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Research Article

Dyslipidaemia and Oxidative Stress in Primary Knee Osteoarthritis: Analysis of Clinical and Serological Features

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Abstract

Background: Osteoarthritis (OA) is a prevalent and debilitating joint disorder with complex etiology, leading to substantial morbidity worldwide. This study aimed to provide a comprehensive and integrative analysis of the clinical and serological features of OA, focusing on primary knee OA as a representative model.

Methods: A large and diverse cohort of 100 OA patients and 120 healthy controls from India were recruited and subjected to rigorous assessments, including radiological, clinical, functional, and biochemical measurements. Serological evaluations encompassed the quantification of creatinine, uric acid, lipids, and MDA as markers of lipid peroxidation and oxidative stress. Additionally, antioxidant enzyme activities, CRP levels, and inflammatory markers were assessed. Results: Statistical analyses revealed significant differences between OA patients and controls, indicating dyslipidemia and increased oxidative stress in OA. Furthermore, significant correlations between some parameters and OA severity or pain scores were found, suggesting potential prognostic roles.

Conclusion: This study provides valuable insights into the clinical and serological features of OA, highlighting the potential for novel biomarkers and personalized interventions for OA management and prevention.

Keywords: Clinical and Serological Features; Dyslipidemia and Oxidative Stress; Biomarkers; Antioxidant Enzymes; Inflammatory Markers; Comprehensive Analysis; Integrative Assessment.

1.Introduction

Osteoarthritis is a prevalent and debilitating joint disorder that affects millions of people worldwide [1]. The exact causes and mechanisms of OA remain elusive, despite extensive research, hindering the development of effective treatments and preventive measures. Therefore, unravelling the complex interplay of factors that contribute to OA onset and progression, such as genetic, environmental, biomechanical, inflammatory, and oxidative stress components, is an urgent need [2-6]. This study provided a comprehensive and integrative analysis of the clinical and serological features of OA, focusing on primary knee OA as a representative model. A large and diverse cohort of 100 OA patients and 120 healthy controls from Kanpur and nearby regions, India, was recruited and subjected to rigorous assessments of their radiological, clinical, functional, and biochemical status. Various validated tools and scales were employed to quantify OA severity, pain, disability, and quality of life, such as the K-L score, WOMAC, VAS, and ACR classification. Relevant clinical parameters, such as BMI and BP, were also measured, and detailed information on patient history, physical activity, disease awareness, and treatment adherence was collected through specific questionnaires. Serological assessments were conducted to investigate the biochemical alterations associated with OA using advanced colorimetric and enzymatic methods. Statistical analyses were performed on the obtained data using GraphPad Prism and STATISTICA software. One-way ANOVA was used to compare values between groups, and Pearson correlation analysis was used to assess associations among variables. The results revealed significant differences in clinical and serological parameters between OA patients and controls, indicating dyslipidemia and increased oxidative stress in OA patients. Significant correlations between some of these parameters and OA severity or pain scores were also found, suggesting potential causal or prognostic roles. The implications of the findings for the identification

of novel biomarkers and therapeutic targets for OA management and prevention are discussed. By combining comprehensive clinical assessments with state-of-the-art analytical techniques, this study contributed significantly to the understanding of this complex joint disorder and provided valuable insights into the clinical and serological features of OA. This study highlights the potential for personalized and multidisciplinary interventions based on individual clinical and serological status, aiming to improve joint health and function. The identification of novel biomarkers and therapeutic targets may pave the way for innovative approaches in OA management and prevention. While acknowledging some limitations, including the cross-sectional design, restricted study population, and reliance on self-reported measures, this study lays the groundwork for future longitudinal and interventional investigations with larger and diverse samples. The findings underscore the importance of continued research in elucidating the complex pathogenesis of OA, facilitating improved patient care and quality of life.

2. Clinical and serological assessment

2.1. Inclusion and exclusion criteria

We enrolled 120 healthy controls and 100 OA patients in this study. Controls were medical college or departmental staff, free of OA or other systemic diseases, and matched with patients for sex, age, weight, and height. Patients were diagnosed with primary knee OA according to the ACR criteria and recruited from outpatient departments of various hospitals in Kanpur and nearby regions, India. We excluded participants with trauma, joint disease, smoking, obesity, hypersensitivity, cardiovascular disease, ligament instability or other forms of arthritis. We obtained written informed consent from all participants and ethical approval from the institutional committee. We assessed the radiological, clinical, and functional status of OA patients using the K-L score, WOMAC, VAS and ACR classification. We also collected data on patient history, physical activity, treatment history, relevant clinical parameters (BMI and BP), disease awareness and treatment adherence using a HAQ and a modified DAS-28 with CRP score questionnaire.

2.2. Investigating parameters and analytical methods

We collected 5.0 ml of blood from fasting participants using a standard clinical procedure. We isolated DNA from heparinized blood and measured serum creatinine, uric acid, and lipids from plain blood. We used a colorimetric method to determine serum creatinine levels by measuring the absorbance of an orange-coloured complex formed by the reaction of creatinine with picric acid in an alkaline environment [7]. We used the formula creatinine (mg/dl) = ΔAT X 2 ΔAS and converted the values to SI units (µmoles/L) by multiplying by 88.42. We used another colorimetric method to measure serum uric acid levels by measuring the absorbance of a red-coloured quinoneimine dye formed by the reaction of uric acid with a phenolic compound and 4-aminoantipyrine, catalysed by uricase and peroxidase [8]. We used the formula: Uric acid (mg/dl) = AbsorbanceT X 8 Absorbance$S$. We estimated LDL cholesterol using the equation LDL cholesterol = Total cholesterol – (HDL cholesterol + Triglycerides). We estimated HDL cholesterol using the PEG precipitation method and measured the absorbance of the supernatant after centrifugation. We used the formula HDL cholesterol (mg/dl) = Absorbance of T X 200 Absorbance of S. We measured plasma lipids, including cholesterol and triglycerides, using a coupled enzyme assay and separated HDL and LDL/VLDL using a precipitation buffer [9]. We measured the absorbance of the reaction mixture after incubation and used the following formula: Total lipids (mg/dl) = *amount of lipids* X 2 total volume of sample. We estimated cholesterol using the CHOD-POD method by measuring the absorbance of a red-coloured quinoneimine complex formed by the oxidation of cholesterol and its reaction with phenol and 4-aminoantipyrine, catalysed by cholesterol esterase, cholesterol oxidase, and peroxidase [9, 10]. We used the formula Cholesterol (mg/dl) = Absorbance of T X 200 Absorbance of S. We estimated MDA using the method described by Ohkawa by measuring the absorbance of a pink-coloured complex formed by the reaction of MDA with TBA in an acidic medium [9]. We used the formula MDA (µmol/l) = OD532 x 1.75/0.156 and expressed the results as µmol/l/mg protein by an MEC of 1.56 x 105 M-1 cm-1 [11]. We performed a qualitative analysis of CRP using a latex agglutination test. We observed the presence or absence of visible agglutination after mixing serum samples with CRP-latex reagents on a test slide and rotating it for 2 minutes. We interpreted agglutination as a positive result indicating a CRP concentration equal to or greater than 6 mg/L. We estimated triglycerides using the GPO-POD method by measuring the absorbance of hydrogen peroxide formed by the hydrolysis of triglycerides and their subsequent phosphorylation and oxidation, catalysed by lipoprotein lipase, glycerokinase, and glycerol phosphate oxidase [12]. We used the formula: Serum Triglycerides (mg/dl) = Absorbance of T X 200 Absorbance of S. We determined SOD activity using the method described by Mishra and Fridovich by measuring the absorbance of adrenochrome formed by the oxidation of epinephrine in the presence of SOD [10]. We expressed SOD activity as U/gHb, with one unit defined as the amount of enzyme required to exhibit 50% inhibition in the conversion of epinephrine to adrenochrome under specified conditions. We calculated the specific activity of the enzyme (SOD) = units per ml enzyme Hb gm/dl and percentage inhibition (%) = X×100 A, where x represents the absorbance change in the experimental reaction minus the absorbance change in the control/blank reaction, and A represents the absorbance change in the experimental reaction. We determined catalase activity using the method described by Sinha by measuring the absorbance of chromic acetate formed by the reaction of dichromate/acetic acid reagent with residual H2O2 after catalase action [12]. We expressed catalase activity as U/gmHb, with one unit defined as the amount of enzyme that decomposes 1 µmole of hydrogen peroxide per minute under specified conditions. We prepared a standard curve using different amounts of H2O2 ranging from 40-160 µmoles and plotted it between O.D. and the amount of H2O2. GSH activity was quantified by the Teitze method, which involved spectrophotometric detection of TNB at 412 nm as an indicator of the total reduced and oxidized glutathione in the sample [13]. TNB was formed by the oxidation of GSH by DTNB, followed by the

reduction of GSSG by glutathione reductase using NADPH as the reducing equivalent [14]. GST activity was measured by the Habdous method, which involved spectrophotometric detection of NADPH consumption at 340 nm as a reflection of GST catalysis in the sample [15]. GST activity was assayed using CDNB as the standard substrate and reduced glutathione as the substrate. We expressed GST activity in U/L, corresponding to the transformation of one µmole of substrate per minute at room temperature. We compared the values of three independent groups using one-way ANOVA. We tested the homogeneity of variance among groups using Ψ2 tests prior to ANOVA. We performed Pearson correlation analysis to assess the association among variables in both groups. We compared the proportion of males and females among the three groups using the Ψ2 test. Data analysis was performed using STATISTICA (version 6.0) and GraphPad Prism (version 3.0) software [16, 17].

3. Results

3.1. Demographic Studies

We obtained approval from the institutional ethical committee and written informed consent from the participants. We enrolled 100 OA patients (37 males and 63 females) and 120 controls (52 males and 68 females) in this study (see Fig. 1). Patients were diagnosed with primary knee OA according to the ACR criteria and without any comorbidities. Controls were asymptomatic and recruited from the neighbourhoods. X-ray examination showed that 78 patients had OA grade II and 22 had grade III changes (see Table 1). Their range of motion was 0-140/142 degrees with pain (see Table 1 Their pain on the visual analogue scale in squatting was 4.7±1.4 (see Table 1). Their total mean WOMAC score was 44.2±9.8 (see Fig. 1) (WOMAC score 0-100 with 0 as the worst and 100 as the best). Fig. 1 show demographic parameters such as sex ratio, age, and BMI of the participants.

3.2. Serological Parameters

The serological parameters of 28 OA patients and 36 healthy controls were measured and are summarized in Table 2 [18]. OA patients showed lower uric acid and HDL levels and higher LDL, total lipids, cholesterol, MDA, CRP, and triglyceride levels than controls. Serum creatinine was also significantly increased but within the normal range in OA patients ($p<0.05$) (see Table 2). LDL, HDL, cholesterol, MDA, CRP, and triglyceride levels were significantly different between the two groups ($p<0.05$ or $p<0.01$). Uric acid levels did not show a significant difference ($p>0.05$) [19].

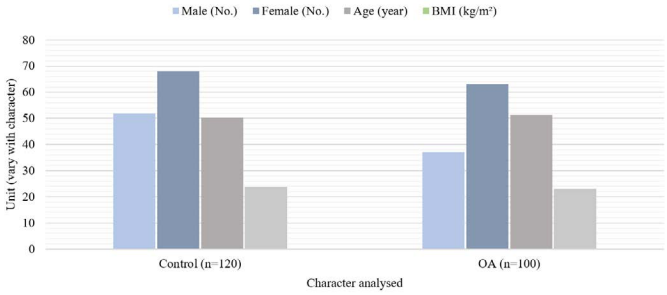

Figure 1: Graphical representation of demographic characteristics of healthy control and patients.

Table 1: Inclusion criteria for diagnosis of OA.

Characteristic	Magnitudes
X-ray Grade	Grade II-78
	Grade III-22
Duration of symptoms (yr)	3.3±1.49
Range of movements	0-140/42± 20.2
VAS pain on movement (cm)	4.7±1.4
WOMAC score	
1- Difficulty	31.0±8.1
2- Pain	9.2±2.2
3- Stiffness	4.2±1.3
4- Total	44.2±9.8

3.3. Status of Antioxidatisve enzymes

Lipid peroxidation, as indicated by MDA production and ESR, was elevated in OA patients (see Table 2), suggesting increased ROS production during chronic inflammation. Lipid peroxides are formed at the site of tissue injury due to inflammation and diffuse into blood, where they can be measured in serum or plasma [20]. The changes occurring in OA may be due to alterations in the balance of oxidants and antioxidants in the system. ROS may play a major role in the pathogenesis of OA due to their generation and inefficiency of the system to eliminate them. We observed decreased enzymatic activity of SOD in OA patients, suggesting inappropriate quenching of superoxide anions (O2-) that may play a role in pathogenesis. We also observed downregulation of catalase and a reduction in GSH levels in OA patients and increased activity of GST (see Table 2 and Fig. 2).

Table 2: Serological parameters and antioxidant enzyme levels (mean±SD) of healthy controls and OA patients. (AP<0.05, bp<0.01 vs. Control).

Parameters	Control (n=36)	OA (n=28)
Creatinine (µmoles/L)	69.42 ± 3.95	78.16 ± 3.72a
Uric acid (mg/dL)	5.05 ± 0.29	4.70 ± 0.28
LDL (mg/dl)	54.81 ± 4.27	96.39 ± 4.93b
HDL (mg/dl)	67.10 ± 1.61	52.93 ± 1.58b
Total lipid (mg/dl)	900.31 ± 53.20	959.80 ± 54.59
Cholesterol (mmol/L)	3.45 ± 0.13	4.25 ± 0.16a
MDA (nmoles/mg protein)	0.76 ± 0.11	1.21 ± 0.11a
CRP (mg/L)	0.41 ± 0.04	2.62 ± 0.05b
Triglyceride (mg/dl)	86.48 ± 4.26	135.82 ± 5.38b
ESR (mm/h)	22.4±1.22	45.5±1.37
SOD (U/gHb)	1.582±0.219	1.276.06±0.139
CAT (U/gHb)	123.70±4.27	102.30±18.40
GSH (mg/gHb)	3.98±0.72	3.14±0.60
GST (U/L)	4.30±0.45	5.00±0.37

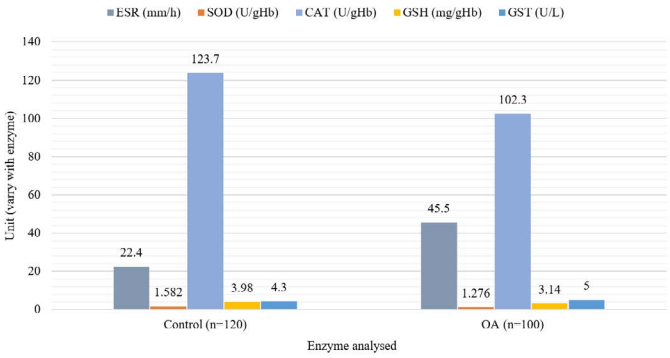

Figure 2: Graphic representation of key enzymes expression in healthy control and OA patients.

4. Discussion

OA is a joint disease with unknown aetiology. Aging-induced degenerative changes have been considered to play an important role in OA. OA was previously regarded as a degenerative and noninflammatory disease. However, increasing evidence supports the role of inflammation, dyslipidaemia, and oxidative stress in the pathophysiology of OA. OA patients had high WOMAC scores, and high VAS scores compared to controls, indicating that they had problems and pain in their knees. The present study aims to evaluate the changes occurring in OA by serological analyses, inflammatory markers, oxidative stress, scavenging enzymes, and proteome profiling using mass spectrometry and NMR. The study also evaluates the association of TGF-β and MTHFR genes with OA by genome-wide association studies and gene expression analysis through microarray.

4.1. Serological component analyses

Serum uric acid levels did not differ significantly (p>0.05) between OA patients and controls, indicating no alteration in uric acid metabolism in OA. Previous studies have found a positive association between serum uric acid and generalized OA [18] but not with knee OA. Serum creatinine, a marker of kidney function, was significantly elevated (p<0.05) in OA patients compared to the control group. However, the levels were still within the normal physiological range (~44-106 µmolesl-1) in OA patients, suggesting mild effects on kidney function. Further studies are needed to confirm whether the changes are related to OA [19]. NMR analyses of metabolite concentrations also revealed increased serum creatinine levels in OA patients compared to controls. Thus, both serological and NMR analyses showed consistent changes in creatinine concentration in OA subjects. Furthermore, OA patients exhibited pathological levels of inflammation and had considerably higher CRP levels than controls.

4.2. Lipid and MDA profile analyses

Inflammation affects lipids, which might be responsible for other complications in OA. Our study shows high total cholesterol, LDL, and triglycerides, which were significantly higher (P <0.05) in OA patients. Earlier associations have been observed between high serum cholesterol and both generalized and knee OA [21]. In our study, HDL levels were significantly decreased in OA patients compared to controls. In OA, an increase in the severity of disease might be related to higher triglyceride and cholesterol levels and lower HDL. These patterns of lipids indicate dyslipidemia. Altered lipids, such as high cholesterol, may induce oxidative stress, resulting in free radical formation, which promotes lipid peroxidation [22]. In hypercholesterolemia, high amounts of lipids and phospholipids accumulate, leading to high release of arachidonic acid along with prostaglandins with the help of cyclooxygenase enzymes and phospholipase A2. Our NMR data also revealed a low HDL/LDL ratio, confirming the serological findings that lipids are altered in OA. Dyslipidemia is the result of inflammatory changes occurring in OA. MDA, the product of lipid peroxidation, provides indirect evidence

of LDL oxidation. Oxidative stress results in a high increase in aldehyde levels, which participate in numerous pathological conditions, such as arthritis, cancer, arthrosclerosis, and cardiac diseases [23]. MDA levels were high in patients with OA ($p<0.05$) compared to controls. Previous studies have shown that synoviocytes from OA patients have high synthesis of HNE and MDA [19, 24].

4.3. Oxidative stress analyses

Our results of high oxidative stress and dyslipidemia are in line with the report by Pawlowska et al. that OA has immunological involvement [24]. The first reactive oxygen radicals are usually superoxide radicals (O2-), produced by superoxide dismutase, which may be further converted to hydroxyl radicals (OH) and hydrogen peroxide (H2O2) [25]. Knee joint destruction is mediated by ROS, which target both invading pathogens and host cartilage [26]. ROS degrade aggrecan, a major ECM component that maintains cartilage hydration and integrity [27]. ROS also modify collagen, the fibrillar network that confers mechanical strength and resists swelling pressure, by direct cleavage or by increasing its susceptibility to proteases [28]. Hydroxyl radicals can directly cleave collagen into small peptides in the presence of oxygen. Interestingly, ROS may also activate matrix metalloproteinases (MMPs), the enzymes that participate in the catabolism of matrix macromolecules [29]. Glutathione reductase, which forms reduced glutathione from oxidized glutathione, has a main role in the scavenging and detoxification of oxygen free radicals [30]. The study by Ostalowska et al. showed increased SOD, glutathione peroxidase and glutathione reductase activity in the synovial fluid of patients with primary or secondary OA of the knee joint [26]. Superoxide can degrade collagen as well as synovial fluid, depolymerize hyaluronic acid, inactivate antiproteinases, and convert arachidonic acid into active components, which further cause joint tissue injury and associated clinical symptoms [31]. Regan and colleagues showed that joint fluid obtained from OA patients had decreased SOD activity and reduced glutathione levels [32]. Our findings are consistent with their findings, as our study also showed reduced activities of SOD and catalase. Afonso studied the role of SOD in scavenging the production of free radicals that may have a role in joint inflammation [33]. Kalpakcioglu reviewed the interactions between antioxidants and free radicals in patients with osteoarthritis [34]. Antioxidative enzymes such as glutathione reductase, catalase, glutathione peroxidase, and superoxide dismutase are known to scavenge free radicals. In our study, the serum of OA patients had significantly decreased levels of SOD and glutathione [32]. The synovial fluid (SF) of affected joints had reduced activity and levels of antioxidants with intense localized inflammatory reactions occurring in the active stage of osteoarthritic joints. These reactions may lead to variable clinical signs and symptoms (pain, swelling, cripitus, etc.) experienced by osteoarthritic patients. Higher GSH levels are supposed to be beneficial for good health; the significance of low GSH status in elderly individuals has been revealed [35].

5. Conclusion

This study provided a comprehensive and integrative analysis of the clinical and serological features of primary knee osteoarthritis (OA), a prevalent and debilitating joint disorder. The findings indicated dyslipidaemia and increased oxidative stress in OA patients, implying the involvement of inflammation and free radicals in OA pathogenesis. Serological markers, such as MDA, CRP, and lipid profiles, may complement radiological and functional assessments for enhanced diagnosis and monitoring. These results underscore the potential for personalized and multidisciplinary interventions based on individual clinical and serological status, aiming to improve joint health and function. The identification of novel biomarkers and therapeutic targets may pave the way for innovative approaches in OA management and prevention. Despite some limitations, this study laid the groundwork for future longitudinal and interventional investigations with larger and diverse samples, emphasizing the importance of continued research in understanding OA pathogenesis for improved patient care and quality of life.

Abbreviation

ACR	-	American College of Rheumatology
ANOVA	-	Analysis of Variance
BMI	-	Body Mass Index
BP	-	Blood Pressure
CAT	-	Catalase
CRP	-	C-Reactive Protein
ESR	-	Erythrocyte sedimentation rate
GSH	-	Reduced Glutathione
GST	-	Glutathione-S-transferase
HDL	-	High-Density Lipoprotein
HNE	-	4-Hydroxy-2-nonenal
K-L	-	Kellgren-Lawrence
LDL	-	Low-Density Lipoprotein
MDA	-	Malondialdehyde
MEC	-	Molar extinction coefficient
OA	-	Osteoarthritis
SOD	-	Superoxide Dismutase
VAS	-	Visual Analog Scale
WOMAC	-	Western Ontario and McMaster Universities Osteoarthritis Index

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