ORIGINAL RESEARCH

To determine the relation between severity of knee osteoarthritis and lipid peroxidation biomarker (MDA) in synovial fluid

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ABSTRACT

Aim: The level of the lipid peroxidation biomarker known as MDA in synovial fluid was shown to have a correlation with the severity of primary knee osteoarthritis. **Materials and methods:** 40 Patients aged 46 and older with acute osteoarthritis symptoms (knee effusion) were included in this study. Knee osteoarthritis was graded using Kellgren-Lawrence (K-L) Radiographic rating system. Grade 1: Dubious, Grade 2: Mild, Grade 3: Moderate and Grade 4: Severe. The Kellgren-Lawrence grading system was used in order to analyse the radiographs of the knee joint. The grading of the knee was connected with oxidative stress measures (the levels of MDA in the synovial fluid), and this was done so that probable correlations between the oxidative stress-induced damage and the course of the illness could be found. **Results:** The MDA levels of subjects who were in grades 3 and 4 were found to be considerably ($p< 0.001$) higher than the MDA levels of individuals who were in grades 1 and 2. (Mean MDA 4.44 and 4.69). Tukey's Honestly significant difference procedure was used to conduct pair wise comparisons of the means, which revealed that grade 3 (Mean MDA 5.87) and grade 4 (Mean MDA 6.11) had significant comparisons: subjects in grades 3 and 4 reported that their MDA levels were significantly ($p<0.001$) higher than subjects in grades 1 and 2 (Mean MDA 4.69). It was determined that there was not a significant difference between grades 1 and 2 ($p=0.33$). **Conclusion:** There was a positive relationship between the Kellgren-Lawrence grade and synovial MDA. Antioxidant supplementation in individuals with early osteoarthritis may help delay the progression of the disease by increasing the antioxidant status of the knee. This rise in antioxidant status helps to neutralise the formation of free radicals and prevent the breakdown of cartilage.

Keywords: Kellgren-Lawrence grade, synovial MDA, Knee, osteoarthritis

INTRODUCTION

Around 4% of the global population is affected by osteoarthritis (OA), making it the 50th most prevalent sequelae of illnesses and injuries[1.] It continues to inflict a large health and economic cost on society, particularly among the elderly, and it is a more major source of nonfatal burden than other burdens. The most prevalent kind of arthritis accounts for 83 percent of all cases of osteoarthritis of the knee [1]. It has a significant frequency among the older population, ranging from 22 percent to 39 percent in India. Because of this, it is one of the primary causes of morbidity, pain, and disability[2]. 45% of women over the age of 65 have symptoms of osteoarthritis, and 70% exhibit radiological features [3, 6]. Women are more likely than men to have the condition. It is a complicated condition with an unidentified root cause that affects a wide variety of joints and is one of the most prevalent reasons why people are unable to work.

OA is a multifactorial and difficult to diagnose condition. Its aetiology is connected to the activation of molecular pathways that contribute to the progression of articular injury, which is influenced by a number of hereditary and environmental factors that have an effect on its aetiology [7]. Osteoarthritis is a degenerative disorder that, over the course of time, may produce symptoms such as pain, stiffness, effusion, restricted mobility, oedema, crepitus, and disability. The pain associated with osteoarthritis is the most conspicuous clinical characteristic [8].

It is characterised by morphological, biochemical, molecular, and biomechanical changes of both cells and extracellular matrix (ECM), which leads to softening, fibrillation, ulceration, loss of articular cartilage, synovial inflammation, and sclerosis of subchondral bone, formation of osteophytes, and subchondral cysts. Additionally, it can lead to the formation of osteophytes and subchondral cysts. When it comes to osteoarthritis, the most clinically important location of involvement is the knee. The degenerative joint disease known as osteoarthritis is a complicated and multifaceted condition. The current pathogenesis hypotheses for OA postulate that OA is caused by a change in the homeostatic balance between the breakdown and synthesis of bone and cartilage [9]. Earlier studies have shown that oxidative stress plays a part in the beginning stages of osteoarthritis (OA) as well as its progression [10,11]. An key part of the pathogenic process is the reactive oxygen species, often known as ROS. These ROS come from a variety of different sources. With osteoarthritis, it might be difficult to halt the disease's development. The highly reactive chemical substances known as reactive oxygen species (ROS) include nitric oxide, superoxide anion, hydrogen peroxide, and the hydroxy radical. These reactive oxygen species prey on molecules including protein, lipid, and nucleic acids, hence causing damage to biological components. Cellular damage leads to structural and functional abnormalities in chondrocytes, as well as damage to extracellular matrix and tissue, all of which have the potential to play a role in the aetiology of osteoarthritis (OA) [12-14]. Nevertheless, oxidative damage will not occur until the antioxidant system is compromised, at which point ROS production will have beyond the capacity of the antioxidants [15,16].

The production of reactive oxygen species is the primary cause of the breakdown of cell membranes and cartilage that occurs in osteoarthritis (produced by free radicals oxidising lipids). All of this occurs because the antioxidant system is no longer able to neutralise the lipid peroxidative stress. Elevated levels of lipid peroxide cause a loss of homeostasis in the maintenance of healthy articular cartilage, which ultimately leads to pathologic articular cartilage degradation in OA as people get older [17].

Antioxidant system: The body has its own endogenous homeostasis, which helps to lessen the effects of oxidative stress; nonetheless, maintaining a healthy balance is essential. There are many intracellular and extracellular antioxidant defences that work together to protect tissues from harm caused by reactive oxygen species (ROS).

The purpose of this investigation was to determine whether or not there was a correlation between the severity of primary knee osteoarthritis and the presence of the lipid peroxidation marker (MDA) in synovial fluid, as well as to investigate whether or not it would be possible to use synovial MDA as a marker for the severity of osteoarthritis disease.

MATERIALS AND METHODS

After receiving approval from the hospital's ethics council, a hospital-based cross-sectional observational research was carried out within the orthopaedic department. Participants for the study were selected from among the forty individuals who sought care at either the outpatient or the inpatient Orthopaedics department over the course of the research. The sample was done using a process called consecutive sampling.

INCLUSION CRITERIA

40 Patients aged 46 and older with acute osteoarthritis symptoms (knee effusion) were included in this study. Additionally, patients undergoing intra-articular pharmacological injection therapy, patients undergoing knee replacement surgery, and patients undergoing arthroscopic lavage were also included.

EXCLUSION CRITERIA

Individuals who have undergone prior surgery on the same joint as well as those who suffer from inflammatory joint disease are candidates for this procedure. Individuals who are using steroids or other medications for an extended period of time and who are suffering from pain as a consequence of a traumatic experience Other systemic illnesses, such as severe liver, renal, or cardiac disease, are also potential causes of an increased oxidative stress level.

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)

The Kellgren-Lawrence grading system was used in order to analyse the radiographs of the knee joint. The grading of the knee was connected with oxidative stress measures (the levels of MDA in the synovial fluid), and this was done so that probable correlations between the oxidative stress-induced damage and the course of the illness could be found.

Synovial fluid samples were obtained from the patients' effused knees at the outpatient department (OPD). The orthopaedic surgeon conducted an arthrocentesis and aspiration of the afflicted joint after obtaining good findings from a bulging test. The point of entrance was sterilised, then a suitable gauge needle was affixed to a syringe, and the injection was

performed. Arthrocentesis was performed using a two-part method, with the initial puncture being made through the skin, followed by a second push into the synovial capsule. This was done in order to collect fluid from the joint. When the fluid was collected and the needle was removed from the joint, the end cap was put on the tip of the syringe, and a new needle was inserted into the syringe. In order to determine the MDA concentration, a sample of synovial fluid was drawn into a vacutainer that included potassium ethylenediamine tetra-acetate (K3 EDTA) as an anticoagulant.

After placing the sample of synovial fluid on the icebox, it was immediately centrifuged at 3000 g for thirty minutes at a temperature of 4 degrees Celsius to remove any cells and particle debris. After being separated, the supernatant was stored in a freezer at -70 degrees Celsius for up to four weeks before undergoing examination.

The thiobarbituric acid (TBA) reaction discovered by Dahle and his colleagues in 1962 was used for the spectrophotometric analysis of synovial fluid and tissue fluid. The number of micromoles per litre is the unit of measure for the amount of MDA present. The normal value of MDA in serum is less than 0.7 nanomoles per millilitre or less than 0.7 micromoles per litre. [19]

STATISTICAL ANALYSIS

For the analysis of all of the data, SPSS20 was used. The data that was collected was subjected to statistical analysis, in which a correlation coefficient Pearson's test and an ANOVA test were used, respectively, to examine the degree to which K-L groups differed from one another.

RESULTS

According to Kellgren-Lawrence, the forty people who participated in the research were classified into four different categories with primary knee osteoarthrosis (KOA). With higher K-L grading of osteoarthritis, patients had a longer disease duration, which was also associated with an increase in mean age, MDA, and VAS score (Table 1). With increasing K-L grading and increasing K-L, the average MDA concentration, expressed as uM/L, rose (Table 1)

The impact of MDA on the severity of osteoarthritis in grade 1 (doubtful), grade 2 (mild), grade 3 (moderate), and grade 4 knee osteoarthritis was investigated using a one-way analysis of variance (ANOVA) comparing participants with knee osteoarthritis (severe). According to the results of a one-way analysis of variance, the mean levels of MDA were significantly different from one another ($F(4, 47) = 71.25$, $p < 0.001$).

The MDA levels of subjects who were in grades 3 and 4 were found to be considerably (p 0.001) higher than the MDA levels of individuals who were in grades 1 and 2. (Mean MDA 4.44 and 4.69). Tukey's Honestly significant difference procedure was used to conduct pair wise comparisons of the means, which revealed that grade 3 (Mean MDA 5.87) and grade 4 (Mean MDA 6.11) had significant comparisons: subjects in grades 3 and 4 reported that their MDA levels were significantly ($p < 0.001$) higher than subjects in grades 1 and 2 (Mean MDA 4.69). It was determined that there was not a significant difference between grades 1 and 2 ($p=0.33$). The variations in MDA mean scores across groups were found to be statistically significant ($F=71.63$, $P < 0.001$). The MDA concentration was shown to be statistically substantially greater in grades 3 and 4 of osteoarthritis when compared to grade 1 osteoarthritis, as demonstrated by a Tukey's post hoc test. Spearman's rank order correlation (Non-parametric): For the purpose of determining the nature of the association, a Spearman's rank order correlation was carried out. There was a significant positive correlation between MDA and K–L grade (rs 0.96, $p<.001$), VAS score (rs 0.74, $p<0.001$), and length of illness (rs 0.71, $p<.001$). Furthermore, there was a correlation between MDA and VAS score (rs 0.74, $p<0.001$). Table2.

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)	10	50.11	4.44	5.14	1.77
Grade 2 (Mild)	10	51.37	4.69	6.85	2.36
Grade 3 (Moderate)	10	64.59	5.87	7.39	6.01
Grade 4 (Severe)	10	70.85	6.11	8.11	7.66
Total	40	58.98	5.01	6.88	4.88

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	Spearman's correlation coefficient (r)	Sig (2 tailed) P- Value
K - L Grading	0.96	0.001
VAS Score	0.74	0.001
duration of diseases	0.71	0.001

DISCUSSION

The K-L grading system was used to individuals diagnosed with KOA in order to categorise them into groups according to the radiological severity of their condition. To the best of our knowledge, no prior research has performed a subgroup analysis of oxidative stress in various degrees of severity of knee osteoarthritis using synovial fluid from an OA knee. This is something that we believe should be done.

Primary knee osteoarthritis (KOA) is a chronic disorder that causes a disturbance in the metabolism of cartilage, which ultimately leads to cartilage deterioration and, as a consequence, knee damage. Examples of ROS include the degradation of articular cartilage and joints that is mediated by superoxide anion. It has been found that the oxidant levels in the synovial fluid of these patients are frequently significantly higher [20,21].

Patients who suffered from osteoarthritis of the knee were found to have considerably higher levels of the lipid peroxidation product known as the mean level of malondialdehyde (MDA) in their synovial fluid. MDA levels in synovial fluid measured in moles per litre likewise showed a significant upward trend with increasing K-L severity (grade 1=4.44, grade 2=4.69, grade 3=5.87, and grade 4=6.11, F 71.25, $p<0.001$). The findings of the current study are consistent with those of an earlier study conducted by Indranil Dawn et al.[22], which demonstrated a statistically significant increase in the levels of MDA found in the synovial fluid of KOA patients and demonstrated that the levels of synovial MDA increased along with the K-L grade. An increase in MDA might be caused by an increase in the formation of reactive oxygen species (ROS), which is caused by the excessive oxidative damage produced in these individuals. In turn, these oxygen species are able to oxidise a wide variety of other significant biomolecules, including the lipids found in membranes. Patients with osteoarthritis had plasma MDA levels that were significantly greater than those of healthy controls, as shown by a research that was carried out by Tanyawan S et al[23]. Serum MDA levels in patients with rheumatoid arthritis and osteoarthritis were found to be 4.64±0.22 and 2.19±1.21, respectively, which were higher than the control (1.68±0.99 nMol/ml, $P<0.001$), according to the research conducted by Shweta Dwivedi and colleagues [24]. Free radicals may be produced by interactions that do not involve enzymes, such as those between oxygen and organic molecules, or through ionising radiation. During the process of oxidative phosphorylation, the process that does not need enzymes may also take place in the mitochondria. Throughout the course of their analysis, they found that individuals with RA and OA had much greater levels of MDA in their serum than controls did. Studies conducted by Mezes M et al.[25], Sarban S[26], Surapaneni KM et al.[27], and Seven et al.[28] all came to the same conclusion: individuals suffering from osteoarthritis and RA had greater levels of MDA.

The levels of MDA in the synovial fluid of individuals suffering from knee osteoarthritis were significantly elevated on average. According to an increasing body of data derived from both experimental and clinical research[16, 26, 27 29-30], oxidative stress seems to be one of the primary causative variables that contributes to the development of osteoarthritis (OA).

Because of this, the present research came to be, the purpose of which was to investigate the connection between the level of lipid peroxidation in synovial fluid and the severity of primary knee osteoarthritis. The results of our study have the potential to shed light on the importance of oxidative stress in individuals who have varying degrees of primary knee osteoarthritis. In order to measure the oxidative damage induced by lipid peroxidation in synovial fluid MDA, we investigated the likely redox imbalance that was the root of the problem.

There was a significant correlation between Kellgren-Lawrence grade and synovial MDA. The K-L grading revealed that there was a significant positive correlation between the amount of lipid peroxidation MDA and the severity of the illness process. Supplementation with exogenous antioxidants may be helpful in lowering oxidative stress, which is an important contributor to the etiopathogenesis of osteoarthritis (OA). It is possible that the amount of MDA present in the synovium might be utilised as a marker to assess the severity of osteoarthritis.

The findings of this study are compelling 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 osteoarthritis (OA) and other age-related diseases. [Clinicians] may be able to persuade patients that antioxidant treatment in the early stages of the disease or as patients get older may be a useful way to prevent. When administered in the early stages of osteoarthritis, antioxidant treatment has the potential to help reduce the course of the disease. In the future, researchers working in this field will be able to offer a better picture of how to utilise the MDA level in synovial fluid as an early signal for evaluating oxidative stress in the knee joint rather than using serum indicators.

CONCLUSION

There was a positive relationship between the Kellgren-Lawrence grade and synovial MDA. Antioxidant supplementation in individuals with early osteoarthritis may help delay the progression of the disease by increasing the antioxidant status of the knee. This rise in antioxidant status helps to neutralise the formation of free radicals and prevent the breakdown of cartilage. To verify this theory, however, more placebo-controlled multicenter studies would need to be conducted.

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