Increased Malondialdehyde Levels in Coronary Heart Disease

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Abstract

Objective: To assess and compare the status of lipid peroxidation, both in control subjects and in coronary heart disease patients.
Methods: Serum total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and malondialdehyde levels were determined in 46 patients with coronary heart disease and 50 age matched control healthy subjects. 29 male coronary heart disease patients were divided into smoker (n=19) and nonsmoker (n=l0) groups, to observe the effect of smoking on lipid peroxidation in coronary heart disease patients.
Results: Malondialdehyde and lipid parameters were found significantly high (P< 0.001) with the exception of high-density lipoprotein cholesterol which was significantly low (P< 0.001) in coronary heart disease patients. Smokers with coronary heart disease showed significantly increased (P< 0.025) malondialdehyde levels as compared to nonsmokers with coronary heart disease.
Conclusion: Elevated serum levels of malondialdehyde indicate increase in the level of production of oxygen free radicals, suggesting their possible role in atherogenesis, leading to coronary heart disease (JPMA 50:261, 2000).

Introduction

Free radicals are reactive chemical species. All of the major class of biomolecules may be attacked by free radicals, but lipids are probably the most susceptible. Cell membranes are rich source of polyunsaturated fatty acids, and are readily attacked by oxidizing radicals. The oxidative destruction of polyunsaturated fatty acids is known as lipid peroxidation. It proceeds as a self--perpetuating chain reaction[1,2]. Free radical species derived from oxygen are named as reactive oxygen species (ROS)3 and the damage inflicted by these ROS is referred to as oxidative stress[4,5]. Oxidant stress is known to increase the production of free radicals. Both the oxidative stress and the production of ROS intracellularly, have been implicated in the pathogenesis of a variety of disease states[6,7], like cancer and cardiovascular disease[7].
Coronary heart disease (CHD) is a health problem of major proportion of population, and is a serious threat to life[8]. It is the single leading cause of death for adults worldwide, and is expected to be the leading cause of morbidity and mortality in the western world well into the 21st century[9]. CHD is the most common cause of lethal atherosclerotic disease[8]. The most important risk factors for CHD include hypertension, hypercholesterolaemia, cigarette smoking[10,11], diabetes mellitus and high-fat diet[11]. Increased serum cholesterol, low-density lipoprotein cholesterol (LDL-c) and low high-density lipoprotein cholesterol (HDL-c) concentrations are associated with increased risk of premature atherosclerosis[12,13], During past few years considerable amount of evidence, has accumulated indicating the crucial and causative role of free radicals in the pathogenesis of atherosclerosis[14,15]. While the mechanisms for atherosclerosis

are not completely understood, it is suggested that[16], free radical modification of low-density lipoprotein (LDL) within the arterial wall renders it more atherogenic, and has been implicated as an early step in atherogenesis[16-20]. According to oxidation theory[21], oxidation of LDL assists in the formation of foam cells, and contributes to various proatherogenic processes[21,22]
Oxidative activity of free radicals can be determined by measuring their oxidative products in a biological system[23]. Malondialdehyde (MDA) is the breakdown product of lipid peroxidation and its assessment is considered as a reliable marker of oxidative damage[24-26]. The purpose of our study was to assess and compare the level of MDA in patients with CHD and in control healthy subjects.

Patients and Methods

This study included 46 patients (32-61 years) with CHD and 50 control healthy subjects (32-56 years). Control subjects were non-smokers, having no cardiovascular, or any other disease. Diagnosis of CHD was based on documented evidence of attack of myocardial infarction (recent or past) or documented evidence of angina with subsequent sudden death or congestive heart failure[27]. The patients with CHD having had first attack of myocardial infarction with in the last week were included. Patients receiving lipid lowering drug therapy were excluded.
Male to female ratio was 28:22 for control and 29:17 for CHD groups respectively. The male patients having CHD were divided into two groups on the basis of smoking. The first group (n=l0) included CHD patients with no history of smoking in the past or present, whereas the second group (n=19) comprised of CHD patients who were smokers, with a history of smoking 20-25 cigarettes per day for the last 10-30 years. All female CHD patients were non¬smokers.
Blood samples were collected after an overnight fast and sera separated and stored at -20°C till analysis. Serum total cholesterol, triglycerides and HDL-c were measured by enzymatic calorimeteric method using the kits supplied by Bio-Systems, Spain. LDL-c was calculated by Friedwald formula[28].
Malondialdehyde content of samples was determined by the thiobarbituric acid (TBA) activity by using the method recommended by Buege and Aust[29]. MDA of the serum sample reacts with TBA to form a coloured pigment, the absorption of which is measured by spectrophotometer at 535nm.
The results are expressed as mean±S.E.M. and all statistical calculations were made by applying paired Student's t-test with the significance level at $P<0.05$.

Results

The serum lipid profile and MDA levels were measured in control subjects and in patients having CHD. The control and CHD groups were age-matched, with mean and S.E.M. of 45.08±0.98 years (control group) and 46.87±1.14 years (CHD group). The body mass index (BMI), and the serum levels of MDA, cholesterol, triglycerides and LDL-c were significantly high ($P< 0.001$), whereas the levels of HDL-c were significantly low ($P< 0.00$) in CHD patients as compared to the control subjects (Table 1).

Table 1. Serum lipid and Malondialdehyde concentrations in control and CHD patients.

	Control (n=50)	CHD (n=46)
Gender (Male/Female)	28/22	29/17
Age (years)	45.08±0.98	46.87±1.14 #
BMI (Kg/m^2)	23.9±0.31	29.22±0.43 ‡
Cholesterol (mmol/L)	4.42±0.1	6.59±0.1 ‡
Triglycerides (mmol/L)	1.43±0.02	2.03±0.02 ‡
HDL-c (mmol/L)	1.46±0.02	0.92±0.01 ‡
LDL-c (mmol/L)	2.31±0.1	4.75±0.1 ‡
MDA (mmol/L)	2.24±0.1	3.83±0.1 ‡

The CHD patients who were non-smokers and the group of CHD patients who were smokers, when compared showed no statistically significant difference in all the lipid#
P value non-significant as compared to control subjects.
P< 0.001 as compared to control subjects.
Table 2. Serum lipid and Malondialdehyde concentrations in CHD patients (Both non-smokers and smokers).
Table 1. Serum lipid and Malondialdehyde concentrations in control and CHD patients.
= P value non-significant as compared to CUD patients who were non¬smokers.
t = P< 0.025 as compared to CHD patients who were non-smokers.
parameters, except for the MDA levels, where a significant increase (P< 0.025) was observed in CHD patients who were smokers as compared to the CHD patients who were non-smokers

(Table 2).

Table 2. Serum lipid and Malondialdehyde concentrations in CHD patients (Both non-smokers and smokers).

	CHD patients (Non-smokers) (n = 10)	CHD patients (Smokers) (n = 19)
Age (years)	49±2.98	46.05±1.85 #
Cholesterol (mmol/L)	6.54±0.1	6.55±0.11 #
Triglycerides (mmol/L)	2±0.03	2.03±0.04 #
HDL-c (mmol/L)	0.93±0.03	0.9±0.02 #
LDL-c (mmol/L)	4.69±0.1	4.73±0.11 #
MDA (mmol/L)	3.57±0.14	3.91±0.11 †

Discussion

Coronary heart disease (CHD) is caused by long-term deposition of lipids in coronary arteries, which lead to atherosclerosis and necrosis of the heart tissue3°. The basic lesion of atherosclerosis is the intimal plaque. The development of a plaque involves, accumulation of lipid (both within macrophages and free in the tissues), proliferation of smooth muscle cells, and the formation of collagen. All these processes lead to the formation of a plaque, which consists of a core of extracellular lipid contained in a fibrous collagenous capsule. Disruption of the atherosclerotic plaque and the superimposed formation of platelet-rich thrombus, produces subtotal or total occlusion of coronary circulation. This leads to myocardial ischaemia[31]. Rapid restoration of the blood flow to the ischaemic myocardium lessens cardiac damage and improves the early and long-term morbidity and mortality[32]. Myocardial ischaemia can result from a number of causes, as atherosclerosis or thromboembolism, or can be produced during surgical interventions such as, percutaneous trans lum ina coronary angiop lasty, coronary artery bypass or transplantation. Whatever is the source of ischaemia, consequences are always the same, i.e., lack of oxygen to the myocardium and a lack of suitable substrate for metabolism[33]. Oxygen free radicals have been implicated in cardiac ischaemic injury. These free radicals (superoxide anions and hydroxyl radicals) are produced in the body by reduction of oxygen. In normal circumstances they are removed by the different scavenger systems present in blood and tissues. In case of myocardial ischaemia, which can lead to myocardial infarction, excessive free radicals may be generated[34]. Pucheu et al[35] suggested that measurement of malondialdehyde is a good marker of radical stress during reperfusion of the ischaemic myocardium, and also showed significantly increased malondialdehyde concentrations in group of CHD patients who were subjected to intravenous thrombolysis than those who had not been subjected to thrombolysis. Oen and colleague's[36] showed significantly increased levels of lipid peroxides in patients suffering from coronary heart disease as compared to the control subjects. Belch et a and Dincic

et al[37] showed evidence -of increased free radical activity in patients with myocardial ischaemia than the control subjects. Our results also show significantly increased concentrations of malondialdehyde, as an index of lipid peroxidation, in CHD patients.

Cigarette smoking adversely affects the lipid profile, leading to lower levels of HDL-c and higher levels of LDL¬c and triglycerides. Production of oxygen free radicals is increased with smoking, which may play a role in atherosclerosis, leading to CHD. In general, smokers have about twice the risk of developing CHD as do nonsmoker's[38]. Wang and associates[39] studied healthy men (smokers and nonsmokers) and showed increased level of oxygen free radicals in smokers as compared to nonsmokers. We studied smoker and nonsmoker patients with CHD, and found significantly elevated level of oxygen free radicals in smokers with CHD than nonsmoker CHD patients.

Wu et al[30] reported no significant differences in total cholesterol, triglycerides and LDL-c concentrations in both control and CHD patients. However, he found lower levels of HDL-c in patients with CHD. Hargreaves and co-workers[40] found no relationship between serum total cholesterol concentration and CHD. He also showed no significant change in the total cholesterol levels of subjects with and without CHD whereas, the HDL-c concentrations were significantly lower and LDL-c concentrations were significantly higher in patients with CHD as compared to control subjects. He pointed out that both increased LDL-c and decreased HDL-c contribute to the development of Cl-ID. Sandkamp and colleagues[41] reported significantly elevated levels of total cholesterol, LDL-c and decreased levels of HDL-c in patients with CHD as compared to control subjects. Our results also show significantly increased concentrations of total cholesterol, LDL-c and significantly decreased concentrations of HDL-c in CHD patients than in control subjects.

In conclusion, our study demonstrates a significant relationship between elevated level of malondialdehyde and CHD. Elevated levels of malondialdehyde indicate increase in the level of production of oxygen free radicals, suggesting their possible role in atherogenesis, leading to coronary heart disease.

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