Original Research Article

Correlation of primary knee osteoarthritis severity with the lipid peroxidation biomarker (MDA) in synovial fluid: A pilot study

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Abstract

Introduction: Osteoarthritis (OA) is 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 reason for medical advice. Methods: A hospital-based cross-sectional study was conducted on patients attending the Outpatient Department of Orthopedics. A total of 50 individuals with primary knee osteoarthritis in the age range of 45-90 years were chosen at random for the research (26 females and 24 males). 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 MDA levels in the synovial fluid of all 50 individuals were measured by using the Thiobarbituric acid technique. The severity of knee OA was compared to oxidative stress measures and synovial fluid MDA levels in order to determine if there was a link between oxidative stress-induced damage and disease development. **Results:** Grades 1, 2, 3, and 4 have MDA values of 3.9±0.4, 4.3±0.5, 5.4±0.2 and 5.96±0.2, respectively, in synovial fluid. MDA mean levels in synovial fluid increased with the severity of knee osteoarthritis (K-L grading), which was statistically significant (p.001). **Conclusions:** Kellgren-Lawrence grading and synovial MDA had a favourable connection. In osteoarthritis patients, free radical-induced lipid peroxidation was high, as evaluated by synovial fluid MDA concentration, and it increased with the severity of osteoarthritis. It suggests that oxidative stress is important in the etiopathogenesis of OA and that synovial MDA could be employed as a biomarker to determine the severity of the illness.

Keyword: Knee osteoarthritis; Malondialdehyde; Synovial fluid; Oxidative stress.

Introduction

Osteoarthritis (OA) is the 50th most common sequel of diseases and injuries, affecting over 4% of the world's population[1]. It is a more significant cause of nonfatal burden, continues to impose an enormous health and economic burden on society, especially among the elderly. Knee OA constitutes 83% of the most common type of arthritis [1]. It has a high prevalence (22% to 39% in India) in the elderly population. And because of that, it is a leading cause of disability, suffering and morbidity[2]. Women are more often than males to have OA; 45% of women over 65 have symptoms, and 70% show radiological characteristics[3- 6]. It is a complex disorder of unknown aetiology that affects many different joints, a common cause of disability in the population.

OA is a polygenic and complex illness. Several genetic and environmental variables impact its aetiology, which are linked to the activation of molecular pathways that contribute to the advancement of articular damage[7]. Osteoarthritis is a degenerative condition that causes pain, stiffness, effusion, limited motion, oedema, crepitus, and impairment over time. The most prominent clinical characteristic is osteoarthritic pain[8].

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It is characterized by morphological, biochemical, molecular and biomechanical changes of both cells and extracellular matrix (ECM), leading to softening, fibrillation, ulceration, loss of articular cartilage, synovial inflammation, and sclerosis of subchondral bone, formation of osteophytes and subchondral cysts. The knee is the most clinically significant site in osteoarthritis involvement.

Osteoarthritis is a complex and heterogeneous form of joint deterioration. Current OA pathogenesis theories propose a disruption in the homeostatic balance between bone and cartilage degradation and synthesis[9]. Previous research has shown that oxidative stress has a role in the development and progression of OA[10,11]. The reactive oxygen species (ROS), which have multiple sources, are an essential element in the pathogenic process. Stopping the progression of osteoarthritis is challenging. **Oxidative stress:** Reactive oxygen species (ROS- nitric oxide, superoxide anion, hydrogen peroxide, and hydroxy radical) are extremely reactive chemical compounds that attack molecules such as protein, lipid, and nucleic acids and cause cellular damage. Cellular damage causes structural and functional alterations in chondrocytes, extracellular matrix, and tissue damage, all of which could have a role in the aetiology of OA[12-14]. However, only when the antioxidant system is disrupted, and ROS generation surpasses antioxidant capacity does oxidative damage occur[15,16].

Role of oxidative stress in the pathogenesis of OA

The principal mechanism of the cell membrane and cartilage breakdown in osteoarthritis is reactive oxygen species (produced by free radicals oxidizing lipids). All of this happens as lipid peroxidative stress is no longer neutralized by the antioxidant system, and elevated levels of lipid peroxide cause a loss of homeostasis in the maintenance of healthy articular cartilage, leading to pathologic articular cartilage degradation in OA as people become older[17].

Antioxidant system: The body has its endogenous homeostasis to reduce oxidative stress, but balance is required. Several lines of antioxidant defence exist intra and extra-cellularly to protect tissues against damage from ROS.

The goal of this study was to see if there was a link between the severity of primary knee osteoarthritis and the presence of the lipid peroxidation marker (MDA) in synovial fluid and look into the feasibility of utilizing synovial MDA as a marker for osteoarthritis disease severity.

Materials and methods

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 for 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

Study population:

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

A thorough evaluation of each patient, including demographic information, disease duration, and a visual analogue scale assessment of pain intensity (0-10). American College of Rheumatology (ACR) was used for the diagnosis of knee OA[18]. Knee osteoarthritis was graded using Kellgren-Lawrence (K-L) Radiographic rating system.

- Grade 1: Dubious (minute osteophyte doubtful significance)
- Grade 2: Mild (definite osteophyte: normal joint space)
- Grade 3: Moderate (moderate joint space reduction)
- Grade 4: Severe (joint space significantly reduced, subchondral sclerosis)

Knee joint radiographs were evaluated with the Kellgren-Lawrence grading scale. Grading of the knee was correlated with oxidative stress parameters (synovial fluid MDA levels) to find out possible associations between the oxidative stress-induced damage and the disease progression.

Synovial fluid sample collection

Synovial fluid samples in OPD were taken from effused knee from those patients. After finding positive results with a bulge test, the orthopaedic surgeon was performed an Arthrocentesis and aspirated the affected joint. An appropriate gauge needle was attached to a syringe, and the entry site was cleaned. A two-step process was employed for Arthrocentesis in which the first puncture was made through the skin followed by a second thrust into the synovial capsule. After the fluid was aspirated and the needle withdrawn from the joint, the needle was removed and an end cap placed on the tip of the syringe. The synovial fluid sample was collected into a vacutainer containing tripotassiumethylenediamine tetra-acetate (K3 EDTA) as an anticoagulant for MDA level estimation.

The sample of synovial fluid was immediately placed on the icebox and centrifuged at 3000 g for 30 minutes at 4°C to eliminate cells and particulate debris. The supernatant was separated and kept frozen at -70°C for up to 4 weeks before analysis.

Estimation of Malondialdehyde levels (MDA)[19]

Dahle's LK (1962) thiobarbituric acid (TBA) reaction was utilized for tissue fluid (synovial fluid) spectrophotometric assay. The concentration of MDA is expressed in µMole/L. Normal value of MDA in serum- <0.7 nmoles/ml or <0.7 µ Moles /L.

Statistical analysis

SPSS20 was used for the analysis of all data. The data obtained was analyzed for statistical significance using correlation coefficient Pearson's test 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, MDA, VAS score, and disease duration were increased with increasing K-L grading of osteoarthritis (Table 1). The mean of MDA concentration (µM/L) was rising with K-L grading and K L (Fig 1)

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

Grading of Osteoarthritis	Case (n)	Age (years)	MDA (µml/L)	Vas score	Duration of disease (years)
Grade 1 (Dublous)	12	49.09	4.01	4.83	1.5
Grade 2 (Mild)	13	50.19	4.18	6.38	2.25
Grade 3 (Moderate)	15	63.3	5.4	7.21	5.67
Grade 4 (Severe)	10	69.27	5.93	8	7.1
Total	50	57.57	4.86	6.6	4.07

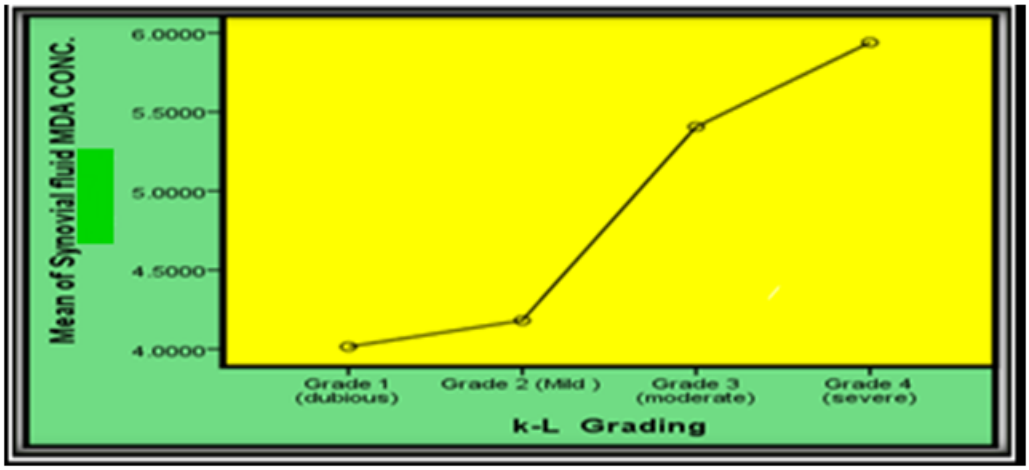


Fig 1: Mean plot of grading of Knee osteoarthritis groups and MDA activity

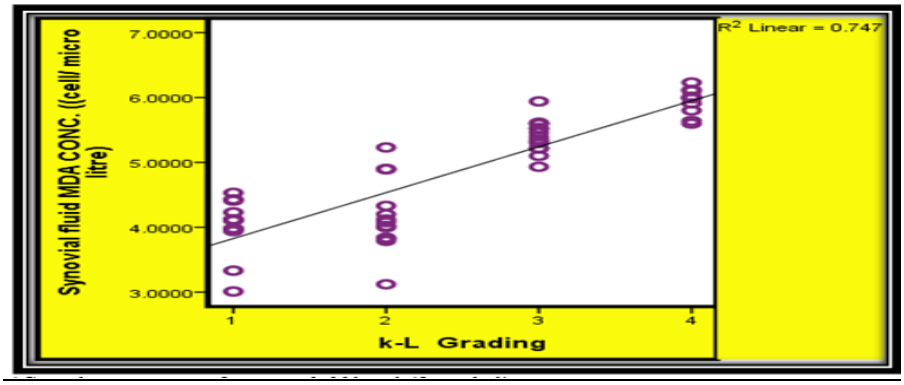
A one-way ANOVA between subjects of Knee Osteoarthritis was conducted to see the effect of MDA on the severity of osteoarthritis in grade1 (dubious), grade 2 (mild), grade 3 (moderate), grade 4 (severe). The mean of MDA levels were unequal according to a one way ANOVA, F (3, 46) =66.100, p<0.0001.

Subjects in grades 3 and 4 reported that their MDA levels were significantly (p0.0001) higher than subjects in grades 1 and 2 (Mean MDA 4.01). Pairwise comparisons of the means using Tukey's Honestly significant difference procedure revealed that grade 3 (Mean MDA 5.10) and 4 (Mean MDA 5.93) had significant comparisons: subjects in grades 3 and 4 reported that their MDA levels were significantly (p0.0001) higher than subjects in grades 1 and 2 (Mean MDA (Mean MDA 4.18). The difference between grades 1 and 2 was not significant (p=0.299). The MDA mean differences across groups were statistically significant (F =66.10, P=.000). A Tukey's post hoc test revealed that the MDA concentration was statistically significantly higher in grades 3 & 4 compared to grade 1 Osteoarthritis.

Spearman's rank-order correlation (Non-parametric): Spearman's rank-order correlation was run to determine the relationship. There were a strong positive correlation of MDA with K-Lgrading(rs 0.862, p<.0001), VAS score (rs 0.671, p<.0001)and duration of disease(rs 0.674, p<.0001). (Table 2)

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

Correlation of synovial fluid MDA concentration with	Spearman's correlation coefficient 'r.'	Sig (2 tailed) P-value
K-L grading	0.862*	0.0001
VAS score	0.671*	0.0001
Duration of disease	0.674*	0.0001



*Correlation is significant at 0.01level (2- tailed)

Fig 2: Spearman's RHO correlation of K-L grading with synovial fluid MDA concentration

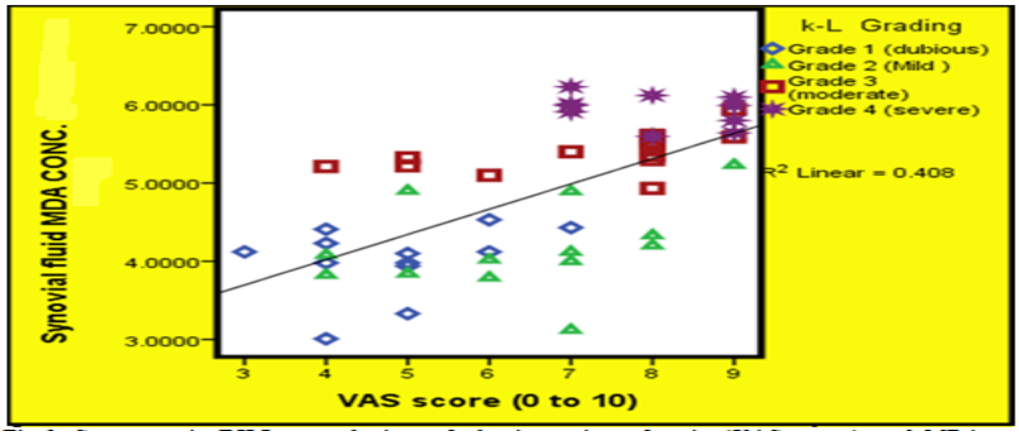


Fig 3: Spearman's RHO correlation of the intensity of pain (VAS score) and MDA concentration in KOA cases

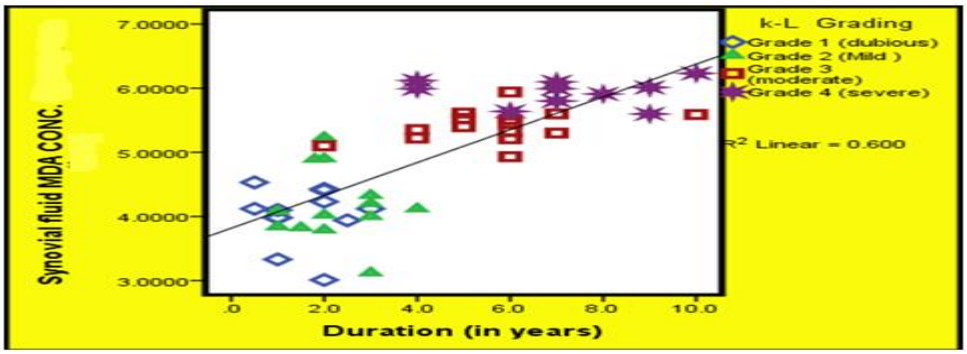


Fig 4: Spearman's RHO correlation of duration of disease grading with synovial fluid MDA concentration

Discussion

The K-L grading system was used to divide patients with KOA into groups depending on their radiological severity. To our knowledge, no previous study has used synovial fluid from an OA knee to perform subgroup analysis of oxidative stress in different levels of severity of knee osteoarthritis.

Primary KOA is a chronic condition that causes a disruption in cartilage metabolism, resulting in cartilage degradation and, as a result, knee injury. Superoxide anion-mediated articular cartilage and joint destruction are examples of ROS. In the synovial fluid of these patients, oxidant levels are frequently substantially greater[20,21].

In the present study, the lipid peroxidation product, i.e., Mean MDA level, had increased significantly in synovial fluid of patients with Osteoarthritis of the Knee (table 1). MDA levels (µMole/L) in Synovial fluid also significantly increased with severity (with K-L grading) of KOA (grade1=4.015, grade 2=4.18, grade 3=5.40, grade 4=5.93, F 66, p<.0001). The present study results agree with the previous research done by Indranil Dawn et al.[22], which showed a significant rise in MDA levels in synovial fluid in KOA patients, and the synovial MDA increased with increasing K-L grade. A rise in MDA could be due to the increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. In turn, these oxygen species can oxidize many other important biomolecules, including membrane lipids. A study conducted by Tanyawan S et al[23]. showed that plasma MDA levels were higher in OA patients than those in healthy controls (P< 0.001).

Shweta Dwivedi et al[24]. found that the serum MDA levels in patients with rheumatoid arthritis and osteoarthritis were 4.64±0.229 and 2.19±1.21, respectively, which were higher than the control (1.68±0.994 nMol/ml, P0.001). Non-enzymatic interactions of oxygen with organic molecules and those started by ionizing radiations can yield free radicals. The non-enzymatic process can also occur in the mitochondria during oxidative phosphorylation. Serum MDA levels were observed to be considerably higher in RA and OA patients than in controls in their investigation. Mezes M et al.[25], Sarban S[26], Surapaneni KM et al.[27], and Seven et al.[28], all found similar results of higher MDA levels in patients with osteoarthritis and RA[28].

In synovial fluid patients with knee osteoarthritis, the mean MDA level had increased considerably. Oxidative stress appears to be one of the key causal elements in the development of OA, according to a growing body of evidence from both experimental and clinical research[16, 26, 27 29-30].

This led to the creation of the current study, which looked at the relationship between lipid peroxidation in synovial fluid and the severity of primary knee osteoarthritis. The findings of our investigation could shed light on the significance of oxidative stress in patients with different degrees of primary knee osteoarthritis. To quantify lipid peroxidation synovial fluid MDA, we looked at the probable redox imbalance that caused the oxidative damage.

Kellgren-Lawrence grading and synovial MDA had a positive connection. According to the K-L grading, there was a substantial positive association between the degree of lipid peroxidation MDA and the severity of the disease process. Exogenous antioxidant supplementation can help to reduce oxidative stress, which is a key factor in the etiopathogenesis of OA. MDA in the synovium could be used as a marker to determine the severity of osteoarthritis.

The findings of this study are strong enough to persuade clinicians that antioxidant treatment in the early stages of the disease or as patients get older may be a useful way to prevent free radical-mediated musculoskeletal tissue degradation in OA and other age-related diseases.

Antioxidant therapy in the early stages of osteoarthritis may help to slow the disease's progression. Future work in this area will provide a clearer picture to use synovial fluid MDA level as an early marker for measuring oxidative stress in the knee joint instead of serum markers.

Conclusion

In osteoarthritis patients, free radical-induced lipid peroxidation was high, as evaluated by synovial fluid MDA concentration, and it increased with the severity of osteoarthritis. It suggests that oxidative stress is important in the etiopathogenesis of OA and that synovial MDA could be employed as a biomarker to determine the severity of the illness. Kellgren-Lawrence grading and synovial MDA had a favourable connection.

Antioxidant supplementation to early Osteoarthritis patients may help slow the evolution of the illness by boosting the antioxidant status of the knee, which helps to neutralize free radical production and prevent cartilage destruction. However, further multicenter placebo-controlled trials are required to prove it.

Limitations

The small sample size and lack of synovial fluid samples from healthy controls were important limitations of our investigation. Further prospective studies on random samples from multiple centres with larger sample sizes are necessary to validate our data.

Statement

The manuscript has been reviewed and approved by all of the authors, and feels that the article reflects honest work.

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