CHANGES IN SERUM HOMOCYSTEINE LEVEL FOLLOW TWO DIFFERENT TRENDS IN PATIENTS DURING EARLY POST MYOCARDIAL INFARCTION PERIOD

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Abstract

The evolution of homocysteine (Hcy) changes after acute myocardial infarction is still not elucidated. Serum Hcy concentration has been shown to increase between acute and convalescent period after myocardial infarction and stroke. Also a decrease in serum Hcy during acute phase was observed. It is still not clear whether the Hcy is a culprit or an innocent bystander in cardiovascular diseases. Addressing the discrepancies in Hcy changes in patients with acute myocardial infarction might give insight in Hcy role in cardiovascular diseases and offer implications both for the clinical interpretation and patients risk stratification. The aim of the study was to evaluate serum Hcy concentration changes during early post myocardial infarction. The study included 55 patients with AMI from the Clinics for Heart Diseases and Rheumatism at University of Sarajevo Clinics Centre. For Hcy analysis blood was collected on day 2 and 5 after the AMI onset. Serum Hcy concentration was determined quantitatively with fluorescent polarisation immunoassay on AxSYM system. Cluster analysis revealed two groups of AMI patients with different trends of serum Hcy changes. Increase in serum Hcy concentration was observed in 33 (60,0%) patients (AMI 1 group), while in 22 (40,0%) patients a decrease was observed (AMI 2 group). On day 2, patients in AMI 2 group had significantly higher mean Hcy concentration compared to AMI 1 group of patients (15,27±0,96 and 11,59±0,61 μmol/L $p<0,05$). On day 5, no significant difference in mean Hcy level between AMI 1 and AMI 2 group of patients was observed (14,86±1,1 vs. 12,75±0,74 μmol/L respectively). Significant differences between AMI 1 and AMI 2 patients were observed in VLDLC levels and CK-MB activity on day 2.

Patients in AMI 1 group had significant increase in platelets count from day 2 to day 5 (230,1±11,6 vs. 244,2±11,0; p<0,05). Our study of serial Hcy changes in patients with AMI revealed two different patterns of Hcy changes in early post infarction period which might reflect two distinct populations of AMI patients. Although further research is necessary, possible explanation for the observed findings could be a different genetic background, vitamin and oxidative status of patients with AMI.

KEY WORDS: homocysteine, acute myocardial infarction, post myocardial period, risk factor

Introduction

Homocysteine is a sulfur-containing amino acid that functions as a key intermediate in methionine metabolism. It is produced as a by-product of methyl-transfer reactions, which are important for the DNA synthesis, methylated proteins, neurotransmitters and phospholipids (1). Hcy might be involved in initiation and progression of atherosclerosis through several mechanisms including increased production of reactive oxygen species, endothelial dysfunction, inflammation and smooth muscle cell proliferation (2). Elevations in serum Hcy levels have been associated with cardiovascular diseases (3). This standpoint mostly relies on results obtained from case-control studies which have consistently related serum Hcy elevation with myocardial infarction, stroke and atherothrombosis. Prospective observational studies, on the other hand, have been less convincing (4). In patients with acute myocardial infarction (AMI) and stroke, subsequent tissue damage might be responsible for elevation of serum Hcy levels and thus explain observed association between hyperhomocysteinemia and atherosclerosis in patients with cardiovascular diseases (5). Several studies have evaluated changes in serum Hcy concentration in patients presenting with acute coronary syndrome and stroke (6, 7, 8, 9). It has been observed that an increase in serum Hcy levels between acute and convalescent period in AMI patients might have influence on the evolution and the degree of myocardial injury (7), but the results on Hcy levels changes during post myocardial infarction are conflicting. Recent study reported a decrease in serum Hcy concentration in a group of patients with AMI but the underlying cause is still not understood (10). Addressing the discrepancies in Hcy behaviour during the early post myocardial infarction period has implications both for the clinical interpretation and patients risk stratification. During myocardial infarction acute phase reactions could alter biochemical and laboratory parameters and also Hcy levels. Therefore, the aim of the study was to evaluate serial serum homocysteine concentration during early post myocardial infarction and to identify possible determinants of different homocysteine concentration changes.

Materials and Methods

Patients

The study included 55 patients with AMI (38 males and 17 females), admitted within 12 hours of symptom onset to the Intensive Care Unite at the Clinics for Heart Disease and Rheumatism at University of Sarajevo Clinics Centre. The diagnosis of acute myocardial infarction was based according to the World Health Organization criteria (11) including one of the main criteria: typical rise and gradual fall in troponine levels or cratine kinase (CK) elevation of at least twice the upper normal limit, with CK-MB isoenzyme concentration of at least 6% of the peak CK value with the at least one addition minor criteria: presence of ischemic chest pain of at least 30 minutes duration, dynamic ST-segment elevation or depression of 1 mm or more in at least 2 adjacent leads or prior coronary intervention. The exclusion criteria were diabetes mellitus, renal failure (creatinine levels ≥133 μmol/L), hypothyroidism, malignant disease, treatment with anticonvulsants, theophylline, niacin or hormonal therapy which might influence Hcy levels and established deficiency of vitamin B12 and folate. Approval for the study was obtained by the local Ethics Committee. All procedures on human subjects were performed in the accord with the Helsinki Declaration of 1975. All subjects included in the study singed informed consent upon careful explanation of the study procedure. Subjects underwent history and clinical examination. Clinical details included risk factor assessment for coronary artery disease. Smoking status, history of hypertension or diabetes mellitus and details of treatment received before the admission was recorded. Routine blood chemistry including serum lipid profile, uric acid and fibrinogen level as well as international normalized ratio (INR) and partial thromboplastin time (aPTT) were carried out on admission in all patients, while serum glucose, creatinine, urea, blood cell count, hematocrit, sedimentation rate, liver enzymes, C-reactive protein and troponine I, CK-MB and CK were determined on 2nd and 5th day upon the AMI onset using standard techniques. Serum blood samples for Hcy measurements were obtained on 2nd and 5th day after AMI onset. Fasting blood samples

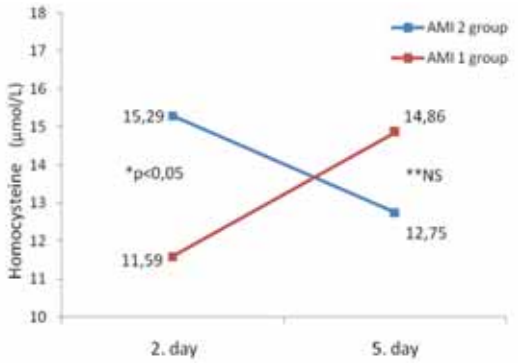

FIGURE 1. Serum homocysteine concentration in patients with different trend in homocysteine changes during early post myocardial infarction.
*statistical significance in Hcy values on day 2 in AMI 2 compared to AMI 1 group
** statistical significance in Hcy values on day 5 in AMI 2 compared to AMI 1 group; NS-no significance

were drawn from antecubital vein into siliconized tubes. Tubes were immediately put on ice and transferred to Central Laboratory for clinical biochemistry at University of Sarajevo Clinics Centre. Blood samples were centrifuged within 15 minutes at room temperature at 3000 g and kept at -80°C until analysis. Cluster analysis revealed two different trends of serum Hcy changes from day 2nd to day 5th. For analysis AIM patients were divided into two subgroups: AIM 1 group comprised of 33 (60%) AIM patients with increase in serum Hcy and in AIM 2 group there were 22 patients (40%) with the decrease in Hcy levels from day 2nd to day 5th.

Hcy assay

Serum Hcy was measured by fluorescence polarization immunoassay (Homocysteine; Abbott) on an automated analyzer (IMx system; Abbott). Optimal procedures in blood sample collection and handling were followed to prevent the passage of Hcy from red cells to plasma and thus ensure reliable measurements. The reference ranges for Hcy concentration in our laboratory is 5,9-16,0 µmol/L for males and 3,36-20,44 µmol/L for females. Hyperhomocysteiemia were defined as serum Hcy concentration ≥ 97,5 percentiles of our reference values which is 15,01 µmol/L for males and 12,44 µmol/L for females.

Statistical analysis

For normal distributed variables, values are expressed as mean±SEM. Significant differences in serial Hcy values were tested using a t-test for paired samples since Hcy values followed normal distribution. Differences in mean between groups were tested using a t-test and differences in median by use of Mann-Whitney's test. Associations between continuous variables were tested with Spearman's rank or Pearson correlation analysis where appropriate. Two-tailed p values <0,05 were considered statistically significant. Statistical analysis was performed using SPSS statistical software system (version 16.0, SPSS Inc, Chicago, Illinois, SAD).

Results

Mean serum homocysteine concentration in AMI patients enrolled in the study increased by 7,2 % from day 2 to day 5 but the difference was not statistically significant (13,07±0,58 vs. 14,01±0,73 µmol/L; p=0,11). Significant correlation coefficient was observed between mean Hcy values on day 2 and day 5 (r=0,624; p<0,001). Hyperhomocysteinemia was observed in 16 (29,1%) patients. In AMI 1 group of patients mean serum Hcy concentration significantly increased by 28,2%, while in AMI 2 group a significant decrease by 19,9% from day 2 to day 5 was observed (p<0,001). Mean serum Hcy values between the AMI 1 and AMI 2 group were significantly different only on day 2 (Figure 1.).

	AMI 1 group (n=33)	AMI 2 group (n=22)	p-value
Age (years)	62,8±1,9	58,9±1,4	NS
BMI (kg/m2)	27,6±0,7	27,8±4,7	NS
Male gender	20 (60,6%)	18 (81,8%)	NS
Prior/current smoking	18 (54,5%)	13 (59,1%)	NS
Hypertension	11 (33,3%)	8 (36,4%)	NS
Prior AMI	5 (15,2%)	5 (22,7%)	NS
Cholesterol (mmol/L)	5,7±0,18	5,35±0,26	NS
Tryglicerides (mmol/L)	2,14±0,16	1,78±0,12	NS
HDLC (mmol/L)	1,0±0,05	1,35±0,24	NS
VLDLC (mmol/L)	1,04±0,8	0,72±0,1	p<0,05
LDLC (mmol/L)	3,78±0,16	3,45±0,21	NS
Fibrinogen (mmol/L)	14,1±0,87	12,86±1,00	NS

Data are presented as mean±SEM. AMI-acute myocardial infarction; BMI-body mass index; HDLC-high density lipoprotein cholesterol, VLDLC-very low density lipoprotein cholesterol; LDLC-low density lipoprotein cholesterol

TABLE 1. Baseline characteristics of patients with different trend of serum homocysteine changes during early post myocardial infarction period

	AIM 1 group (n=33)	AIM 2 group (n=22)	p-value
Troponine 2. day (μgr/L)	49,6±10,1	67,5±18,6	NS
Troponine 5. day (μgr/L)	4,4±0,9	3,3±1,3	NS
LDH 2. day (U/L)	1027,3±111,2	1138,6±138,4	NS
LDH 5. day (U/L)	605,5±40,0	690,8±72,2	NS
CK 2. day (U/L)	1286,4±208,7	1634,7±337,9	NS
CK 5. day (U/L)	105,6±12,2	128,9±14,1	NS
CK-MB 2. day (U/L)	80,6±15,7	180,2±42,9	p<0,05

Data are presented as mean±SEM. LDH-lactate dehydrogenase, CK-creatine kinase; CK-MB-izoform creatine kinase

TABLE 2. Cardiac markers in patients with different trends of serum homocysteine changes during early post myocardial infarction period.

There was no significant difference in baseline characteristics, blood cell count, serum glucose, fibrinogen, C-reactive protein, urea, creatinine and uric acid levels or liver enzymes activity between AMI 1 and AMI 2 group of patients. Mean serum VLDLC values were significantly higher in AMI 1 compared to AMI 2 group of patients ($p<0,05$) In AMI 1 group significant increase in platelets count (230,1±11,6 vs. 244,2±11,0; $p<0,05$) and decrease in serum urea concentration (6,96±0,44 and 5,95±0,35 mmol/L, $p<0,05$) was found during the study period. Although cardiac markers were higher in patients from AMI 2 group on day 2, significant difference was observed only for CK-MB values (Table 2).

Discussion

The results of our study showed two different models of Hcy changes in patients with AMI which may represent two distinct populations. Serum Hcy concentration significantly increased in 60% of patients from day 2 to day 5 after the myocardial infarction, while in 40 % of patients a significant decrease in Hcy concentration was observed. An increase in Hcy concentration has been reported in patients after AMI and stroke (7, 8, 12). Al Obaidi et al. (12) examined variations in plasma Hcy concentration on admission, on day 2, 7, 28 and again after the 6 months in patients presenting with acute coronary syndromes (11,7, 11,5, 12,1, 12,4 and 12,1 μmol/L respectively) and found significant increase only between day 2 and day 7. Sucu et al. (6) also found significant increase in serum Hcy concentration in patients during post myocardial infarction period. Possible reasons for Hcy increase during post myocardial infarction period can be explained with the increased demands for DNA synthesis during the reparation of damaged tissue. These repair processes require the methylation of DNA, RNA and proteins – reactions that lead to the generation of Hcy as the end point in the methylation pathway. Our results can partially support this view since the patients with higher Hcy values on day 2 also had significantly higher CK-MB activity on day 2 compared to other group of patients. CK-MB activity reflects the cardiac tissue damage and increased Hcy concentration might be a marker of reparatory processes in cardiac tissue. Lower serum Hcy levels on day 2 might correspond to patients with no previous ischemic heart disease, whose myocardial cells are not already affected. The rise in Hcy levels on days after the infarction would then be explained by the post infarction presence of a "frontier area" of cardiac cells between infracted and healthy tissue. It may be that these cells are not necrotized but remain in a situation of hypoxemia, with a reduced capacity to metabolize Hcy. In contrast, patients with elevated Hcy levels on day 2 of the infarction may have a history of coronary atherosclerosis with asymptomatic myocardial ischemia that had already affected their myocardial cells (13). The subsequent decrease in Hcy levels on days after infarction could be explained by a reduction in total number of ischemic myocardial cells due to post MI necrosis. Our results showed that patients with an increase in Hcy levels during early post myocardial infarction also had a significant increase in platelets count during the study period. Although, the mechanism responsible for this is not clear Li et al. (14) showed that the L-arginine/NO pathway is one of the various targets of Hcy in human platelets which could be disturbed by Hcy. Authors suggested that Hcy diminishes NO production through decreased uptake of L-arginine resulting in increased platelet reactivity. In this context increased Hcy concentration might be responsible for increased platelets count during post infarction period and not the other way around. It is known that Hcy levels are elevated in patients with impaired folate and vitamin B12 status and hence the different models of Hcy changes might be due to vitamin deficiency in our AMI patients. Osorio et al. (10) have assessed the issue in AMI patients with different Hcy behaviour and found no significant difference in folate and vitamin B12 levels. Furthermore, there is a possibility that AMI patients with increased Hcy levels might reflect a population with MTHRF mutation with lowered capacity for Hcy metabolisation in conditions accompanied by increased synthesis such as post myocardial infarction. Early post myocardial period is characterized by increased ROS production and decreased antioxidant capacity (15). Considering the fact that Hcy is metabolized into glutathione, main intracellular antioxidant, the decrease in Hcy levels during this period might be reflective of patients with preserved capacity for transsulfuration pathway with glutathione as final product. Other group of AMI patients might be the

population with different antioxidant resources. Further research is necessary to examine possible reasons for the existence of two possibly distinct AMI populations with different changes in Hcy concentrations.

Conclusion

Our study of serial Hcy changes in patients with AMI revealed two different patterns of Hcy changes in early post infarction period which might reflect two distinct populations of AMI patients. Although further research is necessary, possible explanation for the observed findings could be a different genetic background, vitamin and oxidative status of patients with AMI.

Acknowledgments

This work was funded by Ministry of Health of Sarajevo Canton.

List of Abbreviations

Hcy	-	homocysteine
AMI	-	acute myocardial infarction
MTHRF	-	methylene tetrahydrofolat reductase
ROS	-	reactive oxygen species

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