

Total Antioxidant Activity and its Relation with Endothelial Dysfunction in Radiological Knee Osteoarthritis Patients.

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Abstract

Background: Progressive deterioration of chondrocytes due to enhanced production of free radicals facilitates the physical inability to radiological knee osteoarthritis patients (OA). Moreover, oxidative stress mediated endothelial dysfunction has been found to be associated with cardiac complications.

Aim: The objectives of present study were to ascertain the plasma total antioxidant activity (TAA), nitric oxide (NO) and serum malondialdehyde (MDA) levels in knee OA, and to determine their cumulative effect in the development of cardiovascular complications in knee OA patients.

Methodology: The study group parameters were estimated in 75 knee OA patients (40-65 years) by using standard methods. Knee OA patients were categorized into three groups (n=25 in each group; on the basis of KL grading scale) and statistically compared it with that of 25 healthy controls by using student's t-test.

Result: Plasma TAA levels were significantly low ($p < 0.05$, $p < 0.001$) in Group II & III, as compared to healthy controls. Similarly, marked depletion in plasma NO level ($p < 0.05$) were observed in Group III patient only subjects as compared to healthy controls, whereas serum malondialdehyde levels (MDA) were increased significantly ($p < 0.05$, $p < 0.001$) in Group II and III with respect to controls.

Conclusion: Thus, assessment of plasma TAA and MDA along with NO levels is imperative diagnostic parameters in knee OA patients with severity of disease for early determination of secondary complications. Therefore, treatment of grade IV knee OA should include adoption of antioxidant rich diet along with anti-inflammatory and analgesic drugs, preventive approach against traditional risk factors and regular monitoring of cardiac markers for early prediction and to reduce the burden of cardiac complication in knee OA patients.

Keywords: Total antioxidant activity, endothelial dysfunction, reactive oxygen species, lipid peroxidation.

Introduction

World Health Organization (WHO) has set the goal to reduce the risk of premature mortality among middle age and elderly population from non-communicable diseases such as cardiovascular complications by 25% by 2025 [1]. In this context, numerous studies have been carried out to reduce the burden of CVD, globally. It is well accepted that enhanced oxidative stress is an emerging

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and independent risk factor for the development of OA and CVD as well. However the underlying mechanism is not clear. Free radicals mediated oxidative stress is characterized by various deteriorating events such as progressive loss of chondrocytes, endothelial dysfunction and increased lipid peroxidation. Moreover, increased production of free radicals in Knee OA either in combination with these events or alone may lead to the development of secondary complication such as cardiovascular disease [2, 3].

Association of oxidative stress with the etiopathogenesis of cardiovascular complications, musculoskeletal diseases and various age related complications are well documented [4]. Oxidative stress ensues when large amount of reactive oxygen species are produced in the cells during smoking that can evade or overwhelm the

antioxidant protective mechanism of cells and tissues, and produce major interrelated impaired cell metabolism including DNA strand breakage, rises in intracellular free Ca^{2+} , damage to membrane ion transporters and other specific proteins leading to cell death [5]. Prime target to free radicals attack are the polyunsaturated fatty acids in the membrane lipids, causing lipid peroxidation, have been found to be a major event in the production of vascular disorders and other cardiovascular complications. Malondialdehyde is the most abundant among the reactive aldehydes derived from lipid peroxidation. It has been suggested that these aldehydes released from cell membrane and increase the risk of hypertension and cardiac complications not only by disturbing endothelial cells of the blood vessels but also by inducing oxidative modification to the cell and in LDL [6].

Antioxidant defense system provides protection against these harmful free radicals by means of antioxidant enzymes and non-enzymic antioxidants. Total antioxidant activity (TAA) is a complex trait reflecting homeostasis of redox metabolism, affected by the relative contribution of each antioxidant and the stress of oxidative free radicals [7]. TAA may have a significant role in the physiochemical alterations in knee osteoarthritis and received much attention in preventing osteoarthritis associated complications such as cardio vascular disease and hypertension. Interestingly, there is no far conclusive evidence on alteration in plasma total antioxidant status and lipid peroxidation in relation with endothelial dysfunction in knee osteoarthritis. Therefore, the overall objectives of present study were to ascertain the plasma levels of TAA, serum MDA and plasma nitric oxide levels (NO) in knee OA and to determine their cumulative effect in the etiopathogenesis of cardiac complication in knee osteoarthritis.

Material and Method

75 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 [8]. These patients were divided into 3 groups (subjects in each group) on the basis of KL grading scale of II to IV (as Group I, Group II and Group III) and 25 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 in the department thoroughly. 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 $\text{BMI} = \text{weight (Kg)} / \text{Height (metre}^2\text{)}$.

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 [9]. 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) [10].

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.

Fasting blood samples (approximately 6 ml) were collected in sterile plain vecutainer (4 ml) and EDTA vecutainer (2 ml) by venous arm puncture after overnight fasting for serum and plasma preparation. Markers of oxidative stress i.e. serum lipid peroxidation (MDA) and plasma total antioxidant activity (TAA) and marker of endothelial dysfunction (NO) were estimated in controls as well as in knee OA subjects. Plasma total antioxidant activity was estimated spectrophotometrically by the method involving reaction of standardized solution of iron EDTA complex with hydrogen peroxide i.e. Fenton type reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of TBARS. Antioxidants from the added plasma cause the suppression of production of TBARS. The reaction was measured spectrophotometrically at 532 nm [11].

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. In this method, the heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine colored substance, which was measured spectrophotometrically at 532 nm [12].

The measurement of plasma NO is difficult because this radical is poorly soluble in water and has a short half-life in tissue (10-60 s), but its half-life may be as long as 4

minutes in the presence of oxygen. For these reasons, the end products of the phenomenon, nitrate and nitrite, are preferentially used in clinical biochemistry. Plasma total nitrate and nitrite levels were measured with the use of Griess reagent as described earlier [13].

Statistical Analysis

Values were entered manually in MS windows excel sheet and expressed as Mean \pm 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

Anthropometric indices along with mean blood pressure of the study group subjects are represented in Table 1. The observation made reveal significant changes in the marker of oxidative stress (levels of plasma TAA and serum malondialdehyde) and marker of endothelial dysfunction in Group II and Group III patients with respect to control group (Table 2). Plasma total antioxidant activity was found to be significantly low ($p < 0.05$ & $p < 0.001$) in Group II and III patients i.e. 29.82 % and 36.84 % low as compared to controls. Similarly, plasma nitric oxide levels were decreased significantly ($p < 0.05$ & $p < 0.001$) in Group II and III patients i.e. 21.55 % and 29.10 % low as compared to controls. On the other hand, erythrocyte MDA levels were increased significantly ($p < 0.001$) in Group II and III patients i.e. 41.48 % and 58.14% high respectively. However, these levels were altered insignificantly ($p < 0.1$) in Group I patients.

Discussion

Reactive oxygen species that are produced in increased amount have been implicated in the pathogenesis of many disease process such as osteoarthritis, hypertension, diabetes and cardiovascular disease [5,14]. In knee osteoarthritis, excessive production of superoxide radical is occur via activation of NADPH oxidase activity in neutrophils that leads not only the depletion of antioxidant enzymes but also amplify further deterioration by producing H_2O_2 , highly reactive hydroxyl radical, peroxynitrite anion and hypochlorous acid (HOCl) [15]. The mechanisms whereby these free radicals may exert cytotoxic effect related to cardiovascular disease in knee osteoarthritis include alteration in antioxidant defense system, damage to cell membrane via lipid peroxidation, endothelial dysfunction and electrolyte imbalance [16].

Disturbance in the antioxidant defense system of the body is characterized by reduction in total antioxidant activity (TAA), which could be due to decrease in individual antioxidants [7]. In the present study, plasma TAA levels decrease continuously with subsequent increase in disease complexity ($p < 0.05$, $p < 0.001$) in knee osteoarthritis patients which clarify the contributory effect of reduced antioxidant status due to augmented oxidative stress. Similarly, marked alteration in antioxidants and antioxidant enzymes are well documented in knee OA subjects with severity of disease [14,17].

Excess oxidative stress is well characterized by lipid

Table 1 : Anthropometry Indices along with Blood Pressure of Patient and Control Group (Mean \pm SD).

	(n=25)			
	Control Group	Group I	Group II	Group III
Age (years)	52.1 \pm 4.0	53.2 \pm 4.2	54.5 \pm 4.3	55.0 \pm 3.8
M:F ratio	11 : 14	13 : 12	10 : 15	10 : 15
Height (meter)	1.60 \pm 0.05	1.67 \pm 0.07	1.65 \pm 0.07	1.62 \pm 0.06
Weight (Kg)	60.2 \pm 4.0	70.7 \pm 6.2	77.0 \pm 7.0	75.4 \pm 6.2
BMI (Kg/m ²)	23.2 \pm 1.3	24.7 \pm 1.1*	27.4 \pm 1.0**	28.6 \pm 0.95**
Systolic blood pressure (mmHg)	107.6 \pm 3.0	111.5 \pm 3.5	114 \pm 3.4	115.7 \pm 2.7
Diastolic blood pressure (mmHg)	75.3 \pm 2.4	74.6 \pm 1.6	75.5 \pm 2.1	77.4 \pm 2.0
VAS pain (mm)	0.0	34.3 \pm 5.2	54.5 \pm 5.0**	72.3 \pm 7.1**

where,

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

Table 2 : Makers of oxidative stress and endothelial dysfunction in Patients and Control group subjects. (Mean \pm SD)

	Control group	Group I	Group II	Group III
TAA level (m mol/L)	1.14 \pm 0.15	95 \pm 0.13*	80 \pm 0.11**	0.72 \pm 0.09***
Malondialdehyde (μmol MDA/ml)	2.70 \pm 0.21	2.98 \pm 0.20*	3.82 \pm 0.23**	4.27 \pm 0.23***
NO level (μ mol/L)	8.35 \pm 2.21	7.12 \pm 1.50*	6.55 \pm 1.35**	5.92 \pm 1.43**

(n=25)

where,

- * p<0.1 = Non-significant
 ** p<0.05 = Significant;
 *** p<0.001 = Highly significant

peroxidation. Lipid peroxidation is a deleterious process leading to structural modification of complex lipid protein assemblies associated with cellular malfunction [18]. Prithviraj & Mishra reported that oxidation of LDL inhibits endothelial production of nitric oxide and prostacyclin, well known vasodilators and inhibitors of platelet aggregation; which reflects the role of lipid peroxidation in vascular disorder leading to hypertension [19]. In the present study, serum malondialdehyde levels, the most abundant reactive aldehyde derived from lipid peroxidation, were also found to be significantly high in grade II, III and IV knee OA patients along with subsequent marked reduction in plasma NO levels (Table 2) which authenticate the contention that disease complexity in knee OA patients is associated with the development of secondary complications, characterized by lipid peroxidation mediated destruction in cell membranes, ion transporters, subcellular organelles and endothelial dysfunction. However, in order to get concrete decision in finalizing the current concept for early intervention of cardiac complication in knee OA patients with severity of disease, there is a need to carry out these studies in a larger sample size.

Conclusion

Our findings indicate that alteration in plasma total antioxidant status along with oxidative stress (via MDA production) may be responsible for biomolecular deterioration and disturbance in homeostatic control leading to the etiopathogenesis of HT in knee OA, as characterized by reduction in the levels of plasma NO. Thus, assessment of plasma TAA and MDA along with NO levels with severity of knee OA may not only be an excellent marker of oxidative stress in knee OA but also important strategy for early interpretation of

cardiovascular complications and therapeutic interventions in knee OA. Therefore, treatment of grade IV knee OA should include adoption of antioxidant rich diet along with anti-inflammatory and analgesic drugs, preventive approach against traditional risk factors and regular monitoring of cardiac markers for early prediction and to reduce the burden of cardiac complication in knee OA patients.

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