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A Comparative Study between Synovial Superoxide Dismutase per Oxidation Marker and the Severity of Knee Osteoarthritis

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Abstract: *Osteoarthritis (OA) 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 (mean of 54.5) 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 .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, synovial fluid superoxide dismutase peroxidation (SOD) activity and to find out possible association between them. Results showed that there was a significant increase in SOD activity in patients with knee joint osteoarthritis as compared to the controls. Synovial SOD showed positive correlation with Kellgren–Lawrence grading and negative correlation with knee joint space width. Synovial SOD increased with increasing Kellgren–Lawrence grade.*

Keywords- *Knee Osteoarthritis, Kellgren–Lawrence grading scale,Synovial fluid, superoxide dismutase peroxidation marker.*

I. INTRODUCTION

Osteoarthritis (OA) is the most common type of arthritis. [1]. Its high prevalence, especially in the elderly, and the high rate of disability related to disease makes it a leading cause of disability, suffering and morbidity. It is characterized by low-grade inflammation resulting in pain in the joints, caused by wearing and destruction of the cartilage that covers and acts as a cushion inside joints 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 [2]., 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 [3]

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

One key antioxidant enzyme implicated in the regulation of ROS-mediated tissue damage is extracellular superoxide dismutase (EC-SOD). EC-SOD is found in the extracellular matrix of tissues and is ideally situated to prevent cell and tissue damage initiated by extracellularly produced ROS. It has been shown

that SOD inhibits articular issue damage in osteoarthritis. Previous study also shows that EC-SOD is highly expressed in cartilage and secreted by chondrocytes when they were grown in alginate bead culture.

II. SUBJECTS

The present study is based on 81 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[5] .The procedures followed were in accordance with the principles of the Declaration of Helsinki in 1975, as revised in 1983. (Ethical committee)

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 X-ray beam was aimed at the lower pole of the patella and kept parallel to the joint surface. The target–film distance was 36 inches. 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 .[6]

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.

Estimation of synovial SOD

SOD estimation was done as described by P. Kakkar, B. Dasand, P.N. Viswanathana in Indian journal of Biochemistry and Biophysics[7]

Principle

It was based on chromogen production using phenozinemethosulphate(PMS), Nitrobluetetrazolium (NBT) in presence of SOD enzyme.

III. RESULT

The obtained data was analyzed using SPSS software.

The Table 1 displays the comparison of mean synovial SOD in cases and controls**.** The analysis of the different between the means was done with independent sample t test. Synovial fluid SOD was found to be significantly higher in the cases compared to the control.

In the Table 2 the data of the knee joint space width, synovial fluid SOD of the entire subject categorized as per the Kellgren–Lawrence grading scale. It is evident from the data that the mean of the knee joint space width is diminishing with increasing severity of knee joint osteo arthritis.

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. (at p value < 0.001).

From the Table 4 it is evident that synovial SOD has a positive correlation with radiological grading and negative correlation with knee joint space width.

The relationship between the Kellgren–Lawrence grading and the synovial SOD is shown in box whisker plot 3. : The synovial SOD increase with increasing Kellgren–Lawrence grade.

IV. DISCUSSION

To scrutinize our findings related to the possible imbalance in the redox status responsible for the oxidative damage, parameters for antioxidant activity were selected and estimated. Estimations of synovial superoxide dismutase were performed to assess the overall antioxidant status of individual.

In the present study, the antioxidant enzymes', i.e., of SOD activities have been increased significantly in patients with osteoarthritis (table 1), Similar results of raised SOD activities have been reported in patients with rheumatic diseases. SOD is an important antioxidant enzyme having an antitoxic effect against superoxide anion. The over-expression of SOD might be an adaptive response.[8] Compensatory regulation in response to increased oxidative stress that results in increased dismutation of superoxide to hydrogen peroxide. Ostalowska *et al* have reported increased activities of SOD, glutathione peroxidase and glutathione reductase in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint [9]

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).

Positive correlation between synovial SOD activity and Kellgren–Lawrence grading and Negative correlation with knee joint space width may be an adaptive response (Table 4). Synovial SOD activity was increase with increasing Kellgren–Lawrence grade. (Box whisker plot 1).

Previous studies have shown that superoxide is an important mediator of inflammation and tissue injury.[10,11]Superoxide can degrade synovial fluid and collagen, depolymerize hyaluronic acid and inactive antiproteinase, and convert arachidonic acid into biological active products, which result in further joint tissue injury and cause clinical symptoms.[10-12] SOD can protect the organism against the toxic effect of the superoxide radical by catalyzing its dismutation to molecular oxygen and hydrogen peroxide.[12,13]
So increase severity may lead to increase oxidative stress and increase consumption of synovial SOD.

V. CONCLUSION

The present study conducted an analysis on anti oxidant enzymes in osteoarthritis patient's and how it related with disease severity. Antioxidant enzyme plasma SOD activity was significantly higher in the osteoarthritis patients as compared with the control population as indicated by the synovial SOD levels. The increased activities of antioxidant enzymes plasma SOD may be a compensatory regulation in response to increased oxidative stress. 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 synovial SOD and the severity of the disease process as indicated by the knee joint space width and radiological grading. Synovial SOD could be used as a marker to assess the disease severity of osteoarthritis. SOD could be used to complement knee joint space width.

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Table1: Independent *t*-test for SOD between the cases and controls.

	Synovial SOD *mean*+ S.D activity(U/ l)
Cases	**19.63+2.6**
controls	**17.08+3.8**
P values	**<0.001**
t value	**5.78**

Table 2: Knee joint space width and Synovial SOD of the study population according to the Kellgren–Lawrence grading scale

	GRADE 1		GRADE 2		GRADE 3		GRADE 4	
Width of knee joint	Mean S.D Maximum Minimum range	4.062 0.696 5.5 3.0 2.5	Mean S.D Maximum Minimum range	3.125 0.679 4.5 2.0 2.5	Mean S.D Maximum Minimum range	2.47 0.915 4.00 1.00 3.0	Mean S.D Maximum Minimum range	1.55 0.4972 1.00 2.5 1.5
Synovial SOD	Mean S.D Maximum Minimum range	17.27 1.444 19.81 14.70 5.11	Mean S.D Maximum Minimum range	19.23 2.019 22.76 16.22 6.54	Mean S.D Maximum Minimum range	21.11 1.51 23.50 18.20 5.30	Mean S.D Maximum Minimum range	23.56 0.561 24.40 22.80 1.60

Table 3: The 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 5 Bivariate correlation analysis between knee joint space width, radiological grading ant synovial SOD

Variable	Pearson's correlation coefficient r	P value
R1,2	-0.742	<0.001
R1,3	0.800	<0.001

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

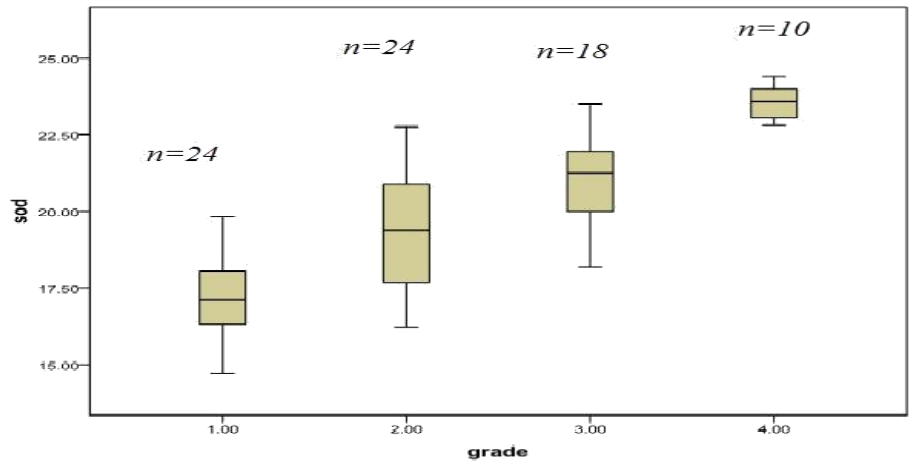

Fig. 1. Box-whisker plot showing distribution of synovial SOD and Kellgren–Lawrence grade The horizontal bars inside the boxes signify median values, the limits of the boxes denote the 25th and 75th percentiles and the upper and lower whiskers .represent the range. n = the number of samples in each group.