CLINICAL COURSE OF ISCHEMIC HEART DISEASE DEPENDENT ON THE CONCENTRATION OF SULPHUR CONTAINING AMINO ACIDS IN BLOOD PLASMA

*Hayane Akopyan[1,3], Iryna Nazarko[1], Evgeniy Andreev[2]

[1] Institute of Hereditary Pathology of National Academy of Medical Sciences, Lviv, Ukraine
[2] O. O. Bohomolets National Medical University, Kiev, Ukraine
[3] Institute of Obstetrics and Emergency Medicine, University of Rzeszow, Rzeszow, Poland

* Corresponding author e-mail: akopyan.h@ihp.lviv.ua

S u m m a r y. A relation between blood plasma concentration of homocysteine, methionine, cysteine, and the parameters of well-established criteria for cardiovascular risk (clinical, laboratory, instrumental) was studied in Ukrainian patients with ischemic heart disease (IHD) of different course. The data evidenced on significant relation between a profile of sulphur containing amino acids (mainly cysteine) with the indices of informative criteria for myocardial infarction risk. Correlation analysis of the dynamics of concentration of homocysteine, cysteine, and methionine in the group of patients with IHC of high cardiovascular risk revealed a prevailing redirection of homocysteine into transsulfonation along with its restricted remethylation into methionine.
K e y w o r d s: ischemic heart disease, homocysteine, methionine, cysteine, homocystinuria.

INTRODUCTION

According to modern concept, hyperhomocysteinemia (HHC) is among important and independent risk factors for the development of ischemic heart disease, as far as the relation between high concentration of HC and structural-functional changes of the myocardium could be observed, even in an absence of other potentially deleterious factors [15, 16]. Moderate hyperhomocysteinemia affects 16% adolescents and more than 30% adults, frequently accompanies multifactor pathologies, and exerts an expressed pathogenic effect [14, 18].

Homocysteine is generated by demethylation of methionine in methionine cycle, and physiologic reduction of its concentration is achieved via its transsulfonation resulting in generation of cystathionine. In case of "methionine deficiency" homocysteine is converted into methionine by remethylation in the folate cycle with the use of methyl group of 5-methyltetrahydrofolate. All stages of methionine/homocysteine metabolism are controlled by special enzymes, and their genetically predetermined malfunction is considered to be a crucial factor for hyperhomocysteinemia (HHC) development. The impact of *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms in HHC development has been studied widely, but the results of these studies are contradictory [1, 5, 9, 13, 14].

For the first time a relation between endothelial dysfunction and vascular lesions and increased homocysteine level was proposed by K. S. McCully who investigated hereditary homocystinuria [12]. A direct effect of HHC on the increased risk of IHD development has been supported by the data of recent studies [2, 7, 11, 13, 17]. One could believe that in such cases a therapeutic correction based on folic acid and other group B vitamins (B2, B6, B12) could be considered pathogenetically valid for the prophylaxis of cardiovascular complications. However, the results of many multicenter studies evidenced on low effectiveness of this approach in the prophylaxis of myocardial infarction, even at the conditions of decreased blood homocysteine concentration by 3.3 mMole/L [10, 18].

Evidently, a discrete study of homocysteine and conventional genetic testing of known polymorphisms will not allow effectively to solve the problem of objective prognosis of cardiovascular

complications. The aim of the present research was to analyze the dependence between the profile of sulphur containing amino acids and the parameters of established cardiovascular risk criteria (clinical, clinical-laboratory, clinical-instrumental).

MATERIALS AND METHODS

The investigation was conducted among 53 patients diagnosed with ischemic heart disease (IHD), stable effort angina of I-IV functional class (by Canadian Cardiovascular Society Grading System). The patients were treated in Olexander Municipal Clinic of Kyiv (Ukraine). Clinical and clinical-instrumental examinations were performed with consultative service provided by The Chair of Internal Medicine № 2, O. O. Bohomolets National Medical University. The patients were divided according to the criteria of European Society of Cardiology into the group of high cardiovascular risk - 25 patients, and low cardiovascular risk group - 28 patients [6, 8]. The control group comprised 53 healthy persons of corresponding age and gender.

The diagnosis of IHD was established on the basis of typical complaints, data of anamnesis, results of clinical, clinical-instrumental and laboratory examinations. Considering anamnesis, the following parameters were evaluated: the presence of extensive myocardial infarction, hypertonic disease, indices of lipid exchange, the results of electrocardiography, coronarography and loading test, left ventricular ejection fraction values, the indices of metabolism and blood clotting. Contraindications for inclusion into the examined group were as follows: decompensated diabetes mellitus, significant renal failure, overt heart failure of class IV NYHA, valvular heart disease, left ventricular ejection fraction < 35%, and pathology which excluded a possibility of loading test performance.

The concentrations of sulphur containing amino acids (mMole/L) in the blood plasma were determined by immunoenzymatic analysis with the use of immunoenzymatic analyzer "Stat Fax 2100" (USA) and reagents (Axis-Shield, UK) in R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine. Correlation coefficient (r) and statistical significance (p) were calculated with the use of parametric Pearson's criterion and three criteria for nonparametric statistics: Spearman's criterion, Goodman-Kruskal gamma criterion, and Kendall's criterion. These statistical tests were used to characterize the dependences between parameters with normal distribution of samplings or these different from normal ones, and to perform correlation analysis of quantitative and qualitative indexes.

RESULTS AND DISCUSSION

Table 1 presents systematized cases of significant linear correlation between the concentration of sulphur containing amino acids and clinical-laboratory indices of cardiovascular risk. In all clinical groups moderate positive correlation between hypertonic disease grade and the concentration of blood plasma cysteine was determined. The strongest dependence between HD grade and blood plasma cysteine concentration was observed in the group of patients at high cardiovascular risk (r=0.52; t=2.74, p=0.006). In the group at low risk and control group these values were similar: r=0.44, t=2.53, p_2=0.018, and r=0.45, t=2.55, p=0.017 respectively.

The dynamics of cysteine concentration in the control group was found to be significantly dependent on the indices of systolic blood pressure – SBP (r=0.47, t=2.73, p=0.011). Similar dependence was detected in the group at low cardiovascular risk (r=0.36, t=2.01, p=0.045). Also, in the group at high cardiovascular risk, the concentration of cysteine significantly correlated with the indices of diastolic blood pressure – DBP (r=0.33, t=2.01, p=0.044).

Table 1. Cases of significant dependence between clinical-laboratory indexes of cardiovascular risk and concentration of sulphur containing amino acids in blood plasma.

Dependence between the parameters	Positive correlation		
	Control	IHD Low risk	IHD high risk
HD grade – concentration of cysteine	+	+	+
SBP – concentration of cysteine	+	+	—
DBP – concentration of cysteine	—*	—*	+
Fibrinogen level – concentration of cysteine	—	+*	—
INR index – concentration of cysteine	+*	—	+**
Smoking habit – concentration of cysteine	—	+	+*
Obesity grade – concentration of cysteine	—	—	+
TC – concentration of cysteine	+*	—	—

Concentration of homocysteine – cysteine	—	—	+*
	Negative correlation		
MET – concentration of cysteine	—	—	+*
EF – concentration of homocysteine	—	+*	—
Obesity – concentration of homocysteine	—	+*	—
TC – concentration of methionine	—	+*	—
NSV – concentration of cysteine	—	+*	—
Concentration of methionine – cysteine	—	—	+

Notes: HD – hypertonic disease, SBP – systolic blood pressure, DBP – diastolic blood pressure; INR – international normalized ratio for blood clotting evaluation; MET – metabolic index; EF – ejection fraction; TC – total cholesterol; NSV – number of stenotic vessels. (–) no dependence; (+) moderate correlation ($0.25<r< 0.75$; $p<0.05$); (+*) moderate-to-strong correlation ($r\geq0.5$; $p<0.05$); (+**) strong correlation ($r>0.75$; $p<0.05$). Numerical indexes are presented in the text.

In other studied sulphur containing amino acids, the concentrations of methionine, or homocysteine were not found to be related to HD grade or blood pressure indices. This is inconsistent with the data on the correlation between increased blood homocysteine content in the Ukrainian patients with IHD and manifestations of hypertonic disease in these patients [3].

Among probable effects of increased concentration of cysteine in the group at high cardiovascular risk it is necessary to mention a direct relation to the development of obesity (r=0.38; t=2.14, p_3=0.032). Also, in the patients at high risk a significant inverse relation between cysteine concentration and metabolic indices was found (MET) (r=-0.50, t=2.75, p=0.011). It should be noted that in the group at high cardiovascular risk all revealed relations for informative clinical-laboratory features of IHD were found only to blood plasma concentration of cysteine, but not homocysteine or methionine. Along with this, only in the group at high cardiovascular risk, a significant relation between the concentrations of the studied amino acids was determined, i.e. direct for homocysteine and cysteine (r=0.53, t=3.05, p=0.006), and inverse for methionine and cysteine (r=-0.42, t=2.30, p=0.03).

In the group of patients at low risk, a direct moderate-to-strong relation between concentration of cysteine and fibrinogen content was determined (r_1=0.60, t_1=2.57, p_1=0.024 ($p<0.05$), and an inverse relation between the dynamics of cysteine concentrations and the number of stenotic vessels: r_2=-0.58, t_2=2.25, p_2=0.048 ($p<0.05$); r_3=-0.70; t_3=2.29, p_3=0.022 ($p<0.05$); r_4=-0.51; t_4=2.29, p_4=0.022 ($p<0.05$). Only in this group of patients pathologic effects of increased concentration of homocysteine inversely dependent on the development of obesity (r_3= -0.60; t=2.00, p=0.046), and ejection fraction value (r=-0.50, t=2.95, p=0.006) was observed. Earlier, 30% decrease of ejection fraction was reported in the Ukrainian patients with HHC [3, 4]. Another specific characteristic of this group of patients is inverse correlation between total cholesterol content and blood plasma methionine (r=-0.57, t=2.59, p=0.021 ($p<0.05$).

A special feature of the control group was the presence of a significant moderate-to-strong direct relation between total cholesterol content and the concentration of cysteine (r=0.68, t=2.61, p=0.03). Unexpectedly, a strong correlation between cysteine content and INR indices that reflect blood clotting capability (r=0.71, t=2.46, p=0.049) was found to be characteristic for the group at high cardiovascular risk (r=0.80, t=4.59, p=0.0006). In the control group no tendency for elevated cysteine concentration in the blood plasma of smokers was found, but the difference was significant in the group at low-risk patients (r=0.43, t=2.10, p=0.036), and it reached even higher statistical significance in the group at high cardiovascular risk (r=0.59; t=3.02, p=0.0025).

Summarizing the above mentioned data, one should emphasize much evidence of a significant relation between the profile of sulphur containing amino acids and indices of well-established cardiovascular risk criteria (clinical, laboratory, instrumental). In the control group and patients with IHD common direct linear correlations were found: a) between the concentration of cysteine and hypertonic disease grade; b) between the concentration of cysteine and INR index (blood clotting capability), and the demonstration of strong correlation in the group with a complicated disease course.

In the control group and the patients at low-risk the dynamics of cysteine concentration was shown to be significantly related to the indices of systolic blood pressure, while in the high-risk group a significant relation with the indices of diastolic blood pressure was noted. These data point to more ominous consequences of increased blood concentration of cysteine for the risk of HD development, with prevalent systolic blood pressure increase in healthy individuals and low-risk patients while diastolic blood pressure increase in the patients at high cardiovascular risk.

A common feature of IHD cases (absent in the control group) was the dependence between the concentration of cysteine and smoking habit, which provides an argument that at metabolic level, smokers could be counted among the group at cardiovascular risk.

A special feature revealed in the group of patients at low cardiovascular risk was shown to be a direct moderate-to-strong relation between the concentration of cysteine and fibrinogen content, and also the presence of three inverse relations of clinical-laboratory indices: a) between the number of stenotic vessels and concentration of cysteine; b) between ejection fraction value and concentration of homocysteine; в) between total cholesterol content and concentration of methionine.

Only in the group at high cardiovascular risk a direct relation between the concentration of cysteine and obesity, and an inverse relation between the concentration of cysteine and metabolic index (MET) were detected. These relations point to significant metabolic changes in the patients with predisposition to complicated IHD course. Only in this group of patients a direct relation between concentrations of homocysteine and cysteine was observed, which could explain the prevalence of moderate HHC among the patients at high cardiovascular risk. The cases of increased cysteine concentration (hypercysteinemia) were observed exclusively in this group. Along with an inverse relation between blood the content of cysteine and methionine, these facts confirm predominant redirection of homocysteine onto the way of transsulfonation and its restricted remethylation into methionine. The insufficient utilization of homocysteine via remethylation could be caused by structural changes (polymorphisms) of folate cycle genes resulting in functional dysfunction of corresponding enzymes [1, 5, 9, 13, 14].

CONCLUSIONS

A direct correlation between higher concentrations of blood cysteine and development of hypertonic disease was found. This relation is the strongest in the group of patients at high cardiovascular risk where it is significantly associated with the indices of diastolic blood pressure, while in the control group and low-risk group with systolic blood pressure indices. A direct correlation between cysteine concentration and smoking habit in the group of patients with IHD provides arguments that at metabolic level smokers could be counted among the group at cardiovascular risk. Among other evidence of disturbed metabolism of cysteine one could mention a direct correlation between the concentration of cysteine and obesity and an inverse relation with metabolic indices. Stable changes in the profile of sulphur containing amino acids in the blood plasma of Ukrainian patients with IHD evidence on significant impact of disturbed metabolism of cysteine on the pathogenesis of early manifestations of cardiovascular diseases.

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