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Homocysteine and lipid peroxidation markers in patients with coronary heart disease

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Several potential mechanisms of homocysteine (Hcy) influence on the pathogenesis of atherosclerosis including lipid peroxidation are described.

The aim of this study was to determine a possible relationship between blood serum Hcy levels and lipid peroxidation in patients suffering from coronary heart disease of various stages.

Serum Hcy level was determined by the fluorescence polarization immunoassay (FPIA) method with an IMx ABBOTT analyser. Malondialdehyde (MDA) concentration and catalase (CAT) activity were measured spectrophotometrically. Patients were grouped into: 1) individuals suffering from acute myocardial infarction (AMI, n = 32); 2) patients with unstable angina pectoris (USAP, n = 23); 3) individuals with stable angina pectoris (SAP, n = 25). Eighteen healthy volunteers comprised the control group.

The results showed that there were no differences in Hcy levels among all study groups, although the level of the circulating marker of oxidative stress – MDA – was elevated in patients with AMI and with SAP if compared with the control group (3.22 ± 1.27, 3.74 ± 0.73, 2.73 ± 0.29 μmol/l, respectively). A weak positive correlation was found between blood Hcy and MDA levels in patients with SAP (r = 0.13). Total Hcy concentration was shown to have a weak tendency to correlate with blood serum CAT activity in patients with USAP (r = 0.27) and SAP (r = 0.15). Patients in all groups showed a higher CAT activity than the control individuals (53.03 ± 22.47, 52.32 ± 18.43, 52.77 ± 16.41, and 30.63 ± 11.98 nmol/l/min, respectively).

The adaptive increase of antioxidative enzyme activity (in the case of catalase) is suggested to be related to oxidative stress as a result of homocysteine action in lipid peroxidation.

Key words: atherosclerosis, lipid peroxidation, malondialdehyde, catalase activity, homocysteine

INTRODUCTION

Mortality from coronary heart diseases is higher in Lithuania than in West European countries (Fig. 1). Traditional risk factors cannot explain these differences in mortality if taken separately. Therefore, a number of studies are directed to determine additional factors that cause such a high mortality rate and to identify new diagnostic markers that could enable to detect the atherosclerotic process at its initial stage.

In recent years, there has been an increased number of reports concerning Hcy, a recognized important risk factor for development of atherosclerosis in coronary, peripheral, and cerebral arteries [1, 2]. Several studies have demonstrated elevated plasma total Hcy levels in patients with coronary heart disease compared with controls. This elevation was suggested to be an important independent risk factor for coronary artery disease and myocardial infarction [3, 4]. No relationship was found between serum Hcy concentration and classical cardiovascular risk factors. Tanriverdi et al. [5] have reported that plasma Hcy may have a strong association with the genesis of coronary heart disease. It has been proved that the cardiovascular disease of adults starts in childhood. There is evidence indicating that maternal hypercholesterolemia during pregnancy is associated with a greatly increased fatty streak formation in human fetal arteries and accelerated progression of atherosclerosis in childhood [6]. The results obtained by Szymczak et al. [7] indicate that in hypercholesterolemic children with a positive family history for coronary heart disease, the concentration of Hcy can be considered as a separate predictive risk factor for premature cardiovascular disease. Different authors have demonstrated that even moderate hyperhomocysteinemia is associated with an increased risk of premature vascular disease in coronary, cerebral and peripheral arteries [1].

However, the mechanisms of vascular disease induction by hyperhomocysteinemia are not well defined, despite some evidence of a certain role of reactive oxygen species (ROS). It has been proposed that during oxidation of the sulfhydryl group of Hcy hydrogen peroxide is formed, which promotes oxidative stress and lipid peroxidation. Hydrogen peroxide may also have a direct harmful effect on vascular endothelium [8]. The results of Hagar [9] suggest that hyperhomocysteinemia aggravates my-

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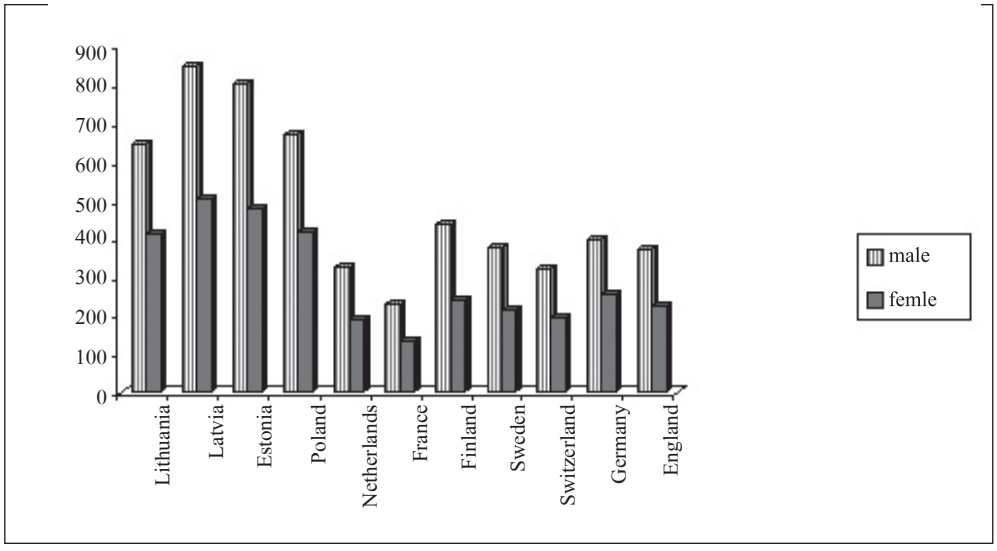

Fig. 1. Mortality rate from coronary heart disease in East and West European countries

ocardial infarction via the oxidative stress mechanism and that lowering the Hcy level can ameliorate the detrimental effects of hyperhomocysteinemia and reduce the risk of myocardial infarction. In addition, studies suggest that Hcy can induce oxidative modification of low-density lipoproteins [10]. This suggestion is relevant because lipoprotein oxidation is supposed to play a key role in the development of atherosclerosis [11–13].

The purpose of this study was to determine a possible relationship between blood serum levels of Hcy and MDA as a marker of lipid peroxidation in patients with coronary heart disease throughout the acute phase of acute coronary syndromes such as unstable angina, myocardial infarction, as well as stable angina.

MATERIALS AND METHODS

Eighty patients aged between 35 and 76 (mean 58.8 ± 1.4 years) with coronary heart disease, admitted to the Vilnius University Hospital, were included in the study. Of them, 55 were hospitalised with acute coronary syndrome and 25 suffered from stable angina pectoris (Fig. 2). Patients were grouped into: 1) individuals suffering from myocardial infarction (AMI, n = 32); 2) patients with unstable angina pectoris (USAP, n = 23); 3) individuals with stable angina pectoris (SAP, n = 25). Eighteen healthy volunteers were the studied in control group. The mean age of the control group was 35.8 ± 2.3 years.

Serum homocysteine (Hcy), malondialdehyde (MDA) levels and catalase (CAT) activity were determined both in patients and control groups. MDA (analysed by the thiobarbituric acid test) concentration and CAT activity (based on the ability of hydrogen peroxide to form a stable stained complex with molybdenum salts) were measured spectrophotometrically [14, 15]. Plasma total Hcy level was estimated by the fluorescence polarization immunoassay (FPIA) method with an IMx ABBOTT analyser [16] at the Center of Laboratory Diagnostics, Vilnius University Hospital "Santariškių klinikos".

All experimental data were processed employing the software STATICTICA and Microsoft Excel. All data are presented as mean ± deviation. Statistically significant difference was set as $P < 0.05$.

RESULTS AND DISCUSSION

Epidemiological studies have linked plasma Hcy elevation with coronary heart diseases, if to compare the patients and con-

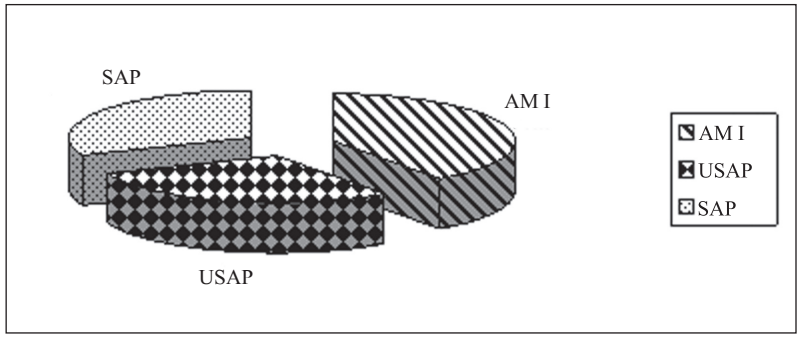

Fig. 2. Study groups of coronary heart disease patients

trol groups. The dynamics of Hcy concentration in the abovementioned diseases differs as reported in various studies. Our results showed that there were no statistically significant differences in Hcy levels while comparing all three patients' groups with controls (Table).

Table. **Total homocysteine (Hcy), malondialdehyde (MDA) levels and catalase (CAT) activity in patients and control group (mean ± SD)**

Group	n	Hcy µmol/l	MDA µmol/l	CAT nmol/l/min
Control	18	10.76 ± 2.55	2.73 ± 0.29	30.63 ±11.98
AMI	32	13.07 ± 5.35	3.22 ± 1.27	53.03 ± 22.47*
USAP	23	12.05 ± 3.89	2.82 ± 0.92	52.32 ± 18.43*
SAP	25	12.26 ± 3.56	3.74 ± 0.73 *	52.77 ± 16.41*

* P < 0.05.

The results of Jonasson et al. [17] showed that 36% of coronary heart disease patients had normal Hcy levels. Cavalca et al. [18] found a moderate increase of Hcy to be associated with cardiovascular diseases, but it was noted that Hcy at the detected values (10.2 µmol/l) could not be considered completely responsible for oxidative damage. A single prospective study on the relation of Hcy with vascular diseases found no association between this parameter and myocardial infarction or stroke. But dietary consumption of folic acid was higher in this population and, as a result, Hcy concentration was determined to be much lower than in other studies [19]. Serum Hcy level is especially dependent on folate nutritional status. Folic acid lowers plasma Hcy [20].

Investigation of lipid peroxidation *in vivo* and *in vitro* shows that Hcy exerts a pro-oxidant effect. Domagala et al. [21] reported a significant relationship between plasma Hcy level and lipid peroxidation detected as an increase in thiobarbituric acid reactive substances (TBARS) in men and women during hyperhomocysteinemia induced by oral methionine load. Similar results were obtained with animal models. Different experimental studies have demonstrated that hyperhomocysteinemia (as determined by the oral methionine loading test) is associated with enhanced lipid peroxidation (as evaluated from plasma conjugated dienes, lipoperoxides and TBARS indices) [22]. Histological analysis of the aorta showed typical atherosclerotic changes. The results obtained in our study indicate that the level of a circulating marker of oxidative stress – MDA – is elevated in patients with AMI and with SAP compared to the control group (Fig. 3A). This elevation was more significant in SAP patients ($p < 0.01$). Increased serum MDA concentration is an index of increased lipid peroxidation. Malaia et al. [23] have found that the time-course of MDA is characterized by a constant increase reaching the maximal values at the height of the destructive phase of myocardial infarction. Also, a remarkable increase of MDA level has been reported in blood red cells of patients with acute coronary syndromes [24]. Cardiac MDA was significantly increased after izoprenaline-induced myocardial infarction [9]. According to Domanski et al. [25], elevated MDA levels in patients with acute myocardial infarction may reflect secondary disorders of cellular metabolism and late appearance of degradation products of lipid peroxides. The results of Thiele et al. [26] indicate that MDA level in the serum of SAP patients is increased only slightly. Kostner et al. [27] confirmed that MDA concentration was significantly higher in USAP patients as compared with SAP patients and the control group. Patients of all groups in the present study had a higher CAT activity than the control individuals (Table).

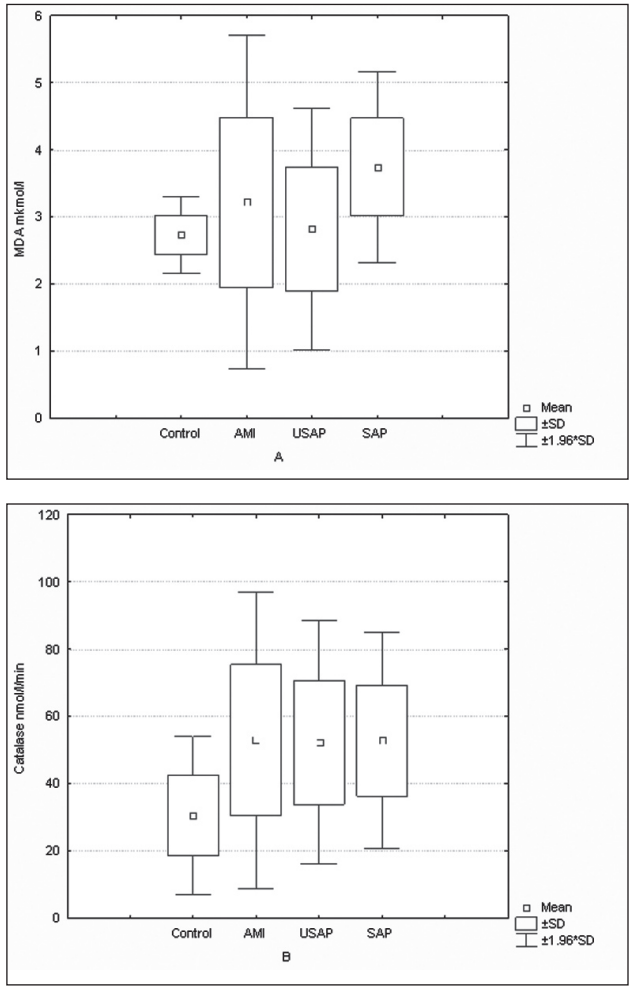

Fig. 3. Serum MDA level (A) and catalase activity (B) in coronary heart disease patients

In addition, our results indicate that the total concentration of Hcy has a weak positive correlation with blood serum MDA in SAP patients ($r = 0.13$) (Fig. 4B). This is consistent with reports of other authors. Ventura et al. [28] found a significant positive correlation ($r = 0.47$, $P < 0.05$) between Hcy and MDA in human plasma. There was no apparent relation between serum Hcy and MDA in other groups (Fig. 4A).

Data of Moat et al. [29] show that the activity of erythrocyte superoxide dismutase and plasma glutathione peroxidase was elevated in samples with plasma total Hcy > 20 µmol. Results suggest that the elevated plasma total Hcy represents the state of oxidative stress, resulting in an adaptive increase of antioxidant enzyme activity in the circulation. Another research revealed that a high methionine diet resulted in a significant increase of aortic antioxidant enzyme activity [30]. High-dose methionine

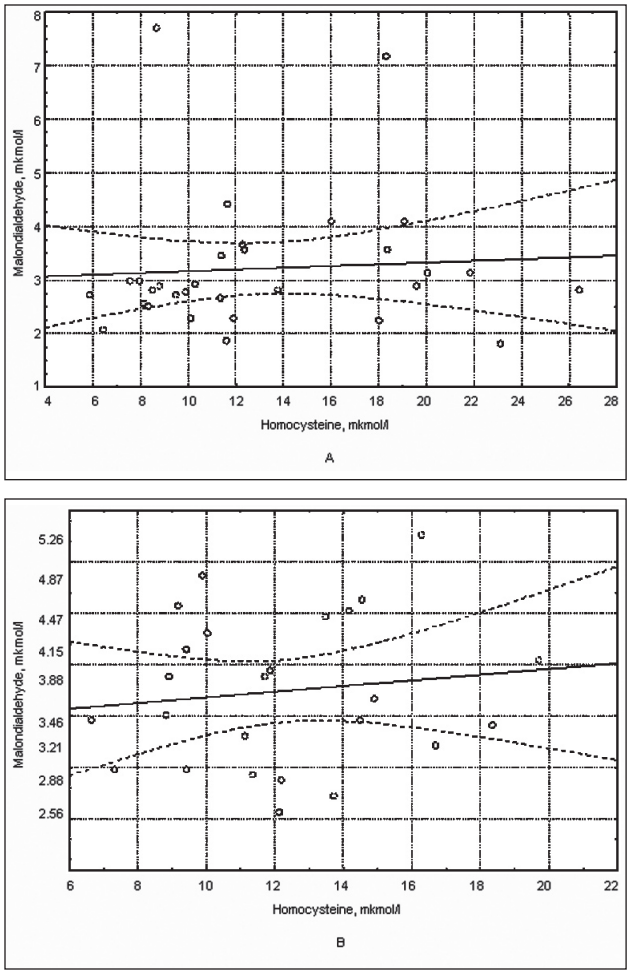

Fig. 4. Correlation between plasma Hcy and MDA level in patients suffering from AMI (A) and with SAP (B)

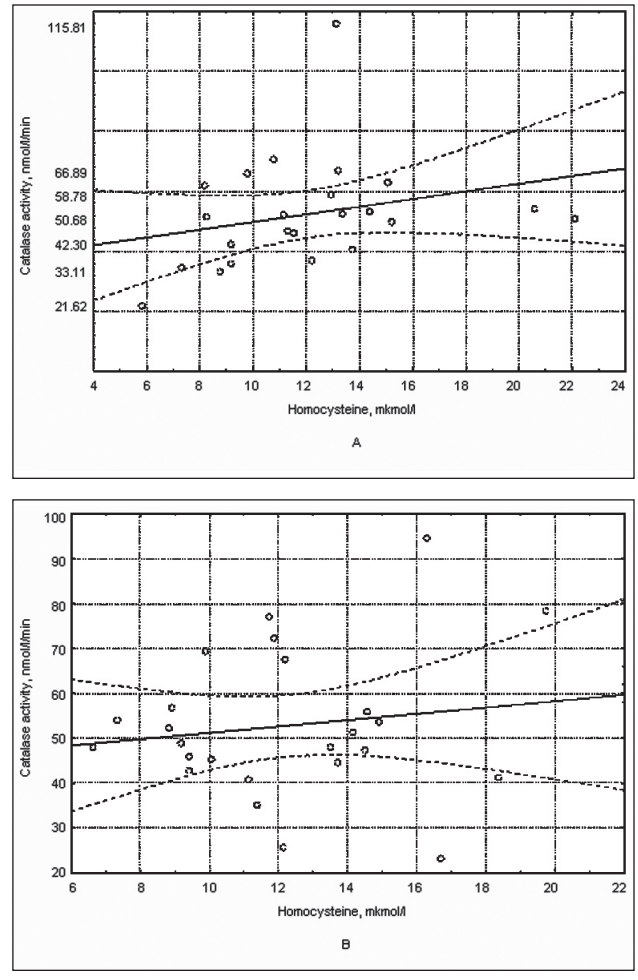

Fig. 5. Correlation between Hcy level and CAT activity in patients with USAP (A) and SAP (B)

administration significantly increased Hcy concentration [31]. However, the total plasma antioxidant capacity was decreased [28]. Our results showed a significant elevation of CAT activity in patients as compared with the control group, but no significant differences were determined while comparing all groups of patients (Fig. 3B). An increased antioxidative defence in plasma may protect against lipid peroxidation. A significant decrease in CAT activity was found in patients with AMI before and after thrombolytic therapy [30]. According to L. Galuzienė et al. [32], the total antioxidant status in patients with ischaemic heart disease throughout the acute phase of acute coronary syndrome was slowly decreasing. CAT activity in the blood serum of some patients increased with increasing Hcy levels. The results obtained in our study indicate that the total Hcy concentration has a weak correlation with blood serum CAT activity in patients with USAP (r = 0.27) and SAP (r = 0.15) (Fig. 5A, 5B).

CONCLUSIONS

A statistically significant increase in catalase activity was determined in patients with myocardial infarction, unstable and stable angina pectoris while malondialdehyde levels were found to be elevated only for stable angina pectoris patients. No significant differences were observed for homocysteine levels comparing all patients' groups studied and the control group, most possibly as a result of medical treatment.

Plasma homocysteine concentration showed a weak correlation with blood serum malondialdehyde in patients with stable angina pectoris.

The correlation between homocysteine and catalase activity was weakly positive in USAP and SAP patients.

The adaptive increase of antioxidative enzyme activity (in the case of catalase) is suggested to be related to oxidative stress as a result of homocysteine effects on lipid peroxidation.

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HOMOCISTEINO KONCENTRACIJOS IR LIPIDŲ PEROKSIDACIJOS RYŠYS SERGANČIŲJŲ KORONARINE ŠIRDIES LIGA KRAUJO SERUME

S a n t r a u k a

Homocisteino aterogeninis poveikis gali būti aiškinamas įvairiais mechanizmais, tarp jų ir sąveika su lipidų peroksidacija. Šis poveikis nustatomas ne tik tada, kai yra ryški hiperhomocisteinemija, bet ir esant nedideliam homocisteino koncentracijos padidėjimui.

Šio darbo tikslas – ištirti ligonių, sergančių širdies ir kraujagyslių ligomis, homocisteino koncentracijos kraujo serume ryšį su lipidų peroksidacija.

Bendras homocisteino kiekis nustatytas imunofermentiniu metodu, malondialdehido koncentracija ir katalazės aktyvumas – spektrofotometriniu metodu.

Į tris grupes suskirstyti pacientai (I – sergantieji miokardo infarktu (MI, n = 32); II – nustatyta nestabili krūtinės angina (NKA, n = 23); III – nustatyta stabili krūtinės angina (SKA, n = 25)) palyginus su sąlygiškai sveikų asmenų (kontroline) grupe (n = 18).

Padidėjęs antioksidantinio fermento katalazės aktyvumas nustatytas visose tirtose ligonių grupėse, tuo tarpu malondialdehido koncentracija kraujo serume buvo padidėjusi tik tų, kuriems diagnozuota SKA. Tyrimo metu nerasta didesnių bendro homocisteino koncentracijos skirtumų tirtose ligonių grupėse lyginant su kontroline grupe. Nustatyta silpna teigiama koreliacija tarp bendro homocisteino kiekio ir malondialdehido koncentracijos kraujo serume pacientų, sergančių SKA, ir bendro homocisteino kiekio bei katalazės aktyvumo tiek sergančiųjų SKA, tiek NKA.

Antioksidantinio fermento katalazės aktyvumo padidėjimas vertintinas kaip apsauginė kompensacinė reakcija, kurią sukėlė lipidų peroksidacija pasireiškiantis aterogeninis homocisteino poveikis.

Raktažodžiai: aterosklerozė, lipidų peroksidacija, malondialdehidas, katazės aktyumas, homocisteinas