LIPID PEROXIDATION AND CORONARY HEART DISEASES
Lipid peroksidasyonu ve koroner kalp hastalıkları

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Summary: *In order to investigate lipid peroxidation suggested an etiological factor in coronary heart diseases (CHD), lipid peroxidation was determined in plasma samples and erythrocyte suspensions obtained from patients with acute myocardial infarction (AMI), angina pectoris (AP), and healthy controls. The degree of lipid peroxidation was appreciated according to malondialdehyde (MDA) formation, accepted as an index of lipid peroxidation. When compared to controls, MDA levels were found to be higher in plasma and erythrocytes of total patients and also separately in patients with AP and AMI. But the increase in MDA levels in both plasma and erythrocytes of patients with AMI was higher than those of AP group. When regression analysis were performed, it was seen that there was a positive correlation between plasma-and erythrocyte-MDA values in both patient groups and healthy controls. It was concluded that lipid peroxidation may be an important factor in appreciation of CHD.*

Key Words: *Lipid peroxidation, Acute myocardial infarction, Angina pectoris*

Özet: *Bu çalışmada, koroner kalp hastalıklarında (KKH) etiyolojik bir faktör olarak öne sürülen lipid peroksidasyonunu araştırmak amacıyla, akut miyokard infarktusu (AMI)'lu ve anjina pektoris (AP)'li hastalardan ve sağlıklı kişilerden elde edilen plazma örnekleri ve eritrosit süspansiyonlarında lipid peroksidasyonu tayin edildi. Değerlendirme, lipid peroksidasyonunun bir endeksi olarak kabul edilen malondialdehit (MDA) üzerinden yapıldı. Kontrollerle karşılaştırıldığında, total hasta grubunda ve ayrı ayrı AP'li ve AMI'lü hastalarda daha yüksek bulundu. Fakat, hem plazma hem de eritrosit MDA seviyelerindeki artış, AP'li gruba göre, AMI'lü hasta grubunda daha yüksekti. Regresyon analizleri yapıldığında, her iki hasta ve sağlıklı kontrol gruplarında, plazma ve eritrosit-MDA değerleri arasında pozitif bir ilişki olduğu tespit edildi. KKH'ların değerlendirilmesinde, lipid peroksidasyonunun önemli bir faktör olabileceği sonucuna varıldı.*

Anahtar Kelimeler: *Lipid peroksidasyonu, Akut miyokard infarktüsü, Anjina pektoris*

Among coronary heart diseases (CHD), acute myocardial infarction (AMI) and angina pectoris (AP) are the main disease patterns, due to high frequency and death rate (6,19). For many years several investigates are performed for early diagnosis of CHD and suggested that coronary atherosclerosis is the principal cause of these diseases (18). Although atherosclerotic lesion is believed to originate from intimal injury through an unidentified event, the importance of intimal smooth-muscle proliferation as the key event in the development of the advanced lesions of atherosclerosis is emphasized (18, 21).

On the other hand, it has been known that oxygen free radicals are important mediators of several forms of tissue damage (7). Although the role of oxygen free radicals in atherogenesis is not definitely demonstrated (10), the results of recent studies have suggested involvement of free radical induced lipid peroxidation in the pathogenesis of atherosclerosis (4,16,24); therefore, lipid peroxidation may play a significant role in the initation and progression of atherosclerotic plaqe, and then lead to CHD.

In the present study lipid peroxidation was determined in plasma and erythrocytes of patients with AMI and AP to provide clinical support to earlier

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data indicating that lipid peroxidation is an important factor in CHD, and also to investigate whether lipid peroxidation may be represented as an useful index of the severity of CHD.

METHODS

Subjects: A total of 40 consecutive patients (34 men and 6 women) with AMI and AP, aged 38 to 73 years (mean age 55.3 years), admitted to the intensive cardiac care unit of Erciyes University Faculty of Medicine were included in the present study. 20 of them were AMI patients (18 men and 2 women; mean age, 58.8 years; range, 38 to 73 years); and the others were AP patients (16 men and 4 women; mean age 52.9 years; range, 38 to 69 years) presenting with clinical and electrocardiographic evidence of myocardial ischemia without acute infarction. The diagnosis of AMI was suspected on clinical findings and confirmed by the presence of appropriate new-onset electrocardiographic changes and elevated creatine kinase MB isoenzyme (19). Presentation to the hospital occured within 8 hours of the onset of chest pain. In both groups criteria for exclusion from the study were age (above 75 years), liver and kidney diseases, primary coagulopathy, chronic malignant or systemic inflammatory disease.

The normal control group consisted of 20 healthy adult volunteers (17 men and 3 women; aged 36 to 68 years; mean age, 54.2 years), without clinical or laboratory evidence of any disease.

The time from onset of chest pain to blood collection was similar in both patient groups; at inital presentation, before treatment with any medication. Heparinized blood obtained from patients and controls was kept at 4 °C for 30 minutes, then centrifuged at 1500 x g for 15 minutes to separate plasma and erythrocytes. Plasma samples were stored at -70 °C until analysis; the packed cells were washed three times with ice-cold 154 mM NaCl. The susceptibility of erythrocytes to lipid peroxidation was determined immediately.

Lipid Peroxidation Analysis: The degree of lipid peroxidation was assessed by methods measuring malondialdehyde (MDA) levels in plasma samples and erythrocytes. The principle of the measurements is based on measuring the concentration of the pink chromogen compound which forms when MDA couples to thiobarbituric acid (TBA) (26).

A spectrophotometric assay improved by Stocks and Dormandy (22) and modified by Jain(8) was used to determine the susceptibility of erythrocytes to lipid peroxidation. Hemoglobin concentrations of erythrocyte suspensions were also measured with Drabkin's reagent. The MDA levels of groups were expressed in nmol MDA per gram of hemoglobin (nmol MDA/g Hb).

Plasma MDA levels were assayed by a spectrophotometrical method (25) which was partly modified in our laboratory, without the high pressure chromatography seperation step. Briefly, the mixture of plasma, phosphoric acid and TBA was boiled at 100 °C for 60 minutes. After cooling, equal volumes of n-butanol were added to the medium and mixed vigorously. The butanol-phase was separated by centrifugation and absorbance was measured at 532 nm. The appreciation was performed using a standard curve obtained from the reaction between varying MDA concentrations and TBA as described above. The CV value of the modified method presented was found to be 3.54 % in our laboratory. MDA levels were expressed as nmol MDA per ml (nmol MDA/ ml).

Statistical Analysis: To test the differences between groups, analysis of variance (ANOVA) were applied; and F value was found to be significant; differences between means were then analyzed using post-ANOVA (Scheffe's procedure) test. The Student's t test was also used in statistical comparison of the data from total patients and controls (3). Probability values equal to or less than 0.05 were considered significant. In addition, correlation coefficient was determined by linear regression analysis performed between plasma-and erythrocyte-MDA levels, in all groups (3).

RESULTS

MDA levels measured in plasma samples and erythrocytes obtained from the healthy controls, and patients with AP and AMI are represented in Table

I. When compared to controls, MDA levels were found to be higher in plasma and erythrocytes of total patients ($p<0.001$) and also separately in patients with AP ($p<0.05$) and AMI ($p<0.01$). But the increase in MDA levels in both plasma and erythrocytes of patients with AMI was higher than those of AP group ($p<0.01$).

When regression analysis were performed, it was seen that there was a positive correlation between plasma-and erythrocyte-MDA values in both patient groups (r: 0.883 for AP patients, $p<0.001$; r: 0.826 for AMI patients, $p<0.001$) and healthy controls (r: 0.791, $p<0.001$) (Table 2).

Table 1. Plasma-and Erythrocyte-Malondialdehyde (MDA) Levels of Healthy Controls, and Patients with Angina Pectoris (AP) and Acute Myocardial Infarction (AMI).

		Lipid Peroxidation	
Groups	n	Plasma (nmol MDA/ml)	Erythrocyte (nmol MDA/g Hb)
Control	20	1.804 ± 0.258	6.552 ± 0.978
Total patients	40	2.834 ± 0.910*	16.276 ± 8.476*
AP group	20	2.338 ± 0.650^{o}	11.291 ± 2.790^{o}
AMI group	20	3.330 ± 0.870$^{\bullet,a}$	21.263 ± 9.340$^{\bullet,a}$

MDA values are expressed as mean and standard deviation (X±SD).

Significances:

- *Total patients compared to controls (*: $p<0.001$; Student's t test)*
- *Between control and patient groups (Control vs AP group and control vs AMI group) (o: $p<0.05$; •: $p<0.01$; post-ANOVA test)*
- *Between patients with AP and AMI (a: $p<0.01$; post-ANOVA test)*

n: The number of subjects.

Table 2. The Correlation Between Plasma-and Erythrocyte-MDA values in Both Patient Groups and Healthy Controls

Groups	n	r	y= a+bx	p
Healthy controls	20	0.791	1.2 + 3.0 x	< 0.001
AP patients	20	0.883	2.4 + 3.7 x	< 0.001
AMI patients	20	0.826	-8.2 + 8.8 x	< 0.001

n: The number of subjects.

DISCUSSION

Evidence shows that toxic oxygen free radicals have been implicated as important pathologic mediators in many clinical disorders (2,7). There has been added interest in the concept that oxygen free radicals and subsequent peroxidative processes play a role in the pathogenesis of myocardial ischemia and infarction (5), which are generally originated from atherosclerosis (18).

Acording to the response-to-injury hypothesis of atherogenesis, "injury" to the endothelium is the initiating event in atherogenesis (18). Therefore, atherosclerotic lesions can be initiated and enhanced by substances that damage the arterial wall. A possible constant source of damaging compounds is the reaction of oxygen with poly-unsaturated lipids that are present in plasma and the arterial wall (2).

Although the role of oxygen free radicals in atherogenesis is poorly defined, there is both some direct and indirect evidence for a pathogenetic role of oxygen derived free radicals; and lipid peroxidation is now thought to be important in the pathology of atherosclerosis (16,17,21,24).

The key pathophysiologic process mediated by lipid peroxides in the coronary circulation might represent progressive vascular injury in vascular beds. This injury ultimately leads to a profound change in the mode of interaction of blood vessels and the blood, and also the endothelial and smooth-muscle cells (6).

When the lipid peroxides formed accumulate to a certain degree, they leak from the organ or tissue into the bloodstream; and the increased lipid peroxides in the blood primarily attack the endothelial cells of blood vessels and then intact organs or tissues as well. Even slight injury to the endothelial cells of the artery can initiate atherogenesis (27). On the other hand, due to the injury to the endothelium, platelets become aggregated and adhere to the sites of injury. This is known to be the initial event in the process of atherogenesis (24). Oxygen free radicals may contribute to acceleration of the atherosclerotic process by enhancing leucocyte chemotaxis and activation, and platelet deposition. Furthermore, oxidation of polyunsaturated fatty acids, important components of atherosclerotic plaque, can result in the formation of lipid peroxides which may damage arterial wall components such as protein, mucopolysaccarides, or result in the generation of additional peroxides.

Another important toxicological aspect of hydroperoxides is their role in the modulation of enzyme activities of the arachidonic acid cascade. Fatty acid hydroperoxides are intermediates in the biosynthesis of prostaglandins, leucotriens, and thromboxane. Regulation of the vascular prostacyclin/thromboxane ratio is considered an important factor in the development of several vascular diseases. Enzymes that are responsible for the synthesis of these substances are differentially inactivated by fatty acid hydroperoxides (5,6). Hence, the balance between lipid peroxide production and metabolism maintains an adequate level of prostacyclin which inhibits platelet aggregation and adherence. But increased levels of lipid peroxides may cause reorientation of arachidonic acid cascade towards intensified thromboxane synthesis, owing to the inhibitory effects of prostacyclin synthesis (10,16); and therefore, modulate neutrophil stimulation, superoxide production and platelet deposition (16).

On the other hand, although all cells contain an effective defence system against free radical overproduction, the actual activity differs largely from cell type to cell type. Thus, cell types that possess a rather low defence capacity such as in heart muscle, will be most susceptible to free radical overflow (10). Further, endothelial cells contain a large amount of ferritin, xanthine oxidase and cyclo-oxygenase activity; and are enriched in fatty acids with 5 and 6 double bonds (5,10). In addition, endothelial cell layer is constantly undergoing mechanical stress (10).

It has been well established that the actual toxicity of free radicals is dependent on the presence of free iron in the heart tissue; and xantine oxidase is able to mobilize iron from ferritin (10). Salonen et al (20) have found elevated plasma ferritin levels in patients with AMI and suggested that a high stored

iron level, as assessed by ferritin concentration in plasma, is a strong risk factor for AMI.

Piotrowski et al (16) have demonstrated an increase in lipid peroxidation in atherosclerotic human tissue, and Ledwozyw et al (11) have also shown that MDA levels are increased in plasma and in the arterial wall as well as there is existence of a positive correlation between them; and suggested that the increased MDA level is evidence of intensification of lipid peroxidation processes in patients with atherosclerotic lesions, which may be the cause of chronic stress for endothelial cells. Increased plasma lipid peroxide levels (in terms of MDA) in myocardial ischemia (9,15,24,27) and in infarction (12,13,29) were found in other resent studies. The level of lipid peroxides was also observed to be increased with the severity of the disease (9). Our results showing high plasma MDA levels in patients with AP and AMI are in good agreement with these studies. Moreover, that higher MDA levels in AMI than that of AP may show a relationship between MDA and the severity of CHD in the present study. All of these data strongly indicate that lipid peroxides increased in the blood initiate and promote atherogenesis; thus, they provide an useful information for the prognosis of CHD.

The consequences of increased plasma lipid peroxide levels are mostly observed in susceptible target tissues. Erythrocyte was an early model for studies of oxidative stress (2). It has been suggested that erythrocyte should be prone to oxidative reactions because of relatively high oxygen tensions, the presence of Hb, and plasma membrane rich in polyunsaturated lipids (1). In addition, abnormal susceptibility of erythrocyte lipids to peroxidation is believed to reflect a similar abnormality in other organs and tissues (23). It was reported that lipid peroxidation was enhanced both in the whole blood and erythrocytes in CHD (14). The increase in MDA levels in erythrocytes of patients with AP and AMI was also observed in our study, depending on the severity of the disease. These results suggest that erythrocyte membrane integrity is impaired in these patients and lipid peroxides are involved in damaging erythrocytic membrane structure and function, thus promoting the progression of CHD.

These data, the existence of a strong positive correlation between plasma-and erythrocyte-MDA values in both patients with AP and AMI, indicate that the increase in lipid peroxidation in erythrocytes similar to that of plasma may reflet a profound damage and progressive vascular injury in those patients.

In conclusion, lipid peroxidation may play a dominant role in the development and the severity of various pathological events associated with myocardial ischmeia and infarction.

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