Serum MDA as a Diagnostic's Biomarker in Stable Coronary Heart Disease

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Abstract: The recent studies showed that Malondialdehyde (MDA) is the important marker of lipid peroxidation and progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements. The aim of this study was to investigate relationship between MDA and lipoprotein concentrations in male patients with Coronary Artery Disease (CAD). This case control study was performed 51 male patients with angiographically confirmed CAD admitted to Shahid Madani Hospital in Tabriz city of Iran as a study group and 60 healthy males that matched in age and sex as the control group. The serum level of MDA was measured by colorimetric method using thiobarbituric acid reaction and lipid and lipoproteins concentrations were determined by standard enzymatic methods. The findings were analyzed using Pearson's correlation test for measurement of relation between MDA with other variables and t-test statistical method for comparison in two groups. The result showed that serum concentrations of cholesterol, triglyceride, LDL-C and LDL-C/HDL-C ratio and level of MDA in patients group were higher than healthy group (p<0.05). There was positive and significant relationship in patients group between serum MDA, triglyceride, cholesterol, LDL-C and LDL-C/HDL-C ratio, but the correlation between MDA and HDL-C was negative and meaningful (p<0.05). No relationship was observed between MDA with BMI and age in the patients group (p>0.05). There is an increasing acceptance that oxidative modification of lipid and lipoprotein is a crucial step in the development of atherosclerosis. In view of the relationship between changes in the serum levels of lipids and lipoproteins and that of MDA in patients with CAD it was concluded that simultaneous control of both dyslipoproteinemia and lipid peroxidation may be of equal importance in prevention of CAD. The independent association of MDA levels with stable CHD indicates a possible role of these parameters as biomarkers of CHD patients.

Key words: CAD, MDA, lipid, lipoprotein, male

INTRODUCTION

Atherosclerotic disease is the leading cause of mortality in countries, with coronary artery disease being the number one killer of both men and women. Epidemiological and clinical studies established a link between dietary saturated fat, serum cholesterol and Coronary Artery Disease (CAD) mortality (Keys, 1980). High levels of oxidized LDL are found in patients with different acute coronary syndromes, indicating that oxidized LDL might be a marker for atherosclerosis (Holvoet et al., 1998; Ehara et al., 2001; Toshima et al., 2000). The relationship between oxidative stress parameters and inflammatory species suggest their strong mutual involvement in atherosclerosis development that leads to CAD progression (Kotur-Stevuljevic et al., 2007). Among many traditional risk factors for Coronary Artery Disease (CAD) development (hypertension, hyper-lipidemia, diabetes, age, obesity, cigarette smoking), oxidative stress (Cai and Harrison, 2000; Landmesser et al., 2002) and inflammation (Ostend and Bjorklid, 2003 and Pearson et al., 2003) are now being considered as significant and novel risk factors for CAD and other diseases. Lipid and lipoprotein abnormalities play a major role in the development and progression of coronary artery disease. Atherosclerosis, which is the basis of Coronary Heart Disease (CHD), is believed to be an inflammatory disorder. Inflammation must smolder for decades before resulting in a clinical event like angina or acute myocardial infarction (Libby 2003). Generation of free radicals and oxidative stress may have an important role in atherogenesis (LaRosa et al., 1990). It has been suggested that oxidative stress, especially oxidative modification of Low-Density Lipoproteins (LDL), may play a causative role in the pathogenesis of athero-sclerotic coronary artery disease (Chisolm and Steinberg, 2000). Patients with CAD also had higher MDA level than the controls, which

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represents the oxidative damage products of lipid and proteins (Tosukhowong *et al.*, 2003). Some investigators have found a correlation between prooxidant-antioxidant imbalance and the severity of coronary lesions (Riemersma *et al.*, 1991; Regnström *et al.*, 1992; Jayakumari *et al.*, 1992). Conflicting reports have discussed the role of oxidative stress in the production of atherosclerotic lesions in patients with CAD (Croft *et al.*, 1992; Virella *et al.*, 1993; Van de Vijyer *et al.*, 1996; Van de Vijyer *et al.*, 1998). However, oxidative-stress-related studies in patients with CAD have been usually done by measuring lipid peroxidation and/or antioxidant power in plasma (Stringer *et al.*, 1989; Weinbrenner *et al.*, 2003), LDL (Weinbrenner *et al.*, 2003) and atherosclerotic vessels (Piatrowski *et al.*, 1990; Mehrabi *et al.*, 1999; Jachec *et al.*, 2003). Therefore, the aim of our study was to investigate MDA as diagnosis indicator in patients with stable CAD.

MATERIALS AND METHODS

Subjects: Subjects of this study consist of two groups, including Fifty one males under 55 years old with angiographically confirmed CAD admitted to Shahid Madani Hospital in Tabriz city of Iran as a study group and the other consisted of 60 healthy individuals that matched in age and sex as the control groups in 2004. Details of patients recorded at the time of admission included age, height and weight, smoking habits, history of metabolic disease and drug using, also BMI (body mass index) was evaluated with measuring height and weight. Exclusion criteria for all subjects were diabetes mellitus and using of lowering serum lipid drugs. The control group consisted of 60 males aged 44±9 years. They had normal electrocardiography findings. Their histories were negative for diabetes, hypertension, cancer, heart disease and their serum cholesterol and triglyceride levels were within the normal levels.

Blood sampling: Following a 18 h fast period, blood samples were collected by venipuncture and EDTA-plasma and sera were obtained by centrifugation at $1500 \times g$ for 20 min. Materials were stored at -20°C until they were analyzed. Serum lipids (total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol) were measured by an automated analyzer using commercial kits.

Plasma Malondialdehyde (MDA) levels: MDA were measured by Slater method. Lipid peroxidation in the plasma was evaluated by the spectrophotometric method based on the reaction between MDA and thiobarbituric acid (Buege and Aust, 1978). 0.5 mL plasma and 0.5 mL

deionized water and 2.0 mL reagent (26 mmol L^{-1} thiobarbituric acid, 0.92 mol L^{-1} trichloroacetic acid in 0.25 mol L^{-1} HCl) were added to each tube and heated in a boiling water bath for 15 min. After cooling, the flocculent precipitate was removed by centrifugation at $1000 \times g$ for 10 min. The absorbance of the sample was determined at 532 nm against a blank. The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard.

Statistical analyses: The finding were analyzed using Pearson's correlation test for measurement of relation between MDA with other variables and t-test statistical method for comparison in two groups, also p-value less than 0.05 was considered as significant.

RESULTS

General characteristics of CAD patients and controls are shown in (Table1). The table show higher BMI and weight in patients as compared to controls elevation are not significant. The CHD patients exhibited an elevated state of oxidative stress (MDA concentration) when compared to the control population. Patients with compared to healthy men had significantly higher levels of total serum cholesterol, LDL cholesterol, trigly-cerides and lower levels of HDL cholesterol. Serum concentrations of cholesterol, triglyceride, LDL-C and LDL-C /HDL-C ratio ($p<0.05$) and level of MDA ($p<0.01$) in patients group were higher than healthy group, but HDL-cholesterol levels were less than ($p<0.05$). At the same time with increase of MDA in patients increased other parameters such as cholesterol, triglycerides, LDL-C and LDL-C /HDL-C ratio, but decreased HDL cholesterol. There was positive and significant relationship in patients group between serum MDA and triglyceride, cholesterol, LDL-C and LDL-C /HDL-C ratio, but the correlation between MDA and HDL-C was negative and meaningful ($p<0.05$) (Fig. 1-4). No relationship was observed between MDA with BMI and age in the patients group ($p>0.05$)

Table 1: Baseline characteristics and oxidative stress biomarkers of patients with stable CAD and healthy control subjects

Parameter	Healthy person	CAD
Weight (kg)	70.9±8.6	73.55±8.2
BMI (kg m^{-2})	24.8±2.8	25.5±4.2
LDL- C (mg dL^{-1}) *	114.5±18.9	206.9±59.9
HDL-C (mg dL^{-1}) *	44.7±5.1	40.3±5.2
LDL-C/HDL- C*	2.7±. 7	5.3±2.2
Triglycerides (mg dL^{-1}) *	164.4±26.5	176.6±68.8
Cholesterol (mg dL^{-1}) *	187.0±16.4	281.7±59.4
MDA (µmol L^{-1}) **	2.8±0. 6	5.4±1.4

Data presented as mean±SD, *Indicates significant difference between two group at the 0.05 level, **Indicates significant difference between two group at the 0.01 level
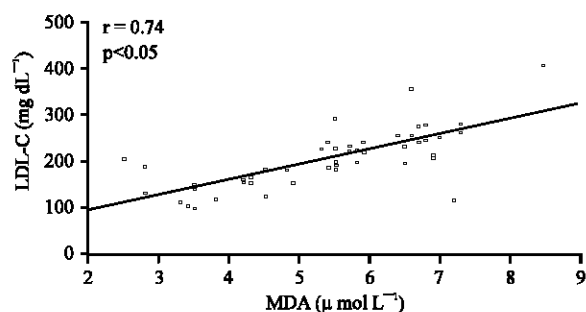
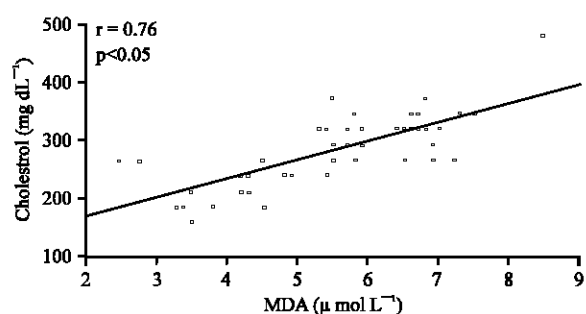Fig. 1: Relation between MAD and LDL-C in CAD patients

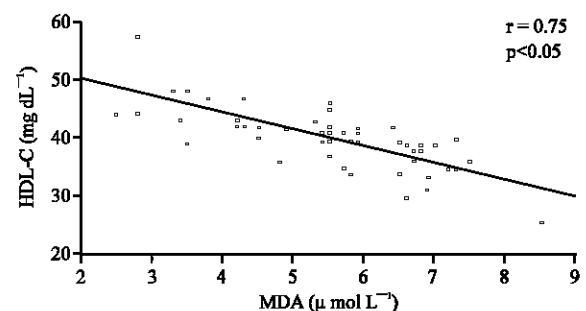Fig. 2: Relation between MAD and cholesterol in CAD patients

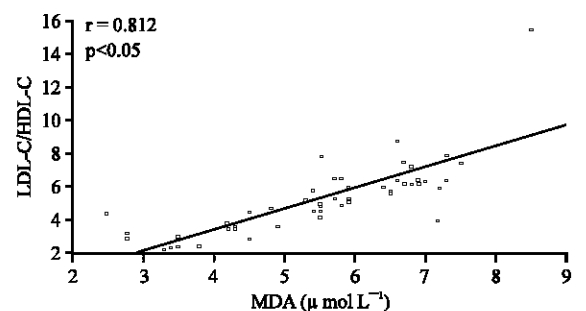Fig. 3: Relation between MAD and HLDL-C in CAD patients

Fig. 4: Relation between MAD and LDL-C/HDL-C in CAD patients

DISCUSSION

In the present study, we have determined several oxidative stress biomarkers in stable CAD patients and healthy volunteers. Our results showed that oxidative stress to be high in CAD patients despite being clinically stable and under medical treatment. Lipid peroxidation was studied extensively to investigate oxidative stress in patients with CAD during the last decades. Some investigators have found a relationship between elevated plasma lipid peroxides and LDL oxidation and the severity of coronary lesions (Riemersma *et al.*, 1991 and Craig *et al.*, 999). However, controversial data have also been obtained in patients with CAD (Croft *et al.*, 1992; Van de Vijyer *et al.*, 1998). In this study, plasma MDA levels were increased in stable CAD patients that are as diagnosis indicator these patients. The relationship between oxidative stress parameters and inflammatory species suggest their strong mutual involvement in atherosclerosis development that leads to CAD progression (Kotur *et al.*, 2007). The few human studies comparing stable coronary heart disease with healthy subjects yielded opposite results (Holvoet *et al.*, 1998; Ehara *et al.*, 2001; Toshima *et al.*, 2000). We observed increased plasma levels of LDL-C in patients with stable CAD. Our results agree with those obtained by Holvoet (Holvoet *et al.*, 1998; Toshima *et al.*, 2000). We found a significant correlation between LDL-C and MDA in plasma in the total study group which was even stronger in CHD patients. This might indicate that LDL-C concentration itself influences the oxidation of the lipoproteins. The possibility exists that LDL particles of CHD patients are more susceptible to oxidation than LDL from healthy volunteers (Rajman *et al.*, 1994) suggesting that the quality of LDL (i.e. LDL composition) influences the oxidation process.

CONCLUSION

In summary, the higher total cholesterol, triglycerides, LDL-cholesterol and MDA levels and lower HDL-cholesterol in compared to healthy controls, suggest an enhanced oxidative stress situation in CHD patients. The independent association of MDA levels with stable CHD indicates a possible role of these parameters as biomarkers of CHD patients. There is an increasing acceptance that oxidative modification of lipid and lipoprotein is a crucial step in the development of atherosclerosis. In view of the relationship between changes in the serum levels of lipids and lipoproteins and that of MDA in patients with CAD it was concluded that

simultaneous control of both dyslipoproteinemia and lipid peroxidation may be of equal importance in prevention of CAD. The independent association of MDA levels with stable CHD indicates a possible role of these parameters as biomarkers of CHD patients

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