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Activities of Anti-Oxidative Enzymes, Catalase and Glutathione Reductase in Red Blood Cells of Patients with Coronary Artery Disease

M. Firoozrai, H. Mehrabi, A. Ehsani, M. Najafi and M. Ghaffari
Department of Biochemistry and Cardiology, School of Medicine,
Iran University of Medical Sciences, Tehran, Iran

Abstract: Free radical scavenging enzymes are an important part of body anti-oxidative system. The aims of this study were to evaluate the enzymatic activities of anti-oxidative enzymes of erythrocytes, catalase (CAT) and Glutathione Reductase (GR), that might be indicators of protective mechanisms involved in atherosclerosis and also to evaluate the serum lipids and lipoproteins which are thought to be correlated with these two anti oxidative enzymes. The study population consisted of 90 patients with angiographically proved coronary stenosis in surgery section of Tehran Rajaee cardiovascular center and 30 subjects without any coronary heart disease used as control. Glutathione reductase and catalase activities in erythrocytes were assayed. Patients had not significantly decreased glutathione reductase activity compared to that in control subjects. However the catalase activity was significantly decreased in erythrocytes of the atherosclerotic patient. Atherosclerotic smoking patients had similar catalase activity compared to that in nonsmoking patients. However the glutathione reductase activity was lower in erythrocytes of the atherosclerotic smoking patients. No significant correlations were found between serum lipids and two anti-oxidative enzymes activities in patients and control subjects.

Key words: Coronary artery disease, anti oxidative enzymes, catalase, glutathione reductase, lipids, lipoproteins

INTRODUCTION

In the routine body metabolism, a number of potent oxidants are generated. In addition, drug metabolites, smoking and exposure to ionized and non ionized radiations seem to be associated with increase in oxidant capacity. Oxidative agents interact with proteins, nucleic acids, lipids and other molecules, leading to cell damage, disturbances in metabolism and function of some macromolecules and perturbations in the immune cells (Desmond and Griendling, 2006). One of important macromolecules involved in atherosclerosis is Low Density Lipoprotein (LDL) (Chisolm and Steinberg, 2000). In absence of suitable balance between antioxidants and pro-oxidants, LDL subparticles are more readily oxidized, propagated in intima and more rapidly promoted atherosclerotic process (Ross, 2004).

The part of antioxidant defense system includes a number of enzymes such as superoxide dismutase, catalase (CAT) and Glutathione Reductase (GR), as reported in some studies (Zhou *et al*., 2004; Guemouri *et al*., 1991). These enzymes convert hydrogene peroxide and scavenge lipid hydro-peroxides. Since, oxidation of LDL may be related to activities of the indicated enzymes, thus, we evaluated activities of catalase and glutathione reductase in erythrocytes of patients with Coronary Artery Disease (CAD) and investigated their correlations with levels of lipids and lipoproteins.

Corresponding Author: Mohsen Firoozrai, Department of Biochemistry and Cardiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran Tel: 98218809317 Fax: +982188058742

MATERIALS AND METHODS

The study population consisted of 90 patients (50 males and 40 females) aged 55.17±10.18 years with angiographically proved coronary artery stenosis who had been referred to Tehran Rajaee Heart Center during 2005. The patients were selected at random from those attending the center. Ten patients had stenosis in one (group I), 22 patients in two (group II) and 58 patients in three (group III) major coronary arteries. Twenty five patients had history of smoking (>10 cigarettes/day) for last three years. Thirty normal healthy subjects were recruited from Iran Medical Science University staff aged 47.50±12.65 years. They had no history of any cardiovascular disease and diabetes. Three healthy subjects had a history of smoking (>10 cigarettes/day).

Venous blood samples of all subjects were collected in plain tubes in the morning after overnight fasting. Each sample immediately was divided into 3 aliquots. One was allowed to clot at room temperature and serum was separated immediately by centrifugation in 3000 rev min^{-1} for 30 min for estimation of lipids and lipoproteins. Second aliquot placed in a tube containing sodium citrate for estimation of catalase activity (Goldberg and Spooner, 1983) and third aliquot placed over EDTA for analysis of hemoglobin and glutathione reductase activity (Andersen *et al*., 1997).

Serum levels of Total Cholestrol (TC), High Density Lipoprotein-Cholestrol (HDL-C) and triglyceride (TG) were estimated by the enzymatic methods using an auto-analyzer (Technical R A 1000), Low Density Lipoprotein-cholestrol (LDL-C) was estimated using friedewald equation. To determine Hb content, the third aliquot of blood was hemolyzed in distilled water and analyzed spectrophotometrically with Drabkins reagent.

Values were reported as mean±SD. The statistical differences were performed by use of t-student test.

RESULTS

The mean activity of CAT was significantly lower in patients than in healthy subjects ($p<0.05$) (Table 1).

The activity of CAT in two groups of patients (I, III), specifically in group III, was significantly lower than in healthy subjects. In patients all together (n-90), decreased activity, also, was significant ($p<0.008$).

The mean activity of GR in patients erythrocytes compared with that of healthy subjects was insignificantly different (Table 1). GR activity was lower in current smoking patients than in non smoking patients ($p<0.05$) (Table 2). However the activity of GR in smoking and non smoking healthy subjects was not significantly different.

Table 1: Activities of erythrocyte catalase and glutathione reductase in 3 groups of patients*

Variables	I n = 10	II n = 22	III n = 53	Patients n = 90	Control n = 30
Catalase (k/gHb)	253.13±44.34[a]	279.23±44.64	262.69±36.70[b]	265.67±40.00[c]	278.82±50.44
Glutathione reductase (U/gHb)	12.86±1.96	12.48±1.64	11.79±1.74	12.07±1.77	11.92±1.56

Data given as mean±standard deviation, [a]$p<0.001$, [b]$p<0.008$, [c]$p<0.05$; *I = Stenosis in 1 vessel, II = Stenosis in 2 vessels, III = Stenosis in 3 vessels

Table 2: Comparison of erythrocyte catalase and glutathione reductase activities between smoking and nonsmoking patients

Variables	Smoking n = 25	Nonsmoking n = 65	p-value
Catalase (k/gHb)	292.95±39.79	266.72±40.34	>0.05
Glutathione reductase (U/gHb)	11.23±1.86	12.40±1.63	<0.05

Data given as mean±standard deviation

Table 3: Correlation between age and lipids and erythrocyte catalase and glutathione reductase activities

	Patients		Control	
Variables	CAT	GR	CAT	GR
Age (year)	0.01	0.07	0.28	0.023
Cholesterol	-0.09	0.02	0.20	0.16
Triglyceride	-0.17	0.01	0.11	0.14
HDL-C	0.03	0.15	-0.31	-0.07
LDL-C	-0.04	0.00	0.025	0.13
VLDL-C	-0.02	0.01	0.11	0.15
LDL-C/HDL-C	0.49*	0.10	-0.09	0.19

Data given as correlation coefficients(r) *p<0.005

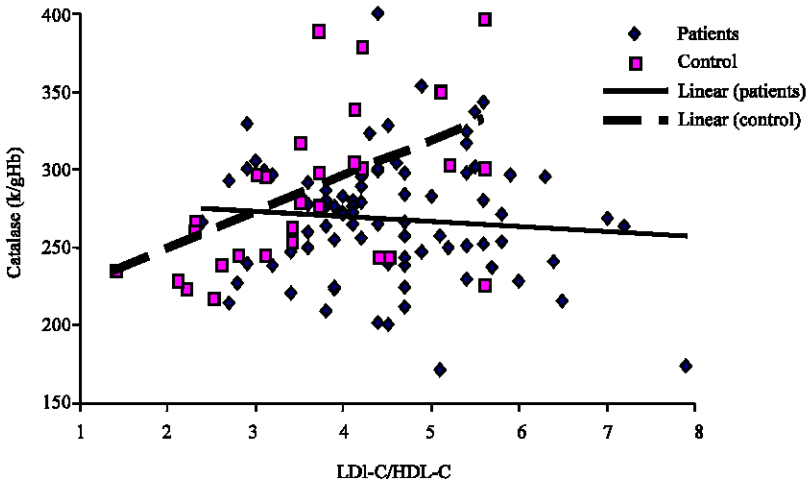

Fig. 1: Correlation between LDL-C/HDL-C and catalase activities in patients and normal subjects Normal subjects: r = 0.4921 patients: r = -0.0919 k is constant coefficient for velocity equation. Sum of k is more significant than V in the assessment as described by Andersen *et al*. (1997)

TC, LDL-C and atherogenic index (LDL-C/HDL-C) in patient were higher than in the healthy subjects ($p<0.002$), although, there were no significant differences in TG, HDL-C and very low density lipoprotein-cholestrol (VLDL-C) between controls and patient groups (not shown). No relationship was found between CAT and GR activities and blood lipid parameters including TC, TG, HDL-C, LDL-C, VLDL-C and atherogenic index (Table 3). The results showed, while there was no significant relation between CAT activity and atherogenic index in patients, in healthy subjects the relation was significant ($p<0.05$) (Fig. 1).

GR activity of erythrocytes in patients and healthy subjects was not correlated with age, TC, TG, HDL-C, LDL-C, VLDL-C and atherogenic index.

DISCUSSION

Present results showed activities of catalase and glutathione reductase in erythrocytes of coronary atherosclerotic patients were different. Data showed that CAT activity compared to that in normal subjects was significantly lower. Reduced CAT activity might be an evidence of both the increased oxidative capacity and oxidized LDL in blood leading to the coronary atherosclerosis in patients. On the contrary of our finding anderson reported a significant increase in the catalase activity in CAD patients with 3 vessels stenosis (Andersen *et al*., 1997).

Present results showed higher GR activity was not statistically significant. It is important to mention that Yegin in Turkish individuals with coronary atherosclerosis (Yegin *et al*., 1997) and Schettler in German patients (Schettler *et al*., 1998), also, could not find any significant differences in GR activity in erythrocytes. However, some reports were shown a link between GR-1 activity and CAD prospectivity, but it was obscure (Ramachandran, 2006). GR activity in smoking patients compared to that of non smoking patients was significantly lower. GR is an indirect anti-oxidative enzyme which produces GS-H, this, in turn, acts as an anti-oxidant, whereas, CAT as a direct anti-oxidative enzyme scavenges hydrogen peroxide, Thus, We assume the role of CAT activity in suitable oxidant balance is more important than GR activity. Furthermore, effects of smoking on GR in patients raised the assumption that some materials within cigarette may reduce GR activity. We also found no significant differences in CAT activities between smoking patients and smoking normal subjects which supports the finding of Volkovova (Volkovova *et al*., 1996).

Significant differences in cholesterol concentration and LDL-C/HDL-C ratio between patients and normal subjects raised the question whether there might be correlations in CAT and GR activities and levels of lipids in the two groups. we could not find any correlation. However, increase in LDL-C/HDL-C ratio was associated with increase in CAT activity in normal subjects, which is some what contradictory.

In conclusion, the results markedly showed reduced CAT anti-oxidative enzyme activity in patients with CAD. However, we did not study reasons of its primary or secondary reduction, but, we assume it is one of the potent factors in promotion of atherosclerosis.

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