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Malondialdehyde as an Indicator of Ischaemic Heart Disease

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Abstract

Lipids are the most involved class of molecules among the many targets of oxidative stress. Lipid oxidation produces a number of secondary products. The principal and most studied product of polyunsaturated fatty acid peroxidation is malondialdehyde (MDA). Beside being a highly toxic molecule, malondialdehyde is also considered as a marker of lipid peroxidation. This study was intended to determine the lipid endoperoxide levels of some Turkish patients with ischaemic heart disease findings at coroner angiography and verify whether malondialdehyde could be an indicator of ischaemic heart disease. For this purpose lipid peroxide levels of the control and test groups were measured with spectrophotometric method which is based on the reaction between malondialdehyde and thiobarbituric acid. Statistical analysis of our results revealed a significant difference between the mean serum lipid peroxide levels of the patiens and the control group being 3.74±0.44 nmol/ml vs 2.72±0.53 nmol/ml respectively, and p=0.258. According to these results we concluded that presence of MDA in serum at certain levels may predict the insurgence of vascular pathologies.

Key Words: Oxidative stress, malondialdehyde, lipid peroxidation, ischaemic heart disease

Introduction

Oxidative stress has been related to the several chronic diseases and plays a role in many of the pathophysiologies associated with cardiovascular diseases such as atherosclerosis (1) and the long-term complications of diabetes (2) and aging (3). Of the many biological targets of oxidative stress, lipids are the the most involved class of biomolecules. These products are mainly aldehydes, with the ability to provoke oxidative damage (4).

A growing body of evidence suggests that many of the effects of vascular dysfunction on cardiovascular diseases are mediated by products of nonenzymatic reactions, such as peroxidative degradation of polyunsaturated fatty acids (lipid peroxidation) (5-7) and glucose-protein or glucose-lipid interactions (glycation) (8), and oxidative modification of amino acids (amino acid oxidation). These reactions lead to the formation of unstable, reactive aldehydic intermediates that readily form intra- and inter-molecular covalent adducts with various biomolecules, such as proteins and phospholipids (9,10).

Lipid peroxidation proceeds by a free radical chain reaction mechanism and yields lipid hydroperoxides as major initial reaction products. Subsequently, decomposition of lipid hydroperoxides generates a number of breakdown products that display a variety of damaging actions. A number of reactive aldehydes derived from lipid peroxidation have been implicated as causative agents in cytotoxic processes initiated by the exposure of biological systems to oxidizing agents.

Glycation also generates highly reactive carbonyl compounds from the glucose moiety of the intermediates through multiple dehydration and rearrangement reactions. Compared to free radicals, the aldehydes are stable and can diffuse within or even escape from the cell and attack targets far from the site of the original event. Some of these aldehydes have been shown to exhibit mild reactivity with various biomolecules, including proteins, DNA, and phospholipids, generating stable products at the end of reactions that are thought to contribute to the pathogenesis of diabetes and vascular diseases such as atherosclerosis. In addition, it has been found that some of the aldehydes are responsible for the effects of lipid peroxidation and glycation on signaling/transcription regulation. This finding suggests that reactive aldehydes may play a role as a regulatory molecules of vascular dysfunction (11-14).

Polyunsaturated fatty acids in chlesterol esters, phospholipids, and triglycerides are subject to free radical -initiated oxidation and can participate in chain reactions that amplify damage to biomole-

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cules. A key feature of lipid peroxidation is the breakdown of these polyunsaturated fatty acids to yield a broad array of smaller fragments, three to nine carbons in length, including aldehydes.

The important reactive aldehydes orginated from lipid peroxidation are ketoaldehydes, including malondialdehyde (MDA) and glyoxal. MDA is in many instances the most abundant individual aldehyde resulting from lipid peroxidation and has been shown to disturb aminophospholipid organization in the membrane bilayer of eryhrocytes (10).

Atherosclerotic lesions of varying severity from human aorta contain material recognized by antibodies raised against the lipid peroxidation-specific aldehydes (9). The chronic inflammatory-fibroproliferative process of atherosclerosis is triggered or modulated by various substances accumulating in the damaged vessel wall, which have been shown to exert specific effects on transcriptional systems or signal transduction cascades. One of these substances is oxidized LDL that has been found to display both positive and negative effects on gene expression. The spesific substances such as reactive aldehydes within the oxidized LDL molecule have been suggested to be responsible for its effects on signaling/transcriptional regulation (15,16).

The aim of the present study is to measure the lipid endoperoxide levels of some Turkish patients with ischaemic heart disease findings and determine whether malondialdehyde could serve as a marker for the prediction of ischaemic heart disease.

Materials and Methods

In this study, serum lipid peroxide and cholesterol levels of 20 patients with ischaemic heart disease findings at coroner angiography having mean age of 49 and the normal healthy individuals having mean age of 50 were compared.

Following an overnight starvation, 10 ml of venous blood was collected from the patiens and the control group and centrifuged at 3500 rpm for 15 min. Biochemical blood parameters of two groups were recorded using an autoanalyzer (Roche, Cobos Mira Plus).

Lipid peroxide measurements were done according to the method employed by Ohkawa and co-workers (17). This spectrophotometric method is based on the reaction between malondialdehyde and thiobarbituric acid (TBA), which ends up with a coloured product (18). Intensity of the colour is proportional to the amount of the end product. Sample tubes of the patient and control groups were prepared as indicated below:

	Blank, ml	Sample, ml
SDS (8.1 %)	0.2	0.2
Acetic acid (20 %; pH 3.5)	1.5	1.5
TBA (0.8%)	1.5	1.5
Serum	--	0.5
Bidistilled water	1.1	0.6

Following an incubation at 90°C for 1 h, the tubes were cooled under running water and centrifuged at 4000 rpm for 10 minutes. Clear supernatants were collected and absorbance measurements were carried out on a spectrophotometer (Boehringer Mannheim 4010) at 532 nm. A stock solution containing 20 nm/ml of 1,1,3,3' tetraacetoxypropane ($C_{11}H_{24}O_4$; 97%) was used for the preparetion of standards. Standards contained 1.25, 2.5, 5, and 10 nmol/ml of 1,1,3,3' tetraacetoxypropane. The mean absorbances produced by the standards were plotted vs the 1,1,3,3' tetraacetoxypropane concentrations. The concentrations of the lipid peroxide in the original samples were obtained using the standard curve in units of nmol/ml.

SDS, TBA, and 1,1,3,3' tetraacetoxypropane were obtained from Sigma, while acetic acid was from Merck.

Student -t test was employed for the statistical evaluation of the differences between means, while r-correlation test was used for the correlation analysis (19).

Results and Discussion

According to the statistical comparisons, it seems that no meaningful association exists between serum lipid peroxide and cholesterol levels of the patients because of the determined r and p values (r= 0.4198; p= 0.065). On the other hand, mean lipid peroxide and cholesterol levels of patients were somewhat higher than those of the control group (3.74± 0.44 nmol/ml vs 2.72±0.53 nmol/ml, p= 0.258 for lipid peroxide; 202.15±47.98 mg/dL vs 171.50±25.63 mg/dL, p=0.374 for cholesterol). Serum lipid peoxide levels of the patients has revealed a statistically significant increase. This conclusion is also true for cholesterol levels. In our another study, it was also found that there is a meaningful inverse association between lipid per-

oxide and serum selenium levels of these patients (r=-0.6137; p=0.004) (20).

Many agents have apperared to be potential sources of intracellular oxidative stress. The lipid peroxidation-derived aldehydes have been shown to induce intracellular peroxide production and our results is consistent with previous studies showing that increased MDA levels is an indication of induced lipid peroxidation (21-27), and increased lipid peroxidation is in relation with coronary heart disease (28-33).

It is therefore likely that reactive aldehydes tend to trigger the formation of reactive oxygene species or are oxidants themselves and potentiate oxidative stress in the cells. Excess oxidative stress is toxic exerting cytotoxic effects, causing membrane damage, and activating pathways of cell death (apoptosis and/or necrosis) (34).

MDA is also related to innate genotoxicity. This molecule may be derived by lipid peroxidation, but it can also be generated by physiological metabolisms (35), and a highly mutagenic product. MDA is therefore, more than a simple marker, but a clear alarm of high risk of mutation (36-38).

Another aspect of MDA is its toxicity towards the cardiovascular system. MDA action on lipoproteins has been related to atherogenesis and, probably its reactivity towards collagen is responsible for the stiffening of the cardiovascular tissue (2).

In our opinion, further studies are needed to observe whether the presence of this molecule at certain levels may predict the insurgence of vascular pathologies. Thus, for its significance as a marker of lipid peroxdation and for the several insulting actions exerted against health, the assessment of MDA with new reliable assays and strategies to reduce oxidative stress in nutritional and medical trials should be encouraged.

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