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Does Antioxidant Superoxide Dismutase levels Decline with Advanced primary Knee Osteoarthritis in Synovial Fluid ? A Pilot Study

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ABSTRACT

Introduction: Osteoarthritis (OA) is a progressive, degenerative disease that leads to joint pain, tenderness, stiffness, locking, effusion, reduced motion, swelling, crepitus, and disability. The pain in OA is the most significant clinical feature and impacts function, mobility, quality of life, and the reason for medical advice. **Methods:** Fifty individuals with primary knee OA in the age range of 45–90 years were chosen at random for the research (N=50). The American College of Rheumatology's diagnostic criteria were employed to diagnose osteoarthritis, and a visual analogue scale was utilized to score the severity of pain. Knee OA was graded using the Kellgren-Lawrence (K-L) radiographic assessment method. The antioxidant levels of superoxide dismutase in the synovial fluid were measured by using a spectrophotometric assay. **Results:** Grades 1, 2, 3, and 4 have SOD activity values of 1.43±0.55, 1.44±0.72, 0.92±0.52, and 0.87 ±0.52 U/ml, respectively, in synovial fluid. Synovial fluid SOD activity was higher in grades 1 & 2 of KOA as compared to grades 3 &4 and the difference was statistically significant ($p<0.05$). **Conclusions:** There was a link between K-L grade and synovial antioxidant activity level. In the late stages of knee osteoarthritis, the antioxidant enzyme (SOD) activity was reduced.According to the results of this study, regular antioxidant supplementation to early osteoarthritis patients may delay disease progression by improving the antioxidant status of the knee, which neutralises free radicals and thus prevents cartilage damage.

KEYWORDS: Antioxidant, Knee osteoarthritis, Oxidative stress, Superoxide dismutase, Synovial fluid.

INTRODUCTION

Primary knee osteoarthritis (KOA) is the most common degenerative progressive multifactorial joint disease, which is characterized by persistent pain, stiffness, effusion, restricted mobility, oedema, crepitus, and functional impairment and morbidity [1, 2]. Knee osteoarthritis is most common in the elderly and has a negative impact on quality of life [3]. It is a severe issue for both people and society [4].

OA is a multifactorial and complicated disease. A variety of genetic and environmental factors influence its etiology, which is connected to the activation of molecular pathways that contribute to the progression of articular injury [5]. This condition is caused by changes in both cells and the extracellular matrix (ECM), which cause softening, fibrillation, ulceration, loss of articular cartilage and synovial inflammation, as well as the formation of osteophytes and subchondral cysts. It is also caused by changes in the way cells and the ECM work together. Previous studies have found that oxidative stress contributes to the development and progression of OA [6, 7]. ROS, which can come from a variety of sources, play an important role in the pathogenic process. Oxidative stress Reactive oxygen species (ROS) (nitric oxide, superoxide anion, hydrogen peroxide, and hydroxy radical) are highly reactive chemical substances that attack and destroy proteins, lipids, and nucleic acids. When cells are damaged, chondrocytes' structural and functional properties can be changed, as well as their extracellular matrix and tissue damage. All of these things may play a role in the development of OA [8–10]. Overproduction of ROS and oxidative stress in chondrocytes are two significant contributions to OA pathogenesis [11]. People who have high levels of lipid peroxide as they get older don't keep their articular cartilage in good shape, which

leads to OA [12].This antioxidant system includes superoxide dismutase, catalase, glutathione peroxidase, glutathione, NADPH, Ubiquinone oxidoreductase, paraoxonases, vitamin C, E, and carotenoids [13, 14]. They react spontaneously and scavenge a wide range of ROS, therefore maintaining an intracellular redox environment [15]. However, as individuals age, the antioxidant system becomes less efficient at neutralizing lipid peroxidative stress, and high levels of lipid peroxide induce a breakdown of homeostasis in the maintenance of healthy articular cartilage, leading to pathologic articular cartilage degradation in OA [16]. As a result, in this work, we looked at possible changes in the antioxidant enzyme activities of SOD and calculated their link to the grade (pain and severity) and duration of osteoarthritis. The study will also pave the way for more research into this topic and help figure out how antioxidant supplements can help people with knee osteoarthritis lessen the pain and severity of their symptoms.

METHDOLOGY

Study design: Hospital-based cross-sectional observational study

Ethics approval: The institutional ethics committee approved the study, and informed written consent was obtained from all the patients.

Sample size: Initially, 60 patients who visited the Orthopaedics outpatient and inpatient department during the study period were enrolled in the study. Out of these five patients who refused to participate, two were diagnosed with rheumatoid arthritis. Two were excluded as they had diabetes mellitus, and one patient was left out as he had a joint infection.

Sampling method: Consecutive sampling method was used for sampling. **Study period:** One year

Inclusion criteria: This study comprised patients aged 45 years and older with acute osteoarthritis symptoms (knee effusion), patients undergoing intra-articular pharmacological injection therapy, patients undergoing knee replacement, and arthroscopic lavage.

Exclusion criteria: Patients who have had previous surgery on the same joint, as well as those who have inflammatory joint disease, Patients who are using steroids or other long-term drugs, who have experienced pain as a result of a traumatic event Other systemic disorders that may produce elevated oxidative stress, such as serious liver, renal, or heart disease.

METHODOLOGY:

Each patient is thoroughly evaluated, including demographic information, illness duration, and a visual analogue scale assessment of pain severity (0-10). The American College of Rheumatology (ACR) criteria were utilized to diagnose knee OA.

The K-L grading system was used to grade knee joint radiographs [17]. To investigate probable links between poor antioxidant activity and disease development, knee grading was associated with antioxidant level parameters (synovial fluid SOD activity).

Synovial fluid sample collection: Synovial fluid samples were collected in the OPD from those patients' effused knees. After aspirating the fluid and withdrawing the needle from the joint, the needle was removed, and an end cap was put on the tip of the syringe. The synovial fluid sample was taken in a vacutainer without anticoagulant to determine the amount of SOD activity.

SOD levels estimation: To remove cells and particle debris, the synovial fluid sample was immediately put on ice and centrifuged at 3000 g for 30 minutes at 4°C. Before analysis, the supernatant was separated and stored at -70°C for up to 4 weeks. A spectrophotometric test based on SOD activity was performed using the technique proposed by Marklund and Marklund (1994) [18].

Unit definition: One unit of SOD is defined as the quantity of enzyme necessary to block pyrogallol auto-oxidation by 50% in 3ml of test mixture. Normal serum SOD level = 1.95-4.60 unit/ml.

Statistical analysis: SPSS20 was used to analyze all the data. The gathered data was examined for statistical significance using Pearson's correlation coefficient and an ANOVA test to analyze group variance in K-L groups.

RESULTS

Fifty study subjects were divided into four groups of primary knee Osteoarthrosis (KOA) based on Kellgren-Lawrence. Mean age, SOD, VAS score, and disease duration were all increased with increasing K-L grading of osteoarthritis Table 1. The mean SOD concentration (μM/L) was rising with K-L grading and K-L. Figure 1 and Figure 2 .

A one-way ANOVA was performed on participants with knee osteoarthritis to determine the amount of antioxidant superdioxide dismutase activity in relation to the severity of osteoarthritis in grade 1 (dubious), grade 2 (mild), grade 3 (moderate), and grade 4 (severe). According to a one-way ANOVA, the mean of SOD activity levels was uneven, $F(3, 46) = 3.784$, $p<0.03$.

Those in grades 3 and 4 reported substantially ($p<0.03$) lower SOD activity levels than subjects in grades 1 and 2. (Mean SOD = 1.17). Pairwise comparisons of means using Tukey's honestly significant difference procedure revealed that grades 3 (Mean SOD 0.93) and 4 (Mean SOD 0.87) had significant comparisons: subjects in grades 3 and 4 reported that their SOD activity levels were significantly (p0.05) lower than subjects in grades 1 (SOD activity is 1.43), and 2 (SOD activity 1.43). (Mean SOD values is 1.44.) The difference between grades 1 and 2, as well as grades 3 and 4, was not significant ($p = 0.958$ and $p = 0.813$, respectively). The

variations in SOD activity mean levels across groups were statistically significant (F = 3.78, P = 0.017). Post hoc tests by Tukey found that SOD levels in grades 3 and 4 osteoarthritis were significantly lower than in grades 1 and 2.

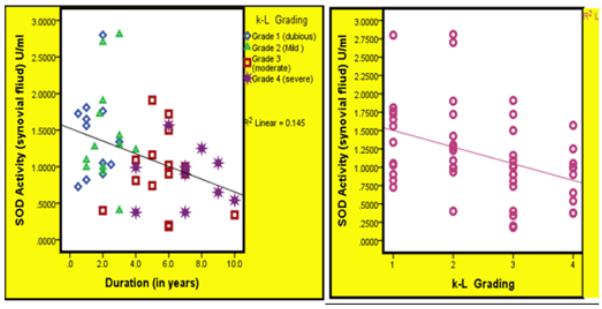

Figure 1: Spearman's RHO correlation of duration of disease, K-L grading with synovial fluid SOD activity

Non-parametric Spearman's rank-order correlation was used to determine the relationship. SOD activity was correlated with K-L grading (rs -0.415, p.003) and illness duration (rs -0.351, p.002) but not with VAS score (rs-0.246, p =.085).Table 2 At 0.05, the correlation is significant (2- tailed).

Correlation of synovial fluid SOD activity	Spearman's correlation coefficient 'r.'	P-value
K-L grading	-0.415*	0.003
VAS score	-0.246*	0.085
Duration of disease	-0.351*	0.012

Table 2: Spearman's RHO correlation of K-L grading, VAS score, duration of disease with synovial fluid SOD activity concentration

DISCUSSION

The K-L grading system was developed to categorize individuals with KOA based on their radiological severity. To our knowledge, no prior work has employed synovial fluid from an OA knee to do subgroup analysis of antioxidant activity (SOD) at different degrees of severity of knee osteoarthritis.

Primary KOA is a chronic disorder that disrupts cartilage metabolism, causing cartilage degeneration and, as a result, knee damage. ROS may be seen in the breakdown of articular cartilage and joint damage caused by superoxide anions. These people typically have much higher oxidant levels in their synovial fluid [18, 19].

Estimates of synovial fluid superoxide dismutase were used to determine an individual's total antioxidant status. SOD activity increased in grades 1 and 2 but dropped in grades 3 and 4 (grade 1 =1.430.55, grade 2 =1.440.72, grade

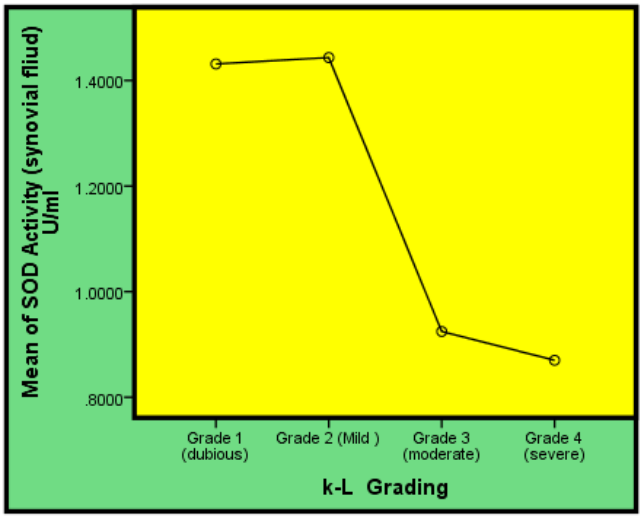

Figure 2: Mean plot of grading of Knee osteoarthritis groups and SOD activity

3=0.920.52, grade 4 = 0.870.52 & overall mean = 1.170.6, F =3.79, p.016). SOD activity was increased in early KOA (grades 1 and 2) to minimize oxidative stress. Early OA may be able to boost or sustain SOD secretion. SOD is the most important ROS scavenger in extracellular spaces and synovial fluids. In the late stage, SOD levels are lowered. Late-stage OA joints appear to fail to minimize oxidative stress due to antioxidant depletion in later stages. The current study's findings are consistent with prior research by E. A. Regan, M.D. et al. [20], who found a substantial increase in SOD activity in the synovial fluid of injured/painful knee (early acute osteoarthritis) patients but a reduction in late osteoarthritis patients.Tercic and Bozic [21] discovered that high levels of ROS in SF can stimulate SOD activity locally, protecting articular cartilage from the damaging effects of ROS [22]. Shweta et al [23] observed that serum SOD levels were greater in rheumatoid arthritis and osteoarthritis patients (2450 40.1 and 2377 38.5 units/ml), respectively than in controls (2166 145, P 0.001). According to Recklies et al. [24], SOD is the first line of defense against ROS because it catalyzes the dismutation of the superoxide anion into hydrogen peroxide. Indranil et al. [25] discovered a substantial increase in SOD activity in individuals with knee joint osteoarthritis versus controls. The connection between synovial SOD and K-L grading was shown to be positive. It is likely that the discrepancy in antioxidant status between our study and other investigators' investigations is attributable to variations in illness stage. Acute inflammation may up-regulate antioxidant defenses, but chronic joint illness may deplete them. The negative connection between SF antioxidant-enzyme activity and the duration of KOA may imply that as the illness progresses, the induction of antioxidant enzymes and, subsequently, their activities in SF diminish.

The current study additionally looked at the Spearman's "rho" association between antioxidant (SOD) levels in KOA patients and pain severity (VAS Score). Synovial SOD activity

Grading of Osteoarthritis	Case (n)	Age (years)	SOD (U/mL)	Vas score	Duration of disease (years)
Grade 1 (Dublous)	12	49.09	1.43	4.83	1.5
Grade 2 (Mild)	13	50.19	1.44	6.38	2.25
Grade 3 (Moderate)	15	63.3	0.92	7.21	5.67
Grade 4 (Severe)	10	69.27	0.87	8	7.1
Total	50	57.57	1.17	6.6	4.07

Table 1: Mean value of age, SOD, VAS score & duration of disease according to K-L grading

(table No. 2, fig. 3) showed a non-significant inverse relationship with VAS score (r = -0.246, p =.085) and a significant inverse relationship with illness duration (r = -0.351, p = 0.012)(Table No. 2fig 2).

Exogenous antioxidant supplementation can aid in the reduction of oxidative stress, which is a critical element in the etiology of OA. OA and other age-related illnesses may be able to be kept at bay by taking antioxidants in the early stages of the disease or as people get older. This study shows that this could be a good idea.

In the early stages of osteoarthritis, antioxidant treatment may assist in decreasing the disease's development.It will be easier to understand how to use synovial fluid SOD activity levels as an early sign of oxidative stress in the knee joint rather than blood tests.

CONCLUSION

The current study demonstrated the importance of redox species and their imbalance, which can cause oxidative stress and particular illnesses. Early stages of knee osteoarthritis were associated with increased synovial fluid SOD activity, indicating a possible response to increasing ROS in the synovial fluid. However, the antioxidant enzyme (SOD) activity was reduced in the advance stages of knee osteoarthritis.

Clinical utility: Antioxidant supplements and a diet high in antioxidants may delay the early onset of osteoarthritis.This may help to delay the progression of the condition by increasing the antioxidant status and aiding in the neutralization of free radical formation and the prevention of cartilage degradation.

Limitations: As a result, we can offer a simple and cost-effective technique for preventing and treating primary knee osteoarthritis. However, more randomized, placebo-controlled studies are necessary to demonstrate it.The limited sample size and absence of synovial fluid samples from healthy controls were significant limitations of our study. Further prospective studies with larger sample sizes on random samples from several centers are required to validate our results.

REFERENCES

1. Hunter DJ, Bierma-Zeinstra S, Osteoarthritis. Lancet. 2019;393:31034380–31034380.
2. Silman AJ, Hochberg MC. Epidemiology of the rheumatic diseases. Oxford: Oxford University Press ; 2001,.
3. Liu C, Zhou WQ, Feng W, Shang X, S. Factors associated with balance function in patients with knee osteoarthritis: an integrative review. Int J Nurs Sci. 2017;4(4):402–411.
4. Martín-Fernández J, García-Maroto R, Sánchez-Jiménez FJ, Bau-González A, Valencia-García H, Gutiérrez-Teira B. Validation of the Spanish version of the Oxford knee score and assessment of its utility to characterize quality of life of patients suffering from knee osteoarthritis: a multicentric study. Health Qual Life Outcomes. 2017;15(1):186–186.
5. Jordan JM, Roos AJD, Renner JB, Luta G, Cohen A, Craft N et al. A case-control study of serum tocopherol levels and the alpha- to gamma-tocopherol ratio in radiographic knee osteoarthritis: the Johnston County Osteoarthritis Project'. Am J Epidemiol. 2004;159(10):968–77.
6. Hamerman D. The biology of osteoarthritis. N Engl J Med. 1989;320(20).
7. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239–286.
8. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 1956;11(3):298–300.
9. Gey KF. On the antioxidant hypothesis with regard to arteriosclerosis. BiblNutrDieta. 1986;37(37):53–91.
10. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage'. 2003;11(10):747–55.
11. Bolduc JA, Collins JA, Loeser RF. Reactive oxygen species, aging and articular cartilage homeostasis. Free Radic Biol Med. 2019;132:73–82.

12. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986;29(8):1039–1088.

13. Mathy-Hartert M, Hogge L, Sanchez C, Deby-Dupont G, Crielaard JM, Henrotin Y. The title differs from PubMed. Suggestion: 'Interleukin-1beta and interleukin-6 disturb the antioxidant enzyme system in bovine chondrocytes: a possible explanation for oxidative stress generation. Osteoarthritis Cartilage'. 2008;16(7):756–63.

14. Henrotin Y, Kurz B, Aigner T. Oxygen and reactive oxygen species in cartilage degradation: friends or foes. Osteoarthritis Cartilage. 2005;13:643–54.

15. Gibson JS, Mccartney D, Sumpter J, Fairfax TP, Milner PI, Edwards HL et al. Rapid effects of hypoxia on H+ homeostasis in articular chondrocytes. Pflugers Arch. 2009;458(6):1085–92.

16. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthrosis. Ann Rheum Dis. 2000;16(4):494–502.

17. Sandy JD, Flannery CR, Neame PJ, Lohmander LS. The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. J Clin Invest. 1992;89(5):1512–1518.

18. Marklund SL, 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(3):469–74.

19. Gutteridge J. Free radicals in disease processes: a compilation of cause and consequence. Free Radic Res Commun. 1993;19(3):141–58.

20. Dawn DI. A study to assess relationship between synovial fluid lipid peroxidation marker and the severity of knee osteoarthritis. IOSR-JDMS;3(6):60–63.

21. Regan EA, Bowler RP, Crapo JD. Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury. Osteoarthritis Cartilage. 2008;16(4):18203633–18203633.

22. Tercic D, Bozic B. The basis of the synovial fluid analysis. Clin Chem Lab Med. 2001;39(12):1221–1227.

23. Komiya S, Tsuzuki K, Mangham DC, Sugiyama M, Inoue A. Oxygen scavengers in simple bone cysts. Clin OrthopRelat Res. 1994;308(308):199–206.

24. Dwivedi S, Singh S. Lipid-peroxidation and antioxidant status in osteoarthritis and rheumatoid arthritis patients. Int J Contemp Med Res. 2016;3(6).

25. Recklies AD, Poole AR. Pathophysiologic aspects of inflammation in diarthroidal joints. In: JA B, TA E, SR S, editors. Orthopaedic basic science: biology and biomechanics of the musculoskeletal system ; 2000,. p. 489–530.

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