Relationship between Prooxidant-Antioxidant Balance and Severity of Coronary Artery Disease in Patients of Imam Khomeini Hospital of Tehran, Iran

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Received: 1 Dec. 2012; Accepted: 29 Apr. 2013

Abstract- The balance between reactive oxygen species production and antioxidant activity has an important role in oxidative stress associated diseases including coronary artery disease. In this study, the prooxidant–antioxidant balance (PAB) and its correlations with serum lipid levels, uric acid levels, and severity of coronary artery involvement were examined. The aim of this study was to determine the diagnostic value of PAB as a predictor in coronary artery disease (CAD). Seventy two patients and 68 healthy subjects were selected. PAB was determined using standard solutions and ELISA. Triglyceride, total cholesterol, LDL- cholesterol, HDL-cholesterol and uric acid levels were measured by enzymatic method. Mean PAB was 66.4 ± 2.84 (HK units) in healthy people, 77.37 ± 33.51 (HK units) in patients with one vessel CAD, 63.76 ± 29.47 (HK units) in patients with two vessel CAD and 68.59 ± 24.51 (HK units) in patients with three or more vessel CAD. There was no significant difference between PAB values in different severity groups (P=0.41). PAB significantly and indirectly correlated with uric acid level in two vessels CAD. The study shows that PAB can be a predictor of CAD associated with other risk factors, but not alone.

Acta Medica Iranica, 2014;52(2):116-121.

Keywords: Coronary artery disease; Lipid prooxidation; Antioxidant; Oxidative stress

Introduction

In humans, there is, a balance between the production and removal and of reactive oxygen species (ROS), as prooxidants (Prooxidants, are chemical complexes that induce oxidative stress and perform this action either by production of ROS or by inhibiting antioxidant systems). Prooxidants are firmed either from the metabolic processes or from external sources (1,2). Antioxidants are responsible for their removal, before biological molecules are damaged (3). Oxidative stress is the result of an imbalance between the production of prooxidants and antioxidant defenses in favor of prooxidants (4-7).

Cardiovascular diseases (CVD) including coronary artery disease (CAD), hypertension, congenital heart failure and infarction are considered major causes of mortality around world (8). Major and independent risk factors for CVD include smoking, hypertension, elevated total cholesterol and LDL, low HDL level, diabetes mellitus and age (9).

However, these factors are not the only risk factors for development of CVD. So it seems that other factors may play roles in the progression of atherosclerosis (10).

Based on previous findings oxidative stress and inflammation have been considered as the major risk factors for cardiovascular diseases (11-15).

ROS can lead to proatherogenic events, such as LDL oxidation, endothelial dysfunction and proliferation and migration of vascular smooth muscle cells. Thus, oxidative stress is sometimes the main mechanism of action for CVD risk factors. Antioxidants that are effective against ROS can play a major role in limiting atherosclerosis and clinical problems such as myocardial infarction. Clinical and experimental studies show that antioxidant supplementation can be an effective measure in preventing and treating diseases (16).

In this study, the prooxidant–antioxidant balance (PAB) and its correlations with serum lipid levels, uric acid levels, and severity of coronary artery involvement were examined.

The aim of this study was to evaluate PAB and its relationship with uric acid, as a natural antioxidant, and number of vessels involved in CAD patients.

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Materials and Methods

In this cross-sectional study, we enrolled 140 subjects who presented at the Cardiac Clinic of Imam Khomeini Hospital (Tehran, Iran) with signs of myocardial ischemia and were suspected for coronary artery disease. Of these individuals, 72 were patients (43 males and 29 females), and 68 were healthy subjects (39 males and 29 females). Selection of patients was based on diagnostic angiography and their interpretation by a cardiologist. Patients were aged 55-75 years. Fasting blood samples were taken and serum was separated in room temperature. Aliquots were stored at -20°C. For measuring PAB, we used a method previously developed by Alamdari *et al.* (7). Standard solutions were prepared by mixing varying proportions (0-100%) of 1 mM hydrogen peroxide with 6 mM uric acid (in 10 mM NaOH).

The following standard solutions were prepared separately: vitamin C (0-800 μM), Trolox (0-800 μM), uric acid (0-6 mM in 10 mM NaOH), glutathione (0-2500 μM), albumin [0-1000 mM (68 g/l)], hydrogen peroxide (0-1000 μM) and tert-butylhydroperoxide (0-1000 μM). TMB (5,5',3,3'-tetra methylbenzidine, 2 HCl) solution was prepared by dissolving one TMB tablet in 10 ml of substrate buffer (0.05 mM phosphate citrate buffer, pH 5). Eighteen μl of fresh chloramine T (10 mM) solution was added to 1 ml of TMB solution for preparation of TMB cation, incubated for 20 min and 1.25 U of peroxidase enzyme solution was added to 9 ml of TMB solution. The working solution was prepared by mixing the two latter solutions. Ten μl of each sample, standard or blank (distilled water) was mixed with 200 μl of working solution, in each well of a 96-well plate, which was incubated in a dark place for 12 min at room temperature; at the end of the incubation time, 100 μl of HCl (2 N) was added to each well. The plate was then incubated for 45 min in a dark place and measured in an ELISA reader at 450 nm with a reference wave length of 620 or 570 nm. PAB readings are expressed in HK units, which reflect the percentage of hydrogen peroxide in the standard solution multiplied by 6. The PAB values of samples were then calculated based on the standard curve (7).

Determination of uric acid was performed by a colorimetric method in alkaline medium. Uric acid in alkaline medium was oxidized by phosphotungstic acid and converted to allantoin and CO_2. In this reaction, phosphotungstic acid was reduced to a blue tungsten compound.

Determination of cholesterol and triglyceride were performed by routine colorimetric methods. Determination of HDL-cholesterol was performed by a precipitative enzymatic method.

Determination of LDL-cholesterol was performed while it was protected from enzymatic reaction, using supporting agents.

Levels of VLDL-cholesterol were calculated using the equation by Friedwald (1972).

Statistical analysis was done with SPSS version 17. To study the relationships between quantitative variables, Pearson correlations were determined. ANOVA and t-test to were used to compare means. *P*-values < 0.05 were considered as significant results.

Result

Of the 140 individuals studied, 68 were healthy subjects (48.6%), 72 patients that were 21 subjects (15%) had single vessel CAD, 17 subjects (12.1%) had two vessels CAD and 34 subjects (24.3%) were diagnosed with three or more vessel CAD.

The mean age in group of healthy subjects was 60.93±11.51 years. The mean age of the patients was 65.26 years. 60.62±10.62, 67.71±9.67 and 66.91±8.96 years in the single, two and three or more vessel CAD groups, respectively. Mean PAB was 66.4±23.46 HK units in healthy participants; among patients, it was 77.37±33.51 HK units in the single vessel CAD, 63.76±29.47 HK units in those with two vessel involvement and 68.59±24.51 HK units in the third groups of patients.

Lipid profile, uric acid and PAB value in healthy subjects group and patients group in according to severity of coronary involvement were analyzed with ANOVA. To investigate the relationship between severity of coronary involvement with parameters Pearson correlation test was used. Analysis for determination the relationship between quantitative variables was done by regression analysis.

There was a significant inverse correlation between uric acid levels and number of blocked vessel only in two vessel group (r=0.747, *P*=0.001) (Table 1).

The correlation between cholesterol and triglyceride values with number of coronary vessel involvement was not significant (Table 1).

Correlations between the number of blocked vessels and other parameters are also shown in table 1.

Studying PAB value relationship with other variables showed negative significant correlations with uric acid, triglyceride, cholesterol: HDL ratio and LDL: HDL ratio (Table 2).

Table 1. Demographic and clinical characteristics of CAD positive and negative

	Case			Control		
		r	***P***		**r**	***P***
BMI (kg/m^2)	26.46 ± 3.60	-0.17	0.884	26.39 ± 4.66	0.055	0.657
Age (year)	60.93 ± 11.51	-0.008	0.946	65.26 ± 9.96	0.149	0.226
Uric Acid (mg/dl)	6.24 ± 2.15	-0.399	0.001*	6.50 ± 2.04	-0.305	0.11*
Total cholesterol (mg/dl)	186.81 ± 45.37	-0.189	0.113	180.86 ± 43.88	-0.127	0.301
Triglycerides (mg/dl)	178.22 ± 101.46	-0.254	0.031*	159.58 ± 99.90	-0.1	0.418
HDL- cholesterol (mg/dl)	40.57 ± 10.82	0.196	0.099	40.92 ± 13.23	0.1	0.416
Cholesterol /HDL	4.89 ± 1.70	-0.245	0.038*	4.74 ± 1.67	-0.174	0.157
VLDL- cholesterol (mg/dl)	34.57 ± 20.45	-0.222	0.063	30.13 ± 16.39	-0.062	0.614
LDL- cholesterol (mg/dl)	90.75 ± 24.63	-0.161	0.178	88.22 ± 25.79	-0.079	0.521
LDL/HDL	2.41 ± 0.98	-0.251	0.033*	2.33 ± 0.9	-0.155	0.207

Table 2. Demographic and clinical characteristics of coronary artery disease accordingly vessels involvement

	Single Vessel Disease			2-Vessel Disease			3-Vessel Disease		
		r	***P***		**r**	***P***		**r**	***P***
BMI (kg/m^2)	27.33 ± 5.31	-0.377	0.092	25.35 ± 3.46	0.250	0.333	26.34 ± 4.76	0.060	0.734
Age (year)	60.62 ± 10.62	0.170	0.461	67.71 ± 9.67	-0.195	0.453	66.91 ± 8.96	0.047	0.791
Uric Acid (mg/dl)	6.36 ± 2.01	-0.338	0.174	6.26 ± 2.52	-0.747	0.001*	6.70 ± 1.84	-0.191	0.278
Total cholesterol (mg/dl)	177.38 ± 42.37	-0.072	0.756	195.88 ± 33.21	-0.336	0.188	175.50 ± 48.65	-0.212	0.229
Triglycerides (mg/dl)	125.95 ± 55.01	-0.229	0.319	166.76 ± 614.85	-0.267	0.3	176.76 ± 110.41	-0.230	0.190
HDL- cholesterol (mg/dl)	41.67 ± 12.39	0.090	0.697	43.88 ± 18.66	0.329	0.197	38.97 ± 10.29	0.185	0.295
Cholesterol /HDL	4.438 ± 1.16	-0.122	0.598	5.14 ± 2.24	-0.331	0.194	4.72 ± 1.61	-0.249	0.155
VLDL- cholesterol (mg/dl)	25.24 ± 11.07	-0.228	0.321	30.06 ± 19.21	-0.156	0.563	33.18 ± 17.41	-0.180	0.308
LDL- cholesterol (mg/dl)	88.67 ± 25.56	-0.145	0.531	97.88 ± 19.23	0.989	0.004	83.12 ± 27.96	-0.208	0.237
LDL/HDL	2.24 ± 0.74	-0.179	0.439	2.58 ± 1.11	-0.234	0.367	2.26 ± 0.88	-0.267	0.127

Discussion

CAD is one of the major causes of mortality in developed and developing countries with risk factors such as hypertension, dyslipidemia and diabetes mellitus (17).

Oxidative stress is defined as an imbalance between the production of prooxidants and antioxidant defenses in favor of prooxidants. This imbalance is usually related to the increased formation of ROS, and is thought to play a pivotal role in the development and pathogenesis of CVD and its complications. It has been suggested that oxidative stress may be a strong and independent prognostic predictor of cardiovascular events (8). In patients with CVD elevated levels of both oxidative stress status parameters (superoxide anion and malonaldehyde) and reduced protective activities of superoxide dismutase have been reported (15).

ROS may lead to the modification of several molecules including the oxidation of LDL. Oxidized LDL may lead to endothelial dysfunction, increased vascular smooth muscle cell growth, and monocyte migration and activation (19-21). All of which may be involved in atherogensis and plaque destabilization. The oxidant species mediate signaling pathways, which lead to initiation of fatty streak development through atherosclerotic lesion progression to ultimate plaque rupture (22,23). Moreover, oxidative stress is the unifying mechanism for many CVD risk factors (15). It has also been reported that isoprostanes, which are the markers of lipid peroxidation and reduced antioxidant capacity, are related to increased risk of CVD and the number of cardiovascular risk factors (24). Moreover, in CVD patients elevated levels of both oxidative stress status parameters such as superoxide anion and malonaldehyde (MDA) and reduced protective superoxide dismutase (SOD) activities have been reported (15).

On the other hand, ROS such as superoxide anions are produced during normal periods of cell life and high reactive properties of these species leads to oxidation of lipids and other vital molecules.

A balance between ROS production and antioxidant activity is necessary in pathogenesis of diseases related to oxidative stress. Studies show that ROS is one of the risk factors in pathogenesis of some diseases (25). Atherosclerosis shows a state of increased oxidative stress that lipid and protein oxidation has occurred in

vessels. Oxidative changes hypothesis indicates LDL oxidation occurs before atherogenesis (26). In this study PAB values were determined in healthy subjects and patients with various involvements in coronary arteries. In addition, uric acid, triglycerides, total cholesterol, HDL, LDL, VLDL levels and their relations were found among control group and patients. There was significant correlation between uric acid and prooxidant antioxidant balance in patients with two vessels coronary involvement inversely. Tatly *et al*. showed that a significant association between uric acid and risk of acute myocardial infarction (27). Torun *et al.* showed that uric acid level is increased in patients with CAD compared to healthy controls (28).

Our study also shows a significant association between uric acid level and PAB in patients with two vessel coronary involvement. Impaired in endothelial function, is the first stage for atherosclerosis (29). Serum uric acid has antioxidant properties and trapping free radicals in human serum.

When uric acid reacts with peroxynitrite, it decreases oxidative damage by peroxynitrite (30). Thus, uric acid can be suppose a protective agent against oxidative stress, but it can be directly or indirectly lead to vascular lesions. Reports indicate uric acid stimulates vascular smooth muscle proliferation and regulates the expression of platelet derived growth factor (31). Hypoxanthine is converted to uric acid via xanthine oxidase. This reaction can be catalyzed by dehydrogenase and oxidase, where the latter enzyme produces uric acid and superoxide. Therefore, it is possible that in certain circumstances, uric acid is accompanied with increased production of ROS.

The importance of free radicals in making changes in lipids (lipid peroxidation) has led to many efforts to determine the best index in biological fluids. Lipid peroxidation products are very complex and can be divided into primary lipid hydroperoxides and secondary products. Among primary products of lipid peroxidation (lipid hydroperoxides), we can name the β-cleavage of an esterified fatty acid in phospholipids that lead to formation of 4-hydroxyalkenal. Isoprostanes are formed for reduction endoperoxide by phospholipase A_2 (32). The increased lipid peroxidation associated with CAD, confirms the role of uncontrollable peroxidation in the pathogenesis of CAD (26,33). However, there are studies that have demonstrated different results. For instance, Croft *et al*. showed no difference oxidation parameters between patients and controls (34). Van de vijver *et al.* were unable to present an inverse relationship between atherosclerosis and LDL oxidation in patients with acute CAD (35). Studies have shown that hypercholesterolemia significantly correlates with the severity of coronary artery lesions (36). In present study we observed a correlation between increased plasma cholesterol and risk of CAD, but there was no significant difference between cholesterol level and PAB among healthy subjects and patients with different degrees of vascular involvement. A possible explanation of this effect is that a history of heart disease in cases with more severe CAD might have affected their life style. In other words, such a history and patient awareness of their condition may have motivated them to follow a healthier diet and better life style; as a result, risk indicators such as HDL had improved by the time of this study.

In this study, there was no statistical difference in PAB levels between healthy subjects and patients. Also, PAB did not correlate with the number of involved vessels. Triglyceride and cholesterol levels had no effect on PAB; there was no significant correlation between total cholesterol, HDL-cholesterol, LDL–cholesterol and PAB. On the other hand, there was an inverse and significant relation between PAB and serum uric acid in patients and healthy subjects. Alamdari *et al.* also showed a significant relationship between PAB and serum uric acid (7). This study also shows that PAB in patients with single vessel involvement is further increased in comparison with healthy subjects and patients with multi-vessel involvement. According to these findings, it can be concluded that perhaps uric acid is not a risk factor for heart failure, but as an antioxidant agent, it may even have a protective or preventive effect.

Evaluation of PAB along with other risk factors can be useful in predicting the prognosis of cardiovascular events but cannot serve as a predictive factor for patients with single or multi-vessel involvement.

Acknowledgements

The authors would like to thank the Research Vice Chancellor of Tehran University of Medical Sciences for their financial support.

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