ORIGINAL ARTICLE

EVALUATION OF SERUM HOMOCYSTEINE AS AN INDEPENDENT RISK FACTOR FOR MYOCARDIAL INFARCTION IN YOUNG PATIENTS

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ABSTRACT

Introduction: Abnormal Homocysteine (HCY) level appear to contribute to atherosclerosis by direct toxic effect that damages the arterial linings, interfering with the clotting factors and oxidation of LDL. Some 10% to 20% of cases of Coronary heart disease have been linked to elevated level of serum Homocysteine.

Objective: This case control study was designed to study the Homocysteine levels in young patients with MI & to carry out statistical analysis to evaluate Homocysteine as an independent risk factor for MI.

Methods: The mean serum Homocysteine in patients of MI and control was calculated and its association with Total cholesterol, LDL cholesterol and HDL cholesterol.

Results: The mean serum Homocysteine in case and control was 29.77 μmol / L and 11 μmol / L respectively with S.D of 6.97 μmol / L and 1.96 μmol / L respectively with a significant p value of 0.0001. The difference in Homocysteine levels observed between patients with LDL-C ≥ 100 mg% and those with LDL-C < 100 mg% was not significant. The difference in Homocysteine levels observed between patients with HDL-C ≥ 40 mg% and those with HDL-C < 40 mg% was not significant. The difference in Homocysteine levels observed between patients with S. Cholesterol ≥ 200 mg% and those with S. Cholesterol < 200 mg% was significant (P value = 0.0001).

Conclusion: From the above findings, in this study the low levels of LDL-C and high levels of HDL-C did not protect the patients against the Homocysteine induced coronary artery disease. Also it shows that in patients who did not have high levels of total cholesterol, the higher levels of Serum Homocysteine triggered the coronary artery disease.

Keywords: Homocysteine (HCY), MI, LDL, HDL.

INTRODUCTION

Although coronary artery disease (CAD) primarily occurs in patients over the age of 40, younger men and women can also be affected[1-2]. Most studies have used an age cut-off of 40 to 45 years to define "young" patients with CAD or Acute Myocardial Infarction (AMI). The prevalence of CAD in younger subjects is difficult to accurately establish since coronary atherosclerosis is frequently a silent process. In the Framingham heart study, the incidence of MI over 10 year follow up was 12.9/1000 in men 30-34 years old and 5.2/1000 in women 35-44 years old[3]. In other studies, 4 to 10 percent of patients with MI were ≤ 40 or 45 years of age. Although CAD is an uncommon entity in young patients, it constitutes an important problem for the physician and the patient because of the devastating effect of this disease on the more active lifestyle of young patients. Homocysteine, a sulphur containing amino acid was first described by Vigneaud in 1931. Elevated plasma levels of Homocysteine have been associated with vascular disease. The hallmarks of homocystinuria[4] are Ectopic lentis, Marfanoid appearance, vascular manifestations, Musculo- skeletal and CNS manifestations. In blood only about 1% of total Homocysteine is free reduced form. The major part of Homocysteine in plasma is oxidized and either colvalently bound to proteins or occur as disulfides. The determinants of total Homocysteine in plasma include several genetic enzyme defects especially cystathionine beta synthase and MTHFR polymorphism[5-6], age and gender, drugs, vitamin status, diabetes, steroid hormones, thyroid disease etc. Homocysteine has been found to induce vascular injury by multiple mechanisms: - Homocysteine promotes leucocyte recruitment by up regulating monocyte

chemoattractant protein –I and interleukin-8 expression and secretion[7]. It also causes oxidation of LDL which has lipid peroxidation effect. Homocysteine increases smooth muscle cell proliferation and enhances collagen production[8]. And it also causes direct endothelial injury[9]. Prothrombotic effects of Homocysteine, which have been demonstrated in patients with acute coronary syndromes and stroke, include attenuation of endothelial cell tissue plasminogen activator binding sites, activation of factor VIIa and V, inhibition of protein C and heparin sulphate, increased fibrinopeptide A and prothrombin fragments 1 and 2, increased blood viscosity, and decreased endothelial antithrombotic activity due to changes in thrombomodulin functions. Also prolonged exposure of endothelial cells to Homocysteine reduces the activity of dimethylarginine dimethylaminohydrolase, the enzyme that degrades asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase; this impairs the production of nitric oxide. This may contribute to impaired endothelium dependent vasodilatation of both conduit and resistance vessels. Several studies[10-11] have showed that's there is a clear dose response relationship between Homocysteine concentrations and cardiovascular mortality in patients with confirmed coronary artery disease. Normal Homocysteine concentrations range between 5 and 15 μmol / L and hyperhomocysteinemia has been classified as follows; moderate- 15 – 30 μmol / L, intermediate- 30 – 100 μmol / L, and severe- more than 100 μmol / L. In our study the difference in the homocysteine levels between the case and the control groups was highly significant (p=0.0001) suggesting homocysteine as an important predictor of coronary artery disease. The difference in homocysteine levels observed between patient with LDL-C ≥ 100mg% and those with LDL-C < 100mg% was not statistically significant (p=0.1325). Hence in this study, the low levels of LDL-C did not protect the patients against the homocysteine induced CAD. The difference in homocysteine levels observed between patients with HDL-C ≥ 40mg% and those with HDL-C < 40 mg% was not statistically significant (p=0.5964) suggesting that high levels of HDL-C did not protect the patients against the homocysteine induced CAD. Also the difference in homocysteine levels observed between the patients with S. Cholesterol ≥ 200mg% and those with S. cholesterol < 200mg% was statistically significant (p=0.0001) suggesting that in patients who did not have high levels of Serum Cholesterol, the higher levels of serum homocysteine triggered the coronary artery disease.

METHODOLOGY

The Study Population:

The study population consisted of 30 patients having Acute Myocardial Infarction and diagnosed by clinical signs and symptoms, 12 –lead ECG and biochemical markers like CK-MB. Control group included 30 young individuals without Ischemic Heart Disease.

Inclusion Criteria:

All patients aged between 20 – 40 years.

- Severe chest pain lasting for > 30 minutes and not responding to sublingual nitroglycerine tablets significantly.
- Presence of pathological Q wave along with ST segment elevation and subsequent T wave inversion appearing in anterior leads, inferior leads or right leads corresponding to anterior wall, inferior wall and right wall myocardial infarction respectively.
- Significant rise in CK-MB isoenzyme on 1st or 2nd day.

After admission detailed history and clinical examination was carried out and 12 lead ECG was taken in every patient of MI. Investigations like Hemogram, Urine examination, Lipid Profile, Serum CK-MB, Serum Homocysteine were carried out in all the patients and controls.

Sample collection and Preparations[12]:

The Serum Homocysteine levels and Lipid Profiles were measured 8 to 12 weeks after the attack of Myocardial Infarction. Serum Homocysteine levels and Lipid Profiles were measured in a 12 hour fasting blood sample in both the case and control groups. A patient, who has had a serious illness such as MI or stroke, should not be tested for 8 to 12 weeks after the event because there is a potential for the Homocysteine level to be abnormally high during that time period. The reason for this has not been elucidated. 5 ml of blood was collected in a plain bulb for the estimation of Homocysteine and Lipid profile. 2 ml of blood was collected in Fluoride bulb for blood sugar estimation. 10 to 12 hours of fasting prior to sample collection is required. EDTA plasma or serum is preferred. Blood should be centrifuged within 30 minutes or kept on ice until centrifugation.

Methods for different Parameters:

Serum cholesterol was estimated by using Cholesterol Oxidase peroxidase (CHOD-PAP) method. Serum triglycerides estimated by GPO method. Serum HDL-C was estimated by using Phosphotungstic Acid (PTA) method, whereas Serum LDL-C was calculated by using Friedwald's Equation. Serum Homocysteine was measured by Fluorescence Polarization Immunoassay method by AxSYM Assay system using the principle of conversion of oxidized Homocysteine to reduced form and then converting into S-adenosyl-L-Homocysteine (SAH)

Data Analysis:

The data were obtained and plotted using a bar diagram. Four important data were obtained indicating the comparison of serum Homocysteine in case and

control group as well as comparing serum Homocysteine with LDL-C, HDL-C, and cholesterol in case groups. Statistical analysis was done and p value was calculated using t-test.

RESULTS

The table 1 shows that the mean serum homocysteine in case and control groups was 29.77 μmol/L and 11 μmol/L, respectively. The standard deviation in case and control groups was 6.97 μmol/L and 1.96 μmol/L, respectively.

Table 1: S. Homocysteine Levels in Case and Control Groups with Myocardial Infarction

S. Homocysteine μmol/L	Cases	Control
Mean	29.77	11.00
S.D	6.97	1.96

p value= 0.0001

Table 2: Relation of Homocysteine to LDL–C in Case Group with Myocardial Infarction

LDL	>100 mg %	<100 mg %
No of Patients	18	12
HCY mean	32.01	26.41
HCY S.D.	10.22	8.83

p value = 0.1325

The table 2 shows that the mean homocysteine level in patients with LDL-C > 100 mg % was 32.01 μmol/L and in patients with LDL-C < 100 mg % was 26.41 μmol/L whereas the S.D were 10.22 and 8.83 respectively.

Table 3: Relation of Homocysteine to HDL–C in Case Group with Myocardial Infarction

HDL-C	<40 mg %	>40 mg %
No. of Patients	5	25
HCY mean	31.88	29.34
HCY S.D.	11.65	9.31

p value = 0.5964

The table 3 shows that the mean homocysteine level in patients with HDL-C > 40 mg % was 29.34 μmol/L and in patients with HDL-C < 40 mg % was 31.88 μmol/L and S.D was 11.65 and 9.31 respectively.

Table 4: Relation of Homocysteine to Total Cholesterol in Case Group with Myocardial Infarction

S. Chol	>200 mg %	S. Chol <200mg %
No. of Patients	4	26
HCY mean	19.0	31.43
HCY S.D.	1.36	5.43

p value = 0.0001

The table 4 shows that the mean homocysteine level in patients with S. Cholesterol > 200 mg % was 19.0 μmol/L and in patients with S. Cholesterol < 200 mg % was 31.43 μmol/L. and S.D were 1.36 and 5.46 respectively.

DISCUSSION

High Homocysteine levels appear to be clearly associated with an increased risk of cardiovascular disease. JAMA 2002 reports a metaanalysis[13] in that evaluated data from 30 prospective and retrospective studies. After adjustments for known cardiovascular risk factors, a 25% lower Homocysteine concentration in the prospective studies was associated with a lower risk of IHD. Schwartz SM et al[14] found that women with Homocysteine ≥ 15.6 μmol / L were at approximately twice the risk of Myocardial Infarction as compared with women with homocysteine ≤ 10 μmol / L. Genest JJ Jr et al[15], reports that homocysteine levels were higher in the CAD group compared with controls (13.9+/- 6.7 vs. 10.9+/- 4.9, p<0.001). The difference between the two groups in our study was highly significant (p = 0.0001), suggesting homocysteine as an important predictor of MI.

Relation of homocysteine to LDL–C in case group with myocardial infarction: The difference observed between the 2 groups in MI was not significant in our study. (p value = 0.1325. Hence, in this study, the low levels of LDL-C did not protect the patients against the homocysteine induced coronary artery disease.

Relation of homocysteine to HDL–C in case group with myocardial infarction: The difference observed in the 2 groups in MI was not statistically significant. (p value = 0.5964). Hence in our study, the higher levels of HDL-C in did not protect the patients against the homocysteine induced coronary artery disease.

Relation of homocysteine to total cholesterol in case group with myocardial infarction: The difference observed in the 2 groups of MI was statistically significant (p value =0.0001) suggesting that patients with lower concentrations of serum cholesterol had significantly higher levels of serum homocysteine levels compared with those who had higher levels of S. Cholesterol. This shows that in patients who did not have high levels of S. Cholesterol, the higher levels of serum homocysteine triggered the coronary artery disease.

CONCLUSION

From this study it can be concluded that in young patients with myocardial infarction the low levels of LDL-C and high levels of HDL-C did not protect against the homocysteine induced MI and homocysteine is an important and independent predictor of myocardial infarction.

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