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ORIGINAL ARTICLE

Relation of Endothelial Dysfunction with Systemic Inflammation in Grade 2 Knee Osteoarthritis Patients

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ABSTRACT

Background: A number of pathophysiological conditions, including hypertension, atherosclerosis, myocardial infarction, and inflammatory joint disorders, are characterized by the presence of systemic inflammation. Although previous research has produced a wealth of literature regarding the mild inflammation in patients with Grade 2 Knee Osteoarthritis (G2KOA), there is insufficient explanation for the change in nitric oxide levels along with markers of systemic inflammation and lipid peroxidation.

Aim: Thus, the objective of this study was to measure the levels of C-Reactive Protein (CRP), Malondialdehyde (MDA), and Nitric Oxide (NO) in G2KOA subjects and to establish their relationship to the likelihood of future cardiovascular disorders.

Methodology: In this study, 30 patients of either gender suffering from G2KOA (aged 35 to 50) and 30 healthy normal individuals (as controls) were taken. The above mentioned parameters were measured using standardized techniques and were compared for patients and controls using the Student's t-test and Pearson Correlation Coefficient.

Result: Apart from the pain, the MDA in erythrocytes and CRP levels in plasma of G2KOA patients were significantly high ($P<0.001$ & $P<0.05$) and the levels of plasma NO were significantly low ($P<0.001$) and were inversely correlated with the levels of MDA and CRP.

Conclusion: Based on the findings it can be said that the combined effect of oxidative stress and inflammation leading to the endothelial dysfunction plays

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an important role in increasing the future cardiovascular complications risk in G2KOA characterized by increased CRP and MDA and decreased nitric oxide. As a matter of routine examination in G2KOA patients, these markers need to be tested for early detection and prevention of cardiac disorders.

KEYWORDS

• Pain • Oxi-Inflammatory Stress • Endothelial Dysfunction • CRP

INTRODUCTION

Osteoarthritis of the knee joints is internationally acknowledged as a metabolic problem and a public health concern, which is also highly related to the increased risk of heart disease. The sedentary behavior is commonly caused by joint pain and limited movement of the knee OA, worsening the cardiometabolic risk factors like obesity, high blood pressure, and hyperlipidemia. Moreover, the current scientific consensus states that knee OA and atherosclerosis are diseases that have similar pathophysiologic mechanisms caused primarily by persistent low-grade systemic inflammation and metabolic syndromes.[1,2]

In addition, there is also speculation that risk factors for cardiovascular diseases, including obesity, lack of exercise, smoking, diet, and psychological stress due to inflammation, can trigger the activation of innate immunity.[4]

One of the age-related conditions that led to interest in C-reactive protein (CRP), a liver-derived biomarker of systemic inflammation, is osteoarthritis in the knee joint. The relationship between systemic inflammation and plasma CRP with cardiovascular risks has already been proved before.[5,6]

From the precursor L-arginine, all human body cells produce nitric oxide (NO), a powerful endothelium-derived vasodilator. It has anti-aggregant qualities, contributes to blood pressure regulation, prevents mast cell degranulation, and controls vascular tone. Additionally, it prevents leukocyte and platelet adhesion as well as smooth muscle cell proliferation.[7,8] The development of cardiac problems is significantly influenced by a decrease in NO levels.

One pathogenic cause of arthritis and its associated secondary consequences, such as cardiovascular disease, has been identified as oxidative stress. Vascular alterations in patients with grade 2 knee osteoarthritis were caused by an increase in free-radical responses.[9,10] Lipid peroxidation is one of the many harmful processes caused by free radicals. It involves the breakdown of polyunsaturated fatty acids found in LDL or in the cell membrane into a range of aldehydes, primarily malondialdehyde (MDA). Hypertension may result from these aldehydes' alteration of endothelial function and inhibition of NO synthase activity.[11]

While there is little data on systemic inflammation in G2KOA patients, there is currently no documentation of changes in serum CRP levels, endothelial dysfunction markers, or oxidative stress in G2KOA patients on a single platform. Thus, the current study's goals were to measure serum CRP, nitric oxide, and erythrocyte MDA levels in G2KOA patients and ascertain how these factors relate to the prediction of cardiovascular problems.

MATERIALS & METHODS

Thirty G2KOA patients of either sex who were between the ages of 35 and 50 were included in the current investigation, along with thirty age-matched, healthy controls. All participants underwent either the general information or pre-experimental questionnaire about demographic details, family history, and physical examination including measuring their blood pressure after obtaining their consent and approval of the procedure from the college's ethics committee.

Inclusion criteria: Age of 35 to 50 years and also newly diagnosed and untreated G2KOA patients served as the inclusion criteria. According to American College of Rheumatology, grade 2 knee osteoarthritis was determined.[12] The requirement for the patients was to have at least a VAS pain score of over 5 cm and being in pain for more than half of the days in the month. Analgesic usage was also

observed in the patients during the experiment using grades (0 = never use; 1 = seldom use; 2 = few days in a week; 3 = regularly in a week; 4 = daily). Those who did not consume any vitamin supplements in the previous month participated in the experiment.

Exclusion criteria: Patients under steroids, amiodarone, lithium, antioxidant vitamins, NSAIDs, antihypertensive drugs, and other medicines at the time of the study were not included, along with those suffering from grade 3 and 4 knee osteoarthritis, cardiovascular, hepatic, diabetic, tuberculosis, and renal diseases.

Anti-cubital veins of the study participants were utilized for withdrawing the fasting blood samples, which were subsequently analyzed immediately in EDTA vials. Levels of plasma CRP were assessed through commercially available ELISA kits (R&D Systems, USA) as per the recommendations by the manufacturer. For calculating the pain score, Visual Analog Scale (VAS) was used.

As discussed earlier, NO is an unstable molecule with a short half-life of 10-60 seconds and is not very soluble in water, thus making estimation of plasma NO difficult. In presence of oxygen, however, its half-life can be extended to four minutes. It is due to this reason that nitrate and nitrite, which are the end products of the phenomena, have been considered appropriate in clinical biochemistry. As mentioned earlier, nitrate and nitrite levels were determined by the use of Griess reagent.

Erythrocyte malondialdehyde (MDA) levels were determined as thiobarbituric acid reactive substances after preparing hemolysate. Malondialdehyde reacts with thiobarbituric acid (TBA) on heating and produces colored trimethine substances which can be estimated spectrophotometrically at 532 nm.[14]

Statistical analysis: Data obtained from the study subject group and control group were presented as mean ± SD and analyzed using Student's t-test and Pearson correlation coefficient test (r).

RESULTS

Beside the abnormalities of the biochemical markers as erythrocyte MDA, plasma CRP, and nitric oxide levels in the study participants, Table 1 presents the pain assessment score of the current study. The pain score measured through VAS was significantly high (P<0.001) in the sick group compared to the healthy controls. There was significantly low (p<0.05; 26.07% low) plasma nitric oxide levels in the G2KOA patients as compared to the healthy controls while erythrocyte MDA and plasma CRP levels were significantly high (P<0.001, 38.48% and 29.17% high). The assessment of oxidative stress, systemic inflammation, and a marker of endothelial dysfunction may serve as a significant predictor of the future cardiac disorders in the G2KOA patients based on these findings. Moreover, the correlation coefficient analysis of plasma NO levels and erythrocyte MDA, plasma CRP, and pain score was carried out. Table 2 highlights the inverse association between endothelial dysfunction and oxidative stress, systemic inflammation, and pain in the G2KOA patients (p<0.05; r = -0.548; -0.651 and -0.489).

Table 1: Clinical outcomes for the study cohort: evaluation of pain levels, oxidative stress, systemic inflammation, and endothelial impairment (expressed as Mean ± SD)

Particulars	Control Group (n=30)	Patient group (n=30)	Level of Significance
Visual analog scale (VAS-pain score in mm)	0.0	37.28 ± 5.20	p < 0.001
CRP (mg/L)	3.29 ± 0.10	4.25 ± 0.12***	P < 0.001
Malondialdehyde (µmol MDA/ml)	2.78 ± 0.11	3.85 ± 0.14***	P < 0.001
NO level (µ mol/L)	8.36 ± 2.0	6.18± 1.9**	P < 0.05

where,

* p<0.1: Non-significant, ** p<0.05: Significant, *** p<0.001: Highly significant

Table 2: Statistical correlation of nitric oxide (NO) levels with diverse biomarkers in individuals with grade 2 knee osteoarthritis

Particulars	MDA	CRP	VAS
Nitric oxide	- 0.548 **	- 0.651**	- 0.489**

where,

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

DISCUSSION

However, despite remarkable success in the diagnosis, treatment, and prevention of knee osteoarthritis, it remains one of the major reasons for the development of cardiovascular problems and causes huge socioeconomic burden worldwide. In recent studies, it has been revealed that people having knee osteoarthritis have high level of oxidative stress, which results from excess production of free radicals.[15] With the help of lipid peroxidation, free radicals contribute in destroying cell membranes. These are some of the harmful substances generated by this route – lipid radicals, lipid peroxides, lipid hydroperoxides, and highly reactive aldehydes, all of which have a major contribution in the formation of inflammation and age-related diseases such as knee osteoarthritis. Free radicals via lipid peroxidation cause grade 2 knee osteoarthritis and related cardiac complications.[16,17] The levels of malondialdehyde, a product of lipid peroxidation, is significantly higher in patients compared to healthy control subjects. This high level is negatively correlated with nitric oxide, showing the link between oxidative stress and endothelial dysfunction in patients with knee osteoarthritis.[18] In conclusion, this damaging effect of lipid peroxidation is directly related to the vascular disease connection between knee osteoarthritis and cardiac disorders because the lipid peroxides themselves are toxic to the cells.[19] From the analysis of the findings presented above, it can be said that there is definitely a connection between oxidative stress, inflammation, and the development of cardiac complications in G2KOA patients. According to the literature, in patients with G2KOA, the ischemia-reperfusion causes oxidative stress, resulting in vascular cell apoptosis and inflammatory necrosis.[20,21] Inflammatory conditions result due to the formation of free radicals and intermediates from the process of inflammation through proteolysis of enzymes, reduction of antioxidants in body fluids, oxidative degradation of lipids, and blocking of catabolic enzymes. In particular, the fact that G2KOA patients have much higher concentrations of plasma C-reactive protein (CRP) ($p < 0.001$) proves this point. In addition, this increase indicates how the oxidative stress and inflammation interact with each other leading to endothelial dysfunction that leads to the general pathophysiology and cardiac complications of G2KOA.

CONCLUSION

Overall, this study indicates that there is a negative association between increased concentrations of plasma C-reactive protein (CRP) and malondialdehyde (MDA) and endothelial functions. The aforementioned systemic endothelial dysfunction acts as a key factor behind the development of cardiovascular problems in G2KOA subjects. Through testing MDA and CRP, in addition to nitric oxide (NO), healthcare providers can use these parameters as an extremely efficient marker of oxidative stress and inflammation leading to blood vessels impairment in patients suffering from osteoarthritis. In order to prevent such consequences, the findings point out that it is crucial to consider prophylactic treatment in this case. More specifically, regular joint pain assessment and consumption of antioxidant-containing food will be essential ways to overcome the negative impact of physiological and oxo-inflammatory stress on G2KOA patients.

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