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Oxidative stress mediated electrolyte imbalance in 30 known cases of knee osteoarthritis patients: A clinical approach

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ABSTRACT

Background: Osteoarthritis (OA), also known as degenerative arthritis, is the leading cause of chronic disability in elderly. Although, it is a process of progressive deterioration of articular cartilage with ageing which involves numerous risk factors, it is conceivable that oxidative stress mediated electrolyte imbalance may have a crucial role in the development of hypertension risk in OA elderly. **Aims & objective:** The present study was designed to assess the association of oxidative stress and altered serum electrolyte levels in OA elderly patients and to determine their effect in predicting hypertension (HT) risk. **Methods:** Total antioxidant activity (TAA), lipid peroxidation (malondialdehyde; MDA) and serum mineral (Na^+, K^+, Mg^{2+} and Ca^{2+}) levels were estimated in 40 OA elderly patients by using standard methods and statistically compared it with that of 40 healthy normal individuals of same age group (55-70 years). **Result:** Plasma TAA, serum potassium, magnesium and calcium levels were significantly low in patient group ($p<0.05$) as compared to healthy controls whereas erythrocyte MDA levels were significantly high in OA subjects. However, serum sodium levels were increased insignificantly ($p<0.1$) in OA subjects. **Conclusion:** Our findings indicate that oxidative stress plays a significant role in shaping the OA older population to develop future hypertension, characterized by altered serum minerals levels. Therefore, consumption of diet rich in antioxidants, proteins and minerals with low dietary salt should be increased with senescence in order to prevent disease complexity.

Key words: Elderly, Total antioxidant activity, Potassium, Magnesium, Malondialdehyde

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INTRODUCTION

Osteoarthritis (OA), the most common arthritic condition and the leading cause of chronic disability in the elderly, has been found to be associated with variety of common risk factors of hypertension such as age, sex, increase in body weight and smoking etc.[1,2] In this context, the role of oxidative stress has now been receiving much attention towards solving the conundrum of OA pathophysiology with future risk of hypertension (HT). Oxidative stress mediated by free radicals can evade or overwhelm the antioxidant protective mechanism of cells and may cause cell membrane and cartilage destruction, DNA strand breakage, rises in intracellular free Ca^{2+}, damage to membrane ion transporters and other specific proteins leading to cell death followed by disease development.[3,4]

Physiologically important elements responsible for electrolyte balance include sodium, potassium, magnesium and calcium, and their optimal concentration for proper biochemical and physiological activities of the cell is maintained by ion channels. Interestingly, Garg and Sanchette have well elucidated the role of various ion channels in cardiovascular disease (CVD), central nervous system (CNS), taste sensation, skeletal muscle, renal, respiratory, pancreatic, erectile and platelet function.[5] Inactivation of these ion channels leads to altered ionic homeostasis which may produce various sorts of age

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related disorders including cartilage degradation in knee OA patients and thereby making them not only physical and functional disable but also susceptible to develop secondary complications.

Free radicals attack on polyunsaturated fatty acid in the membrane lipids and thereby causing lipid peroxidation, a major event in the development of HT and OA as well.[3] Malondialdehyde (MDA), the most abundant product of lipid peroxidation, reacts with membrane proteins and ion channels, affecting their normal function and thereby may cause electrolyte imbalance.[1,4]

In order to overcome the load of free radicals, antioxidant defense system of the body (including enzymic and non-enzymic antioxidants) as determined by estimating total antioxidant activity (TAA), plays a dynamic role by regulating free radical production and thereby prevents the development of various sorts of disorders with advancing age such as hypertension and arthritis etc. Amusingly, alteration in antioxidant defense system in knee osteoarthritis patients and in older population has been well documented.[1,6] However to best of our knowledge; this is the first study addressing the relation of oxidative stress mediated electrolyte imbalance in knee OA elderly patients to predict future HT risk. Therefore, the overall objectives of present study were to ascertain the marker of oxidative stress and serum minerals (Na^+, K^+, Mg^{2+} & Ca^{2+}) levels in knee OA older patients and their role in prediction of HT risk.

MATERIAL AND METHODS

In the present study, 40 healthy subjects of both sex (20 males & 20 females) served as controls and 40 patients of knee OA aged 55-70 years attending the OPD, were included after taking their informed consent and approval of study by ethics committee of the institution. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination was completed from all the subjects. 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 informed consent for study, fulfilled American Rheumatism Association Clinical diagnostic criteria for knee OA and had radiographic evidences of narrowing joint space, osteophyte formation and subchondral sclerosis were included.[7] Patients were required to have pain on more than half the days of a month and atleast pain score above 20% using a 5 cm visual analogue scale (VAS).[8] Patients already receiving anti-inflammatory drugs were not excluded if the dosage and regularity of administration was not expected to alter during last three months.

Exclusion criteria

Patients with diabetes mellitus, hypertension (B.P. >120/80 mmHg, renal insufficiency, hepatic disease or any systematic disease other than knee osteoarthritis were excluded. Patients of mental stress induced disorders, obese (BMI >30), smokers, alcoholics and subjects under any other vitamin supplementation were excluded from the study.

Fasting blood samples were collected in plain vial (for serum minerals estimation) and in EDTA vial from anticubital veins avoiding venostasis from each patients and healthy controls. Samples were processed immediately for plasma and serum separation. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate.[9] The heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine coloured substance, which is measured spectrophotometrically at 532 nm.

Plasma total antioxidant activity was estimated spectrophotometricaly 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 thio barbituric acid reactive substances (TBARS). Antioxidants from the added plasma cause the suppression of TBARS production. The reaction was measured spectrophotometrically at 532 nm.[10]

Serum electrolytes (Na^+ and K^+) levels were measured by Sinha methodby using flame photometer in which test sample is aspirated followed by calculation of test sample value from calibration curve of standard solution (i.e. NaCl and KCl solution).[11] Serum magnesium levels were estimated by Neill and Neely method in which protein free filtrate is treated with titan yellow solution. A red color complex is formed which is measured at 520 nm.[12] Serum calcium levels were estimated by Tinder's method. Calcium in an alkaline medium combines with o-Cresolphthalein Complexone to form a purple coloured complex, which is measured at 570 nm.[13]

Statistical analysis

The data collected from patients and control were entered separately in Microsoft Excel sheet of windows 2010 and values were expressed as Mean ± SD. The significance of mean difference between patient and control groups was compared by using Student's t test.

RESULT

The demographic indexes along with clinical profile of knee OA elderly patients and controls are depicted in Table 1. All the OA patients fulfilling inclusion criteria have characteristic pain in knee and VAS score above 20%. Marked reduction ($p<0.05$) in plasma TAA levels were observed in patients group i.e. 19.5% low as compared to healthy controls. Conversely, erythrocyte MDA levels were increased significantly ($p<0.001$; 35.4% high) in knee OA subjects as compared to healthy controls, as represented in Graph 1. Similarly, serum K^+, Mg^{2+} and Ca^{2+} levels were decreased in OA subjects i.e. 16.4%, 23.5% & 20.7% low (Graph 2). Statistically, these levels were altered significantly ($p<0.05$) in OA subjects as compared to controls. However, serum sodium levels were increased insignificantly ($p<0.1$; 9.8% high) in OA subjects with respect to control, as represented in Graph 3.

DISCUSSION

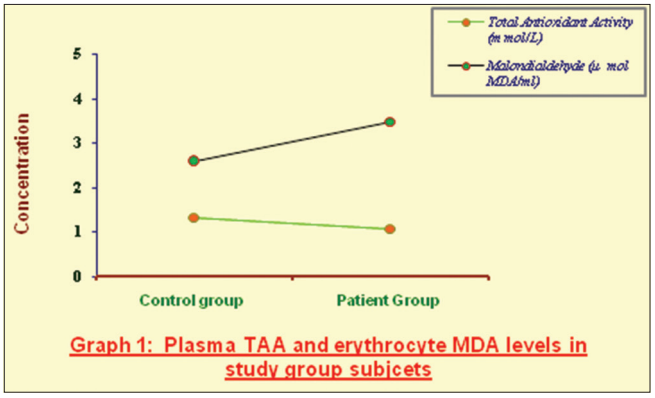
The association of OA progression with excessive body weight, impaired cardiovascular fitness and oxidative stress, have been well documented in previous studies.[1,6,14] In this context, depletion in total antioxidant activity (TAA) indicates the disturbance in the antioxidant defense system of the body, which could be responsible for the initiation of disease development with senescence. In the present study, plasma TAA levels were found to be significantly low ($p<0.05$) in OA subjects which could be explained on the basis of contributory effect of reduced enzymic and non-enzymic antioxidant levels, due to augmented oxidative stress in OA. Recently, marked reduction in TAA in knee OA as well as elderly hypertensive subjects has been documented, which were in concordance with our findings and clarify the common culprit effect of oxidative stress in both the diseases with senescence.[14,15]

Reduced total antioxidant activity therefore means increased production of H_2O_2 or incomplete scavenging of $O_2^{\cdot-}$ leading to further destruction related to HT risk in OA which include membrane damage via lipid peroxidation, ion transporters and electrolyte imbalance. In addition, deleterious role of lipid peroxidation is also implicated to structural modification of complex lipid protein assemblies associated with cellular malfunction.[4] Moreover, lipid peroxidation also contributes local membrane destabilization that alters endothelial or intimal cells architecture of the blood vessels, cell signaling, proper trafficking of intracellular vesicles, phagocytosis,

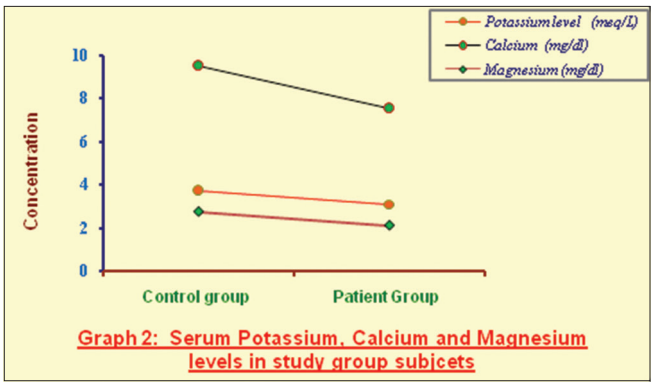
Table 1: Clinical profile of knee osteoarthritis elderly and healthy controls (mean±SD)

S No	Particulars	Control group (n=40)	Group I (n=40)
1)	Age (years)	61±5.1	63±5.3
2)	M:F ratio	1:1	1:1
3)	Height (meter)	1.62±0.03	1.60±0.027
4)	Weight (kg)	58.0±1.8	61.0±2.0
5)	B.M.I. (kg/m^2)	22.4±1.2	24.2±1.3*
6)	Systolic blood pressure (mmHg)	106.5±3.12	110.0±3.24
7)	Diastolic blood pressure (mmHg)	76.0±2.24	76.4±2.40
8)	VAS pain (mm)	0.0	± 4.4

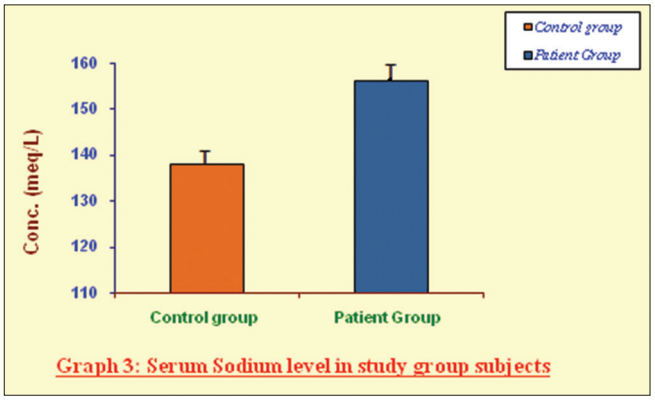
*P<0.1 : Non-significant



Graph 1: Plasma TAA and erythrocyte MDA levels in study group subjects



Graph 2: Serum potassium, calcium and magnesium levels in study group subjects



Graph 3: Serum sodium level in study group subjects

degranulation, antigen presentation and many other processes leading to disease complexity.[16]

Indeed, biologically important elements (Na^+, K^+, Mg^{2+} and Ca^2) have also significant role in maintenance of homeostasis by participating in various physiological activities such as neuromuscular irritability, nerve conduction, inhibition of free radical formation, in proper functioning of enzymes which include Na- K ATPase, Ca-ATPase, enzymes of carbohydrate and fatty acid metabolism; cell division, calcification of bones and teeth, in the synthesis of ATP, DNA, RNA and protein; inhibition of platelet aggregation, absorption of amino acids and in the prevention of development of age related complications.[17-20] Altered levels of these elements may induce series of events such as slow movement, postural abnormality, impaired balance, extensive membrane damage and peripheral vascular resistance i.e. characteristic symptoms of OA and HT as well.[1,21]

In the present study, malondialdehyde levels (marker of lipid peroxidation) were also found to be significantly high in OA subjects ($p<0.001$, Graph 2) in association with significantly altered levels of serum minerals (Na^+, K^+, Mg^{2+} & Ca^{2+}) which indicate that OA patients are not only closely associated with oxidative stress mediated electrolyte imbalance but also susceptible to develop future HT. Similarly, elevated levels of erythrocyte MDA in OA as well as in HT subjects also reported in the study of Singh et al.[3] According to them, lipid peroxidation initiates a complex cascade such as inhibition of NO, enhancement of cytosolic free calcium, electrolyte imbalance and leakage of lysosomal hydrolases via breakdown of lysosomal membrane which cause dystrophic changes in muscle fibers leading to weakness of muscles and difficulty in performing simple tasks. Similarly, Kim and Akera also reported that free radical mediated lipid peroxidation causes electrolyte imbalance not only by injuring Na^+-K^+ ATPase but also by interfering with normal interaction of membrane pumps (including Na–Ka–2Cl co-transporter and K^+ channel) and production of protein radical in lipid membranes that effects normal ion transport,[22] and thereby shaping OA elderly susceptible to develop HT.

CONCLUSION

Therefore, it is obvious from our findings that the oxidative stress plays a crucial role in shaping knee OA elderly more susceptible to develop HT, characterized by alteration in systemic antioxidant status, lipid peroxidation and electrolyte imbalance. However, to validate the findings of the current study, multicenter study with large sample size should be carried out. Furthermore, consumption of diet rich in antioxidant vitamins along with adequate mineral supplements should be increased with advancing age or appearance of disease symptom, which may be effective in the prevention and management of HT in knee osteoarthritis older population.

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Authors Contribution:
JD – Principal investigator, Formulation of study, Facilitate the sample collection, Correction of the write up; **DS –** Planning, Evaluation and Literature collection & Manuscript editing; **RS –** Biochemical analysis, Data collection and writing of manuscript, Statistical analysis, Literature collection.

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