Association of Oxidative Stress and Dyslipidemia with Complexity of Knee Osteoarthritis

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Abstract

Background: The spectrum of abnormalities in knee osteoarthritis (OA) ranges from pain and inflammation in joints to physical disability, identified as disease process. It is conceivable that occurrence of dyslipidemia and oxidative stress enhances the risk of cardiovascular disease (CVD) in knee OA patients. Aim: The present study was indented to estimate the serum lipid profile and marker of oxidative stress in knee OA patients of different KL grade and to determine their role in predicting CVD risk with disease severity. Methodology: In the present study, serum lipid profile, malondialdehyde and uric acid levels were measured in 180 knee OA patients (40-65 years) by using standard methods. Knee OA patients were categorized into three groups (n=60 in each group; on the basis of KL grading scale) and statistically compared it with that of 60 healthy controls by using student’s t-test. Result: Serum total cholesterol, triglycerides, LDL-cholesterol VLDL-cholesterol, uric acid and MDA levels were significantly high (p<0.001) in Group II and Group III subjects as compared to healthy controls. However, these levels were altered insignificantly (p<0.1) in Group I subjects. Conversely, HDL-cholesterol levels were found to be significantly low (P<0.001) in Group II and Group III as compared to healthy controls. Conclusion: Result of this study imply that the abnormal lipid profile along with enhanced oxidative stress and hyperuricemia are more efficient marker in prediction of CVD risk with advancing of knee OA than merely conventional lipid profile parameters. Thus, regular monitoring of these markers along with adoption of preventive strategies can reduce the CVD mortality in knee OA population.

Keywords: Malondialdehyde, uric acid, inflammation, reactive oxygen species, synovitis.

INTRODUCTION

Progressive destruction of articular cartilage results in impaired joint motion severe pain, structural and functional failure of synovial joints. Moreover, formation of osteophytes on the joint margins, periarticular ossicles and narrowing of joint cartilage associated with sclerosis of subchondral bone are hallmark of knee osteoarthritis process which lead to morbidity and its associated secondary complications [1]. Eventually, the spectrum of abnormalities ranges from pain and inflammation in joints to physical disability, identified as disease process. Dyslipidemia or abnormal lipid profile with advancing of disease process is an alarming condition of future health complications predominantly cardiovascular diseases (CVD) such as myocardial infarction, atherosclerosis etc [2].

In addition, reactive oxygen species (ROS) generated as byproducts of biological oxidations, lead to oxidative stress, which induce casual and cumulative oxidative damage to macromolecules characterized by cellular dysfunction, structural and functional failure of synovial joints with age and eventually knee OA disease process [3]. These free radicals may act through several mechanisms to mediate vascular changes, OA progression and major interrelated derangements of cell metabolism such as damage to endothelium, cartilage, membrane ion transporters and other specific proteins, DNA strand breakage and oxidation of LDL i.e. lipid peroxidation [4, 5]. Oxidised LDL served as a substrate for macrophage “scavenger” receptors and supports the
formation of foam cells, a hallmark of atherosclerotic lesions leading to development of CVD among OA patients [6]. Among the reactive aldehydes derived from lipid peroxidation, Malonaldehyde (MDA) is the most abundant and toxic aldehydic end product of lipid peroxidation which mediates the oxidation of cartilage collagen [7].

ROS mediated destructive events are well controlled by antioxidant defense system of the body which includes both enzymic and non-enzymic antioxidants. Apart from enzymic one, various non-enzymatic antioxidants may have a significant role in the regulation of physiochemical alterations during arthritis and, received much attention in preventing age related complications such as osteoarthritis [3, 8]. It is obvious that uric acid is an effective antioxidant in plasma as it scavenges superoxide radical, protects erythrocyte against peroxidative damage and free radical attack [9-11]. However, previous studies reveal its relation with circulating inflammatory markers, vascular injury and endothelium dysfunction, and attract the researchers to clarify its role in OA pathology. Denoble et al. reported that synovial uric acid is a risk factor for OA progression and suggested a potential involvement of pro-inflammatory cytokines (IL-18 and IL-1β) produced by uric acid activated inflammasomes in OA pathology [12, 13].

It is conceivable that there is a close link between oxidative stress, dyslipidemia and inflammation in increasing the frequency of CVD risk in knee OA patients. In order to enhance our understanding on knee OA etio-pathophysiology, the present study was focused on dyslipidemia, oxidative stress and inflammation in osteoarthritic population in order to explore the complex mechanism involved in making knee OA patients more susceptible to develop future CVD. Therefore, the present work was intended to evaluate the extent of dyslipidemia and levels of MDA along with uric acid in the knee OA patients of different KL grades and to determine their role in prediction of CVD risk with increasing severity of disease.

**Material and Method**

In the present study, 180 radiographic knee OA patients (40-65 years) attending outdoor patient department were included from urban area of Delhi – NCR region of North India. Radiographic knee osteoarthritis was defined according to Kellgren Lawrence (KL) grading scale. These patients were divided into 3 groups (60 subjects in each group) on the basis of KL grading scale of II to IV (as Group I, Group II and Group III) and 60 healthy subjects were included from the hospital staff and their relatives, served as control. 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. General information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination was completed from all the subjects after taking their 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 (BMI) was calculated as BMI = weight (Kg) / Height (metre²).

**Inclusion Criteria**

Patients who gave informed consent for study, not taken any vitamin supplements in last one month before study, fulfilled American Rheumatism Association Clinical diagnostic criteria for knee OA 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 [15]. 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) [16].

**Exclusion Criteria**

Patients suffering from conditions that affect lipid profile such as diabetes mellitus, hypothyroidism, liver or kidney disease, obesity (body mass index > 30), smokers and a history of familial dyslipidemia were excluded. In order to remove biasness, knee OA patients having one type of grade in one knee and different grade in another knee, and KL grade I knee OA patients were also excluded from the study.

Fasting blood samples were collected in plain vials from the antecubital vein of the subjects and processed immediately for serum separation. Serum MDA levels were estimated by thiobarbituric acid (TBA) reaction. Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid (pH 2-3) and boiled with thiobarbituric acid which reacts with Malondialdehyde, forming a MDA-TBA to get pink color. The pink colored complex that occurred was refrigerated to room temperature and measured by using a spectrophotometer at 530 nm [17]. Serum uric acid levels were estimated by Caraway’s method in which uric acid react with phosphotungstic acid in alkaline medium forming a blue color complex which was measured at 700 nm [18].

Serum lipid profile contents (Total Cholesterol, Triglycerides and HDL cholesterol) were analysed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). LDL-cholesterol levels were calculated by Friedwald’s formula [19].

\[
\text{LDL-C} = \text{TC} - [(\text{TG}/5)+\text{HDL-C}]
\]

**Statistical Analysis**

Values were entered manually in MS windows excel sheet and expressed as Mean ± SD. The
significance of mean difference between groups was compared by using Student’s t test and distribution of probability (p) in online Graph pad software.

**RESULT**

In the present study, the Demographic indices and clinical profile including mean age and blood pressure of the study group subjects, are depicted in Table-1. Body mass index (BMI) and visual analogue scale (VAS) of pain measurement revealed significant and continuous elevation in Group I, II and III knee OA patients. Serum uric acid levels were found to increased significantly only in Group II and Group III (p<0.001) subjects respectively i.e. 43.2%, and 52.3% high as compared to healthy controls whereas in Group I subjects serum uric acid levels were increased (p<0.1) insignificantly.

Marked alterations were observed in serum lipid profile and markers of oxidative stress in the study subjects as represented in Table-2. Serum TC, TG and VLDL-C levels were found to be increased significantly in Group II (p<0.05 i.e. 21.9%, 21.35% and 21.3% high) and in Group III knee OA subjects (p<0.001 i.e. 29.5%, 27.6% and 27.5% high) respectively. Similarly, LDL-cholesterol levels were found to increased significantly only in Group II and Group III (p<0.001) subjects respectively i.e. 40.7%, and 53.05% high as compared to healthy controls whereas in Group I subjects serum uric acid levels were increased (p<0.1) insignificantly. On the other hand, serum HDL-C levels were decreased significantly in Group II and Group III patients (p<0.05) i.e. 22.09% and 24.94% low respectively while insignificant alteration (p<0.1) in lipid profile were observed in Group I subjects.

Serum malondialdehyde (MDA) levels were also found to be significantly high in Group II (p<0.05) and Group III (p<0.001) knee OA patients i.e 38.8% and 54.2% high as compared to controls. These levels revealed continuous variation with increase in OA severity but statistically these values were altering insignificantly on comparing with each other.

**Table-1: Demographic and clinical profile of study group subjects (Mean ± SD)**

<table>
<thead>
<tr>
<th>S No</th>
<th>Particulars</th>
<th>Control group (n=60)</th>
<th>Group I (n=60)</th>
<th>Group II (n=60)</th>
<th>Group III (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Age (years)</td>
<td>53.3 ± 6.0</td>
<td>54.4 ± 6.2</td>
<td>57.7 ± 3.2</td>
<td>60.3 ± 2.9</td>
</tr>
<tr>
<td>3)</td>
<td>Height (meter)</td>
<td>1.61 ± 0.056</td>
<td>1.69 ± 0.076</td>
<td>1.67 ± 0.078</td>
<td>1.64 ± 0.065</td>
</tr>
<tr>
<td>4)</td>
<td>Weight (Kg)</td>
<td>60.3 ± 4.2</td>
<td>70.5 ± 6.0</td>
<td>77.3 ± 7.3</td>
<td>76.6 ± 6.0</td>
</tr>
<tr>
<td>5)</td>
<td>BMI (Kg/m²)</td>
<td>23.1 ± 1.2</td>
<td>24.5 ± 1.1</td>
<td>27.7 ± 1.1     **</td>
<td>28.2 ± 0.93     ***</td>
</tr>
<tr>
<td>6)</td>
<td>Systolic blood pressure (mmHg)</td>
<td>106.9 ± 3.3</td>
<td>111.2 ± 3.2</td>
<td>114 ± 3.8</td>
<td>116.6 ± 2.8</td>
</tr>
<tr>
<td>7)</td>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.1 ± 2.5</td>
<td>75.8 ± 1.8</td>
<td>75.4 ± 2.5</td>
<td>77.0 ± 2.1</td>
</tr>
<tr>
<td>8)</td>
<td>VAS pain (mm)</td>
<td>0.0</td>
<td>35.5 ± 5.6</td>
<td>58.3 ± 5.1     **</td>
<td>75.4 ± 7.4     **</td>
</tr>
</tbody>
</table>

Where,  
* p<0.1: Non-significant  
** p<0.05: Significant

**Table-2: Marker of oxidative stress and serum lipid profile of knee osteoarthritis patients and healthy controls. (Mean ± SD)**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Particulars</th>
<th>Control group (n=60)</th>
<th>Group I (n=60)</th>
<th>Group II (n=60)</th>
<th>Group III (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Uric acid (mg %)</td>
<td>4.4 ± 0.73</td>
<td>5.1 ± 0.68</td>
<td>6.3 ± 0.71     ***</td>
<td>6.7 ± 0.73      ***</td>
</tr>
<tr>
<td>2.</td>
<td>Malondialdehyde (µmolMDA/ml)</td>
<td>2.73 ± 0.22</td>
<td>3.303 ± 0.23</td>
<td>3.79 ± 0.24    **</td>
<td>4.21 ± 0.23     ***</td>
</tr>
</tbody>
</table>
DISCUSSION

Cardiovascular disease (CVD) is the main cause of mortality and morbidity in patients with knee osteoarthritis (OA). Both diseases share their pathophysiology so closely that CVD can be considered as an extra articular manifestation of OA [20]. Amongst various modifiable risk factors for CVD such as smoking, hypertension, diabetes and overweight, management of dyslipidemia can help to prevent and reduce the CVD burden in knee OA patients. Dyslipidemia is defined as altered serum concentration of lipids (triglyceride and total cholesterol) and their related blood-transporting lipoproteins: HDL cholesterol, LDL cholesterol, and VLDL cholesterol [21]. The present study group subjects revealed a traditional CVD risk factor i.e. an abnormal lipid profile, characterized by an increase in serum total cholesterol, triglycerides and LDL-C levels, and a reduction in HDL-C levels which enhances the CVD risk in knee OA patients with severity of disease. It could be explained on the basis of less physical activity with advancing of disease which may be considered as a secondary impact of senescence related disorders [22,23]. Progressive reduction in HDL cholesterol levels, as observed in different KL grade knee OA patients, also exposed them to CVD risk because HDL particle is known not only for its ability to facilitate reverse cholesterol transport, but also due to its anti-inflammatory, anti-oxidant, anti-thrombotic, anti-oxidant, anti-inflammatory, and endothelium-stabilizing properties that may benefit against atherosclerosis [24, 25]. Our findings were in consistent with the findings of Saxena et al. who also observed marked alteration in lipid profile content in knee OA subjects [26].

Moreover, it has been well established that disturbance in systemic oxidative balance due to uncontrolled ROS production plays a crucial role in increasing the chances to develop CVD complications in knee OA population. ROS produced by endothelial cells and vascular smooth cells not only oxidize low density lipoprotein and initiate atherosclerotic event but also involve in cell membrane damage via lipid peroxidation which in turn play a crucial role in the development and progression of vascular complications in knee OA [27]. In the present study, marked increase in serum MDA levels were observed in Group II and Group III subjects (p<0.001) as compared to healthy controls which clarify the eti-pathogenic role of ROS via lipid peroxidation, in shaping knee OA patients more susceptible to develop future incidence of CVD and its related complications. Similar findings have been reported in previous studies carried out on elderly subjects with osteoarthritis [28]. According to them, lipid peroxides are toxic to the cellular components, and responsible for not only initiation of complex cascade that promotes atherosclerotic plaque formation, prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance and thereby leading to development of CVD complications in knee OA with dyslipidemia.

It is conceivable that dyslipidemia and oxidative stress in knee OA are associated with incidence of inflammation and the mechanism behind its complex interplay is responsible for the development of future CVD risk. In the present study, serum uric acid levels were found to be significantly high along abnormal lipid profile in KL grade III and IV knee OA patients as compared to healthy controls which may be due its potential involvement in the pro-inflammatory cytokines (IL-18 and IL-1β) production by activating inflammasomes leading to OA pathology and progression [12]. Furthermore, in response to an oxidative inflammatory effect, atherogenic complexes of autoantibodies to oxidized LDL are generated which enhances the accumulation of LDL in the endothelial wall and thereby enhances the CVD risk [13]. Interestingly, in contrast to opinion regarding role of uric acid in inducing synovitis and OA pathology, its antioxidant properties in extra cellular fraction are well documented. Ames et al. pointed out the fact that urate provides antioxidant defense against radicals causing cancer and aging in humans which in turn direct the researchers towards the antioxidant role of uric acid in inflammatory diseases including knee osteoarthritis [9]. Our findings were in agreement with the previous

<table>
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<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>VLDL cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>163.2 ± 7.1</td>
<td>121.7 ± 8.3</td>
<td>37.5 ± 5.1</td>
<td>99.9 ± 9.7</td>
<td>21.1 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>189.4 ± 9.7</td>
<td>121.7 ± 8.3</td>
<td>37.5 ± 5.1</td>
<td>99.9 ± 9.7</td>
<td>21.1 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>199.1 ± 6.8</td>
<td>121.7 ± 8.3</td>
<td>37.5 ± 5.1</td>
<td>99.9 ± 9.7</td>
<td>21.1 ± 1.6</td>
</tr>
<tr>
<td>6</td>
<td>211.5 ± 7.1</td>
<td>121.7 ± 8.3</td>
<td>37.5 ± 5.1</td>
<td>99.9 ± 9.7</td>
<td>21.1 ± 1.6</td>
</tr>
</tbody>
</table>

Where,
*p<0.1: Non-significant
**p<0.05: Significant
***p<0.001: Highly significant
findings of Gupta et al., [27]. According to him, altered level of serum uric acid might be due to enhanced oxidative stress in knee OA patients and body is trying to protect itself from the deleterious effects of free radicals by increasing uric acid production.

**CONCLUSION**

Since dyslipidemia predicts the risk of CVD, it is important to consider uric acid levels in knee OA patients for more comprehensive strategic management of risk factors. Moreover, regular assessment of markers of oxidative stress is additional approach to provide clear clinical picture with advancing of disease. Thus, based on these observations, our study concludes that incorporation of malonaldehyde and uric acid along with conventional lipid profile parameters can be included to the battery of routine analysis of CVD risk determination in knee OA patients with severity of disease. Furthermore, counseling of knee OA population to maintain healthy dietary pattern, lifestyle modification, regular exercise and adoption of antioxidant rich diet are essential steps which may help them to reduce the cardiovascular risks and prevent the progression of OA as well.

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**Ethical approval:** Approved

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