Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/z71snh12

NONENZYMIC ANTIOXIDANTS STATUS AND LIPID PEROXIDATION IN KNEE OSTEOARTHRITIS PATIENTS

Dr. Dilutpal Sharma[1], Dr Rahul Saxena[2*]

[1]Additional Professor, Department of Biochemistry, "King George's Medical University ", KGMU, UP.
[2*]Professor & Head, Biochemistry department, Sharda School of Allied Health Sciences, Sharda University, Greater Noida

***Correspondence:** Dr. Rahul Saxena
*Professor & Head, Biochemistry department, Sharda School of Allied Health Sciences, Sharda University, Greater Noida. Email ID: rahulapril@gmail.com; Mobile: 9958255816

Abstract:
Background: Reactive oxygen species (ROS) mediated biomolecular deterioration including lipid peroxidation is generally believed to be a significant factor in the development of knee osteoarthritis (OA) and its consequent sequelae. Although limited information is available about the knee OA disease pathophysiology in relation with oxidative stress, there is a pausity of satisfactory explanation regarding the alteration in the level of nonenzymic antioxidants and lipid peroxidation with severity of knee OA
Aim: Therefore, the overall objective of present study was to estimate the levels of nonenzymic antioxidants and malondialdehyde (MDA) in the knee OA patients of different KL grade and to determine the variation in their levels with disease severity.
Methods: In the present study, nonenzymic antioxidants (ascorbic acid, vitamin E and uric acid) and malondialdehyde levels were measured in 120 knee OA patients (40-60 years) by using standard methods. Knee OA patients were categorized into three groups (n=40 in each group; on the basis of KL grading scale) and statistically compared it with that of 40 healthy controls by using student's t-test.
Result: Serum vitamin C and vitamin E levels were significantly low in Group II ($P<0.05$) and Group III ($P<0.001$) subjects as compared to healthy controls whereas Serum uric acid 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. Study group parameters were altered insignificantly in Group I subjects ($P<0.1$).
Conclusion: Our findings indicate that alteration in nonenzymic antioxidants 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 thereby serve as a preventive strategy in field of knee OA management.

Keywords: Vitamin C, uric acid, vitamin E, free radicals, Malondialdehyde, oxidative stress.

Introduction:

Knee osteoarthritis (OA) is leading cause of chronic disability between fourth and fifth decade of life.[1] The 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 ($O_2^{-\cdot}$), hydroxyl ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and peroxyl free radical ($ROO^{\cdot}$**)**.[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 phosholipid 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 nonenzymic antioxidants (vitamin C, vitamin E and uric acid) 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 nonenzymic antioxidants and malondialdehyde in the Knee OA patients of different KL grades and to determine the variation in their levels with increasing severity of disease.

Material and Methods:

In the present study, 120 radiographic knee OA patients (40-65 years) attending outdoor patient department 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] These patients were

divided into 3 groups of 40 subjects each based on KL grading scale of II to IV (as Group I, Group II and Group III) and 40 healthy subjects were included from the hospital staff and their relatives, served as control. Radiography before inclusion into the study included a weight bearing anteroposterior tibiofemoral view in full extension and skyline patella view. The blinded radiographs were read by an experienced observer. General information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college. 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).

Inclusion criteria: 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) were included. 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). Patients who had not taken any vitamin supplements in last one month before study were included.

Exclusion criteria: 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. 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 plain vials from the anticubital vein of the subjects and processed immediately for serum separation. Serum vitamin C (ascorbic acid) levels were estimated by Mc Cormick and Greene method. Ascorbic acid in serum is oxidized by Cu (II) to form dehydroascorbic acid which reacts with acidic 2,4–dinitrophenyl hydrazine to form a red bishydrazone, which is measured at 520 nm.[12]

Serum tocopherol levels were estimated by Hashim and Schuttringer method [13]. Protein in the serum is precipitated by an equal volume of absolute ethanol. The whole mixture is subjected to extraction by an equal volume of n-heptane. α,α^1-dipyridyl is added followed by ferric chloride reagent to the system which produce light pinkish orange color.

Serum uric acid levels were estimated by Caraway's method in which uric acid react with phosphotungstic acid in alkaline medium forming a blue color complex which is measured at 700 nm [14].

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.[15]

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.

Result:

The anthropometric measurements and the levels of nonenzymic antioxidants along with serum malondialdehyde in the subjects of study group subjects are depicted in Table 1.0 and 2.0 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.

Table 1.0 Demographic details of various KL grade Knee OA subjects and healthy controls (Mean ± SD).

S No	Particulars	Control group (n=40)	Group I (n=40)	Group II (n=40)	Group III (n=40)
1)	Age (years)	52.3 ± 5.75	53.40 ± 5.51	54.60 ± 3.15	50.46 ± 3.42
2)	M:F ratio	16/24	19/21	24/16	21/19
3)	Height (meter)	1.60 ± 0.050	1.67 ± 0.072	1.65 ± 0.071	1.64 ± 0.062
4)	Weight (Kg)	60.5 ± 4.04	69.05 ± 5.2	74.45 ± 5.8	75.72 ± 5.7
5)	BMI (Kg/m^2)	23.10 ± 1.1	24.5 ± 1.2*	26.9 ± 1.1**	28.0 ± 1.3**
6)	Systolic blood pressure (mmHg)	105.72 ± 3.28	112.5 ± 3.15	113.70 ± 3.72	116.2 ± 2.76
7)	Diastolic blood pressure (mmHg)	74.82 ± 2.50	75.62 ± 1.82	74.15 ±2.32	76.75 ± 2.15
8)	VAS pain (mm)	0.0	34.9 ± 5.5	57.5 ± 5.0**	74.9 ± 6.09**

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

Table 2.0 : Levels of nonenzymic antioxidants and Malondialdehyde level of various KL grade Knee OA subjects and healthy controls (Mean ± SD).

S.No	Particulars	Control Group (n=40)	Group I (n=40)	Group II (n=40)	Group III (n=40)
1.	Vitamin E (mg %)	1.48 ± 0.42	1.35** ± 0.32	1.08** ± 0.25	0.98 ** ± 0.24
2.	Vitamin C (mg %)	0.85 ± 0.21	0.76** ± 0.18	0.61** ± 1.4	0.58 ** ± 0.12
3.	Uric acid (mg%)	4.52 ± 1.32	4.8** ± 1.41	5.40 ** ± 1.45	5.74** ± 1.48
4.	Malondialdehyde (µmolMDA/ml)	2.75 ± 0.20	3.15 ± 0.24*	3.75 ± 0.24**	4.20 ± 0.26**

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

Serum vitamin E and C levels were found to be reduced continuously with severity of disease in Group I, II and III knee OA patients (i.e. 8.78%, 27.03% and 33.7% low vitamin E levels and 10.38%, 28.23% and 32.0% low vitamin C level) as compared to controls. Conversely, serum uric acid levels were increased in all three patient group subjects i.e. 6.19%, 19.46% and 26.99% 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 (14.54%, 36.36% and 52.72% 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 2.0, $P<0.05$, $P<0.001$) as compared to healthy controls but these values do not differ significantly in Group I vs Group II and Group II vs Group III.

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.[16] Antioxidants defense system, present in the

body, destroy these free radicals. The primary intra and extra cellular non enzymic antioxidants responsible to scavenge free radicals includes vitamin C, vitamin E and uric acid. [17] Alteration in the levels of vitamin C and vitamin E, as observed in the present study, directly affects the cartilage loss and the vitality of chondrocytes. In addition, decreased levels of these vitamins could not be only due to their role in limiting lipid peroxidation by scavenging free radicals but also due to their utilization in maintaining the body antioxidant reserve, membrane stability and integrity, and in normalization of superoxide formation. 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]

Interestingly, in contrast to opinion regarding role of uric acid in inducing synovitis and OA pathology, its antioxidant properties in extra cellular fraction are well documented. Ames et al. pointed out the fact that urate provides antioxidant defense against radicals causing cancer and aging in humans which in turn direct the researchers towards the antioxidant role of uric acid in inflammatory diseases including knee osteoarthritis [18]. Our findings were in agreement with the previous findings of Gupta et al [19]. According to him, altered level of serum uric acid might be due to enhanced oxidative stress in knee OA patients and body is trying to protect itself from the deleterious effects of free radicals by increasing uric acid production.

Reduced enzyme activity therefore means increased production of H_2O_2 or incomplete scavenging of $O_2^{-\cdot}$ leading to further destruction i.e. lipid peroxidation via formation of highly reactive $OH^{\cdot}$ 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.[9,10,20] MDA level was also found to be significantly high in both the study groups ($P<0.05$, Table 2.0) 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 and cartilage collagen, membrane ion transporters and other specific proteins. [4,6]

. Our findings were also in agreement with the findings of Gupta et al.[19] 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.[21]

Conclusion: On the basis of present study and consistent findings of previous studies, it can be inferred that these nonenzymic antioxidants 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; 5:309-316.
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; 62:1190-3.
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: 505-510.

5) Sen CK. Oxygen Toxicity and antioxidants: state of the art. Ind J Physiol Pharmacol 1995; 39 (3): 177 – 196.
6) Martin J A, Buckwalter J A. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology. 2002;3:257-264.
7) Takeda H., Nakagawa T., Nakamura K., Engebretsen L. Prevention and management of knee osteoarthritis and knee cartilage injury in sports. Br J Sports Med. 2011;45:304- 309.
8) Suantawee T, Tantavisut S, Adisakwattana S, Tanavalee A, Yuktanandana P, Anomasiri W, Deepaisarnsakul B, Honsawek S. Oxidative Stress, Vitamin E, and Antioxidant Capacity in Knee Osteoarthritis. Journal of Clinical and Diagnostic Research. 2013; 7(9): 1855-1859
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 Medical Sciences. 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. International Journal of Contemporary Medical Research 2016; 3(6):1738-1741.
12) Mc Cormick DB, Greene HL. Vitamin: In Tietz Text Book of Clin Chem Burtis CA, Ashwood ER eds. WB Saunders Company USA. 1998, pp. 1025-1027.
13) Hashim SA, Schuttringer GR. Rapid determination of tocopherol in macro and micro quantities of plasma. *Am J Clin Nutr* 1996; 19 : 137 – 144.
14) Caraway WT. Standard methods of clinical chemistry, Academic press, New York. 1963; 4: pp. 239-240.
15) Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta. 1978: 37-43.
16) Saxena R. Aging: Oxidative stress and dietary antioxidants. 1st ed. London: Academic press Elsevier publications; 2014. Chapter 5, Arthritis as a disease of ageing and changes in antioxidant status; p. 49-59.
17) Kadhim AS, and Al-Karawi AS. Investigating the multifactorial correlation between obesity and rheumatoid arthritis: A study of immunological and biochemical markers. Obesity Medicine. 2025, 53:100578-100582.
18) Ames BN, Cathcort R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defence in humans against oxidant and radical caused aging and cancer. *Proc Natl Acad Sci USA*. 1981; 78: 6858 – 6862.
19) Gupta V, Saxena R, Bhattacharya I, Sunita. Assessment of Coronary heart disease risk in knee osteoarthritic North Indian elderly. J Indian Acad Geriat. 2012; 8: 64-71.
20) 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 – 144.
21) 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): A84-A85.