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IMPACT OF SUPEROXIDE DISMUTASE (SOD) AND GLUTATHIONE REDUCTASE (GR) ACTIVITY AS MARKERS OF OXIDATIVE-STRESS AND THEIR ROLE IN INFLAMMATION WITH OSTEOARTHRITIS PATIENTS

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ABSTRACT

Introduction: Osteoarthritis afflicts millions of individuals across the world resulting in impaired quality of life and increased health costs. It is an important issue for both the individual and society. Osteoarthritis is a slow progressive and inflammatory disorder of synovial joints. It is a clinical syndrome in which low grade inflammation results in pain in the joints, caused by abnormal wearing of the cartilage that covers and acts as a cushion inside joints and destruction or decrease of synovial fluid that lubricates those joints. The term 'Oxidative stress' is a physiological condition or to the situation of a serious imbalance between production of reactive oxygen species/reactive nitrogen species (ROS/RNS) and antioxidant defense. Excessive ROS accumulation will lead to cellular injury. Oxygen-derived free radicals are generated during oxidative metabolism and energy production in the body and are involved in regulation of signal transduction and gene expression, activation of receptors, oxidative damage to cell components, as well as in aging and age related degenerative diseases. The disbalance between free radical burden and cellular scavenging mechanisms is a relevant part of OA pathogenesis. Oxidative stress is likely not only to promote cartilage destruction but also to be involved in inflammative transformation, promoting the transition from clinically silent cartilage destruction to apparent OA. **Objective:** To determine the level of blood marker of cellular oxidative stress (SOD,GR) with osteoarthritis patients by using antioxidant enzymes systems, as well as to determine the significant level (p value) of SOD and GR activity of controls and osteoarthritis patients(combined, >45 and<45 age). **Methods:** The present study carried out 20 c0ntrols, and 40 patients, has been suffering from osteoarthritis (inflammatory joint disease). The study protocol, consent form and all recruitment materials were approved by the ethical Board. Patient blood samples were carried out from OPTM research institution. SOD activity assay/ the inhibition rate (%) was determined using a colorimetric method or by abcam® (ab65354) superoxide dismutase activity assay kit at 450nm. GR activity assay was determined using a colorimetric method or by abcam® (ab83461) Glutathione reductase activity assay kit at 405 nm. **Results:** It was observed that SOD activity of osteoarthritis patients in combined group showed a significant increase ($p<0.001$) as compared to controls, and in two separate age groups(age<45 years and age>45 years) showed a significant increase ($p<0.001$; $p<0.001$) as compared to controls, besides it was studied that GR activity of osteoarthritis patients in combined group showed a significant increase ($p<0.001$) as compared to controls, and in two separate age groups(age<45 years and age>45 years) showed a significant increase ($p<0.001$; $p<0.001$) as compared to controls. **Conclusion:** As, this study concerning the significant increase then it indicate that this result may be as a disruption of homeostatic balance between the entire antioxidant and pro-antioxidant causing an increase in oxidative burden, one of the many etiological causes of chronic inflammation.

KEYWORDS: Osteoarthritis; Inflammation; Oxidative stress; Reactive oxygen Species; Reactive nitrogen species; Oxidative metabolism; Antioxidant; Pro-antioxidant; Superoxide dismutase; Glutathione reductase.

INTRODUCTION

Osteoarthritis afflicts millions of individuals across the world resulting in impaired quality of life and increased health costs. Osteoarthritis is a slow progressive and inflammatory disorder of synovial joints. It is a clinical syndrome in which low grade inflammation results in pain in the joints, caused by abnormal wearing of the cartilage that covers and acts as a cushion inside joints and destruction or decrease of synovial fluid that lubricates those joints. As the bone surfaces become less well protected by cartilage, the patient experiences pain upon weight bearing, including walking and standing.

Due to decreased movement because of the pain, regional muscles may atrophy and ligaments may become more lax.[1] The main symptom is pain, causing loss of ability and often stiffness. 'Pain' is generally described as a sharp ache or a burning sensation in the associated muscles and tendons. Some people report increased pain associated with cold temperature, high humidity, and a drop in barometric pressure, but studies have had mixed results.[2]

The disease imparts a profound economic impact on today's society, with healthcare costs exceeding $60 billion per year and OA aggregate costs increasing to $185.5 billion per year based on 2007 data.[3 ; 4] By the year 2030, an estimated 25% of the adult population in the united states will be afflicted with OA resulting in some form of disability.[5,6] While several risk factors have been associated with OA, including genetic predisposition[7], aging[8], obesity[9], and joint malalignment[10], the pathogenesis of OA remains largely unknown.[11; 12;13]

The term 'Oxidative Stress' is a physiological condition or to the situation of a serious imbalance between production of reactive oxygen species/reactive nitrogen species (ROS/RNS) and antioxidant defense. The formation of free radicals, which is secondary to the production of reactive oxygen species, is part of the physiological process of aerobic metabolism. In this manner, cellular metabolism produces free radicals in physiological conditions. These active radicals, in turn can be very useful in acting, as a defense mechanism controlled by molecular stimuli or signals against damage caused by microorganisms. [14] Free radicals are produced from normal cell metabolism in situ or from external sources (pollution, smoke, radiation, toxins, drugs, chemical products etc). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates an oxidative stress condition. This process plays a major part in the development of chronic and degenerative illness or this oxidative damage to essential cell components caused by oxygen free radicals is generally considered a serious mechanism in the pathogeny of many infirmities.[15; 16; 17]

Under normal circumstances, human body has antioxidant defense systems consisting of nonenzymatic antioxidants (vit. A, E, beta carotene) and of enzymes (SOD, catalase, glutathione peroxidase, glutathione reductase) which are capable of metabolizing oxygen free radicals. Human body has several mechanisms to counteract oxidative stress by producing this antioxidants. This endogenous and exogenous antioxidants act as 'free radical scavengers' by preventing and repairing damages caused by ROS and RNS, therefore can enhance the immune defense.[18; 19; 20; 21; 22] Increase in the levels of various biological indicators related to oxidative damage to cell, such as rupture of membrane, rupture of DNA chains and alteration in the structure or function of proteins[23] have been demonstrated in these situation. These modifications may be directly related to routine markers of inflammatory processes.

Oxygen free radicals are lipid-peroxidation-inducing agents that cause the depletion of unsaturated fatty acids of the cell membrane, thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes.[24] Oxidative damage to DNA leads to formation of different oxidative DNA lesions which can cause mutations. Production of reactive oxygen species generally is the result of tissue damage, and in turn, causes tissue damage associated with osteoarthritis.[25] While epidemiological studies are complex and probably require further validated method development, expert to agree that ROS plays a key role in the degradation of cartilage, a key factor in the etiology of OA.[26] Elderly and aging populations, because of lower socioeconomic status, reduced nutritional intake and lower ability to ingest, absorb and digest foods, generally take in reduced levels of antioxidants. [27] Poor intake of antioxidants, in conjunction with oxidative stress, has been associated with chronic disease states in the elderly.[28] The generation of free radicals is increasingly being implicated in both cartilage aging and pathogenesis of OA.[29; 30; 31] In the joints chondrocytes are potent sources of reactive oxygen species, which cause degradation of joint cartilage matrix components such as proteoglycans and collagen, as well as synovial fluid.[32] The body defense itself against free radical damage with an integrated antioxidant defense system that utilizes antioxidants produced naturally within the body such as SOD. This antioxidant can prevent matrix degradation and therefore may have a preventive or therapeutic value in OA.[33; 34]

Superoxide dismutase (SOD) is one of the most important anti -oxidative enzymes, that involved in the defense system against reactive oxygen species. SOD catalyzes the dismutation reaction of superoxide radical anion ($O_2^{\cdot -}$) into hydrogen peroxide and molecular oxygen, which is then removed by glutathione peroxidise or catalase.[35] Thus SOD prevent the formation of highly aggressive ROS, such as hydroxyl radical.

$$2O_2^{\cdot -} + 2H^+ + SOD = H_2O_2 + O_2$$

Glutathione reductase (GR) is a flavoprotein that catalyzes the NADPH-dependent reaction of oxidized glutathione (GSSG) to reduced glutathione (GSH), which plays an important role in the GSH redox cycle that maintains adequate levels of reduced GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress.

GR

$$GSSG + NADPH + H^+ \Longrightarrow 2GSH + NADP^+$$

(Oxidized glutathione)

In osteoarthritis and rheumatoid arthritis, there is a focal loss of cartilage resulting from increased activity of

catabolic pathways. This catabolic activity is stimulated, for the most part, by pro-inflammatory cytokines, like interleukin-1 and tumor necrosis factor alpha. In addition reactive nitrogen and oxygen intermediates are involved in the extracellular matrix degrading activity and may also be responsible for the cartilage damage occurring in osteoarthritis and rheumatoid arthritis.[36] Damaging oxidative species (reactive oxygen, nitrogen) arise as by-products of metabolism and as physiological mediators and signalling molecules.[37] The levels of these oxidative intermediates are held in check by the antioxidant defense system. The components of this defense system are micronutrients, like vit C and E or are dependent on dietary micronutrients (CU/Zn and Mn superoxide dismutase). The antioxidant defense is a coordinated system in which deficiency of the others. A deficiency in these micronutrients leads to oxidative stress, which leaves body tissues open to the damaging effects of the oxidative intermediates seen in arthritis. The copper, zinc and manganese are key components of the two major SOD enzymes which have been shown to fight against reactive intermediaries that are linked to the joint damage in arthritis.[38]

The objective of this study was to determine the effect of blood markers of oxidative stress (SOD, GR) and of antioxidant enzymatic system in inflammation with osteoarthritis patients.

MATERIALS AND METHODS

1. Study population/patients

The present study carried out 20 c0ntrols, and 40 patients, has been suffering from osteoarthritis (inflammatory joint disease). Patients were newly diagnosed and selected from OPTM health care. The study protocol, consent form and all recruitment materials were approved by the ethical Board.

2. Blood samples

5 ml of venous blood samples (with EDTA vial) were collected from osteoarthritis patients. Blood samples were centrifuged at 1000 x g for 10 min at 4°C. Serum aliquots were obtained after centrifuging of blood and stored at -80°C/-20°C until analyses were carried out.

3. Markers of oxidative stress/enzymatic antioxidant system activity analysis

A. Superoxide dismutase activity assay

SOD activity assay/ the inhibition rate (%) of SOD was determined using a colorimetric method or micro-plate reader by abcam® (ab65354) superoxide dismutase activity assay kit at 450nm.

B. Glutathione reductase (GR) assay

Glutathione reductase activity was determined using micro-plate reader (colorimetric) by abcam® (ab83461) GR assay kit at 405nm. This assay was measured GR activity by measuring the amount of reduced glutathione (GSH) generated from the reduction of GSSG (oxidized glutathione).

4. Statistical analysis

Statistical analysis was done by using software (Microsoft Office Excel 2016,add-in statistical tool pack) for the determination of student 't' test at a significant values ($p<0.05$) amount two variables. The't' test was used to compare between two independent means. The data was represented as the mean ± standard deviation.

5. RESULTS

Table 1:Superoxide dismutase assay activity(SOD) [U/ml] in osteoarthritis patients represented in combined groups (all age groups) and two types of separate age groups (<45 and >45 years). [n= no. of total samples; Mean± Standard deviation].

Subjects	SOD activity (U/ml)
Control (combined) [n=20]	17.88± 1.01
Experimental (combined) [n=40]	28.46± 4.95*
Control (<45 age) [n=10]	18.03± 1.00
Experimental (<45 age) [n=20]	28.82± 2.00*
Control (>45 age) [n=10]	17.73± 1.03
Experimental (>45 age) [n=20]	28.11± 6.78*

* $p<0.001$.

Table 2: Glutathione reductase assay activity (GR) [mU/ml] in osteoarthritis patients represented in combined groups (all age groups) and two types of separate age groups (<45 and >45 years). [n= no. of total samples; Mean± Standard deviation]

Subjects	GR activity (mU/ml)
Control (combined) [n=20]	8.84± 1.05
Experimental (combined) [n=40]	18.52± 5.62*
Control (<45 age) [n=10]	8.12± 0.82
Experimental (<45 age) [n=20]	14.08± 4.17*
Control (>45 age) [n=10]	9.56± 0.7
Experimental(>45 age) [n=20]	22.96± 2.42*

*P<0.001.

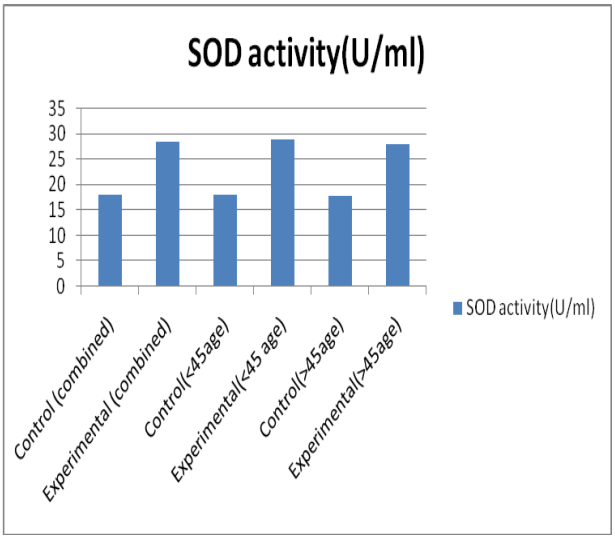

Fig 1: Mean ± SD value of Superoxide dismutase assay activity(SOD) [U/ml] in osteoarthritis patients represented in combined groups (all age groups) and two types of separate age groups (<45 and >45 years).

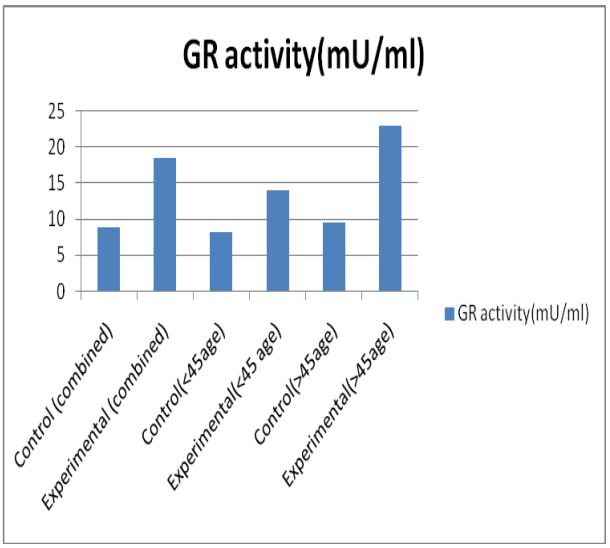

Fig.2: Mean ± SD value of Glutathione reductase assay activity (GR) [mU/ml] in osteoarthritis patients represented in combined groups (all age groups) and two types of separate age groups (<45 and >45 years).

In present study, Superoxide dismutase(SOD) activity and Glutathione reductase(GR) activity levels were estimated. Tables 1 and 2 presents results of oxidative stress markers level observed in the all age groups, <45 and >45 age groups of patients with OA, both in the experimental and in the controls.

It was observed that Mean ± SD value of SOD activity of osteoarthritis patients in combined group (all age group) showed a significant increase ($p<0.001$) as compared to controls and in two separate age groups (age<45 and age>45 years) showed a significant increase ($p<0.001$; $p<0.001$) as compared to controls. Table 1 represent that, there was a statistically significant increase in the SOD activity level in OA patients (combined, age<45 and age>45 years) with compared to controls.

It was observed that Mean ± SD value of GR activity of osteoarthritis patients in combined group (all age groups) showed a significant increase ($p<0.001$) as compared to controls and in two separate age groups (age<45 and age>45 years) showed a significant increase ($p<0.001$; $p<0.001$) as compared to controls. Table 2 represent that there was a statistically significant increase in the GR activity level in OA patients (combined, age<45 and age<45 years) with compared to controls..

6. 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). 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 effectors 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 per-oxidation. It is reported that synovial cavity damage correlates with fluctuating oxygen pressure in the joint, overproduction of free radicals and lack of oxygen-processing enzymes and free radical-scavenging molecules. [39] Free radicals are formed in both physiological and pathological conditions in mammalian tissues. [40] The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies.[41] Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions.[42]

The superoxide radical is the first product of molecular oxygen reduction. In addition to its natural toxicity, it is an important source of hydroperoxides and other reactive free radicals. The activity of superoxide dismutase (SOD), a catalyst for dismutation of superoxide radicals into H_2O_2 and into molecular oxygen, protects cells and tissues from superoxide radicals.[43] and other peroxides, such as lipid peroxides *in vivo*. These enzymes represent the first line of defense against superoxide radicals, and their production is rapidly induced under certain circumstances, such as exposure to oxidative stress.

Different authors have observed that oxidative stress induces the activity of SOD in leukocytes and erythrocytes. [44] Theoretically, since there is no protein synthesis in erythrocytes, there should not be any need for induction of the enzymatic activity. However, in erythrocyte-precursor cells, induction of SOD may occur following the oxidative process. This may explain the increase in erythrocyte SOD activity observed in our study when comparing the whole group of patients, and subgroups of patients, with controls. Since SOD represents the first line of defense in the intracellular

antioxidant defense system, and since its activity may be increased in order to compensate excessive production of superoxide radicals, the increase in enzyme activity suggests an adaptive response of patients against possible damages caused by oxygen free radicals.

The glutathione redox enzymatic cycle represents the most important intracellular defense against toxicity induced by oxygen free radicals. The cycle includes glutathione reductase (GR). The glutathione reductase enzyme reduces oxidized GSH, thus regenerating GSH. Under oxidative stress, there is an excess glutathione redox cycle, and thus an increase in the concentration of oxidized GSH.

Glutathione reductase plays an important role as an intracellular antioxidant in order to maintain a high GSH/oxidized GSH ratio, which is a fundamental condition for protection against oxidative damage. Enzyme activity of plasmatic GR was significantly higher in the whole group of patients when compared to controls. This result suggests an adaptive response in patients facing an increase in oxidative stress, and could be the consequence of a process of enzyme induction. GR, an oxidative stress inducible enzyme, plays a significant role in the scavenging mechanism and in maintaining functional integration of cell membrane.[45] The rise in the activity of SOD and GR could be due to its induction to counter the effect of increased oxidative stress. It appears that increased levels of superoxide and other radicals are not detoxified in patient with OA due to decreased efficiency of antioxidant enzymatic and non-enzymatic mechanism may act as mediators of tissue damage.

7. CONCLUSION

Oxidative stress may be involved in OA. There is a shift in the oxidant-antioxidant balance, which could lead to the tissue damage observed in this disease. The results of our study suggest higher oxygen free radical production, as evidenced by significant increase in SOD and GR activity levels, supports the higher oxidative stress hypothesis in OA. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The results suggest the association of cellular damage caused by oxygen free radicals with the pathogenesis of OA. The increased activity of SOD and GR confirm a reduction of intracellular defense protection against toxicity induced by oxidative stress in these patients. A crucial and causative role in the pathogenesis of these conditions is played by the free radical process, is involved in the oxidative modification of cellular and sub-cellular structures. It was observed that the entire gamut of non-enzymatic and enzymatic antioxidant system along with other relevant antioxidant bio-molecules come into play, trying to circumvent the oxidative stress. It is quite clear that the patients of OA are subject to oxidative stress and increased SOD and GR levels are due to the high oxidative burden on the OA patients.

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