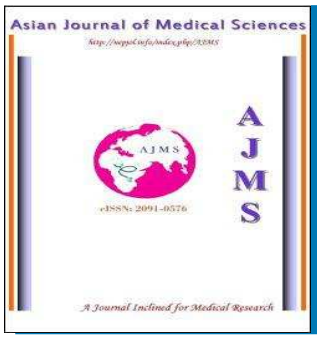

SUSCEPTIBILITY OF KNEE OSTEOARTHRITIS PATIENTS TO DEVELOP CARDIOVASCULAR DISEASE – A CLINICAL STUDY

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"Patients with chronic degenerative joint disorders such as Knee OP have a higher risk to develop CVD with disease severity. Early detection of CVD markers with the progression of Knee OA and possible preventive approach are essential"

ABSTRACT

Background: Osteoarthritis (OA) is a process of progressive destruction of articular cartilage which makes the OA patients physically inactive and increases the probability to develop cardiovascular disease (CVD). Aim: The objectives of present study were to estimate atherogenic index, markers of oxidative stress and inflammation in knee OA patients of different Kellegren-Lawrence (KL) grade and clarify the probability of knee OA patients to develop future CVD risk with disease severity.

Methodology: Atherogenic index, plasma paraoxonase, C-reactive protein (CRP) and erythrocyte malondialdehyde (MDA) levels were estimated by using standard methods in 55 - 70 years aged 76 knee OA patients categorized into three groups (on the basis of KL grading scale) and 24 healthy subjects (control group). The obtained values were compared statistically by using student's t-test.

Result: A significant reduction in the plasma paraoxonase and serum HDL cholesterol levels were observed in ($p<0.05$) Group II & III knee OA patients. Similarly, erythrocyte MDA, Total cholesterol/HDL ratio and plasma CRP levels were increased significantly in ($p<0.05$) Group II and III knee OA patients. However, these levels were found to be altered insignificantly ($p<0.01$) in Group I knee OA subjects as compared to controls.

Conclusion: Thus, the probability of KL grade III and IV knee OA patients to develop future risk of CVD are more as characterized by elevated antherogenic index, systemic inflammation and oxidative stress and reflect the need of antioxodants supplementation along with drug of choice to reduce CVD risk in knee OA patients.

Keywords*:* Atherogenic index, inflammation, malondialdehyde, paraoxonase.

INTRODUCTION

Osteoarthrits (OA) is the most common form of joint disease that affects humans. The incidence of OA increases during every decade of life, and by the age of 65 years, most of the elderly has OA of knee joints.[1] Its prominent feature is the progressive destruction of articular cartilage which results in impaired joint motion, severe pain, structural and functional failure of synovial joints.[2] Consequently, the patients become physically inactive. It has been hypothesized that physically inactive OA patients have at greater risk for manifestations of cardiovascular disease (CVD).[3]

Although precise etiology of this debilitating disease (OA) is poorly understood, probability of OA patient to develop future CVD risk are more due to involvement of some common CVD risk factors such as high body mass index, aging, genetic factor and nutritional factors.[2] Previous studies have also shown an excess of cardiovascular risks, morbidity and mortality in patients with arthritis compared with the general population.[4] Approximately 50% of CVD in the community occurs in the absence of traditional risk factors i.e. increasing age, hypertension and hypercholesterolemia.[5] In particular, increased TC/ HDL-C ratio i.e. atherogenic index, is an important prognostic marker for cardiovascular disease and the risk of myocardial infarction increases considerably when this ratio is higher than five.[6,7] The serum TC and HDL-C levels in knee OA were inversely correlated with disease activity, suggesting a potential role of inflammation in the atherogenic profile and the higher atherosclerotic risk in arthritic patients.[8,9]
Amongst various risk factors, the imbalance between pro-oxidants and antioxidants gives rise to cellular oxidative stress, which plays an important role in the progression of osteoarthritis and CVD as well. Oxidative damage induced by reactive oxygen species (ROS) is caused by increased production of superoxide anion ($O_2^{\circ-}$) and its metabolites or by reduced bioavailability of antioxidant defenses. ROS may act through several mechanisms to mediate vascular changes and major interrelated derangements related to rheumatic disease such as damage to endothelium, cartilage, membrane ion transporters, collagen and other specific proteins, DNA strand breakage and oxidation of LDL i.e. lipid peroxidation. [10,11] Oxidised LDL served as a substrate for macrophage "scavenger" receptors and supports the formation of foam cells, a hallmark of atherosclerotic lesions,[12] leading to development of CVD among OA patients.

Recently, paraoxonase (PON), a calcium-dependent A-esterase synthesized primarily in the liver and secreted into the serum as HDL-associated enzyme that prevents oxidation of low density lipoprotein (LDL), responsible for anti-atherogenic property of high density lipoprotein (HDL) and contribute significantly as antioxidant enzyme in antioxidant defense system of body, has received much attention in CVD as well as in knee OA patients.[13]

In particular, C-reactive protein (CRP), a marker of chronic mild inflammation, has been found to be associated with disease progression in patients with OA.[14] In previous studies, it has been observed that acute systemic inflammation is associated with an increased risk of acute cardiovascular events and cardiovascular mortality.[15,16]

Despite various evidences regarding association between OA progression with inflammation and involvement of inflammation in CVD development,[14-17] there is no far conclusive evidence enlightens the possible cause of CVD development in different KL grade knee OA patients, as best of our knowledge. In addition, it is conceivable that inflammation, alteration in paraoxonase activity, increased levels of lipid peroxides, lipid abnormality and involvement of high body mass index further amplify the probability of CVD development in knee OA patients. Therefore, the overall objectives of

patients. Therefore, the overall objectives of present study were to estimate the levels of plasma C-reactive protein, paraoxonase activity, extent of lipid peroxidation and atherogenic index in knee OA patients and to determine the variation in their levels with subsequent up gradation of disease severity in knee OA patients.

MATERIALS AND METHODS

Radiographic knee osteoarthritis was defined according to Kellgren Lawrence (KL) grading scale. This scale involves the following grades:- grade 0: normal; 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.[18]

In the present study, 72 radiographic knee OA patients (50-70 years) attending OPD were included and divided into 3 groups of 24 subjects each on the basis of KL grading scale (i.e. Group I includes 24 knee OA patients of KL grade II, Group II includes 24 knee OA patients of KL grade III and Group III includes 24 knee OA patients of KL grade IV) and 24 healthy subjects served as control, were included from urban area of Delhi – NCR region of North India. In each group, age matched 1:1 male-female ratio was maintained. Radiography before inclusion into the study included a weight bearing anteroposterior tibiofemoral view in full extension and skyline patella view. The blinded radiographs were read by an experienced observer.

A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination were taken from all the subjects, after taking their written informed consent and approval of protocol by ethics committee of college. Height and weight were measured with subject barefoot and light dressed. The body mass index (B.M.I.) was calculated as B.M.I. = *weight (Kg) / Height (metre*^{2}).

Inclusion criteria: Patients who gave written informed consent for study, fulfilled American Rheumatism Association Clinical diagnostic criteria for knee OA,[19] and had radiological evidence of grade 2, 3 and 4 knee OA in at least one or both of the knees (as per KL grading scale) were included. Patients were required to have pain for more than half the days of a month and at least pain score above 20% using a 5 cm visual analogue scale (VAS).[20] Analgesic and anti-inflammatory drugs usage were monitored during the study as grades (0 = never used; 1= rarely used; 2 = used few days in a week; 3 = used most days in a week; 4 = used daily).

Exclusion criteria: Patients with hypertension, diabetes mellitus, uncontrolled thyroid disease, renal failure, hepatic disease, viral or bacterial infection, gout or any systematic disease other than knee osteoarthritis were excluded. Patients of mental stress induced disorders, obese (BMI >29.9), hypertensives (B.P. >120/80 mmHg), smokers, alcoholics and subjects under any vitamin supplementation were excluded. In addition as per KL scale grading, knee OA grade 0 (None i.e. definite absence of x-ray changes of OA) and grade 1 (Doubtful presence of OA) were also excluded from the study. In order to remove biasness, knee OA patients having one type of grade in one knee and different grade in another knee, and grade I knee OA patients were excluded from the study.

Fasting blood samples were collected in EDTA vials from the anticubital vein of the subjects and processed immediately. Plasma CRP levels, markers of oxidative stress and serum lipid profile were estimated in controls as well as in different grades knee OA subjects. Plasma C-reactive protein (CRP) levels were measured using commercially available ELISA kits (R&D Systems, USA), according to

to manufacturer's instructions. Serum lipid profile contents (total Cholesterol, Triglycerides& HDL cholesterol) were analysed enzymatically. LDL-cholesterol levels were calculated by Friedwald's formula [21] and VLDL cholesterol by dividing Triglycerides level by 5.

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate.[22] The heat induced reaction of MDA with thio barbituric acid (TBA) in the acid solution forms a trimethine coloured substance, measured at 532 nm.

Plasma paraoxonase activity was estimated by Gan et al method using p-nitrophenyl acetate (5.5 mM/l) as a substrate.[23] The increase in the absorbance of p-nitrophenol formed at 412 nm was measured spectrophtometrically. The activity of PON was measured in Tris buffer (20 mM/L; pH 8.0) containing 1mM $CaCl_2$. The generated product p-nitrophenol was calculated by using molar extinction coefficient of 17000 per mole/cm at pH 8.0. Results are expressed as Units/ml (1 nmol p-nitrophenol formed per minute).

***Statistical analysis*:** Data were collected and statistically analysed manually. Values were expressed as Mean ± SD. The significance of mean difference between control group and each patient group was compared by using Student's t - test and distribution of probability (p).

RESULTS

In the present study, age, anthropometry and clinical profile of the control group and knee OA patients are depicted in Table 1. BMI and visual analogue scale of pain measurement revealed significant and continuous elevation in Group I, II and III knee OA patients which indicate their direct relation with disease severity. Plasma CRP levels were found to increased significantly ($p<0.05$) only in Group II and III subjects respectively whereas in Group I subjects plasma CRP level increases insignificantly.

Marked occurrence of atherogenic profile and significant alteration in the levels of plasma paraoxonase and markers of lipid peroxidation were observed in the study group subjects, as represented in Table 2. Serum total cholesterol, Triglycerides, LDL cholesterol and VLDL cholesterol levels were found to be increased significantly ($p<0.05$ & $p<0.001$) in all the three knee OA groups. On the other hand, serum HDL-cholesterol levels were decreased significantly ($p<0.05$) in Group II and Group III patients while insignificant decline ($p<0.1$) observed in Group I subjects. Plasma paraoxonase levels were found to be significantly low ($p<0.05$ & $p<0.001$) in all knee OA groups i.e. 18.9%, 27.4% and 33.5% low as compared to controls. Erythrocyte MDA levels were 13.8%, 31.5% and 43.5% high in Group I, Group II and Group III respectively as compared to healthy controls. These levels reveal continuous variation with increase in OA severity but statistically these values were altering insignificantly, when these levels were compared with in each other.

DISCUSSION

Systemic inflammation is associated with an increased risk of acute cardiovascular events and cardiovascular mortality.[24, 25] In fact, OA and atherosclerosis may share a common predisposition factor. [26] CRP is the common denominator for both diseases.[14] In the present study, plasma CRP level increases with severity of knee OA (Table 2), which may contribute to CVD risk in knee OA patients because it stimulates macrophages to produce tissue factor, a pro-coagulant that is found in atherosclerotic plaques. The presence of CRP in atherosclerotic lesions also suggests a 'cause and effect' relationship between this acute phase reactant and coronary events.[27] Gotto in his study also suggested that

Table 1: Age, anthropometry and clinical profile of different stages of knee OA patients and control group (Mean ± SD)

Particulars	Control group (n=24)	Group I (n=24)	Group II (n=24)	Group III (n=24)
Age (years)	60 ± 4.8	62 ± 5.2	63 ± 5.5	62 ± 5.0
M:F ratio	1:1	1:1	1:1	1:1
Height (meter)	1.59 ± 0.028	1.61 ± 0.031	1.60 ± 0.030	1.62 ± 0.030
Weight (Kg)	58.2 ± 1.7	63.5 ± 2.5	69 ± 2.8	74.7 ± 3.2
BMI (Kg/m^2)	23.0 ± 1.4	24.6 ± 1.5*	27.0 ± 1.7**	28.5 ± 1.8**
Systolic blood pressure (mmHg)	106.5 ± 3.08	110.0 ± 3.14	114.0 ± 3.36	116.0 ± 3.40
Diastolic blood pressure (mmHg)	76.0 ± 2.24	76.4 ± 2.40	78.2 ± 2.46	78.4 ± 2.50
VAS pain (mm)	0.0	35.5 ± 4.2	46.8 ± 4.7**	65.5 ± 5.6**

where, * $p<0.01$: Non-significant ** $p<0.05$: Significant

inflammation is an important determinant for the reduced HDL-C, [28] as observed in present study group subjects.

Association of OA progression with excessive body weight, impaired cardiovascular fitness and oxidative stress are well accepted fact in osteoarthritc research. In this context, we observed a significant impact of continuous increase in body weight on severity of knee OA. Similarly, Magliano observed that high body weight increases the risk of radiographic knee OA,[26] and may contribute to increased cardiovascular morbidity in patients with arthritis.

In addition, present study group subjects revealed an atherogenic lipid profile characterized by an increase of serum TC, LDL-C, TG and VLDL-C levels, and a reduction in HDL-C levels. Thus, an increase in atherogenic ratio of TC/HDL-C, as observed in TO moderate severe to end stage lower extremity OA (i.e. KL grade scale 3 and 4) patients, suggesting that these patients are possibly exposed to a higher risk of CAD. It could be explained on the basis of less physical activity with increase in BMI of Group II and III knee OA subjects and may be considered as a secondary impact of rheumatic diseases. Our findings were in consistent with the findings of Al-Arfaj who also observed marked elevation in lipid profile content in radiograohic OA.[8] However, Borman and Dessein et al. in their studies do not observe a significant difference in serum lipid profile contents in OA patients as compared to healthy population.[25, 29]

Cartilage degeneration related to osteoarthritis may occur because of the loss of viable cells (chondrocytes) due to apoptosis or other cell mechanisms which include oxidative stress mediated biomolecular destruction (proteins, lipids, and nucleic acids) with aging process.[11] In mediated biomolecular destruction (proteins, lipids, and nucleic acids) with aging process.[11] In particular, malondialdehyde (MDA), a toxic

Table 2: Serum lipid profile, and markers of inflammation and oxidative stress in control group and Knee OA patients. (Mean ± SD)

Particulars	Control group (n=24)	Group I (n=24)	Group II (n=24)	Group III (n=24)
Total Cholesterol (mg/dl)	156.84 ± 14.4	187.28 ± 15.9*	193.5 ± 18.1**	**205.0 ± 19.5***
Triglycerides (mg/dl)	105.20 ± 12.32	121.8 ± 13.46*	127.6 ± 14.7**	**133.5 ± 15.8****
HDL cholesterol (mg/dl)	47.0 ± 3.72	40.24 ± 3.17*	36.89 ± 3.10**	**34.9 ± 2.95***
LDL cholesterol (mg/dl)	93.82 ± 12.37	125.19 ± 13.74*	137.38 ± 13.95**	**145.2 ± 14.0***
VLDL cholesterol (mg/dl)	21.82 ± 2.25	25.23 ± 2.38*	27.54 ± 2.47**	**29.8 ± 2.59****
Total cholesterol/HDL cholesterol ratio	3.57 ± 0.72	4.76 ± 1.05	5.38 ± 1.39	**5.93 ± 1.58**
CRP (mg/L)	3.35 ± 0.15	4.13 ± 0.18*	4.60 ± 0.21**	**4.86 ± 0.25****
Paraoxonase (U/ml)	218.5 ± 17.9	177.2 ± 16.0*	158.7 ± 15.3**	**145.3 ± 14.2****
Malondialdehyde (µmolMDA/ml)	**2.67 ± 0.13**	**3.05 ± 0.21***	**3.51 ± 0.27****	**3.84 ± 0.34****

where, * p<0.01 : Non-significant ; ** p<0.05 : Significant; *** p<0.001: Highly significant

aldehydic end product of lipid peroxidation, mediates the oxidation of cartilage collagen. It also initiates a complex cascade that promotes atherosclerotic plaque formation, prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance leading to hypertension.[12] Shah et al also showed that chondrocyte derived lipid peroxidation mediates collagen degradation.[1] In the present study, erythrocyte MDA levels were significantly high (p<0.05 & p<0.001) in study group subjects, which clarify the role of lipid peroxidation in knee OA progression and development of CVD risk.

Oxidation of lipids is well controlled by antioxidant enzymes including plasma paraoxonase, an enzyme found in association with HDL contributing it to anti-atherogenic and antioxidant capability by hydrolyzing specific oxidized phospholipids and cholesterol linoleate hydroperoxides, and by neutralizing hydrogen peroxide. Alteration in the PON activity may have significant effect in CVD development possibly due to increased production of reactive aldehydes.[30] In the present study, plasma PON activity was found to be decreased continuously in KL grade 2, 3 and 4 knee OA subjects which reflects toward the increasing susceptibility of knee OA patient to develop CVD with OA progression. It could be explained on the basis of inactivation of enzyme itself due to interaction of oxidized lipids with the PON free sulphydryl group. Consistent findings have been reported by Saxena et al. in the patients with hypertensive smokers, indicating the association of PON reduction with CVD risk factors.[30]

CONCLUSION

Thus, we conclude that continuous increase in body weight, inflammation, hypercholesterolemia and oxidative stress collectively play a crucial role in the progression of OA along with development of CVD risk. Therefore, suggestions of health professionals and dieticians, and adoption of lifestyle changes, particularly possible regular exercise, should also be considered and evaluated in order to reduce CVD risk in OA. In addition, the

present study is strong enough to convince the physicians that treatment with natural antioxidants in the initial stages of the disease may be used as an effective remedy to sustain the development of CVD risk and progression of knee OA as well.

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