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A study to assess relationship between Synovial fluid lipid peroxidation marker and the severity of knee osteoarthritis

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Abstract: *Osteoarthritis is the most common cause of pain due to arthritis in women. Its high prevalence especially in the postmenopausal women, and the high rate of disability associated with the disease makes it a diagnostic and therapeutic priority. A hospital based cross sectional study was conducted in Burdwan Medical College, West Bengal, India on 76 postmenopausal women aged 45–70 years suffering from Osteoarthritis (OA) of the knee joint, which was diagnosed from symptoms, clinical examinations and radiographic findings. 150 subjects undergoing knee joint arthroscopy for chronic knee pain, meniscal tears or anterior cruciate ligament reconstruction were taken as control .Synovial fluid malondialdehyde (MDA) was measured as an index of synovial fluid lipid peroxidation. The width of knee joint space was measured from the radiographic plates to assess the disease severity. Knee joint radiographs were evaluated with the Kellgren–Lawrence grading scale .Furthermore both were correlated with oxidative stress parameters ,synovial fluid MDA levels to find out possible association between the oxidative stress induced damage and the disease progression. Results showed that there was a significant increase in MDA levels in patients with knee joint osteoarthritis as compared to the controls. Synovial MDA showed positive correlation with Kellgren–Lawrence grading and negative correlation with knee joint space width. The joint space width decreased and synovial MDA increase with increasing Kellgren–Lawrence grad.*

Keywords- *Knee Osteoarthritis, Kellgren–Lawrence grading scale, lipid peroxidation marker, malondialdehyde,.*

I. Introduction:

Osteoarthritis (OA) is the most common type of arthritis.[2]. Its high prevalence (22% to 39% in India) [1], especially in the elderly, and the high rate of disability related to disease makes it a leading cause of disability, suffering and morbidity. Over 40% of the Indian populations in the age group of 70 years or above suffer from osteoarthritis[3] it is characterized by low-grade inflammation resulting in pain in the joints. Pain is caused due to wear and destruction of the joint cartilage as well as destruction or decrease of synovial fluid that lubricates those joints.

Current concepts of the pathogenic mechanisms of OA suggest that there is a shift in the homeostatic balance between the destruction and synthesis of bone and cartilage, with a net progressive destruction of these tissues [4] Recent studies of the biology of chondrocytes show that these cells actively produce reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, hydroxyl radicals, and nitric oxide [5] which may be detected in the synovial fluid of the knee joint.

However, few studies have been done to establish the relationship between oxidative stress and clinical severity of disease. Aim of this study was to analyze the relationship between oxidative stress in the knee joints and the clinical severity of osteoarthritis. The clinical severity of OA was evaluated by measuring the width of knee joint space and by utilizing the Kellgren-Lawrence grading scale. The width of knee joint space was measured from the radiographs of the same. The severity of OA was graded by the Kellgren–Lawrence grading scale [6]which is as follows:

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;
4: large osteophytes marked narrowing of joint space, severe sclerosis and definite deformity Grade of bone contour.

The oxidative stress parameter was measured by analyzing the levels of malondialdehyde (MDA) in the synovial fluid of the knee joints. It was demonstrated that malondialdehyde (MDA), a toxic aldehydic end product of lipid peroxidation, mediates the oxidation of cartilage collagen. MDA and hydroxynonenal (HNE) are specific and major aldehydic products of lipid peroxidation that are believed to be largely responsible for the cytopathologic effects observed during the oxidative stress of lipid peroxidation taken together [7]

II. Methodology

2.1 Subjects

The present study is based on 76 postmenopausal women aged 45–70 years (mean of 54.5) with OA of the knee, which was diagnosed from clinical symptoms, examinations and radiographic findings. Secondary OA patients, such as post-traumatic OA cases, were excluded from the study. 150 subjects undergoing knee arthroscopy for chronic knee pain, meniscal tears or anterior cruciate ligament reconstruction were taken as control. All patients fulfilled the ACR criteria for knee OA [8]The procedures followed were in accordance with the principles of the Declaration of Helsinki in 1975, as revised in 1983.

2.2 Knee joint radiographs

Antero-posterior weight-bearing radiographs of both knees were taken. The bilateral weight-bearing antero-posterior knee radiograph was taken with the patient standing with toes pointed straight ahead, knees fully extended, and weight equally distributed on both feet. The Kellgren–Lawrence grading of radiographs was done by a radiologist who was blinded to the source of subjects. The joint space width of the medial and lateral compartments of the knee joint was measured in millimeters. A vertical line was drawn from the midfemoral medial and lateral condyles to the tibial plateau, and the lesser of the two measurements was taken as the joint space width [9] (figure)

2.3 Knee synovial fluid aspiration

After obtaining informed consent, patient was placed supine on a gurney. A rolled towel was placed below the patient's knee. Then superomedial or superolateral borders of the patella were identified .An 18-G needle was inserted through the midpoint of either set of superior borders. Direct the needle toward the intercondylar notch of the femur. With this approach, the needle enters the suprapatellar bursa. Needle was inserted briskly into the joint space while gently aspirating until synovial fluid enters the syringe (usually 1-2 cm in an adult of average size). The needle was removed and a bandage was applied.

2.4 Selection of marker for lipid peroxidation

Synovial MDA was measured by its reaction with thiobarbituricacid [10].

III. Result

The obtained data was analyzed using SPSS software. The Table 1 displays the comparison of mean synovial MDA in cases and controls**.** The analysis of the different between the means was done with independent sample t test. Synovial fluid MDA was found to be significantly higher in the cases compared to the control. ANOVA of the knee joint space width and synovial fluid MDA of the various groups of subjects categorized clinically as per the Kellgren –Lawrence grading scale shows there is significant difference between them.(Table 2). Analysis of correlation between knee joint space width and Kellgren–Lawrence grading**–**was done by Pearson's correlation (Table 3). It was found that the decrease in the knee joint space width was significant. (p value < 0.001). From the table 4 it is evident that synovial MDA have a positive correlation with radiological grading and negative correlation with knee joint space width. The relationship between the Kellgren–Lawrence grading and the synovial MDA is shown in box whisker plot (fig 1). Here the synovial MDA increase with increasing Kellgren–Lawrence grade. MDA showed variances of 0.384, 0.060, 0.087, 0.012 and skewness of -0.056, 0.086, 0.272, -0.159 for grade 1, grade 2, grade3 and grade 4 respectively.

IV. Discussion

To scrutinize our findings related to the possible imbalance in the redox status responsible for the oxidative damage, parameters for the lipid peroxidation was selected and estimated. Synovial MDA was selected as a marker of lipid peroxidation.

Table 1, 2 showed, the lipid peroxidation product, i.e., synovial MDA levels, has increased significantly in synovial fluid of patients with osteoarthritis. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules, including membrane lipids. Similar results of elevated MDA levels have been reported in patients with rheumatic disease [12].

The severity of the disease process evaluated with the help of measuring knee joint space width and radiological grading. Pearson's bivariate correlation was done between knee joint space width and Kellgren–Lawrence grading, which showed a significant strong negative correlation (Table 3)

Synovial MDA showed a significant strong negative correlation with knee joint space width and positive correlation with radiological grading (Table 4). Synovial MDA was increase with increasing Kellgren–Lawrence grade.(box whisker plot –fig-1)

All data signified that with increasing disease severity of osteoarthritis there was simultaneous increase in the free radical induced lipid peroxidation. ROS contribute to cartilage matrix degradation in OA is through lipid peroxidation. Metabolic process that leads to direct cartilage damage is the oxidative change of arachidonic acid (AA), an essential fatty acid, to F2-isoprostanes, MDA and HNE. All three have been shown to be elevated in OA patients. These molecules [13] have also been shown to act directly to degrade cartilage. Lipid peroxidation generates a variety of hydroperoxide and aldehyde products that are highly reactive with components of the cell and the extracellular matrix. [14]Lipid radicals also function as intracellular signaling molecules, influencing various cellular functions .moreover chondrocytes produce ROS, especially superoxide anion and H2O2. Hydrogen peroxide and downstream products such as hydroxyl groups (-OH) are linked to aggrecan degradation.[15] Exposure of type I cartilage to superoxide generated by xanthine-hypoxanthine system degrades collagen and prevents formation of fibrils by this collagen[16] .Each of these changes has the direct effect of further degrading cartilage.

.This leads to a vicious cycle between the increasing free radical induced lipid peroxidation and worsening of the disease scenario.

V. Conclusion

The present study conducted an analysis on lipid peroxidation product responsible for causing an altered redox status leading to oxidative stress in the osteoarthritis patient's and how it related with disease severity. Free radical induced lipid peroxidation was increased in the osteoarthritis patients indicated by Lipid peroxidation was significantly higher in the osteoarthritis patients as compared with the control population as indicated by the synovial MDA.. The study, hence, suggests that treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage and deterioration of the musculoskeletal tissues in osteoarthritis. But further multicentre placebo controlled trials are needed to establish it. There was a significant positive correlation between the free radical induced lipid peroxidation (TBARS) and the severity of the disease process as indicated by the knee joint space width and radiological grading. Synovial MDA could be used as a marker to assess the disease severity of osteoarthritis. Synovial MDA could be used to complement knee joint space width.

Reference

[1] Chopra A, Patil J, Bilampelly V, Relwane J, Tandle HS. Prevalence of rheumatic disease in rural population in Western India: a who- ilar-copcord study. J Assoc Physicians India 2001; 49:240-46.

[2] Poole AR. An introduction to the pathophysiology of osteoarthritis. Front Biosci. 1999 Oct 15; 4:D662-70.

[3] The burden of musculoskeletal conditions at the start of the new millenium, who technical report series: 919.

[4] Hamerman D. The biology of osteoarthritis. New Engl J Med 1989;320:1322–30

[5] Del Carlo M Jr, Loeser RF. Increased oxidative stress with aging reduces chondrocytes survival: correlation with intracellular glutathione levels. Arthritis Rheum 2003;48:3419–30

[6] Spector TD, Cooper C. Radiographic assessment of osteoarthritis in population studies: whither Kellgren and Lawrence? *Osteoarthritis* Cartilage. 1993 Oct;1(4):203–206

[7] Tiku ML, Allison GT, Naik K, Karry SK. Malondialdehyde oxidation of cartilage collagen by chondrocytes. Osteoarthritis Cartilage 2003; 11:159–66.

[8] 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. Arthritis Rheum 1986;29:1039-49.

[9] Masaaki Takahashi**,** Kenichi Naito**,** Masashi Abe**,** Tomokazu Sawada and Akira Nagano. Relationship between radiographic grading of osteoarthritis and the biochemical markers for arthritis in knee osteoarthritis; 2004, 6**:**R208-R212

[10] Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys. 1962 Aug; 98:253–261.

[11] Kakkar P, Dos B and Viswnathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem., 21: 130-132.

[12] Mezes M, Bartosiewicz G: Investigations on vitamin E level and lipid peroxide status in rheumatic diseases. Clin Rheumatol, 1983;. 2: 259-63.

[13] Basu, S, Whiteman, M, Mattey, DL, Halliwell, B. 2001. Raised levels of F(2)-isoprostanes and prostaglandin F(2alpha) in different rheumatic diseases. Ann Rheum Dis. 60:627-31.

[14] Kasai H. Analysis of a form of oxidative DNA damage 8-hydroxy-2' deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis, Mutat Res 1997; 387:147.

[15] Tiku, ML, Gupta, S, Deshmukh, DR. 1999. Aggrecan degradation in chondrocytes is mediated by reactive oxygen species and protected by antioxidants. Free Rad Res, 30:395-405.

[16] Henrotin, YE, Bruckner, P, Pujol, JP. 2003. The role of reactive oxygen species in homeostatis and degradation of cartilage. Osteoarthritis Cartilage, 11(10):747-755

Table 1: Independent *t*-test for synovial MDA between the cases and controls.

	Synovial MDA *mean*+_ S.D nmol/ml
Cases	**4.8+_ .78**
Controls	**3.85+_ .95**
P values	**<0.001**
t value	**8.9**

Table 2: ANOVA of the knee joint space width, synovial MDA, with Kellgren–Lawrence grading scale

ANOVA

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Width	**Between Groups**	**53.241**	**3**	**17.747**	**33.413**	**.000**
	Within Groups	**38.242**	**72**	**.531**		
	Total	**91.484**	**75**			
MDA	**Between Groups**	**33.929**	**3**	**11.310**	**68.972**	**.000**
	Within Groups	**11.806**	**72**	**.164**		
	Total	**45.735**	**75**			

Table 3: Bivariate correlations between radiological grading and knee joint space width

Knee joint space width	**Radiological grading**
Pearson's correlation coefficient	**-0.761**
P value	**<0.001**

Table 4: Bivariate correlation analysis between knee joint space width, radiological grading and synovial MDA

Variable	**Pearson's correlation coefficient r**	**P value**
R1,2	**-0.763**	**<0.001**
R1,3	**0.827**	**<0.001**

X 1=synovial MDA, X2= knee joint space width, X3=radiological grading and R1, 2 Bivariate correlation between X1and X2, R1, 3 Bivariate correlation between X1and X3.

Fig.1. Box-whisker plot showing distribution of synovial MDA and Kellgren–Lawrence grade The horizontal bars inside the boxes signify median values, the limits of the boxes denote the 25th and 75th percentiles and the upper and lower whiskers .represent the range. n = the number of samples in each group.