Elevation of plasma homocysteine levels associated with acute myocardial infarction

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Abstract

Objective: To study the effect of acute myocardial infarction (AMI) on plasma homocysteine (Hcy) levels, to determine the optimal time to measure this risk factor for coronary artery disease.

Design: A prospective case study.

Setting: The Division of Cardiac Sciences, Grey Nuns Hospital in Edmonton.

Patients: Sixty-two patients (40 men, 22 women) admitted to hospital with AMI.

Intervention: Measurement of Hcy levels within 48 to 72 hours of admission and at 6 weeks after discharge from the Coronary Care Unit. In a second group of 15 patients, the Hcy levels were measured on hospital days 1 and 3.

Main outcome measure: Comparison of the Hcy levels measured at the time of AMI and after discharge.

Results: Mean (and standard error of the mean) Hcy level measured during the AMI (13.6 [0.98] µmol/L) was significantly higher ($p < 0.05$) than at 6 weeks (12.1 [1.01] µmol/L). Based on the 48- to 72-hour and 6-week determinations, 31 and 21 patients, respectively, had abnormal Hcy levels (greater than 12 µmol/L) ($p < 0.001$). In the separate group of 15 patients, the Hcy level measured on day 3 (9.7 [0.6] µmol/L) was noted to be significantly higher ($p < 0.01$) than on day 1 (7.7 [0.8] µmol/L).

Conclusions: There is an elevation of Hcy during AMI that may be related to an increase in the acute-phase reactant proteins. Thus, Hcy measurement should be deferred for 6 weeks in order to determine the true baseline level.

Résumé

Objectif: Étudier l’effet de l’infarctus aigu du myocarde (IAM) sur les taux plasmatiques d’homocystéine (Hcy) afin de déterminer le moment optimal pour mesurer ce facteur de risque de coronaropathie.

Conception: Étude de cas prospective.

Contexte: La Division de cardiologie de l’hôpital Grey Nuns, à Edmonton.

Patients: Soixante-deux patients (40 hommes, 22 femmes) hospitalisés à la suite d’un IAM.

Intervention: Mesure des taux de Hcy dans les 48 à 72 heures suivant l’admission et six semaines après que le patient ait reçu son congé de l’unité des soins coronariens. Dans un deuxième groupe de 15 patients, on a mesuré les taux de Hcy les premier et troisième jours de l’hospitalisation.

Principales mesures de résultats: Comparaison des taux de Hcy mesurés au moment de l’IAM et après le congé.

Résultats: Le taux moyen (erreur type) de Hcy mesuré au cours de l’IAM (13.6 [0.98] µmol/L) était beaucoup plus élevé ($p < 0.05$) qu’à six semaines (12.1 [1.01] µmol/L). Si l’on se fonde sur la détermination établie de 48 à 72 heures et six semaines après l’incident, 31 et 21 patients respectivement présentaient des taux anormaux de Hcy (supérieurs à 12 µmol/L) ($p < 0.001$). Dans l’autre groupe de 15 patients, on a constaté que le taux de Hcy mesuré le troisième jour (9.7 [0.6] µmol/L) était beaucoup plus élevé ($p < 0.01$) que le premier jour (7.7 [0.8] µmol/L).

Conclusions: Il se produit, pendant un IAM, une élévation du taux de Hcy qui peut être liée à une augmentation des protéines réactantes en phase aiguë. C’est pourquoi il faudrait retarder de six semaines la mesure du taux de Hcy afin de déterminer le niveau de référence réel.
Introduction

In the natural history of coronary artery disease (CAD) an acute myocardial infarction (AMI) is often its first clinical presentation. An AMI results in early and late morbidity and mortality. Thus, an assessment of the risk factors for CAD is done at the time of an AMI, because favourable modification of these factors may minimize future coronary events, including repeat infarction and death. Unavoidably, there is much controversy in the search for risk factors for CAD, where the relative influence of independent risk factors is often confounded. Also, the traditional risk factors of smoking, hypertension, diabetes, dyslipidemia and a family history of premature CAD are believed to account for only 30% to 40% of the causes of atherosclerosis. An elevated plasma homocysteine (Hcy) level has recently been indicated as an independent risk factor for CAD, although there is no universal agreement regarding the pathogenetic role of homocysteine in the development of atherosclerosis. Further, there is debate regarding what value should be considered as the upper limit of normal for Hcy, similar to the controversy associated with serum cholesterol levels. It has been shown that AMI is associated with a decrease in serum cholesterol levels. Thus, cholesterol measurements are usually deferred for about 6 weeks after an AMI, although a measurement taken within the first 24 hours after the onset of an AMI may approximate the baseline level. In light of these changes in serum cholesterol, we planned to study the effect of an AMI on Hcy levels in order to determine the optimal time for measuring Hcy after an AMI.

Methods

We prospectively examined 62 consecutive patients (40 men, 22 women, mean [and standard error of the mean] age 63.3 [1.5] years) admitted during a 3-month period with an AMI to the Coronary Care Unit of Grey Nuns Hospital in Edmonton. The diagnosis of AMI was based on the presence of at least 2 of the 3 following criteria: (1) chest pain suggestive of myocardial ischemia lasting 30 minutes or longer; (2) enzymatic evidence of acute myocardial necrosis, as demonstrated by a rise in creatine kinase levels above the upper limit of normal (greater than 170 U/L with the CK-MB fraction being greater than 5%; (3) new electrocardiographic changes that included development of new Q waves or ST-T changes (defined as new or presumed new ST segment depression greater than 1.0 mm or new T-wave inversion greater than 2.0 mm in depth, or both) lasting 48 hours or longer. Informed consent was obtained from each patient.

Measurement of Hcy levels

The Hcy concentration was measured after an overnight fast of 12 hours, with the first sample taken 48 to 72 hours after admission. The delay of 48 to 72 hours was used to allow for the biologic changes associated with an AMI to manifest themselves. The blood was drawn in pre-cooled test tubes containing ethylenediaminetetraacetic acid with the plasma separated within 10 minutes and stored at –20 °C until analysis. The plasma was analysed for homocysteine levels by an enzyme immunoassay, which allowed total plasma homocysteine levels to be measured. Repeat fasting Hcy samples were measured by the same procedure, 6 weeks after discharge from the Coronary Care Unit.

Based on the results of the first part of the study, we decided to investigate prospectively the change in Hcy between day 1 and day 3 after an AMI. In a separate group of 15 consecutive patients admitted with an AMI, a fasting Hcy was obtained within 24 hours of admission and repeated after 72 hours.

Statistical analysis

The Hcy levels together with demographic, clinical and investigational variables were put into a database within the SPSS data management system and analysed with use of the system. The Hcy level at the time of the AMI for each patient was compared with the level determined 6 weeks after discharge from the Coronary Care Unit by a paired t-test. The Hcy level at 6 weeks was further categorized into 2 groups: patients with abnormal and normal Hcy levels. The upper limit of normal for Hcy (as established in the University of Alberta hospital laboratory, which does Hcy assays for all hospitals in Edmonton) was 12.0 µmol/L. Variables obtained from the med-
ical history, clinical course and complications in the Coronary Care Unit, and investigative data were evaluated against the Hcy level. Discrete variables were categorized to ensure that responses would fall into mutually exclusive categories. The cross-tabulations were done on discrete variables using a χ² test. Analyses between abnormal and normal Hcy groups for continuous variables were made using an unpaired t-test. The Hcy levels between day 1 and day 3 in the second part of the study were compared with a paired t-test. For continuous variables, mean (and standard errors of the mean [SEM]) are quoted. A p value less than 0.05 was considered as significant in all analyses.

Findings

Demographic and investigational data

Of the 62 patients, 56 had their fasting Hcy repeated at 6 weeks; 5 patients had died in the interim and 1 patient refused follow-up. The clinical and investigational characteristics for these 56 patients are outlined in Table 1. There were 36 men (64%) and 20 women (36%), with a mean age of 62.3 (1.6) years (range from 38 to 83 years). Eight patients (14%) had a history of AMI. The frequency of independent risk factors for AMI was as follows: hypertension, 26 (46%); known dyslipidemia, 25 (45%); current smokers, 20 (36%); family history of CAD (defined as CAD in a first-degree male relative younger than 55 years or a first-degree female relative younger than 65 years old), 18 (32%); and diabetes, 10 (18%). The locations of the AMIs were: anteroseptal, 18 (32%); inferior or posterior, or both, 33 (59%); lateral, 3 (5%); indeterminate, 2 (4%). The AMIs were Q wave in type in 31 (55%) patients. Twenty-seven patients (48%) received thrombolytic therapy. The highest creatine kinase levels had a mean of 1256 (166) U/L. The mean creatinine level for the group was 100.5 (4.4) µmol/L.

Hcy measurements

The Hcy levels measured 48 to 72 hours after admission and at 6 weeks in the 56 patients were 13.6 (0.98) µmol/L (range from 5.0 to 50.0 µmol/L) and 12.1 (1.01) µmol/L (range from 5.8 to 60.0 µmol/L) respectively (p = 0.009 by a paired t-test). By assessment of the 48- to 72-hour measurement, 31 patients (50%) had an abnormal Hcy level (greater than 12.0 µmol/L). In contrast, evaluation of the Hcy at 6 weeks revealed that 21 patients (38%) had abnormal levels. A cross-tabulation analysis of normal versus abnormal Hcy between the 48- to 72-hour and 6-week levels demonstrated a significant difference (p < 0.001). The magnitude of the change in the plasma homocysteine levels between first and second measurements varied from 10.0 to 18.0 µmol/L. Fourteen patients (25%) demonstrated an increase in the Hcy level during the 6-week period.

Analysis for differences between patients who demonstrated an increase in the Hcy levels and patients who demonstrated a decrease during the 6-week period found that the only significant variable (of 23 evaluated) was a lower prevalence of a history

<table>
<thead>
<tr>
<th>Table 1: Clinical and investigative characteristics of the 56 patients who had acute myocardial infarction (AMI)</th>
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<tr>
<td>Characteristic</td>
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<td>Mean (and SEM) age, yr</td>
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<tr>
<td>Age group (yr), no. (%)</td>
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<td>&lt; 50</td>
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<td>Smoking, no. (%)</td>
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<td>Diabetes, no. (%)</td>
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<td>Hypertension, no. (%)</td>
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<td>Known dyslipidemia, no. (%)</td>
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<td>Family history of coronary artery disease, no. (%)</td>
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<td>Mean (and SEM) maximum creatine kinase, U/L</td>
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<td>Mean (and SEM) serum creatinine, µmol/L</td>
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<td>Location of AMI, no. (%)</td>
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<td>Anteroseptal</td>
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<td>Inferior and/or posterior</td>
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<td>Type of AMI, no. (%)</td>
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of hypertension (24% versus 60%) in those with an increase in Hcy level ($p = 0.019$). Some of the variables that did not demonstrate a significant difference were age, weight, gender, peak creatine kinase level, lipid subfractions, duration of hospital stay, history of diabetes mellitus and history of smoking.

The 5 patients (3 men, 2 women) who died between the sampling points had a mean (and SEM) age of 75.8 (3.2) years (range from 67 to 86 years). The infarctions were anteroseptal in 4 of these patients; 2 patients had non-Q-wave AMIs. The maximum creatine kinase levels ranged from 555 to 3437 U/L, with the ejection fraction ranging from 0.25 to 0.4. The mean (and SEM) 48- to 72-hour Hcy level in this group was 12.7 (2.1).

In the second part of the study 15 patients (13 men, 2 women; mean [and SEM] age 57.9 [3.2] years) were investigated. The mean (and SEM) peak creatine kinase for the group was 1862 (485) U/L. The AMI was Q wave in type in 69%. The locations of the AMIs were as follows: anteroseptal, 25%; inferior or posterior, or both, 51%; lateral, 13%. The mean (and SEM) Hcy levels on day 1 and day 3 following the AMI in this group were 7.7 (0.8) µmol/L and 9.7 (0.6) µmol/L respectively. The mean Hcy value on day 3 was significantly higher than on day 1 for the whole group ($p = 0.007$ by the paired t-test). Thirteen of the 15 patients demonstrated an increase in Hcy levels from day 1 to day 3, ranging from 0.9 to 5.1 µmol/L. Two patients demonstrated decreases in the Hcy of 2.2 and 2.6 µmol/L between day 1 and day 3.

**Discussion**

**Results and implications**

Increased Hcy levels are an independent risk factor for the development of atherosclerosis. In light of the acceptance of this finding, an accurate measurement of the Hcy level is necessary. The present study demonstrates that Hcy measurements taken 48 to 72 hours after the onset of an AMI appeared significantly different from those measured 6 weeks after discharge from the Coronary Care Unit. If this difference was not appreciated, it would result in misclassification of nearly 12% of patients with respect to the normality or abnormality of their plasma homocysteine levels. Although there is debate about the cut-off point between normal and abnormal Hcy, this difference between the 48- to 72-hour level and the 6-week level would persist regardless of the exact value accepted as the upper limit of normal. There was a significant decrease in Hcy levels from the 48- to 72-hour and the 6-week post-discharge measurements; however, a considerable variation was noted, with 25% of the patients demonstrating an increase during the time period. All blood samples were collected approximately at the same time of the day (morning) in pre-cooled test tubes, and the same assay technique was used for both samples. The intra-assay coefficient of variation has been shown to be 2.0%, with an inter-assay coefficient of variation of 5.1%. This suggests that fluctuations in Hcy due to inconsistencies in the assay itself are unlikely to account for the differences. The group who demonstrated an increase in the Hcy level between the first and second measurements (from 48 to 72 hours to 6 weeks) did not show any appreciable difference in clinical and investigational characteristics from the group who demonstrated a decrease with only 1 of 23 variables (history of hypertension), achieving a statistically significant difference between the 2 groups. Although the causes leading to an increase in the Hcy level in some patients remains unclear, this type of nonuniform directional change is not uncommon with biologic variables.

**Possible explanations**

There are 3 possible explanations for the differences in Hcy level noted in this study: (1) elevation of Hcy is associated with an AMI, thus the true level is that measured 6 weeks after discharge from the Coronary Care Unit; (2) the true Hcy is the one measured at the time of the AMI, with a possible decrease in Hcy level 6 weeks after discharge resulting from favourable modification of diet; and (3) an increase in the protein-bound fraction of Hcy during an AMI.

Studies have shown that folic acid is effective in lowering the Hcy level in patients after AMI. Thus, it is possible that the dietary education given at the time of an AMI, with emphasis on increased consumption of vegetables, may have resulted in a decrease in the Hcy levels. However, this is contrary
to expectation, as pure dietary sources generally do not provide a folic acid intake of more than 0.2 mg/d (especially as the bioavailability of folic acid in foods varies widely and is generally low), a level that is generally insufficient to reduce abnormal Hcy levels. This conclusion is also supported by the observation that diets containing cereals fortified with folic acid (at the levels recommended by the United States Food and Drug Administration) have not been shown to be efficacious in reducing Hcy levels. This study demonstrated only a 3.7% reduction in Hcy at the current levels of folic acid supplementation of 127 µg/d.

Evidence for the thesis that an increase in the Hcy occurs at the time of an AMI is provided by the second part of the study. The results indicated a statistically significant increase in the Hcy between day 1 and day 3 following an AMI, with a mean difference of 2.1 µmol/L. Of the 15 patients in this part of the study, 86.7% demonstrated an increase in the Hcy from day 1 to day 3. The mean Hcy at day 3 in the second part of the study (9.7 [0.6] µmol/L) was lower than the value at day 3 in the first part of the study (13.6 [0.98] µmol/L). The exact reason for this is unclear but is likely due to the small sample size with a wide range of Hcy levels obtained in the first part of the study (2 patients had Hcy levels greater than 40 µmol/L, resulting in an increase in the mean value for the whole group). If there is a spurious increase in the Hcy level after AMI, a mechanism must be elucidated to explain this transient change. Homocysteine is a nonessential amino acid produced during the metabolism of the essential amino acid methionine. Homocysteine can either be re-methylated back to methionine or undergo trans-sulfuration with the formation of cysteine. In this model, Hcy concentration is believed to be in equilibrium with plasma methionine concentration. In contrast, the conversion to cysteine is believed to be an irreversible reaction. The Hcy concentration is thus dependent on the cystathionine β-synthase activity, the rate-limiting enzyme in the formation of cysteine. In this model, Hcy concentration is believed to be in equilibrium with plasma methionine concentration. In contrast, the conversion to cysteine is believed to be an irreversible reaction. The Hcy concentration is thus dependent on the cystathionine β-synthase activity, the rate-limiting enzyme in the formation of cysteine, as well as the state of equilibrium with methionine. The equilibrium of methionine is influenced by the activity of several enzymes and the availability of their cofactors, such as folic acid and vitamin B12.

It has been shown that the concentration of free methionine increases during an AMI. This would suggest that Hcy may also increase, in view of the known state of equilibrium between methionine and homocysteine. This conclusion is supported by the previously noted increase in Hcy as a result of a methionine-loading diet. Another explanation for the transient increase in Hcy may be an enzymatic function, or a lack thereof. In genetically caused homocystinuria, there is a mutation in a gene that codes for cystathionine β-synthase, the enzyme that causes homocysteine to condense with serine to form cystathionine. It is possible that the activity of cystathionine β-synthase is compromised during AMI as a result of previously documented decreases in plasma serine concentrations. This decrease would in turn lead to an increase in Hcy concentration owing to a lack of substrate, rather than to a deficient enzyme.

The third possible cause for the increase in Hcy at the time of an AMI is an increase in the protein-bound fraction of Hcy during an AMI. It has been demonstrated that there is an increase in acute-phase reactant proteins at that time. This increase could lead to binding of the free homocysteine on the additional binding sites available on the proteins. During this process, the total concentration of Hcy would increase in proportion to the increase in plasma proteins, while the concentration of free homocysteine may be maintained relatively constant through homeostasis.

In plasma, homocysteine exists in 3 forms: free homocysteine; protein-bound homocysteine (the predominant fraction) with albumin being the major protein, although other proteins too are involved; and cysteine–homocysteine mixed disulfide. Most assays, including the one used in the present study, measure the total homocysteine concentrations. Therefore, the measured concentration of homocysteine would reflect and follow changes in plasma protein concentrations. Thus, in association with an AMI with a rise in the concentration of acute-phase reactant proteins, Hcy could increase and return to baseline levels over the ensuing weeks with resolution of the acute-phase reaction. For the hypothesis to be true, homocysteine must bind to 1 or more of the acute-phase reactant proteins (C-reactive protein, α1-antitrypsin, haptoglobin, ceruloplasmin or α1-acid glycoprotein), as the serum albumin level itself may drop with an AMI.
Although 1 or more of the explanations given above could account for an increase in the Hcy level with an AMI, these explanations should be considered as speculative, requiring further elucidation.

**Literature review**

A literature review on this topic revealed only 2 articles that examined the timing of Hcy measurement after an AMI. Egerton and associates concluded that there was a decrease in the Hcy with an AMI, with the levels determined on the first and third day being lower than those determined on the seventh day. It was suggested that the levels at day 7 or later would accurately reflect the baseline level. As shown in figure 1 of their article, this conclusion appears unjust since the levels measured at 21 days after the AMI as well as on the first and third day appeared lower than the level at day 7. The more plausible conclusion would be that Hcy increases after an AMI (as the values on the first and third day were lower than on the seventh day), reaches a maximum at day 7, and subsequently decreases, which is similar to the time-course of events demonstrated in our study. However, the study by Egerton and associates is limited because only 14 patients completed the day 7 measurement.

A second study, by Landgren and associates, showed that the Hcy concentration 6 weeks after an AMI were significantly higher than at 24 to 36 hours after the onset of the infarction. It was suggested that the difference in level may be due to a change in plasma protein content (due to hemoconcentration), which is believed to be 9% higher in the standing position than the supine position. It was hypothesized that the difference in the Hcy level was owing to the fact that the patients were supine during the in-hospital venipuncture but seated at the 6-week blood collection. However, there was no specified protocol in the methodology to ensure that the subjects were supine for a fixed time before blood sampling in the hospital. The blood samples were collected up to 1200 hours during the hospital stay, making it unlikely that patients would have been supine except at the time of collection. The different results obtained in the study of Landgren and associates compared with our study and one by Egerton and associates remains unclear.

In our study, too, 25% of the patients demonstrated an increase in the Hcy level from the time of the AMI to 6 weeks after discharge from the Coronary Care Unit, although the entire group of patients demonstrated a significant decrease in Hcy levels. Conversely, in the study by Landgren and associates, 5 of the 20 patients in their figure 1 showed a decrease in Hcy levels. Thus, a bi-directional change in Hcy levels during the recovery period after an AMI remains a possibility, with patients who exhibited continued coronary inflammation (at the atherosclerotic plaques) demonstrating persistent increases in the Hcy level. In recent years there has been considerable interest in explaining the etiology and, more importantly, the precipitation of coronary events on the basis of infection or inflammation, or both. In this regard, it would be interesting to follow up those patients who demonstrate an increase in the Hcy levels at 6 weeks (compared with the level at the time of AMI) to assess for a higher incidence of future coronary events than those who demonstrated a decrease. Nevertheless, during the acute phase of the AMI there appears to be a more uniform increase in the Hcy levels, with 13 of our 15 patients demonstrating an increase in the levels from day 1 to day 3.

Homocystinemia is not a single entity; a number of different genetic mutations modified by environmental influences determine the Hcy level for each patient. Further, the genetic mutations resulting in homocystinemia appear to be quite frequent although different ethnic groups demonstrate marked variations in their frequency. Thus, the differences in the study by Landgren and associates, done in Sweden, and our study may be related to differences in the populations studied. The population in Alberta is more diverse with significant proportions of British, German and East European origin, whereas those in Sweden may have been of more uniform ethnic origin.

**Conclusions**

We have demonstrated an increase in Hcy level associated with an AMI, manifesting within 48 to 72 hours after admission to hospital. Therefore, the measurement of Hcy is best deferred for approximately 6 weeks after an AMI to obtain an accurate reflection of the baseline level of homocysteine. Whether a Hcy
measurement taken within 24 hours of admission would reflect the baseline level remains unclear. A preliminary report of the present study has been presented as an abstract.21

References


22. Griffiths J, Nagendran J, Senaratne MP. Spurious elevation of plasma homocysteine levels associated with acute myocardial infarction [abstract]. *J Heart Dis*