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Research Article

Alteration in Plasma Paraoxonase Levels and its Relation with Coronary Artery Disease

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Abstract: The precise etiology and mechanism underlying the development of coronary artery disease (CAD) remain incompletely understood although a number of risk factors have been identified over the past several decades. In this context, an attempt has been made to estimate the extent of oxidative stress mediated lipid peroxidation, alteration in plasma paraoxonase and non-enzymic antioxidants level in co-ordination with conventional CAD risk factors and to determine the role of these antioxidants in CAD patients. Plasma paraoxonase, non-enzymic antioxidants level (ascorbate, tocopherol and uric acid), erythrocyte malondialdehyde (MDA) and serum lipid profile contents were estimated in 50 CAD subjects (30-60 years) and statistically compared it with that of 30 healthy individuals served as control by using student's t-test. Despite existence of conventional CAD risk factors, significant alteration in the levels of aforesaid parameters was observed. Plasma paraoxonase, ascorbate and tocopherol levels were significantly low in CAD subjects as compared to controls whereas plasma uric acid erythrocyte MDA levels were significantly high ($p<0.001$) in CAD subjects. These findings suggest that alteration in plasma antioxidants level, as a consequence of augmented oxidative stress plays an etiopathological role in the development of CAD along with other classic risk factors including dyslipidemia, hypertension and obesity. Thus, exogenous antioxidant supplementation, modification in nutritional behavior and life styles can offer the strong ground in the amelioration of CAD related risk and its consequent sequelae.
Keywords: Ascorbate, Tocopherol, Uric acid, Oxidative stress, Lipid peroxidation

INTRODUCTION

Coronary Artery Disease (CAD), a major cause of morbidity and mortality, has assumed an epidemic proportion today across the globe especially during the second half of the 20th century. It is predicted that in India by the year 2020, CAD would replace infectious disease as the major killer. Lipids and lipoproteins are important risk factors for CAD, however, they do not account for the disease in 30-40% of the population with CAD [1]. Other risk factors include hypertension, diabetes mellitus, drinking habit and obesity. In addition to above mentioned classic risk factors, several novel factors have been implicated as predictors of CAD namely left ventricular hypertrophy, oxidative stress, increased level of fibrinogen, triglycerides, lipoprotein a and homocysteine [2]. Among these, oxidative stress i.e. oxidative damage inflicted by reactive oxygen species (ROS), has been received much attention in connection with the development of CAD. Many physiological processes are known to result in the production of reactive oxygen species (ROS) e.g. enzymatic action (NADPH oxidase and Xanthine oxidase system), electron transport processes within the mitochondria and endoplasmic reticulum, arachidonic acid metabolism and the activation of phagocytic cells. ROS acts through several common mechanism to mediate destructive changes leading to CAD and hypertension including direct action on endothelial cells and vascular smooth cell resulting in structural and functional damage, scavenging of the important vasodilator nitric oxide (NO), production of peroxynitrite (a potent vasoconstrictor and lipid oxidizing radical), effects on endothelial cell eicosanoid metabolism and oxidative modification of LDL i.e. lipid peroxidation [3, 4].

These free radicals are efficiently removed by antioxidant defense system which includes antioxidant enzymes and non-enzymic antioxidants. Recently, paraoxonase (PON), a calcium-dependent A-esterase synthesized primarily in the liver and secreted into the serum as HDL-associated enzyme that prevents oxidation of low density lipoprotein (LDL) and responsible for anti-atherogenic property of high density lipoprotein (HDL), has received much attention as antioxidant enzyme in CAD patients [5].

Moreover, the most important biological antioxidants are ascorbate, tocopherol and uric acid etc play a crucial role in preventing oxidative stress. It was observed that when the radicals were formed initially in the aqueous region, vitamin C, an exogenous, water soluble antioxidant functions as the primary defense against free radicals in plasma disappeared more quickly while vitamin E mainly α-tocopherol, a major lipid soluble, chain breaking antioxidant, is a critical component of antioxidant defense system in all the body tissues. It quenches and reacts with superoxide anion, hydroxyl ($OH^{\bullet}$) and peroxyl radical to inhibit free radical chain reaction and to protect against oxidative stress mediated biomolecules and membrane damage via lipid peroxidation. Both the vitamins act synergistically and inhibit oxidation of LDL i.e. key step in the prevention of Atherosclerosis [6]. In previous experimental studies, vitamin C and vitamin E either alone or in combination with each other produces a significant reduction in age related complications including cardiovascular motrality [7, 8]. Similarly, there has been renewed debate about the nature of association between plasma uric acid and CAD. Ames et al. reported that uric acid is an effective antioxidant in plasma and scavenges superoxide radical, protects erythrocyte against peroxidative damage and free radical attack [9]. However, controversial studies reflect the need of further investigation [10, 11].

Although previous studies provided limited and conflicting literature about their role in CAD, the data regarding the relationship between these antioxidants and CAD risk factors are scanty. Therefore, the overall objectives of present study were to estimate the extent of oxidative stress implying lipid peroxidation, plasma paraoxonase, non-enzymic antioxidants (ascorbate, tocopherol and uric acid) level in co-ordination with conventional CAD risk factors and to determine the role of these antioxidants in CAD patients.

MATERIAL AND METHODS

In the present study, 50 subjects who were diagnosed clinically and angiographically as CAD patients (30-60 years) were recruited after taking their informed consent and approval of protocol by ethics committee of college. 30 age and sex matched normal healthy volunteers were taken as control. Patients who had suffered from first time CAD complication were recruited while the patients with diabetes mellitus, renal insufficiency, hepatic disease, taking lipid lowering drugs or antioxidant vitamin supplements were excluded. Height and weight were measured with the subject barefoot and light dressed. Obesity was expressed as body mass index (BMI) and values more than 25 being taken as cutoff point.

Venous blood was collected in EDTA vial between 7-10 am (fasting sample) from each subject after collecting the information of age, sex, height, weight, blood pressure, drinking habit and confirmation of disease state. Samples were processed immediately after collection. Plasma paraoxonase activity was estimated by Gan et al method using p-nitrophenyl acetate (5.5 mM/L) as a substrate [12. Plasma ascorbic acid level was estimated by plasma ascorbate by Mc Cormick and Greene method [13]. Plasma tocopherol level was measured by Hashim and Schuttringer method and uric acid by Caraway's method [14, 15]. Erythrocyte malondialdehyde level was estimated by thiobarbituric acid reaction after preparation of hemolysate [16]. Serum lipid profile contents were analysed enzymatically. LDL cholesterol and VLDL cholesterol level were calculated by Friedwald formula [17]. These levels were estimated spectrophotometrically. The data obtained from the patient and control groups were expressed as Mean ± SD and compared by using Student's t-test.

RESULTS

The prevalence of CAD risk factors and anthropometric indices of the study group subjects are depicted in Table 1 and 2 respectively. The mean ± SD age of the patients and controls were 45.5 ± 15.7 and 44.2 ± 14.6. Most of the patients were belonged to age group 46-60 years. Amongst 50 CAD subjects, 78.6% were males and 21.33% were females. Based on BMI criteria of NIN, Hyderabad, obesity was present in 56% CAD subjects. Similarly, based on Hypertension criteria of "JNC 7^{th} report on High Blood Pressure", hypertensive CAD subjects were 48% and their mean systolic and diastolic blood pressure were 132 ± 22.0 and 88 ± 7.0 respectively. Serum lipid profile of both patient and control group subjects are represented in Table 3.0. In CAD subjects, total cholesterol ($p<0.05$, 35.72 %), triglycerides ($p<0.05$, 38.48%) LDL-cholesterol level ($p<0.001$, 56.96%) and VLDL cholesterol ($p<0.05$, 39.7%) were significantly increased whereas HDL cholesterol level ($p<0.05$, 28.02%) were significantly reduced in patient group as compared to controls.

The observation made reveal significant changes in plasma paraoxonase and non-enzymic antioxidant status in all the study groups. Alteration in plasma antioxidant status and marker of lipid peroxidation i.e. erythrocyte MDA levels are represented in Table 3. Plasma PON, ascorbate and tocopherol levels in CAD subjects were 167.5 ± 14.2, 0.29 ± 0.06 and 0.47±0.18 respectively which revealed a significant reduction of plasma antioxidant enzyme and antioxidant vitamin status in CAD subjects as compared to controls and reflect the association of CAD risk factors. On the other hand, plasma uric acid and MDA level were significantly high i.e. 7.36±1.34 and 2.92 ± 0.16 in CAD subjects (Table 3).

Table 1: Prevalence of major risk factors in CAD patients

Sl. No.	Risk Factor		CAD patients (%) (n = 50)
1.	Age (years)	30 – 45 46 – 60	08 (16 %) 42 (84 %)
2.	Sex	Men Women	39 (78 %) 11 (22 %)
3.	Body Mass Index (kg/m^2)	Non obese (B.MI ≤ 25) Obese (B.MI ≥ 25)	22 (44 %) 28 (56%)
4.	CAD Family History	Positive Negative	13 (26 %) 37 (74 %)
5.	Hypertensive Normotensive		24 (48%) 26 (52%)
6.	Drinking habit	Alcoholic Non-alcoholic	23 (46 %) 27 (54 %)

Table 2: Age and Anthropometry of Patients and Control group (Mean ± SD)

S.No.	Particulars	Control group (n = 30)	CAD patients (n = 50)	Level of significance
1.	Age (years)	44.2 ± 14.6	45.5 ± 15.7	p< 0.1
2.	Systolic Blood Pressure (mm Hg)	112 ± 8.0	132 ± 22.0	p < 0.001
3.	Diastolic Blood Pressure (mm Hg)	76 ± 9.0	88 ± 7.0	p < 0.05
4.	Height (meter)	1.59 ± 0.08	1.62± 0.07	p< 0.1
5.	Weight (kg)	59.2 ± 9.83	72.17 ± 12.5	p < 0.01
6.	Body Mass Index (Kg/m^2)	23.30 ± 2.83	28.33 ± 5.7	p< 0.05

where, p < 0.001 : highly significant; p < 0.05: Significant; p < 0.1: Non- significant

Table 3: Serum Lipid profile, plasma non-enzymic antioxidants and MDA level in CAD patients and Control group (Mean ± SD)

S.No.	Particulars	Control group (n=30)	CAD patients (n=50)	Level of significance	% Increase	% Decrease
1.	Total Cholesterol (mg/dl)	159.39 ± 16.19	216.34 ± 14.2	p < 0.05	35.72	-
2.	Triglycerides (mg/dl)	101.56 ± 10.78	140.65 ± 8.26	p < 0.05	38.48	-
3.	HDL - Cholesterol (mg/dl)	48.54 ± 2.86	34.94 ± 2.95	p < 0.05	-	28.02
4.	LDL - Cholesterol (mg/dl)	93.38 ± 14.20	146.57 ± 13.2	p < 0.001	56.96	-
5.	VLDL - Cholesterol (mg/dl)	20.18 ± 2.50	28.20 ± 1.62	p < 0.05	39.47	-
6.	Paraoxonase (U/ ml)	234.3 ± 15.5	167.5 ± 14.2	p < 0.05	-	28.5
7.	Ascorbic acid (mg/dl)	0.77 ± 0.13	0.29 ± 0.06	P < 0.001	-	62.27
8.	Tocopherol (mg/dl)	1.41 ± 0.54	0.47 ± 0.18	P < 0.001	-	67.14
9.	Uric acid (mg/dl)	4.61 ± 1.28	7.36 ± 1.34	P < 0.001	59.65	-
10.	Malondialdehyde (µ mol/gm Hb)	1.78± 0.103	2.92 ± 0.162	P < 0.001	64.2	-

DISCUSSION

Prevention is the key to progress in the control of coronary artery disease (CAD). Although a number of risk factors have been identified over the past several decades, the precise aetiology and mechanism leading to the development of CAD remains incompletely understood. Recognition of these epidemic diseases early in their evolution makes possible prompt intervention to slow or arrest the processes or even reverse them. In this context, the percentage prevalence of CAD risk factors and anthropometric indices are reported in Table 1 and 2, which authenticate the fact that age, sex and CAD family history are non-modifiable risk factors. CAD can develop in either of the sex but more prominently in males, in middle age group subjects and those with CAD family history background. In addition, obesity, dyslipidemia, hypertension and drinking habit are modifiable risk factors of CAD. These findings were in concordance with the findings of Gupta et al who have reported that role of increasing mortality from CAD can be reversed by reducing these modifying risk factors [18]. Table 3 indicates that increased levels of all the lipid profile contents except HDL cholesterol level increase the risk of atherosclerosis, and its consequent sequelae. Similar findings have been documented by Sinha and Sharma and showed the importance of estimation of lipid profile contents and serum minerals level in case of CAD subjects [19].

In addition to above mentioned risk factors, several lines of evidence indicate that the development of CAD is related to free radical processes [3, 5]. It has been suggested that oxidation of LDL particles in the arterial wall initiates a complex cascade of events that leads not only to the development of atherosclerotic plaques followed by myocardial infarction but also the development of hypertension by inhibiting the synthesis of NO and prostacyclin and by increasing the cytosolic free calcium and peripheral vascular resistance [20, 21]. In the present study, MDA level i.e. marker of lipid peroxidation, was found to be significantly increased ($P<0.001$, 64.2% high) in CAD patients which clarify the etiopathogenic role of free radical mediated oxidative stress in CAD patients. Our finding was quite similar to the findings of Kharb [22].

Interestingly, ROS mediated lipid peroxidation is well controlled by antioxidant enzymes including paroxonase which is found to be associated with HDL and imparts anti-atherogenic activity. It provides protection against CAD by hydrolyzing specific oxidized phospholipids and by neutralizing hydrogen peroxide [5]. In the present study, plasma PON activity was found to be low in patients group suggesting that PON had a significant effect in prevention of CAD development possibly decreasing the production of reactive aldehydes. Consistent findings have been reported in previous studies on obese females as well as in elderly indicating that individuals with low PON activity are at great risk to develop cardiovascular complications [23, 24].

It is generally recognized that vitamin E can scavenge the ROS present in lipid phase whereas vitamin C and uric acid act in aqueous phase. Oxidation of LDL in vessel wall plays an important role in increase of blood pressure followed by development of atherosclerosis. Heitzer et al. observed that vitamin C alone can afford protection against the oxidant mediated damage to LDL [25]. The additional possible mechanisms through which ascorbate may reduce the risk of CAD are the promotion of endothelial prostacyclin which reduces platelet aggregation, inhibition of free radical mediated inactivation and destruction of Nitric oxide (a well known vasodilator produced by NO synthase activity) and the maintenance of electrolyte balance by protecting the ion channels from peroxidative damage of biomembranes [26]. In the present study, a significantly reduced level of plasma ascorbate was observed ($P<0.001$, 62.27% low) in CAD subjects as compared to healthy controls and statistically, it was inversely associated with CAD risk factors except HDL. Our findings were in agreement with the findings of Yashioka et al. who reported that reduction in vitamin C level is directly associated with increased risk of CAD and suggested that CAD modifiable risk factor can be modified by elevating the plasma level of antioxidants [27]. In addition, Hersey et al. also observed a positive correlation between ascorbic acid and HDL cholesterol in myocardial infarction subjects [28]. Ascorbic acid also acts as a synergist to regenerate α-tocopherol by reducing the α-tocopheroxyl radical formed from reaction of α-tocopherol with free radical [6].

Vitamin E, a universal lipophilic, chain breaking antioxidant and a stabilizer of biological membranes, prevents accumulation of free radicals and decreases lipid peroxidation. Prithviraj and Misra have reported that α-tocopherol not retards only LDL oxidation but inhibits smooth muscle proliferation, platelets adhesion and aggregation, expression and function of adhesion molecule, decreases synthesis of leukotrienes and potentiates the release of prostacyclin through upregulation of cytosolic phospholipase A_2 and cycloxygenase [4]. In the present study, plasma tocopherol level was ($P<0.001$, 67.14% low) significantly low in CAD patients as compared to healthy controls and statistically it was inversely associated with CAD risk factors except HDL cholesterol. Our findings were quite similar to the findings of Keith *et al*. [29]. Decreased level of vitamin E was not only due to its free radical scavenging action but also in maintaining the body antioxidant reserve and normalization of vascular superoxide formation which prevent endothelial dysfunction. Although several studies have suggested a modest benefit of vitamin C and vitamin E intake others have reported no

relationship between vitamin C or vitamin E intake and CAD [8, 10].

Besides these, uric acid is one of the major endogenous, preventive and chain breaking antioxidant in human plasma which contributes about 65% of free radical scavenging action, stabilizes ascorbate, protects erythrocytes from peroxidative damage, inhibits free radical damage to DNA and oxidative degradation of hyaluronic acid [9]. Reduction of NO bioavailability due to free radical mediated endothelial dysfunction appears to have important role in the development and progression of CAD. Plasma uric acid interacts with peroxynitrite to form a stable NO donor, thus promoting vasodilation and reducing the potential for peroxynitrite induced oxidative damage [30]. In the present study, plasma uric acid level was found to be significantly high ($p<0.001$) in CAD subjects and positively associated with CAD risk factors which reflect that body is trying to protect itself from the deleterious effects of free radicals by increasing uric acid production. Pahwa and Seth also observed hyperuricemia in association with low ascorbic acid level in CAD subjects [31]. Despite data linking antioxidant role of uric acid in CAD patients, its controversial role in promoting LDL oxidation in-vitro, in stimulating granulocyte adherence to the endothelium and its association with increased blood pressure, circulating inflammatory markers and vascular injury have been well documented, which authenticates the fact that uric acid has a dual action in cardiovascular system [10, 32].

CONCLUSION

On the basis of prevalence of risk factors of CAD in middle aged subjects and the consistent findings of previous studies, it is obvious that a combination of increased physical activity, life style modification, and adoption of diet rich in fruit, vegetables and low fat dairy products can prevent the development of CAD. Considering the altered plasma paraoxonase level, non-enzymic antioxidant status, elevated MDA level and their relation with CAD risk factors, it is also evident that oxidative stress plays a significant role in the etiopathogenesis of CAD in association with conventional CAD risk factors which can be modified by maintaining high level of vitamin C and E (i.e. marker of oxidative stress) and adoption of prevention strategy.

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