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Elevated concentrations of plasma homocysteine in coronary artery disease patients with normal folate level

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Abstract

Hyperhomocysteinemia is becoming a growing concern as a risk factor in patients suffering from coronary artery disease. The purpose of our study was to evaluate the existence of hyperhomocysteinemia with folate levels along with conventional risk factors. A total of 80 cases (50 patients and 30 controls) were studied. Abnormal 12 lead echocardiogram (ECG) served as criteria for inclusion. Out of 50 patients 42 were men and 8 were women. Controls were age and sex matched. Total plasma homocysteine in patients was 21±1.9 ($p<0.0001$) and controls 11.7±0.7. Folate levels were 8.2±1.2 and 7.4±0.8 ($p<0.323$) in patients and controls respectively. All the other conventional risk factors were found not statistically significant when compared to homocysteine. Despite normal folate levels elevated homocysteine levels exist in patients suffering from coronary artery disease. Since homocysteine is a modifiable risk factor, testing for homocysteine must be included in risk assessment and diagnosis of coronary artery disease.

Keywords: Homocysteine, coronary artery disease, folate

Introduction

Coronary Artery Disease (CAD) is the primary cause of mortality in developed and developing countries that leads to the development of a series of complex coronary events [1]. Control of conventional risk factors (e.g. smoking, lipids, etc.) has brought a decline in incidence of CAD in developed countries. However, despite aggressive control of risk factors in the general population, it is not possible to prevent progression of CAD in all patients. A raised plasma homocysteine level has been found to be an independent risk factor for atherosclerotic and thrombotic disease [2-4]. Acute coronary syndrome encompasses a cluster of diseases that have similar pathophysiology but different forms of clinical manifestation. This is generally due to the occlusion of the coronary arteries. These arteries innervate the cardiac structure and supply oxygen and nutrients. An occlusion in any one of these will result in a hindrance to the supply of oxygen; resulting in ischemia and consequently death of the surrounding tissue [4].

Homocysteine is an important sulfur-containing amino acid that is formed during the metabolism of methionine, an essential amino acid derived from dietary protein. Total plasma homocysteine (tHcy) is the sum of all; free homocysteine, homocysteine - homocysteine disulfide, homocysteine-cysteine disulfide, and protein bound forms. This amino acid is the product of the methionine catabolic pathway. This amino acid is usually metabolized by the transulfuration cycle to cysteine [5]. An increase in homocysteine otherwise termed as hyperhomocysteinemia can be brought about by number of factors involved in this metabolic pathway. Metabolic reasons for this increase are often linked to dietary intake of methionine or other vitamin cofactors [6, 7]. Modest elevation of this amino acid is also seen in persons with deficiency or low intake of vitamins

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(folate and B_{12} both cofactors in the metabolic pathway) that leads to decreased activity of the respective enzymes for which these vitamins are cofactors [7, 8].

The methionine tolerance in some people could be altered due to some other factors such as genetic make up. Genetic defects in synthesis of certain enzymes in the methionine catabolic or methionine remethylation pathway contribute in a major way. Methylene tetrahydrofolate reductase (MTHFR), enzyme responsible for remethylation of homocysteine to methionine has been cloned and sequenced with disease associated mutations identified. In particular, a C → T substitution at nucleotide 677 results in a conservative Ala → Val replacement accounting for thermo labile MTHFR enzyme (tMTHFR). tMTHFR genotype is significantly correlated with decreased enzyme activity and increased homocysteine levels. The hyperhomocysteinemic effects of tMTHFR are variable and appear to be related to folate sufficiency.

Homocysteine induces vascular dysfunction through oxidative stress and also by inhibiting nitric oxide synthesis. Oxidation of homocysteine generates reactive oxygen species (ROS). Homocysteine derived ROS induces vascular dysfunction. Effects of homocysteine derived ROS on endothelium may include bioavailability of nitric oxide (NO) [9]. Homocysteine post translationally inhibits dimethylarginine dimethyl- aminohydrolase causing asymmetric dimethylarginine to accumulate and inhibit nitric oxide synthesis. This may explain the known effect of homocysteine to impair endothelium mediated NO-dependent vasodilation [10].

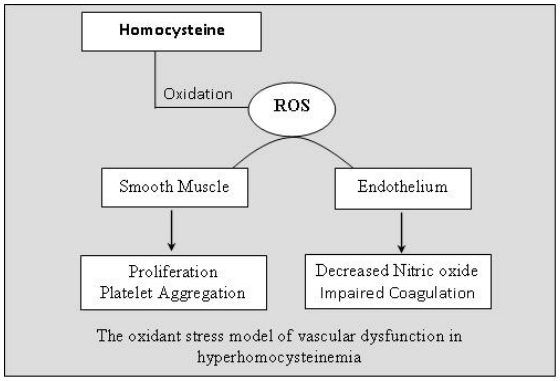
The present study was designed to investigate homocysteine as a good prognostic marker and a modifiable risk factor, hence the need for screening for homocysteine as part of the risk assessment profile. So it would go a long way to show that actually regulating homocysteine levels, by vitamin supplementation, controlled diet and timely diagnostic evaluation can actually help high-risk persons.

Materials and Methods

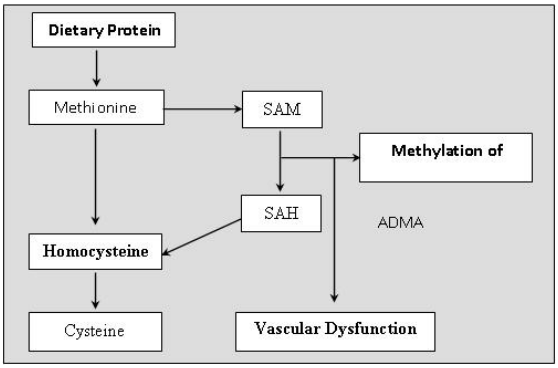
Study Population

The present study was carried out jointly by the Department of Biochemistry and Cardiology, Kovai Medical Center and Hospital, Coimbatore, Tamil Nadu, India. 50 patients were selected cases as and when they were admitted to the Coronary Care Unit with symptoms of chest pain. Myocardial infarction (MI) was confirmed using a standard 12 – lead ECG. Abnormal 12 – lead ECG, absence of vitamins/supplements and prescribed drugs such as anticonvulsants/antiepileptics, no renal disorders (confirmed by urea/creatinine levels) and angiographically proven stenosis (>50%) in two or more vessels are accounted as the inclusion criteria. Age and sex matched healthy individuals (n=50) without clinical evidence of coronary artery disease or family history of cardiac problems and with normal ECG constituted the control group. Inclusion criteria were based on factors such as family history for cardiac problems and normal resting 12 lead ECG. Subjects with renal failure (Crt >/= 3.0gm/dl), hepatic dysfunction, pregnancy, hypothyroidism, and those taking methotrexate, carbamazepine or phenytoin were excluded from the study. The study protocol was approved by the Human Ethics Committee of Kovai Medical Center and Hospital. All the subjects mentioned above gave their consent in writing, and the objectives of the study were fully explained to them in detail prior to taking consent. Blood samples were collected after overnight fasting of more than 10 hrs. Plasma and serum samples were separated and preserved in

accordance with instructions of manufacturers test reagent kit.



The oxidant stress model of vascular dysfunction in hyperhomocysteinemia



Clinical Assessment

On admission, clinical examination was done. Detailed history was taken regarding the duration, frequency and severity of chest pain, exercise tolerance, presence of coronary risk factors and history of previous myocardial infarction. A detailed examination was done to find out the presence or absence of cardiac failure and or presence of associated cardiac lesion. A 12-lead ECG recording was taken and blood samples were taken for the relevant investigations.

Biochemical Analysis

Serum glucose, urea, creatinine, cholesterol, HDL-cholesterol (HDL) were estimated using diagnostic kits made by Roche Germany and Hitachi 912 random access chemistry analyzer. LDL-cholesterol (LDL) was estimated by Friedwalds method. Serum folate level was also analyzed using Hitachi 912 fully automated chemistry analyzer by CEDIA® method, a new recombinant DNA technology assay [17]. Total plasma homocysteine was estimated using microtitre well Enzyme Immuno Assay (EIA) from Axis Shield AS, Norway. Standards ranging from 2.0 – 50.0 were used to obtain a five-point calibration curve required for the assay of homocysteine. This method not only measures free homocysteine but also homocysteine-homocysteine disulfides, homocysteine-cysteine disulfide and protein bound forms.

Statistical Analysis

All results were analyzed using computerized statistical package SPSS 10.0 for Windows. Values are represented as Mean ± SD (standard deviation). Paired t test for unequal samples was used to calculate statistical significance. Statistical significance was noted as $p < 0.05$ at 95% confidence interval.

Results

Demographic, behavioral and nutritional variances in patient and control groups are presented in Table 1. The mean age for the patients group (n=50) was 50±8.7 and for Control group (n=30) was 46±7.5. The patient group consisted of 85% males and 15% females The Control group comprised of 75 % males and 25% were females. In the patients group 72% were non – vegetarians compared to the control group where 82 % were non – vegetarians. 34% in patients and 27 % in controls were diabetic. Hypertensives were 36% and 12 % in patient and control groups respectively. Smokers were higher in patients group (62%) than the control group (16%). 42% in patients and 26% in controls consumed alcohol. In the patient's group anteroseptal MI was seen in 15%, inferior wall MI in 26%, anterior wall MI in 15%, unstable angina in 20% and remaining 24% had symptomatic ischemia. In the patients group 27% of the patients had triple vessel disease and 41 % had more than 70 % stenosis in two of the blood vessels.

Table 2 shows the levels of homocysteine, folate, total cholesterol, triglycerides, HDL and LDL in patient and control groups. Total cholesterol, triglycerides, HDL, LDL and folate levels in patients group when compared with control group showed no statistical significance. Mean homocysteine level 21.5±1.9 μmol/L in patient group compared to 11.7±0.7 μmol/L of control group showed high statistical significance ($p<0.0001$). From the results obtained, there is statistically significant evidence to show relation between elevated levels of homocysteine and coronary heart diseases.

Table 3 shows the mean homocysteine levels between the different age population in the control and patient groups. The mean plasma homocysteine levels of patient population in 25-35, 35-45, 45-55, 55-65 year age groups were 20.12 ± 7.36, 21.42 ± 6.58, 23.17 ± 9.54, 20.92 ± 9.12 μmol/L respectively. The mean plasma homocysteine level in the control population in the age groups 25-35, 35-45, 45-55, 55-65 years were 10.17 ± 3.54, 11.69 ± 4.89, 12.20 ± 6.41 μmol/L respectively. It is clear that there is a marked increase in the mean plasma homocysteine level with increasing age in patients when compared to controls.

Table 4 shows the mean homocysteine levels in the patient population based on the behavioral and clinical factors. There was no significant change noted in the mean plasma homocysteine level in the smokers (11.09 ± 1.86 μmol/L) of the control group compared with non-smokers (11.94 ± 3.46 μmol/L). There was however significant difference when comparing the smokers in patient population with mean plasma homocysteine level of 23.26 ± 10.41 μmol/L with 19.41 ± 8.55 μmol/L in the non-smokers. The differences in the mean plasma homocysteine levels between alcoholics and non-alcoholics, diabetics and non-diabetics, hypertensives and non-hypertensives in the patient and control groups showed no statistical significance.

Discussion

Hyperhomocysteinemia is now recognized as a graded independent risk factor for CAD [11- 13]. Prospective studies done elsewhere showed homocysteine to be a risk factor for atherosclerotic vascular disease, deep venous thrombosis and also for CAD, peripheral arterial occlusive diseases etc., where homocysteine acts individually or in combination with other risk factors [14-20]. Research is currently ongoing to help determine if homocysteine causes cardiovascular disease or is merely a marker associated with it.

Hyperhomocysteinemia is usually defined as an elevation of plasma total homocysteine >15 μmol/L and may be caused by genetic defects, renal insufficiency, certain drugs, or nutritional deficiencies of folate, vitamin B_6, or vitamin B_{12}. Even a mild elevation of plasma tHcy levels within the high-to-normal range may increase cardiovascular risk. Because plasma tHcy can often be lowered by oral administration of folic acid or combinations of B vitamins, there is growing enthusiasm for treatment of hyperhomocysteinemia as a strategy for prevention of cardiovascular disease and its complications [21]. The American Heart Association has recommended that individuals with a family history of heart and cardiovascular disease be tested for plasma tHcy. Other subjects who should be tested are those with premature atherosclerosis or atherosclerosis with no known conventional risk factors such as hypertension or hyperlipidemia. Hypercoaguable profiles now routinely include plasma tHcy [22].

This study shows patients suffering from CAD with hyperhomocysteinemia even in the presence of a normal lipid profile. This is concordant with a study done by Kumar et al [23]. Prospective studies in the United States also showed the same existence of

Table 1: Demographic, behavioral and nutritional variances of study groups

Parameter	Patients (n=50)	Controls (n=50)
Mean age (years)	50±8.7	46±7.5
Sex (%)		
Men	85 %	75 %
Women	15 %	25 %
Non-vegetarian (%)	72 %	82 %
Hypertensive (%)	36 %	12 %
Diabetic (%)	34 %	27 %
Smoking (%)	62 %*	16 %
Alcohol intake (%)	42 %	26 %

Table 2: Homocysteine, folate, total cholesterol, triglycerides, HDL and LDL levels of patient and control groups

Parameter	Patients	Controls	P value
Homocysteine (µmol/L)	21.5 ± 1.9*	11.7 ± 0.7	<0.0001
Folate (ng/ml)	8.2 ± 1.2	7.4 ± 0.8	0.323
Cholesterol (mg/dl)	199 ± 6.3	184 ± 6.1	0.670
Triglycerides (mg/dl)	167 ± 11.4	144 ± 12.8	0.122
HDL (mg/dl)	38 ± 2.8	41 ± 2.2	0.108
LDL (mg/dl)	125 ± 5.5	114 ± 5.2	0.075

Values are given as mean ± S.D from fifty subjects in each group.
Statistical significance was considered as p <0.05 at 95% confidence interval

Table 3: Mean plasma homocysteine levels with correlation to different age population in the patient and control groups

Different age population	Homocysteine (µmol/L)	
	Patients	Controls
25 – 35	20.12 ± 7.36	10.17 ± 3.54
35 – 45	21.42 ± 6.58	11.69 ± 4.89
45 – 55	23.17 ± 9.54	12.20 ± 3.41
55 – 65	20.92 ± 9.12	11.83 ± 3.22

Values are given as mean ± S.D from fifty subjects in each group.
Statistical significance was considered as p <0.05 at 95% confidence interval

Table 4: Mean plasma homocysteine levels with behavioral and clinical factors of patient and control groups

Behavioral and clinical factors	Homocysteine (µmol/L)	
	Patients	Controls
Smokers	23.26 ± 10.41*	11.09 ± 1.86
Non-smokers	19.41 ± 8.55*	11.94 ± 3.46
Alcoholics	21.40 ± 8.18	10.73 ± 3.09
Non -alcoholics	22.87 ± 9.10	12.37 ± 3.28
Diabetics	21.44 ± 9.70	11.73 ± 2.6
Non-diabetics	22.25 ± 10.21	11.79 ± 3.54
Hypertensives	20.51 ± 10.45	11.60 ± 3.37
Non-hypertensives	22.60 ± 9.90	11.93 ± 3.35

Values are given as mean ± S.D from fifty subjects in each group.
Statistical significance was considered as p <0.05 at 95% confidence interval

plasma homocysteine as a risk factor [24, 16]. There is conflicting evidence as far as some of the Indian studies. Some studies suggest there is no association between homocysteine and coronary heart diseases [25-27], whereas other Indian studies with strong evidence definitely showed homocysteine as a risk factor [28, 23]. The latter are in conformance with our study. Moderate elevation of this amino acid is also seen in persons with deficiency or low intake of vitamins: folate and B_{12}. Although hyperhomocysteinemia exists due to decreased folate levels in CAD, our study showed that in spite of normal folate levels in both the patient as well as control groups. The majority of the both populations were non- vegetarians. This suggests that there may be a gross understatement in general requirement of folate in diet. The higher number of non-vegetarians in both groups may be the reason since green leafy vegetables contain high levels of folic acid.

There were increased levels of total plasma homocysteine in the patient group when compared to the control group. Raised homocysteine levels may be due to higher frequency of non-vegetarian diet in turn increasing the methionine intake. The numbers of smokers were higher in the patients group when compared to the control group. This could be another attributable factor for raised homocysteine levels. There is a definite relation between smoking and hyperhomocysteinemia that has been shown by Stein and McBride [19].

There are variations in homocysteine levels in different studies in India when compared to other studies done in foreign countries. These variations could be due to the immense diversity in the dietary as well as behavioral patterns in the Indian populations. Variations could also be due to lack of uniformity in, the sampling and definition of cases, sample size and methodology.

Conclusion

Our study definitely suggests a strong link between elevated homocysteine and coronary heart diseases independent of the folate levels. Hence multi–centered prospective studies involving diverse populations based on risk assessment are

required to bring out the actual role of homocysteine as a modifiable diagnostic marker.

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