Assessment of antioxidant enzyme status and lipid peroxidation in different KL grade knee osteoarthritis patients

Rahul Saxena[1], Jyoti Batra[2,*], Gladys Rai[3], Saurabh Srivastava[4], Rajni Ranjan[5]

[1]Research Scholar, [2,4]Professor, [3]Professor & Head, [5]Associate Professor, [1,2]Dept. of Biochemistry and Orhtopedics, Santosh Medical College & Hospital, Santosh University, Ghaziabad, Uttar Pradesh, [3-5]Dept. of Biochemistry, Medicine and Orhtopedics, School of Medical Sciences & Research, Sharda University Greater Noida, Uttar Pradesh, India

***Corresponding Author:**
Email: jyotivinay89@gmail.com

Abstract

Introduction: Although limited information is available about the pathophysiology of knee osteoarthritis (OA) disease in relation with oxidative stress, there is a paucity of satisfactory explanation regarding the alteration in the level of antioxidant enzymes and lipid peroxidation with severity of knee OA.

Aim: To estimate the levels of antioxidant enzymes and malondialdehyde (MDA) in the knee OA patients of different KL grade and to determine the variation in their levels with severity of disease.

Materials and Methods: Antioxidant enzyme status (superoxide dismutase and ceruloplasmin) and malondialdehyde levels were measured in 150 knee OA patients (35-65 years), by using standard methods. On the basis of KL grading scale Knee OA patients were categorized into three groups (n=50 in each group) and statistically compared it with that of 50 healthy controls by using student's t-test.

Results: Erythrocyte superoxide dismutase activity was significantly low in Group II (P = <0.05) and Group III (P = <0.001) subjects as compared to healthy controls whereas serum ceruloplasmin level was increased significantly only in Group II (P = <0.05) and Group III (P = < 0.001) subjects. Similarly, serum MDA level was increased significantly in Group II (P = < 0.05) and Group III (P = <0.001) subjects as compared to healthy controls.

Conclusion: Study findings indicate that alteration in antioxidant enzyme status and increased production of MDA are excellent marker of oxidative stress in different grades of knee OA patients. Thus, the diet rich in antioxidant or antioxidant supplementation could be beneficial in delaying the progressive destruction of articular cartilage process and serve as a preventive strategy in field of knee OA management.

Keywords: Superoxide dismutase, Ceruloplasmin, free radicals, Malondialdehyde, Oxidative stress.

Introduction

Knee osteoarthritis (OA) is leading cause of chronic disability between fourth and fifth decade of life.[1] Name "osteoarthritis" arose from observation of the striking overgrowth of marginal and subchondral bone by the pathologists and radiologists. According to Hinman RS et al. global statistics, over 100 million people worldwide suffer from OA.[2] In general, the formation of osteophytes on the joint margins, periarticular ossicles and narrowing of joint cartilage associated with sclerosis of subchondral bone are the radiological evidences of knee OA. In addition, there are various ways to define radiographic knee osteoarthritis. Kellgren Lawrence (KL) grading scale is one of the best way to define knee OA.[3] This scale involves the following grades:- grade 0: normal; grade 1: doubtful narrowing of joint space and possible osteophytic lipping; grade 2: definite osteophytes and possible narrowing of joint space; grade 3: moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade 4: large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour.

Numerous biological phenomenon results from tissue damage by free radicals which are characterized by progressive morphological and physiological deterioration of the organs that are often accompanied with frequent attacks of various degenerative diseases such as knee OA.[4] Many physiological processes are known to result in the production of oxygen free radical e.g. enzymatic action (e.g. NADPH oxidase, xanthine oxidase system), electron transport processes within the mitochondria, arachidonic acid metabolism and the activation of phagocytic cells. Antioxidant defense system of body reduces or eliminates the free radicals e.g. superoxide (O_2), hydroxyl (OH), hydrogen peroxide (H_2O_2) and peroxyl free radical (ROO).[5] In early age, the generation of free radicals appear to be approximately in balance with the antioxidant defense system but as the age progresses this balance is upset because of reduction in antioxidant reserve and excessive production of free radicals which play a crucial role in development of knee OA and its consequent sequelae.[6] Excessive musculoskeletal loading, high body mass index, previous knee injury, female gender and muscle weakness are also well-known factors of knee OA.[7] The antioxidant defense system which protects the biomolecules against potentially damaging effects of free radicals include antioxidants and antioxidant enzymes e.g. superoxide dismutase (SOD), ceruloplasmin, uric acid, vitamin C

and vitamin E etc. The imbalance between pro-oxidants and antioxidants gives rise to cellular oxidative stress, which plays an important role in the progression of OA.[8]

Culprit effect of free radical on phospholipid or polyunsaturated fatty acid of membrane of cellular or subcellular organelle is characterized by the process known as lipid peroxidation. Consequently, there is a generation of complex mixture of aldehydes, ketones and polymerization products which react and destroy the biomolecules, enzymes and nucleic acid leading to disease process. Malondialdehyde (MDA) is the most abundant and excellent marker of lipid peroxidation among reactive aldehydes and plays a crucial role in rheumatic diseases.[9,10] Dwivedi et al. in their study on osteoarthritis and rheumatoid arthritis reported a significant alteration in the levels of antioxidant enzymes and concluded it as a one of the important factor in the development of rheumatic diseases.[11]

Although limited information is available about the status of antioxidant enzymes in knee OA patients in relation with oxidative stress, there is a pausity of satisfactory explanation regarding the alteration in the level of antioxidant enzymes (SOD and ceruloplasmin) and lipid peroxidation with increase in disease severity as per KL grading scale in Knee OA patients. Therefore, the overall objective of present study was to estimate the level of antioxidant enzymes and malondialdehyde in the Knee OA patients of different KL grades and to determine the variation in their levels with increasing severity of disease.

Materials and Methods

A cross-sectional study, was conducted at Biochemistry department, Santosh Medical College and Hospital, Ghaziabad and Biochemistry department, School of Medical Sciences and Research, Greater Noida, from September 2016 to August 2018. Ethical clearance was obtained from institutional ethical committee. 150 Radiographic knee OA patients between the age group of 40-65 years, attending OPD were included from urban area of Delhi-NCR region of North India. Radiographic knee osteoarthritis was defined according to Kellgren Lawrence (KL) grading scale[3] and the patients were divided into 3 groups of 50 each on the basis of KL grading scale of II to IV (as Group I, Group II and Group III). 50 healthy subjects were taken from the hospital staff and their relatives included as control. Height and weight were measured with subject barefoot and light dressed. The body mass index (BMI) was calculated as BMI = weight (Kg) / Height (metre^2).

In order to recruit the patients, radiography was carried out which include weight bearing anteroposterior tibio-femoral view in full extension and skyline patella view. The radiographs collected from patients were read by an experienced radiologist. After taking their informed written consent, general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination (blood pressure measurement and pain measurement) was done of all the subjects. Patients who gave informed consent for study, fulfilled American Rheumatism Association Clinical diagnostic criteria for knee OA and had radiological evidence of grade 2, 3 and 4 knee OA in at least one or both of the knees (as per KL grading scale) and those who had not taken any vitamin supplements in last one month before study were included in the study. Patients were required to have pain for more than half the days of a month and at least pain score above 20% using a 5 cm visual analogue scale (VAS).

Smokers or patients suffering from conditions that affect lipid profile such as diabetes mellitus, hypothyroidism, liver or kidney disease, obesity (body mass index > 30) and a history of familial dyslipidemia were excluded from the study. In order to remove biasness, knee OA patients having one type of grade in one knee and different grade in another knee, and KL grade I knee OA patients were also excluded from the study.

Fasting blood samples were collected in EDTA vials from the anticubital vein of the subjects and processed immediately. Plasma ceruloplasmin levels were estimated by Ravins's method (1961). Ceruloplasmin due to its oxidase activity, catalyses the oxidation of substrate p- phenylenediamine chloride into purple coloured oxidation product, measured spectrophotometrically at 530 nm.[12]

Erythrocyte SOD activity was measured by Marklund and Marklund's method (1974). The enzyme SOD inhibits the auto-oxidation of pyrogallol by catalyzing the breakdown of superoxide. The inhibition of pyrogallol oxidation by SOD is monitored at 420 nm and the amount of enzyme producing 50% inhibition is defined as one unit of enzyme activity.[13]

Serum MDA levels were estimated by thiobarbituric acid (TBA) reaction. Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid (pH 2-3) and boiled with thiobarbituric acid which reacts with Malondialdehye, forming a MDA-TBA to get pink color. The pink colored complex that occurred was refrigerated to room temperature and measured by using a spectrophotometer at 530 nm.[14]

Statistical analysis: Values were entered manually in MS windows excel sheet and expressed as Mean ± SD. The significance of mean difference between groups was compared by using Student's t test and distribution of probability (p) in online Graph pad software.

Results

The anthropometric measurements and the levels of antioxidant enzymes along with serum malondialdehyde in the subjects of study group subjects are depicted in Table 1 and Table 2 respectively. Knee

OA was more prevalent in female subjects as compare to males. BMI and visual analogue scale of pain measurement revealed significant and continuous elevation in Group I, II and III knee OA patients.

The erythrocyte SOD activity was found to be reduced continuously with severity of disease in Group I, II and III knee OA patients i.e. 13.71%, 33.47% and 34.54% low as compared to controls. Conversely, plasma ceruloplasmin levels were increased in all three patient group subjects i.e. 17.01%, 31.62% and 41.45% high respectively. Statistically, these values were significant altered only in Group II and III patients ($p<0.05$; $p<0.001$). Marked alteration was observed in serum MDA levels (11.31%, 38.32% and 54.01% high) in all patient groups i.e. KL grade II, III and IV knee OA patients compared to healthy controls. On comparing these levels, it has been observed that these levels were significantly increased in Group II and Group III (Table 2, P = < 0.05, P = < 0.001) as compared to healthy controls but these values do not differ significantly in Group I vs Group II and Group II vs Group III.

Table 1: Age and Anthropometry of various KL grade Knee OA subjects and healthy controls (Mean ± SD)

S. No.	Particulars	Control (N = 50)	Group I (N = 50)	Group II (N = 50)	Group III (N = 50)
1	Age (years)	53.2 ± 5.87	54.46 ± 5.72	57.66 ± 3.17	60.32 ± 2.94
2	M:F ratio	26 / 24	19 / 31	24 / 26	21 / 29
3	Height (meter)	1.61 ± 0.056	1.69 ± 0.077	1.66 ± 0.075	1.65 ± 0.064
4	Weight (Kg)	60.1 ± 4.08	70.08 ± 5.8	76.52 ± 6.7	76.86 ± 5.9
5	BMI (Kg/m^2)	23.02 ± 1.2	24.4 ± 1.1*	27.6 ± 1.2**	28.3 ± 0.99**
6	Systolic blood pressure (mmHg)	106.76 ± 3.25	111.3 ± 3.07	113.74 ± 3.75	116.5 ± 2.80
7	Diastolic blood pressure (mmHg)	74.96 ± 2.53	75.70 ± 1.85	75.12 ±2.47	76.94 ± 2.08
8	VAS pain (mm)	0.0	35.6 ± 5.8	58.4 ± 5.1**	75.5 ± 7.01**

* p = < 0.1: Non-significant, ** p = < 0.05: Significant, *** p = < 0.001: Significant

Table 2: Antioxidant enzymes activities and Malondialdehyde level of various KL grade Knee OA subjects and healthy controls (Mean ± SD)

S. No.	Particulars	Control (N = 50)	Group I (N = 50)	Group II (N = 50)	Group III (N = 50)
1.	SOD (U/gm Hb)	1580.28 ± 97.80	1363.60 ± 97.88*	1051.32 ± 97.73**	1034.4 ± 97.74**
2.	Ceruloplasmi (mg/dl)	27.45 ± 1.87	32.12 ± 1.89*	36.13 ± 1.84**	38.83 ± 2.32**
3.	Malondialdehyde (µmolMDA/ml)	2.74 ± 0.21	3.05 ± 0.23*	3.79 ± 0.25**	4.22 ± 0.25**

* p = < 0.1: Non-significant,
** p = < 0.05: Significant,
*** p = < 0.001: Significant

Discussion

Degradation of the extracellular matrix of the articular cartilage caused by reactive oxygen species including those associated with lipid peroxidation is generally believed to be a significant factor in the etiopathogenesis of knee OA.[15] Antioxidants and antioxidant enzymes, present in the body, destroy these free radicals. The primary intracellular antioxidant enzyme responsible to scavenge free radicals includes SOD, catalase, GSHPx and ceruloplasmin.[16] Alteration in their activities directly affects the cartilage loss and the vitality of chondrocytes. Recently, a novel link between knee OA and the regulation of oxidative stress in chondrocytes has been proposed, indicating the crucial role of oxidative stress in Knee OA development.[9] In the present study, erythrocyte SOD activity was significantly low in group II and group III patients as compared to controls which direct towards its protective and O_2 radicals scavenging action in knee OA patients with increasing severity. The diminished activity of SOD among these subjects could be explained on the basis of its progressive enzyme inactivation by resultant product of dismutation reaction i.e. H_2O_2 or due to increase in the glycosylation of SOD with increase in disease severity and aging process [17]. Findings of this study were in concordance with the findings of Dwivedi et.al, which showed a marked reduced activity of SOD in arthritic patients due to enhanced oxidative stress.[11] Reduction in antioxidant enzyme activities are generally associated with increased risk of secondary complications of knee OA such as cardiovascular disease and diabetes etc.[18-20]

Copper containing enzyme ceruloplasmin has been found to mimick the superoxide anion (O_2) scavenging

action of SOD and thus, scavenges O_2 radicals. In the present study, serum ceruloplasmin level was found to be increased insignificantly in KL grade III and IV knee OA patients (P = <0.05 and (P = <0.001, Table 2) as compared to healthy controls, which indicate that ceruloplasmin also protect the tissues against the deleterious effects of oxygen free radical and to compensate the loss of SOD activity, occur due to continuous increase in oxidative stress with advancing of knee OA. Although disease severity related changes in ceruloplasmin level have been the subject of intensive investigation, its ambiguous property as an antioxidant enzyme as well as acute phase protein are well reported in previous studies.[20-22] However, it is still unclear whether the altered activity of antioxidant enzymes is the cause or the consequence of increased oxidative stress during disease process.

Reduced enzyme activity therefore means increased production of H_2O_2 or incomplete scavenging of O_2 leading to further destruction i.e. lipid peroxidation via formation of highly reactive OH radical as a consequence of Haber- Weiss reaction with H_2O_2. Malondialdehyde (MDA), the most abundant reactive aldehyde derived from lipid peroxidation has been implicated as the causative agents in cytotoxic processes related to joint degeneration and physical inability to move followed by enhanced risk of cardiovascular disease most probably by inducing oxidative modification in cell membrane and low density lipoprotein molecules.[9,10,23] MDA level was also found to be significantly high in both the study groups (P<0.05, Table 2) as compared to control which indicate that knee OA disease pathophysiology is closely associated with oxidative stress mediated major interrelated derangements of cell metabolism such as peroxidation of lipids, degradation of aggrecan (major proteoglycan in the articular cartilage) and cartilage collagen, membrane ion transporters and other specific proteins.[4,6]

The findings were also in agreement with the findings of Gupta et al.[24] Besides this, free radical mediated lipid peroxidation in lysosome membranes leak out lysosomal hydrolases which cause dystrophic changes of muscle fibers, as a result muscle become weak with growing age.[16] Srivastava et al also showed that chondrocyte derived lipid peroxidation mediates collagen degradation as evidenced by enhanced MDA levels with increase in disease severity.[25]

Conclusion

On the basis of present study and consistent findings of previous studies, it can be inferred that these antioxidant enzymes are excellent markers of oxidative stress in different KL grade knee OA patients. Thus, changes occur during knee OA cannot be avoided but can be delayed and controlled to some extent by exogenous antioxidant supplementation. It may prevent or postpone the onset of Knee OA related degenerative changes and secondary complications. The present evidence is strong enough to have convinced physicians that daily consumption of fruits and vegetables rich in antioxidants should be increased with severity of disease in order to sustain the harmful action of free radicals in knee OA and its related complications.

References

1. Lutzner J, Kasten P, Günther KP, Kirschner S. Surgical options for patients with osteoarthritis of the knee. *Nat Rev Rheumatol.* 2009;5:309-16.
2. Hinman RS, Hunt MA, Creaby MW, Wrigley T, McManus FJ, Bennell KL. Hip muscle weakness in individuals with medial knee osteoarthritis. *Arthritis Care Res*. 2010;62:1190-93.
3. Kellgren JK, Lawrence JS. Radiological assessment of osteoarthritis. *Ann Rheum Dis*. 1957;15:494-501.
4. Halliwell B. Oxygen radicals, nitric oxide and human inflammatory joint disease. *Ann Rheum Dis*. 1995;54:505-10.
5. Sen CK. Oxygen Toxicity and antioxidants: state of the art. *Ind J Physiol Pharmacol*. 1995;39(3):177–96.
6. Martin J A, Buckwalter J A. Aging, articular cartilage chondrocyte senescence and osteoarthritis. *Biogerontology*. 2002;3:257-64.
7. Takeda H., Nakagawa T., Nakamura K., ngebretsen L. Prevention and management of knee osteoarthritis and knee cartilage injury in sports. *Br J Sports Med*. 2011;45:304-9.
8. Suantawee T, Tantavisut S, Adisakwattana S, Tanavalee A, Yuktanandana P, Anomasiri W, et. al. Oxidative Stress, Vitamin E, and Antioxidant Capacity in Knee Osteoarthritis. *J Cli Diagno Res*. 2013;7(9):1855-59.
9. Dutta J, Sharma D, Saxena R. Oxidative stress mediated electrolyte imbalance in 30 known cases of knee osteoarthritis patients: A clinical approach. *Asian J Medi Sci*. 2015;6(5):26-30.
10. Bhattacharya I, Saxena R, Gupta V. Efficacy of vitamin E in knee osteoarthritis management of North Indian Geriatric population. *Therap Adv Musculo Dis*. 2012;4(1):11-19.
11. Dwivedi S, Singh S, Jaiswal G. Lipid-peroxidation and antioxidant status in osteoarthritis and rheumatoid arthritis patients. *Int J Contem Medi Res*. 2016;3(6):1738-41.
12. Ravin HA. Photometric method of Ceruloplasmin. *J Lab Clin Med.* 1961;58:61.
13. Marklund S and Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* 1974;47:469–74.
14. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chimica Acta.* 1978;90(1):37-43.
15. Saxena R. Arthritis as a disease of ageing and changes in antioxidant status . In: Preedy VR, editor. Aging: Oxidative stress and dietary antioxidants. 1st ed. London: Academic press Elsevier publications; 2014;49-59.
16. Saxena R, Lal A M. Effect of Aging on antioxidant enzyme status and lipid peroxidation. *J Ind Acad Geriat*. 2006;2(2):53-6.
17. Ceballos-Picot L, Triver JM, Nocale A, Spsinet PM and Thevenin M. Age correlated modification of copper-zinc superoxide and Glutathione related enzyme activities in human erythrocytes. *Clin Chem* . 1992;38:36-70.

18. Saxena R, Batra J, Rai G, Srivastava S, Ranjan R. Evaluation of diabetes risk in knee osteoarthritis patients: A biochemical approach. *Updacon.* 2018;56.
19. Saxena R, Bhattacharya I, Saxena, R. Susceptibility of Knee Osteoarthritic patients to develop Cardio-vascular disease. *Asian J Med Scie.* 2013;4(3):62-8.
20. Louati K, Vidal C,Berenbaum F, Sellam J. Association between diabetes mellitus and osteoarthritis: systematic literature review and meta-analysis. *RMD Open.* 2015;1(1):1-10. Available as https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4613158/pdf/rmdopen-2015-000077.pdf
21. Verma VK. Ramesh V and Tiwari S. Role of Bilirubin, Vitamin C and Ceruloplasmin as antioxidants in Coronary Artery Disease. *Ind J Clin Biochem.* 2005;20(2):68–74.
22. Klipstein GK. Grobee DE and Koster JF. Serum Ceruloplasmin as a Coronary risk factor in the elderly. The Rotterdam study. *Br J Nutr.* 1999;81:139–44.
23. Tandon R. Sinha MK. Garg H and Khanna R. Oxidative stress in patients with essential hypertension. *Natl Med J India.* 2005;18(6):297-99.
24. Gupta V, Saxena R, Bhattacharya I, Sunita. Assessment of Coronary heart disease risk in knee osteoarthritic North Indian elderly. *J Ind Acad Geriat*. 2012;8:64-71.
25. Srivastava S, Saksena AK, Khatri S, Kumar S, Dagur RS. Status of oxidative stress biomarkers in osteoarthritis patients in North Indian population. *Ostoarthritis and Cartilage*. 2015;23(2):84-85.