Original Article

Study of Prooxidant-antioxidant Balance and Some Risk Factors of Coronary Artery Disease

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Abstract

Background: There is evidence that oxidative stress can be considered as a critical event in the development of atherosclerotic complications. In this study, we aimed to assay the values of prooxidant-antioxidant balance (PAB) in patients with coronary artery disease (CAD), as a cardiovascular risk factor, and its relationship with some clinical, biochemical, and anthropometric parameters was examined. **Materials and Methods:** In a case–control study, forty CAD patients and forty age and body mass index (BMI)-matched healthy controls hospitalized in the cardiology section of Imam Ali hospital of Zahedan, Iran, were enrolled. The levels of serum lipid profile, C-reactive protein (CRP), blood pressure, BMI, and waist circumference (WC) were evaluated. The values of PAB were also assayed simultaneously by photometric method, using 3, 3′, 5, 5′-tetramethylbenzidine and its cation, used as an indicator of redox. **Results:** PAB was found to be significantly higher in CAD patients ($P < 0.05$) as compared with control group. The obese patients had higher values than nonobese patients and controls ($P < 0.05$). In CAD patients, a significant positive correlation was demonstrated between WC ($r = 0.56$, $P = 0.05$), high-sensitivity-CRP ($r = 0.65$, $P = 0.04$), cholesterol ($r = 0.36$, $P = 0.052$), and triglyceride ($r = 0.29$, $P = 0.055$) with PAB. **Conclusion:** The study shows that the PAB assay in conjunction with other risk factors can be used as an independent prognostic predictor of CAD, particularly in patients who need antioxidant therapy.

Keywords: Coronary artery disease, risk factors, prooxidant-antioxidant balance

Introduction

Coronary artery disease (CAD) is one of the leading causes of mortality and morbidity in the world,[1,2] which results from atherosclerotic plaques in the coronary arteries wall.[2] CAD is a multifactorial disease, which is caused by several risk factors including high levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C), low levels of high-density lipoprotein cholesterol (HDL-C), diabetes mellitus, obesity, hypertension, and cigarette smoking.[3,4] However, other causes may affect in the development of CAD.[4] It has been reported that oxidative stress and inflammation have important role in the pathogenesis of CAD and its complications.[5-7]

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) as prooxidants and antioxidant defenses.[4] Oxidative stress markers including reduced activity of superoxide dismutase and glutathione, increased levels of malondialdehyde, superoxide anion ($O2^-$), and sulfhydryl groups can lead to proatherogenic events such as LDL oxidation and vascular smooth muscle cell proliferation.[4,8] Recent data indicate that high values of adiposity, especially of the abdominal type, which has a strong association with increased risk of atherosclerosis, hypertension, and dyslipidemia[9] generate chronic low-grade chronic inflammation and increase conditions of oxidative stress. Both inflammation and oxidative stress in vascular and fat tissues contribute to the atherosclerotic process.[10]

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The prooxidant-antioxidant balance (PAB) is evaluated by determination of both the oxidant and antioxidant status. The researchers have recently applied a method which measures the balance of oxidants and antioxidants simultaneously in one reaction (the PAB assay).[11]

In this study, PAB values in patients with CAD were compared with healthy controls, and its relationship with some cardiovascular risk factors such as serum levels of lipids and C-reactive protein (CRP), blood pressure, body mass index (BMI), and waist circumference (WC) was assessed.

Materials and Methods

In this case–control study, forty patients aged 30–80 years hospitalized in the cardiology section of Imam Ali hospital of Zahedan, Iran, who based on diagnostic angiography had 50% or more coronary stenosis at least one major coronary artery were enrolled. Interpretation of angiography was done by a cardiologist. Forty healthy controls 30–79 years of age were selected from among those who referred to the cardiology clinic of Imam Ali hospital of Zahedan, and CAD was not confirmed by cardiologist. All patients and controls with medical history, including diabetes mellitus, thyroid, liver or renal failure, cardiomyopathy, left ventricular systolic dysfunction or severe heart failure, acute or chronic inflammatory disorders, or the recent use of lipid-lowering drugs and corticosteroids or smoking were excluded from the study. Two groups were matched for age and BMI. The sample size in each group was determined based on the similar articles.[12-14]

After 15 min of sitting, blood pressure was measured in all participants, by the mercury sphygmomanometer.

BMI was calculated as weight (kg) divided by the square of the height (m^2). WC was measured midway between the lower rib margin and the iliac crest at the end of a gentle expiration. WC >102 in men and WC >88 in women were considered as prognostic predictors of abdominal obesity.[15]

Peripheral venous blood was drawn from all participants after overnight fasting. Serum was separated, and aliquots were immediately frozen at −70°C until analysis.

The levels of total cholesterol, triglyceride, HDL-C, LDL, creatine phosphokinase (CPK), lactate dehydrogenize (LDH), creatine kinase-muscle/brain (CK-MB), and aspartate transaminase (AST) were measured using standard kits (Pars Azmun, Tehran, Iran) using an autoanalyzer (Hitachi, Japan). Serum high-sensitivity CRP (hs-CRP) was measured using latex-enhanced nephelometry (Behring BN II nephelometer, Germany).

As previously described,[16] the values of PAB "as a oxidative stress marker" was measured simultaneously, by photometric method, using 3, 3′, 5, 5′,-tetramethylbenzidine in an ELISA reader at 450 nm with a reference wavelength of 570 or 620 nm, according to the described method by Alamdari *et al*.[12] The values were expressed in an arbitrary HK units, which show the hydrogen peroxide percentage in the standard solution, and were calculated based on the standard curve.[12]

The study was performed according to the Helsinki declaration rules and was approved by the ethics committee of Zahedan University of Medical Sciences. An informed consent was obtained from all subjects participating in the study (Approval date: December 4, 2016; Code No: IR.ZAUMS.REC.1395.224).

Statistical analysis

Statistical analyses were carried out by SPSS statistical software package program (version 18 for Windows, Chicago, IL, USA). The results were expressed as mean ± standard deviation or mean ± standard error of mean in accordance with their distribution. Unpaired *t*-test or Mann–Whitney U-test was performed for comparison between two groups. Unpaired sample *t*-test was used to check of homogeneity of age and BMI between groups. Spearman correlation coefficients and multivariable regression analysis were used for assessment of correlation between PAB with other variables. $P < 0.05$ was considered as statistically significant.

Results

The mean age and BMI of the patients (20 men and 20 women) were 55.6 ± 13.4 years and 25.1 ± 4.8 kg/m^2, respectively. The mean age and BMI in the healthy controls (23 men and 17 women) were 53 ± 12 years and 25.4 ± 5 kg/m^2, respectively. The results showed that these variables did not have different significance ($P = 0.18$ and 0.79 achieved for age and BMI, respectively).

Based on the WC results, of the 40 patients studied, 29 individuals (72.5%), and of the 40 healthy controls, 19 individuals (47.5%) had abdominal obesity ($P = 0.02$).

Demographic, clinical, and biochemical characteristics of obese and nonobese CAD patients were represented in Table 1. The levels of blood pressure ($P < 0.01$), triglyceride ($P < 0.0001$), cardiac enzyme tests (AST, LDH, CPK, and CK-MB), and hs-CRP in both obese and nonobese CAD patients were significantly higher when compared with control group ($P < 0.0001$). The serum levels of cholesterol were significantly increased in obese CAD patients compared with nonobese patients and obese controls ($P < 0.01$).

The comparison PAB values in CAD patients and controls showed that the values were significantly higher in obese CAD patients than obese controls ($P < 0.05$). No significantly difference was found between nonobese individuals in CAD patients compared with controls.

Overall, a significant positive correlation was found between WC ($r = 0.56$, $P = 0.05$), hs-CRP ($r = 0.65$, $P = 0.04$), cholesterol ($r = 0.36$, $P = 0.052$), and triglyceride ($r = 0.29$, $P = 0.055$) with PAB, in CAD patients.

The multiple regression analysis showed that a positive correlation was found between WC ($\beta = 0.514$, $P = 0.04$) and CRP with PAB ($\beta = 0.429$, $P = 0.052$) in CAD patients [Table 2].

Table 1: Demographic and chemical characteristics of individuals based on the abdominal obesity

Abdominal obesity Parameters	Groups			
	CAD patients (n=40)		Controls (n=40)	
	Nonobese (n=11)	Obese (n=29)	Nonobese (n=21)	Obese (n=19)
BMI (kg/m²)	21±2	27±4.4	22.7±3.4	28.3±4.8
Blood pressure (mmHg)				
Systolic	135±17	139±13**,#	120±6.6	123±13
Diastolic	86±14	90±9.6**,#	77±4.7	83±9
Cholesterol (mg/dL)	177±35	202±52#,§	170±36	163±48
Triglyceride (mg/dL)	157±44†	187±59¶	77±40	138±47
LDL-C (mg/dL)	81±24	95±32‡,‖	81±22	82±25
HDL-C (mg/dL)	42±11	42±14	45±11.5	40±13
AST (U/L)*	87±31†	100±37¶,§	24±2.2	30±1.7
LDH (U/L)*	479±90†	758±1134¶,§	178±14	225±11
CPK (U/L)*	425±141†	716±255¶,§	83±9.5	114±14
CK-MB (U/L)*	54±14.7†	91±38¶,§	16±2	19.7±1.3
hs-CRP (mg/L)*	11.5±5.4†	13.9±4.4¶	1±0.08	1.1±0.05
PAB (HK unit)	88.6±20.7	107±30‡,‖	87±30.5	88±30

Data are presented as mean±SD. *Cardiac enzyme tests (AST, CPK, LDH, CK-MB) and hs-CRP levels in patients are presented as mean±SEM because the data were not normally distributed. **, #P<0.01 between CAD patients and controls (both obese and nonobese), §P<0.01 and P<0.0001 between obese and nonobese CAD patients, †,¶P<0.0001 between CAD patients and controls (both obese and nonobese), ‡P<0.05 between obese and nonobese CAD patients, ‖P<0.05 between obese CAD patients and controls. BMI: Body mass index, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, AST: Aspartate transaminase, CPK: Creatine phosphokinase, LDH: Lactate dehydrogenize, CK-MB: Creatine kinase-MB, hs-CRP: High sensitivity C-reactive protein, PAB: Prooxidant-antioxidant balance, NS: Not significant, CAD: Coronary artery disease, SD: Standard deviation, SEM: Standard error of mean

Table 2: Results of multivariate regression analysis between prooxidant-antioxidant balance and various parameters in coronary artery disease patients

Parameters	Standardized coefficients	P
	Beta	
BMI	0.253	0.146
WC	0.514	0.040
Blood pressure		
Systolic	0.341	0.103
Diastolic	0.259	0.186
Cholesterol	0.246	0.110
TG	0.144	0.272
LDL	0.138	0.384
HDL	−0.105	0.402
hs-CRP	0.429	0.052

BMI: Body mass index, WC: Waist circumference, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, hs-CRP: High sensitivity C-reactive protein, TG: Triglyceride

Discussion

Oxidative stress, which is purposed to be an important contributor to the complications of atherosclerotic vascular, occurs before atherogenesis.[17] PAB is a relatively simple indicator that demonstrates the occurrence of oxidative stress. It measures total prooxidants and the total antioxidants in one experiment.[11] Our findings demonstrated that the values of PAB were significantly higher in CAD patients compared with healthy controls. In previous studies, which have measured the oxidant and antioxidant capacities separately, there was a significant increase in the lipid peroxidation markers and a significant decrease in antioxidant capacity and/or total antioxidant status levels, in CVD patients when compared with controls.[13,18,19]

The studies performed using method of modified PAB assay, as applied in the present study, demonstrated a significant increase of the PAB value in these patients compared to control group.[11,20] The relationship between CAD and oxidative stress can result from reaction between superoxide radicals and other reactive oxygen species (ROS) with nitric oxide, which is a vital endothelial-derived vasodilator. This reaction may lead to endothelium dysfunction, which is associated with atherosclerosis and cardiovascular diseases.[21]

In this study, we found an increase in the PAB values in obese CAD patients, and a significant positive correlation was found between WC and PAB "as an index of abdominal obesity." Moreover, in multivariate regression analysis, we demonstrated an association between serum PAB and WC, after adjusting age and BMI. Several studies suggest that oxidative stress is linked to obesity-associated development of CAD.[6,7] It has also been reported that the obesity increases the oxidative stress and depletes the reserves of antioxidant.[22] Obesity, especially increased levels of visceral fat is considered as a marker of dysfunctional adipose tissue, which can contribute to the development of cardiovascular complications including blood pressure, inflammation, lipids metabolism, and homeostasis of glucose, which is independently linked to the oxidative stress.[9,23,24]

The study demonstrated that in CAD patients, serum levels of hs-CRP, as a systemic inflammatory marker, were markedly

higher than controls, and a positive association with PAB and also with several risk factors of CVD including WC, blood pressure, and triglyceride was shown. After adjustment for age and BMI, in multivariate regression analysis, an association between serum CRP and PAB was revealed.

The relationship between increased serum levels of CRP with PAB and cardiovascular risk factors, suggesting an vital role of inflammation as predictors of coronary events,[6,7] in those who have been diagnosed with history of acute coronary disease, stable angina, and also in apparently healthy individuals.[25]

The evidence demonstrate that accumulation of lipids in arteries induces endothelial dysfunction and contributes to oxidized LDL (ox-LDL) and results in the formation of foam cell, thus plays vital role in the atherosclerotic process.[26,27] An earlier study has indicated that LDL-C concentration can contribute to the lipoproteins oxidation in patients with coronary heart disease (CHD).[14] As well, an increased levels of ox-LDL has been reported in patients with stable CHD,[14] coronary artery stenosis (CAS),[28] and CAD.[29]

In our study, ox-LDL was not measured; however, serum levels of triglyceride (in both obese and nonobese patients) and total cholesterol and LDL-C (only in obese patients) were markedly higher compared with healthy controls. As well, a marginal positive correlation between PAB with serum triglyceride and total cholesterol was found in obese-CAD patients, but not in nonobese individuals. There was no significant correlation between LDL-C and HDL-C levels and PAB in both patients and healthy controls.

Considering the important role of lipids, as one of the main targets of ROS generation,[26] it is possible that in CAD patients in addition to abdominal obesity, increased levels of LDL-C, total cholesterol and triglyceride "as markers of atherogenic with oxidative stress properties," and increased levels of serum hs-CRP can be associated with oxidative stress status. However, HDL concentration, as an important index in prevention or decrease of atherosclerosis, which inhibits the LDL oxidation,[30] was no difference between both the patient and control groups.

The blood pressure is also one of risk factors contributing to the development of cardiovascular disease, which is associated with oxidative stress.[30] In our study, the mean of blood pressure in the obese CAD patients was significantly higher compared with controls. However, we did not find any correlation between oxidative stress and hypertension in CAD patients, which probably the limited sample size, could explain this fact. It was partly in agreement to a previous study.[22]

Further research with larger population is needed to clarify the PAB which is presented as a cardiovascular risk factor.

Conclusion

The study shows that the PAB assay in conjunction with other risk factors can be used as an independent prognostic predictor of CAD, particularly in patients who need antioxidant therapy. However, further studies are needed to examine the possible role of PAB as a cardiovascular risk factor.

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Conflicts of interest

There are no conflicts of interest.

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