



RESEARCH ARTICLE

A STUDY OF SERUM HOMOCYSTEINE LEVELS IN ACUTE MYOCARDIAL INFARCTION PATIENTS

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ABSTRACT

Myocardial infarction is a major consequence of coronary artery disease. In addition to the traditional risk factors of myocardial infarction, hyperhomocysteinemia has emerged as major factor. Plasma homocysteine level was determined in 30 myocardial infarction patients and 20 age matched healthy individuals. Statistically significant differences ( $p < 0.01$ ) were observed in the mean of plasma homocysteine concentrations between the acute myocardial infarction patients ( $25.89 \pm 6.18$  mM/L) and normal healthy individuals ( $13.83 \pm 3.51$  mM/L). Homocysteine in myocardial infarction patients was seen to be significantly high ( $p < 0.01$ ) when compared to that of the controls. The study indicated a strong association between plasma homocysteine and acute myocardial infarction, which establishes plasma homocysteine as a key risk factor for myocardial infarction.

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INTRODUCTION

During the past 20 years, the battle to reduce the incidence of cardiovascular disease has led researchers to the discovery of various clinical markers. In the absence of traditional risk factors in some cases has led the focus on other predisposing factors, which may contribute to myocardial infarction. Researchers have taken effort to find the possible association between plasma homocysteine levels and acute myocardial infarction (AMI). Homocysteine is a thiol containing amino acid and is an intermediate in the metabolism of methionine, linking the methionine cycle to the folate cycle. It is present in plasma in four forms: a free thiol (1%); disulfide (5- 10%); mixed disulfide (5-10%) and protein bound thiol groups (80-90%). The combined pool of all four forms of homocysteine is referred as “total plasma homocysteine” (Lin and Liou 2002). The total homocysteine levels in plasma has been reported to be in the range of 5-15 mM/L in healthy individuals. (Neki 2003) Altered homocysteine metabolism play a potential role in the pathogenesis of atherosclerosis, thromboembolism and vascular endothelial damage. Individuals untreated for hyperhomocysteinemia may have major cardiovascular events before the age of 30 years. (Mudd et al., 1989; McCully 1996) Several studies conducted in different parts of the world have reported that elevated levels of plasma homocysteine are associated with coronary artery disease, independent of other risk factors (Chambers et al., 2000; Boushey et al., 1995). As

the concentrations of homocysteine is also influenced by genetic background there is need to study on homocysteine levels in different ethnic groups. Recent reports on homocysteine suggest that it is an independent single largest risk factor of vascular disease, including stroke and coronary artery disease (Myers et al., 2009).

MATERIALS AND METHODS

30 patients admitted to the intensive care unit of SSG Hospital, Vadodara between January to October 2009 with diagnosed AMI showing characteristic ECG signs and rise in troponin-I concentration were included in this study. Fasting blood was collected from the patients and 20 healthy individuals after obtaining informed consent. Plasma was separated using EDTA coated tubes. Analysis for plasma homocysteine was performed using homocysteine 2 reagent enzymatic photometric assay. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM) catalyzed by a Hcy S-methyltransferase to form methionine and S-adenosylhomocysteine. SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase. The formed Ado is immediately hydrolyzed into Inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD<sup>+</sup>. The concentration of Hcy in the sample is indirectly proportional to the amount of NADH converted to NAD<sup>+</sup> ( $\Delta A$  340nm) (Refsum et al., 2004; Turgeon et al., 2010). All values are

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expressed in mean  $\pm$  S.E. Student 't' test was used to compare the means.

## RESULTS AND DISCUSSION

Myocardial patient show an elevated plasma homocysteine when compared with the control group (Table-1).

**Table 1. Laboratory data of the study subjects**

	AMI patients (n = 50)	Healthy controls (n=25)
Age in years	48.5 $\pm$ 10.3	44.5 $\pm$ 12.8
Sex (M/F)	37 / 13	20 / 5
Homocysteine mM/L	25.89 $\pm$ 6.18	13.83 $\pm$ 3.51

Statistically significant differences ( $p < 0.01$ ) were observed in the mean of plasma homocysteine concentrations between the acute myocardial infarction patients (25.89  $\pm$  6.18 mM/L) and controls (13.83  $\pm$  3.51 mM/L). Studies on the association of hyperhomocysteinemia with coronary artery disease in different populations have yielded conflicting results with some positive evidence for an association (Senaratne *et al.*, 2000; Nair *et al.*, 2002) while others have found none (Deepa *et al.*, 2001; Chacko 1998). This may be attributed to ethnic differences or due to differences in the definition of cases. As genetic background and nutritional intake vary in different populations, the Homocysteine level varies in different ethnic groups and this may be due to the polymorphism seen in the genes encoding enzymes involved in the metabolism of homocysteine. Plasma homocysteine concentration are found to be higher in Indian Asian overseas compared to the North Americans and European whites. (Chambers *et al.*, 2000; Nair *et al.*, 2002; Anand *et al.*, 2000). Hyperhomocysteinemia may also occur due to nutritional deficiency that leads to low blood concentrations of folate, Vitamin-B<sub>12</sub>, or vitamin-B<sub>6</sub> (Ueland *et al.*, 1993). A meta analysis conducted by Boushey *et al.* (1995) showed that homocysteine was an independent, graded risk factor for atherosclerotic disease in the coronary, cerebral and peripheral vessels (McCully 1996; Chambers *et al.*, 2000). Stampfer *et al.* (1992) concluded that moderately high levels of plasma homocysteine are associated with subsequent risk of myocardial infarction. Chamber *et al.* (2000) have reported that elevated plasma homocysteine concentration is a risk factor for coronary heart disease, independent of conventional risk factors (McCully 1996). Homocysteine is known to induce atherothrombosis in many ways: homocysteine thiolactate, a by product of oxidation of homocysteine combines with LDL to form foam cells (Prasad 1999). The LDL rich foam cells embed themselves in the vascular endothelium and become fatty streak, which is the precursor to atherosclerotic plaque. Homocysteine thiolactate probably impairs the oxidative phosphorylation and enhancement of the proliferation and fibrosis of smooth muscle cells (Miller 1996). It may also induce atherosclerosis by affecting endothelial-derived relaxing factor, nitric oxide (NO). NO combined with homocysteine in the presence of oxygen to form s-nitroso homocysteine, which inhibits sulphhydryl dependent generation of hydrogen peroxide. The bioavailability of NO is decreased due to endothelial cell injury. This dysfunctional endothelium may be due to generation of oxygen radicals produced by homocysteine. Homocysteine enhances lipid peroxidation which may decrease the expression of endothelial NO synthase

and directly degrade NO (Stampfer *et al.*, 1992). Auto oxidation of homocysteine results in oxidation of LDL through generation of the superoxide anion radical. Homocysteine may also reduce the antioxidant status which could injure endothelial cells. Homocysteine stimulates platelet generation of thromboxane A<sub>2</sub>, which is a vasoconstrictor and pro-aggregant (Haynes 2000). As the level of plasma homocysteine is very high among the myocardial infarction patients, the findings of this study underscores the importance of determining the levels of plasma homocysteine in individuals who are at high risk of developing myocardial infarction.

In conclusion, our data provide evidence that plasma total homocysteine levels are markedly elevated in acute myocardial infarction patients.

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