antioxidant (SOD, vitamin C and vitamin E) levels. The decrease in the levels of vitamin E, vitamin C and SOD could be linked to their consumption in protective scavenging of ROS, which could also be the reason behind the negative correlation observed between MDA and vitamin C, vitamin E and SOD. The enzymatic and non-enzymatic antioxidants present in the body are capable of scavenging these free radicals. Enzymatic antioxidant such as SOD acts on superoxide radical and catalase converts H<sub>2</sub>O<sub>2</sub> into oxygen and water. The non-enzymatic antioxidant vitamin E would intercept the peroxy free radical and inactivate it before a PUFA can be attacked and Vitamin C stabilizes the hydroxyl radical with its capacity to act on singlet oxygen radical. Vitamin C can also act on vitamin E and revert it back to its active state. These findings are in agreement with the various studies cited earlier<sup>(6,11,12)</sup>. According to Venkatarao *et al.*, in a study conducted to determine the effect of lycopene in OA, the levels of MDA, vitamin C, vitamin E,  $\beta$ -carotene and SOD were measured in cases and controls before and after the treatment. A significant increase in the levels of MDA in OA patients has been reported against those in controls and these levels were seen to have decreased after the treatment. The levels of SOD, vitamin C, vitamin E,  $\beta$ -carotene were significantly decreased in cases than the controls before the treatment and were found to be increased after the treatment<sup>(21)</sup>. In another study by Jokie *et al.*, the levels

of MDA, SOD and catalase in OA cases and controls were estimated, along with the influence of sulphur baths and mud packs application on the activity of SOD and catalase. The level of MDA was increased in patients with OA as compared to the controls before the therapy and was decreased after the therapy. The level of SOD was decreased in OA cases before the therapy and was increased during the therapy<sup>(22)</sup>. In 2007, a study conducted by Alturfan *et al.*, showed a decrease in plasma total antioxidant activity, SOD and reduced glutathione in patients with OA compared to controls<sup>(23)</sup>. According to a report by M Maneesh *et al.*, increased levels of thiobarbituric acid reacting substances (TBARS) was seen as a result of increased lipid peroxidation. It was also reported that there was a significant decrease in the levels of antioxidants like vitamin C, GSH and catalase in patients with OA when compared with normal control group. The increased oxidative stress in OA patients could be the result of decreased levels of antioxidants or due to the increased levels of lipid peroxidation<sup>(10)</sup>.

## CONCLUSION

This study demonstrates a close relationship between oxidative stress and cartilage degeneration in osteoarthritis in the study population from endemic fluorosis belt. More studies involving large population groups from such areas if studied along with the treatments and their effects could provide vital information. Administration of antioxidants along with conventional drugs to patients from such areas could prove beneficial during the course of treatment of OA. More awareness in population from such areas regarding their improvement in diet with sufficient antioxidants could reduce the risk or advancement of OA.

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