



Journal Homepage: -www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI:10.21474/IJAR01/5183
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/5183>



RESEARCH ARTICLE

IMPACT OF GLUTATHIONE PEROXIDASE ACTIVITY (GPX) AS OXIDATIVE-STRESS MARKER AND ITS ROLE ON INFLAMMATION WITH OSTEOARTHRITIS PATIENTS.

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Manuscript Info

Manuscript History

Received: 15 June 2017
 Final Accepted: 17 July 2017
 Published: August 2017

Keywords:-

Osteoarthritis, Osteophytes, Subchondral cysts, Inflammation, Distal interphalangeal (DIP), Proximal interphalangeal joint (PIP), Carpometacarpal joint (CMC), Metatarsophalangeal joint (MTP), ROS, Oxidative stress, Chondrocytes, Free radical scavenging molecule.

Abstract

Osteoarthritis is an important issue for both the individual and society and its public health impact continues to grow due to the ageing population. It is the common form of arthritis and characterized by softening and disintegration of articular cartilage. The deterioration and loss of articular cartilage leading to an impairment of joint structure are the final pathogenic events common to osteoarthritis. Free radicals are produced in large amounts in tissues under physiologic conditions, these are controlled by several antioxidant protective mechanisms. Biologic tissues maintain a balance between antioxidant reserve and ROS or pro-oxidants, but under the pathophysiologic conditions this balance is upset because of an increase in ROS production and an alteration in the antioxidant defense system. This condition is oxidative stress. The objective was to determine the effect of blood glutathione peroxidase activity with OA patients, as well as to determine the p-value of controls, combined (all age groups) and two separate age groups (age < 45; age > 45). The study carried out 20 controls and 40 patients, has been suffering from OA and patient blood samples were carried out from OPTM health care and research institute. It was observed that mean \pm SD value of GPx activity of OA patients in combined group (all age groups) and in two separate age groups (age < 45; age > 45) showed a significant decrease as compared to controls. Then it indicates that the glutathione redox enzymatic cycle is the most important intracellular defense against toxicity induced by oxygen free radicals. This incident could be explained by the reduction of GSH found in OA patients. The enzyme itself may be inactive under conditions of intense oxidative stress, which contributes to low GPx activity. Besides, it was reported that synovial cavity damage correlates with fluctuating oxygen pressure in the joint, overproduction of free radicals and free radical-scavenging molecules. This result confirm the role of oxidative stress in the pathogenesis of OA and suggesting that in this condition treatment is required.

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Introduction:-

Osteoarthritis is an important issue for both the individual and society ^[1] and its public health impact continues to grow due to the ageing population, the rising prevalence of obesity and the lack of definitive treatments to prevent or halt the progress of the disease. ^[2] Osteoarthritis (OA) is the most common form of arthritis and characterized by softening and disintegration of articular cartilage. The progressive deterioration and loss of articular cartilage leading to an irreversible impairment of joint structure are the final pathogenic events common to osteoarthritis. ^[3] Prior to 1986, no standard definition of OA existed, most authors described OA as a disorder of unknown etiology that primarily affects the articular cartilage and subchondral bone in contrast to rheumatoid arthritis, a disorder that primarily affects the synovial membrane. In that year, the subcommittee on osteoarthritis of the American College of Rheumatology Diagnostic and Therapeutic Criteria Committee proposed that following definition of OA: “a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with the defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins”. ^[4]

OA is the most common form of arthritis. Its high prevalence, especially in the elderly, and the frequency of related physical disability make OA one of the leading cause of disability in the elderly, especially with respect to weight bearing functional tasks. ^[5] According to a report on the prevalence of arthritis: ‘By 2020 the estimated number of persons with arthritis is projected to increase by 57 per cent and activity limitations associated with arthritis by 66 per cent’. These projected increases are largely attributable to the high prevalence of OA among older persons and the increasing average age of the US population. ^[6] Epidemiology is the study of disease in populations and its association with characteristics of people and their environments. Epidemiological studies document the burden of disease in society and evaluate risk factors for disease that, if modified, might lead to disease prevention and a lessening of the burden of disability associated with disease.

OA is a disorder of diverse etiologies, which affects both the small and large joints, either singly or in combination. The classification schema for OA developed at the ‘Workshop on Etiopathogenesis of Osteoarthritis’ ^[7] in which idiopathic OA is divided into two forms: localized or generalized; the latter represents the form of OA described by Kellgren and Moore involving three or more joint groups. ^[8] Furthermore, generalized OA may occur with or without Heberden’s and Bouchard’s nodes, that is as either a nodal or non-nodal form. The classification of OA into idiopathic (primary) and secondary forms was based on the knowledge that OA could result from some recognized causative factors. These factors operate largely through two mechanisms: abnormalities of the biomaterials of the joint, usually the articular cartilage; and abnormalities of the biomechanics of the joint, usually due to the abnormal joint structure, resulting in abnormalities in the distribution of loading forces across the joint. Idiopathic OA is the most common form of arthritis and is a debilitating progressive disease that affects 60% of men and 70% of women over the age of 65 ^[9] with enormous socioeconomic costs, rivaling those of ischemic heart disease.

The current definition was developed in 1994 at a workshop entitled ‘New Horizons in Osteoarthritis’ sponsored by the American Academy of Orthopedic Surgeons, the National Institute of Arthritis, Musculoskeletal and Skin Diseases, the National Institute on Aging, the Arthritis Foundation, and the Orthopaedic Research and Education Foundation. ^[10] This definition underscores the concept that OA may not represent a single disease entity:

Osteoarthritis is a group of overlapping distinct diseases, which may have different etiologies but with similar biologic, morphologic, and clinical outcomes. The disease processes not only affect the articular cartilage, but involve the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane, and periarticular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration, and full thickness loss of the joint surface. OA diseases are a result of both mechanical and biologic events that destabilize the normal coupling of degradation and synthesis of articular cartilage chondrocytes and extracellular matrix, and subchondral bone. Although they may be initiated by multiple factors, including genetic, developmental, metabolic, and traumatic, OA diseases involve all of the tissues of the diarthrodial joint. Ultimately, OA diseases are manifested by morphologic, biochemical, molecular, and biomechanical changes of both cells and matrix which lead to a softening, fibrillation, ulceration, loss of articular cartilage, sclerosis and eburnation of subchondral bone, osteophytes, and subchondral cysts. When clinically evident, OA diseases are characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of inflammation without systemic effects.

Prevalence and incidence of OA:-

Osteoarthritis is an extremely common joint disorder in all populations. It often affects certain joints, yet spares others. For example, in the hands, the distal interphalangeal (DIP), proximal interphalangeal (PIP) joints, and the carpometacarpal (CMC) joint of the thumb are frequently involved. Other joints commonly affected include the cervical spine, lumbosacral spine, hip, knee, and first metatarsophalangeal (MTP) joint. The ankle, wrist, elbow, and shoulder are usually spared. Our joints were designed and shaped, in an evolutionary sense, when humans were brachiating apes. Only later did humans develop a pincer grip capability^[11] and full weight-bearing on their legs. These evolutionary differences in joint function and, possibly, differences in the composition of articular cartilage among the different joints, predispose some joints to cartilage breakdown, leading to OA.

Osteoarthritis can be defined in many ways. On the one hand, there is structural change in a joint often assessed by radiograph. Radiographic changes of OA include osteophytes and joint-space narrowing, the latter reflecting cartilage loss. Many persons with radiographic OA do not have joint symptoms. Secondly, one can assess the occurrence of joint symptoms. While this is an appealing definition from a clinical and public health standpoint, many with joint symptoms do not have radiographic changes of disease and may not have OA. Prevalence estimates across studies vary because of the inconsistent definitions of symptoms and radiographic change. The only exception to this is the Kellgren and Lawrence scale, which has been widely used to evaluate the prevalence of radiographic OA in most joints.

Radiographic disease is highly prevalent with radiographic hand OA occurring in approximately 32.5 per cent of adults aged 30 and over.^[12] The prevalence of radiographic knee and hip OA has been best studied in the population surveys of elders. The Framingham Study suggests that radiographic knee OA occurs in 33 per cent of people aged 63 and over, and studies from the United States of America and Europe suggest that radiographic hip OA occurs in roughly 3–4 per cent of elders.^[13] *Symptomatic OA* is generally defined as frequent joint pain plus radiographic change; its prevalence in the knee has ranged in different studies from 1.6–9.4 per cent of adults and in 10–15 per cent of elders. Symptomatic hip OA occurs in anywhere from 0.7–4.4 per cent of adults, and, using British data from the 1960s, symptomatic hand OA occurs in about 2.6 per cent of adults. The prevalence of OA in all joints is strikingly correlated with age. Regardless of how OA is defined, it is uncommon in adults aged under 40 and extremely prevalent in those aged above 60. Radiographic hand OA, for example, was present in only about 5 per cent of adults aged under 35, but was seen in over 70 per cent of those who were aged 65 or older.^[14]

Osteoarthritis has a higher prevalence, and more often exhibits a generalized distribution, in women than in men. Before the age of 50, men have a higher prevalence than women, but after the age of 50 women have a higher prevalence, and this sex difference in prevalence further increases with age.^[15, 16] These gender and age-related prevalence patterns are consistent with a role of post-menopausal hormone deficiency in increasing the risk of OA.

Free radicals are produced in large amounts in tissues under physiologic conditions, these are precisely controlled by several antioxidant protective mechanisms. Biologic tissues maintain a critical balance between antioxidant reserve and ROS or pro-oxidants, but in pathophysiologic conditions this balance is upset because of an increase in ROS production characterized by biomolecular deterioration and an alteration in the antioxidant defense system. This condition is known as oxidative stress. (Fig 1) it plays a crucial role in the development of degenerative diseases, including rheumatoid arthritis and osteoarthritis etc.

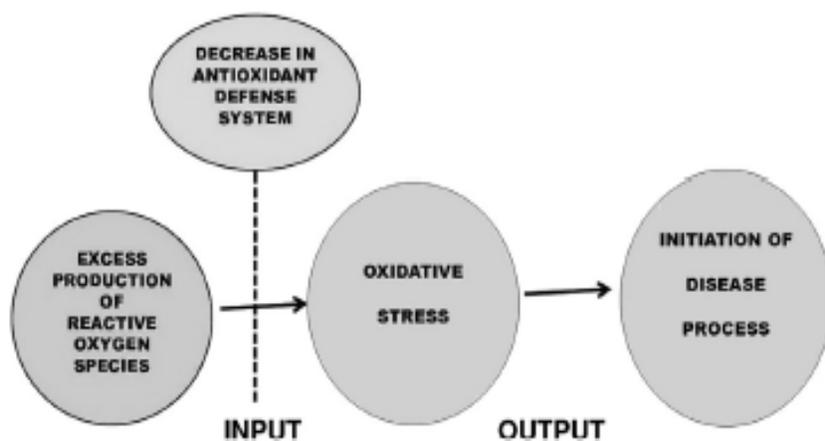


Fig 1:-Overview of oxidative stress in arthritis: increase in free radical production, with subsequent decrease in the antioxidant defense system, leads to oxidative stress which, in turn, initiates the disease process.

Several protective mechanisms have evolved in the biologic system that serve to protect the biomolecules, membranes and cells by preventing the generation, or by scavenging, the free radicals. These include antioxidant enzyme is glutathione peroxidase. Oxidative stress ensues when ROS evade the antioxidant protective mechanisms of synovial tissues. Alterations in the levels of the antioxidant with subsequent bio-molecular deterioration via increased ROS production, can cause degradation of cartilage collagen, loss of homeostasis in chondrocytes leading to impaired chondrocyte function, destructive changes in extracellular matrix, synovitis and cartilage aging, and thereby perpetuate the development of arthritis with senescence.^[17]

Glutathione Peroxidase:-

Glutathione peroxidase (GPx) refers to a family of multiple isozymes. In mammalian tissues, there are six GPx isozymes, namely, GPx1, 2, 3, 4, 5, and 6.^[18] GPx1, 2, 3, and 4 are selenoproteins. All of the GPx isozymes are able to catalyze the reduction of H_2O_2 or organic hydroperoxides (LOOH) to water or corresponding alcohols (LOH) using GSH as an electron donor (Eq.1 and Eq.2). During the reactions, GSH is oxidized to glutathione disulfide (GSSG):

GPx

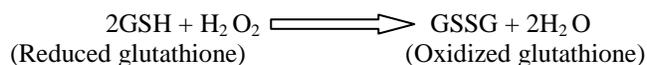


GPx



GPx play a crucial role in the final detoxification of H_2O_2 in blood and tissues.

Glutathione peroxidase



This enzyme spontaneously react with, and scavenge, many forms of ROS, prevent oxidation of lipids and phospholipids, maintain the intracellular redox milieu, replenish a number of crucial antioxidants (vitamins E and C) and thereby prevent free radical mediated biomolecular destruction in arthritis patients.^[19] Depleted levels of GPx in RA and OA patients have been observed in previous studies, clarifying their role in protecting chondrocytes from augmented oxidative stress.^[20, 21]

The objective of this study was to determine the effect of blood glutathione peroxidase activity (oxidative stress marker) in osteoarthritis patients.

Materials And Methods:-

Study population/patients :-

The present study carried out 20 controls, 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.

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.

Marker of oxidative stress:-

Glutathione peroxidase activity assay:-

Glutathione peroxidase assay was determined using colorimetric method or micro-plate reader by abcam® (ab102530) glutathione peroxidase assay kit at 340nm.

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 \pm standard deviation.

Result:-

Table1:- Glutathione peroxidase assay activity (GPx) [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 \pm Standard deviation]

Subjects	GPx activity (mU/ml)
Control (combined)[n=20]	79.97 \pm 5.39
Experimental (combined)[n=40]	30.28 \pm 5.85*
Control (<45 age)[n=10]	78.65 \pm 5.78
Experimental (<45 age)[n=20]	32.59 \pm 4.25*
Control (>45 age)[n=10]	81.30 \pm 4.91
Experimental(>45 age)[n=20]	27.97 \pm 6.41*

* $P < 0.001$. This was considered statistically significant for the result.

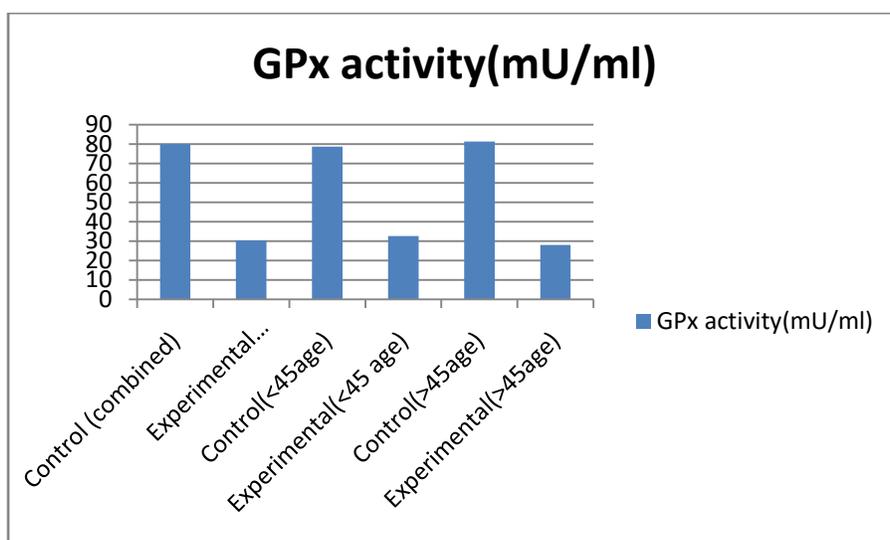


Fig.1:-Mean \pm SD value of Glutathione peroxidase assay activity (GPx) [mU/ml] in osteoarthritis patients represented in combined groups (all age groups) and two types of separate age groups (<45 and >45 years).

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

Discussion:-

The glutathione redox enzymatic cycle represents the most important intracellular defense against toxicity induced by oxygen free radicals. This cycle is the enzyme glutathione peroxidase. This enzyme uses GSH as a substratum in reactions that catalyze reduction of H_2O_2 , of fatty acids, and organic hydroperoxides into water and hydroxylated fatty acids.^[22] During the reduction of peroxides, oxidized form is produced. The significant reduction of GPx activity in the combined age group in <45 and >45 age group of patients when compared to controls could be explained by the reduction of GSH found in osteoarthritis patients, since GSH is a substratum and a cofactor of GSH-Px. Also lower levels of GSH result in lower activity of GPx, which, in turn, may increase vulnerability to oxidative stress. In addition, the reduction of glutathione peroxidase activity may also be caused by the process of enzyme inactivation. The enzyme itself may be inactive under conditions of intense oxidative stress, which contributes to low GPx activity.^[23] It was 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.^[24]

Conclusion:-

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. Patients with systemic OA presented highly marked modifications related to the presence of systemic oxidative stress, characterized by intense lipid and protein per-oxidation and reduced antioxidant defense system. This leads to alteration in the anti-oxidant status which varies with the anti-oxidant depending upon their biochemical action. The result of the study confirm the important role of oxidative stress in the pathogenesis of OA and suggesting that in this condition treatment is required.

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