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THE RELATIONSHIP BETWEEN OXIDATIVE STRESS AND THE ONSET OF CORONARY ARTERY DISEASE

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ABSTRACT

Oxidation of low density lipoprotein (LDL) particles plays a key role in the etiology of atherosclerosis and coronary artery disease (CAD). Oxidative stress enhances the likelihood of LDL oxidation and atherosclerotic plaque development. Paraoxonase (PON1) is an enzyme associated with HDL that metabolizes organophosphates and has antioxidant activity.

In order to investigate the relationship between oxidative stress and the onset of coronary artery disease (CAD), total ferric reducing antioxidant power (FRAP)-as an index of antioxidant capacity of plasma- and the activity of PON 1 were measured in 80 patients over 65 and 80 patients less than 55 years old as late and early-onset CAD groups respectively. Plasma lipids were also determined.

Patients with early-onset CAD had significantly lower serum levels of HDL-C ($p<0.05$) and higher LDL-C/HDL-C ($p<0.01$) than the late-onset group. This may imply the significance of HDL at the onset of CAD. There was no difference in serum levels of TG, TC, LDL-C, PON 1 activity and FRAP values between the two groups. The FRAP value was significantly lower than the reference range for healthy subjects in our laboratory. Although the FRAP value is lower in normal elderly people compared to the younger subjects, there was no difference between the two groups. This indicates that in young CAD patients, oxidative stress may be more important than in the elderly subjects and should be monitored in conjunction with routine lipid measurements.

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INTRODUCTION

Oxidative stress occurs when there is an imbalance between free radical production and antioxidant capacity. This may be due to increased free radical formation in the body and/or loss of normal antioxidant defences. It is well known that oxidative stress is involved in the pathogenesis and development of many human diseases, such as Alzheimer, atherosclerosis and CAD. It has been shown that elevated levels of some free radicals in plasma are associated with the extent and the severity of CAD and with the occurrence of different atherogenic risk factors.[1] Most clinical events in humans with CAD are precipitated by the rupture of plaque in

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intermediate and advanced atherosclerotic lesions. The critical early step in the formation of the atherosclerotic plaque is the oxidation of the LDL particle. This oxidation triggers a number of events that enhance the likelihood of atherosclerotic plaque development. For example, oxidized LDL particles are marked for uptake by macrophages leading to foam cell formation. In fact the oxidized LDL is chemotactic toward monocytes and inhibits macrophage exodus from the artery, induces endothelial cell damage, and stimulates cytokine and growth factor release from cells in the artery wall.[2] The oxidative modification of LDL is clearly inhibited by HDL possibly via paraoxonase.[3] This enzyme that is associated with HDL, can break down lipid peroxides thus preventing their accumulation in LDL and hence may be antiatherogenic.[4] PON has different polymorphic isoenzymes and it has been proposed that clinically PON 1 activity is low when cardiovascular risk is high (diabetes mellitus, chronic renal failure, alphalipoproteinemia) and its activity is reversely associated with premature CAD in many, but not all studies.[5] However, serum PON 1 activity shows wide variations within or among populations of different ethnic backgrounds[6] and its regulation is complex, involving both genetic and environmental factors. It is influenced by gender and inflammation, two important factors in atherosclerosis.[7] CAD prevalence is increased in advance ages. It has been proposed that a decrease of this enzyme activity with ageing could play a part in the high prevalence of cardiovascular diseases in the aged people.[7]

To assess the total antioxidant capacity of human serum or plasma as an index of oxidative stress, several methods have been developed.[8] The development of these different methods is because of the difficulty in measuring each antioxidant component separately and the interactions among different antioxidant components in the serum or plasma. The FRAP assay measures the ferric-to-ferrous iron reduction in the presence of antioxidants and is very simple and convenient in terms of its operation.[9] A significant decrease in FRAP values as an index of total antioxidant capacity was observed in elderly subjects. However, the results of studies investigating oxidative stress in ageing are still controversial and conflicting results on the antioxidant system are also reported in ageing.[10]

Given the association between the oxidative stress with CAD,[1] the association between ageing and increase of CAD and the association between ageing and increase of oxidative stress,[10] we hypothesized that oxidative stress may be a more important risk factor for premature CAD. Accordingly, we hypothesized that patients presenting with early onset CAD would have decreased antioxidant capacity and PON 1 activity compared to those presenting with CAD later in life. To investigate the relationship between oxidative stress and onset age of CAD, we determined plasma antioxidant status, PON 1 activity and lipid profile in early onset and late onset CAD patients.

MATERIAL AND METHODS

Study subjects

Four-hundred and eighty Iranian patients were recruited from the Tehran Heart Center and Amiraalam Hospital of Tehran University of Medical Sciences and Imam Hossein Hospital of Shahid Beheshti University of Medical Sciences. All had ischemic heart disease defined as either a history of MI or angina pectoris with CAD confirmed by angiography demonstrating one or more epicardial coronary vessels with greater than 50% diameter stenosis and were on optimal medical therapy. Patients with familial hypercholesterolemia, diabetes mellitus, kidney, thyroid or liver disease were excluded. Eligible patients were sequentially recruited into two groups: early-onset CAD (n = 80) were those presenting with CAD under the age of 55 years (mean age = 44.9 ± 4.9 yr) and late-onset CAD (n = 80) were patients presenting with CAD for the first time over the age of 65 years (mean age = 71.7 ± 2.5yr). The study was conducted following the approval of our institutional re-

Table I. Fasting serum concentration of lipids and lipoproteins in patients with early- and late-onset CAD.

Variable	Early-onset	Late-onset
Age (years)	44.8±5	71.7±2.5
Total Cholesterol (mg/dL)	230 ± 60	219 ± 47
Triglyceride (mg/dL)	232 ± 104	218 ± 77
HDL-C (mg/dL)	36 ± 14.8	40 ± 10.6
LDL-C (mg/dL)	145 ± 60	144 ± 48.7
LDL-C /HDL-C	5.1 ± 4.1	3.8 ± 1.8

Table II. The FRAP and PON activity in patients with early- and late-onset CAD.

Variable	Early-onset	Late-onset
Paraoxonase (IU/mL)	402 ± 262	430 ± 205
FRAP (μmol/lit)	991 ± 347	948 ±289

view board, and informed consent was obtained from all subjects before inclusion in the study.

Biochemical analysis

Lipid and lipoprotein assays: A blood specimen was collected after overnight fasting for 12 h. Serum total cholesterol (TC) and triglyceride (TG) levels were measured by commercially available enzymatic reagents. HDL cholesterol (HDL-C) was measured after precipitation with phosphotungstic acid and LDL cholesterol (LDL-C) was calculated using Friedwald's[11] formula for serum samples with TG values less than 400 mg/dL. In patients with a TG value greater than 400 mg/dL, LDL-C levels were not calculated. Standard control serum was employed to ensure the accuracy of the measurements throughout the study.

FRAP assay: Plasma antioxidant status was evaluated using FRAP assay.[9] The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method. In this assay, at low pH a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex reduced to the ferrous form, which is blue color and can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is directly proportional to the reducing power of the electron-donating antioxidants present in the plasma. The absorbance change is translated into a FRAP value (in μmol/lit) by relating the change of absorbance at 593 nm of test sample to that of a standard solution of known FRAP value.

Paraoxonase activity assay: PON 1 activity was measured by adding 15 μL serum to 285 μL Tris–HCl buffer (100 mM, pH 8.0) containing 1 mM $CaCl_2$ and 1 mM Paraoxon (D9286 Sigma Chemical Company). The generation of p-nitrophenol was measured at 405 nm by spectrophotometer.[7]

Statistical analysis

Data are expressed as mean ± SD for continuous variables and as counts and percentage for discrete variables. Statistical analyses were conducted with a commercially available software package (SPSS version 10.0; SPSS Inc, Chicago, Illinois). Statistical comparisons were analyzed by using Student's t-test. A value of $p<0.05$ was considered statistically significant.

RESULTS

In this study, the first 160 subjects (120 men, 40 women) that met the inclusion criteria were examined. Eighty patients were less than 55 years old (44.9±4.9) as the early-onset CAD group and 80 patients were over 65 years old (71.7±2.5 ys) as the late-onset CAD group. There were 66 men and 14 women in the early-onset and 54 men and 26 women in the late-onset CAD group. Patients with early-onset CAD had significantly lower serum levels of HDL-C ($p<0.05$) and higher LDL-C/HDL-C ratio ($p<0.01$) than the late-onset group. There was no difference in serum levels of TG, TC, and LDL-C between the two groups (Table I). There was no significant difference in serum levels of FRAP between the two groups. Although PON 1 activity was lower in the early-onset group with respect to the late-onset but the difference was not significant (Table II). We found that in both groups, the FRAP value is significantly lower than the reference range of healthy subjects (1540 ± 500 μmol/lit) in our laboratory.[12]

DISCUSSION

Over the past decade, emerging data have demonstrated that cardiovascular risk factors and oxidative stress play a crucial role in abnormal vasomotor responses. These abnormal responses are probably one of the first events in the atherosclerosis process and have been shown to be an important cause of ischemia in patients with established atherosclerosis. While the use of antioxidants to prevent CAD has not been satisfactorily demonstrated, some studies have recently argued persuasively in favour of continued research, citing a number of limitations and unanswered questions related to earlier attempts to evaluate the oxidation hypothesis in clinical trials.[13] To investigate the relationship between the oxidative stress and onset age of CAD,

we determined and compared the total plasma antioxidant status and PON 1 activity in 80 patient less than 55 years old (early-onset CAD group, mean age= 44.9±4.9 ys) and 80 patients over 65 years old (late-onset CAD group, mean age=71.7±2.5 ys). We also determined total triglyceride (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and LDL-C/HDL-C ratio in these two groups.

In the early-onset group there were only 17.5% women compared to the 32.5% in late-onset. The lower probability of early-onset CAD in young women compared to men has been attributed to the protective effect of estrogen in young (premenopausal) women. Although there was no difference in serum levels of TG, TC and LDL-C between early and late-onset CAD groups, patients with early-onset CAD had significantly lower serum levels of HDL-C ($p<0.05$) and higher LDL-C/HDL-C ratio ($p<0.01$) than the late-onset group. This may imply the importance of HDL compared to LDL in the onset of CAD. In other words, although both groups were on cholesterol lowering medication (optimal medical therapy), either the late onset group responded better to the treatment or they had a lower HDL level prior to starting the medication. The FRAP value is lower in normal elderly people compared to younger subjects[5] but in our study there was no difference between the two groups. This may indicate that in young CAD patients, the oxidative stress may be more important than in the elderly subjects and the evaluation of oxidative stress indices should be monitored in conjunction with routine lipid measurements.[14] We found that in both groups, the FRAP value is significantly lower than the reference range of healthy subjects (1540 ± 500 µmol/lit) in our laboratory. We are uncertain as to whether this may be due to the effect of drug treatment itself or whether patients had lower FRAP levels prior to initiating drug treatment.

In our study PON 1 activity was lower in the early-onset group with respect to the late-onset group but the difference was not significant. Although some studies reported that low PON 1 levels have been associated with CAD,[15] but Rahmani[16] and Azizi[17] reported no significant difference between PON 1 activity in a CAD group compared to healthy control group in Iranian subjects. In our study the relatively lower activity of PON 1 in early-onset CAD may be because of lower level of HDL in these patients and probably by improving HDL level the activity of this enzyme and hence the antioxidant defence may increase. We are not sure whether the differences in the indices of oxidative stress observed in different studies represent a temporal adaptation to the medication or not and it remains to be evaluated.

In conclusion, while the results of studies investigating oxidative stress in aging and some diseases are still controversial and conflicting, our results indicate the importance of HDL and perhaps its antioxidant enzyme PON 1 in young CAD patients. Our study also supports the hypothesis that the evaluation of oxidative stress may represent an additional prognostic predictor in such events and a potential target of future therapeutic interventions.

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