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RELATIONSHIP OF PLASMA HOMOCYSTEINE WITH LIPID PROFILE PARAMETERS IN ISCHEMIC HEART DISEASE

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ABSTRACT

The present study was undertaken to explore the relationship of plasma homocysteine with other biochemical parameters in ischemic heart disease. Plasma levels of total homocysteine was measured by HPLC - fluorescence detection with internal standard in 60 ischemic heart disease patients and were compared with 30 age matched normal healthy controls. The significant increase of plasma homocysteine was observed in both myocardial infarction and chronic stable ischemic heart disease patients when compared with the controls. The hyperhomocysteinemia appears be to due to increased body demand of vitamins such as folic acid, vitamin B_{12}, B_6, B_2 either alone or in combination to regulate normal homocysteine metabolism.

KEYWORDS

Ischemic heart disease (IHD), High density lipoprotein (HDL), High performance liquid chromatography (HPLC), Homocysteine (Hcy), Total cholesterol (TC) and Triglycerides (TG).

INTRODUCTION

Ischemic heart disease (IHD) is a major public health problem in India. Its mortality and disability rises very rapidly with age. It occurs at an early age of life. This cannot be explained by the conventional risk factors like hyperlipidemia, smoking, diabetes mellitus, hypertension, abdominal obesity, stress, physical inactivity, insulin resistance. The discovery of homocysteine (Hcy) as a risk factor in vascular diseases diverted the attention of medical practitioners and researchers from conventional risk factors (1).

It is now well established that elevated circulating level of Hcy is associated with IHD (2,3). The severe hyperhomocysteinemia is seen in 5 - 7 % of general population who do not show clinical signs and symptoms of IHD in their early life (4). Much work is done in Indian population on this aspect (5,6), however, there are conflicting reports on the serum Hcy in Indian population (3,5). The molecular mechanism by which Hcy promotes atherosclerosis is unknown. Hence the line of thought was adopted to elucidate the relationship between Hcy with other biochemical parameters in IHD.

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MATERIAL AND METHODS

The present work is a collaborative study involving the Departments of Biochemistry, Government Medical College, Miraj and Maharashtra Institute of Medical Sciences and Research-Medical College, Latur. Sixty IHD patients, constituting 30 myocardial infarction (MI) and 30 chronic stable IHD patients, were included in the present study. The IHD patients were diagnosed based on clinical examination, laboratory investigations, stress test (TMT), electrocardiogram, angiography and other tests. The chronic stable IHD patients had last episode of MI more than a year back and from then they were symptom less or without any episode of fresh ischemia.

30 normal healthy subjects, having age ranging 20 to 65 years matched with the patients, formed the controls. All the controls were symptom less on clinical examination. All the laboratory tests including TMT were within normal limits. These subjects had no past / family history indicating cardiac disorders and were found to be free from diseases like diabetes mellitus, hypertension, renal, liver or thyroid disorders.

Fasting blood samples were collected from the patients and the controls. The blood samples were collected by vein puncture in EDTA vacutainer and plain bulb using disposable syringe. The blood collected in EDTA vacutainer was immediately

centrifuged and plasma was separated. The plasma was used for the estimation of Hcy by HPLC-Fluorescence detection with internal standard method (7,8) using reagents of M/s RECIPE, Sandstrasse, Germany. The blood collected in plain bulb was kept in tilted position for 30-45 minutes at room temperature and then centrifuged to separate serum. The serum was used for the estimation of total cholesterol (TC), HDL-cholesterol and triglycerides (TG) levels (9-11) using reagents of M/s Bayer Diagnostics. The serum folic acid and vitamin B_{12} were estimated by competitive immunoassay using direct chemiluminescence technique (Bayer Corporation, USA) (12,13). The serum lipid peroxides were measured by colorimetric method (14) as thiobarbituric acid reactive substances (TBARS) using malondialdehyde as a standard. The statistical analysis of results included 't' test and regression analysis for correlation.

RESULTS

In the present study, mean serum TC level was significantly elevated in MI ($p < 0.001$) as compared to the controls. There was no significant change of serum cholesterol level in chronic stable IHD patients ($p > 0.05$) as compared to the controls (Table 1). HDL-cholesterol level was significantly decreased in MI ($p < 0.05$), but no significant change was observed in chronic stable IHD. Serum TG levels were significantly elevated in MI and chronic stable IHD patients ($p < 0.001$) as compared to the controls. The values of lipid profile in the controls were agreeable and within the acceptable range as suggested by National Cholesterol Education Programme (15).

The significantly elevated levels of lipid peroxide were observed in MI ($P<0.001$) and chronic stable ischemic heart disease ($P<0.05$) as compared to normal healthy controls (Table 2). Plasma Hcy levels were significantly elevated in both MI and chronic stable IHD patients ($p < 0.001$) as compared to the controls. There was about three-fold rise in the mean level of plasma Hcy in the IHD patients compared to the controls. There were no significant changes in serum folic acid and vitamin B_{12} of the IHD patients ($P>0.05$) as compared to normal healthy controls. In the present study, there was no significant correlation between plasma Hcy and HDL-cholesterol, total cholesterol and TG in MI and chronic stable IHD patients (Table 3).

DISCUSSION

In the present study, plasma Hcy level was significantly increased ($p < 0.001$) in patients of MI and chronic stable IHD as compared to the controls. Similar results are reported by others in IHD (2), MI (6,16), atherosclerosis (1) and in coronary artery disease (5).

The increased levels of plasma Hcy indicate abnormal homeostasis of Hcy metabolism. There could be several possibilities of hyperhomocysteinemia in MI and chronic stable IHD. One possibility is that there could be decreased utilization of Hcy with normal or increased production of Hcy. If this possibility is considered, then re-cycling of Hcy to methionine could be altered or the conversion of Hcy to cysteine and ultimately its excretion could have been abnormal (Fig). The possibility of the abnormality in the re-cycling of Hcy to methionine is ruled out in view of the observation that serum folic acid and vitamin B_{12} levels are within the normal limits (Table 2) since folic acid and B_{12} are involved as coenzymes for remethylation of Hcy to methionine. The other possibility, that the conversion of Hcy to cysteine and its excretion could have been abnormal. In view of this, urinary amino acid excretion pattern by paper chromatography (unpublished) shows intense spots for cysteine and Hcy in IHD patients than the normal healthy controls. This indicates that the excretion of cysteine along with Hcy is increased in IHD patients.

The second possibility of increased plasma Hcy could be due to deficiency of specific enzymes involved in

Table 1. The lipid profile parameters in the controls and IHD patients

Gr	Study subjects	n	Serum TC (mg/dl)	Serum HDL chol. (mg/dl)	Serum TG (mg/dl)	Serum LDL chol (mg/dl)	Serum VLDL chol (mg/dl)
I	Normal controls	30	172.08±26.80	47.51±14.53	100.88±18.26	110.29±24.79	19.74±3.87
II	MI	30	215.73±8.56*	41.78±4.63**	152.28±66.36*	139.73±37.04*	33.79±21.87
III	Chronic stable IHD	30	184.23±7.12	45.09±16.07	132.07±49.16*	95.60±40.46	26.36±12.32

The results were compared between group I and II, group I and III. The values are mean±S.D. *P < 0.001, **P < 0.05.

Table 2. Plasma Homocysteine and other parameters in the controls and IHD patients

Gr	Study subjects	n	Plasma Hcy (M/L)	Serum Folic acid (mg/dl)	Serum Vitamin B_{12} (mg/dl)	Serum TBARS (nmol/ml)
I	Normal controls	30	10.76±2.77	6.78±4.3	280.60±78.75	1.78±0.19
II	MI	30	32.48±18.99*	6.74±4.8	278.75±85.89	3.70±0.41*
III	Chronic stable IHD	30	30.30±17.72*	6.76±5.2	276.00±98.07	2.03±0.14**

The results were compared between group I and II, group I and III. The values are mean±S.D. *P < 0.001, **P < 0.05.

Table 3. The correlation between plasma Hcy and lipid profile parameters in the patients

Biochemical parameters	MI			Chronic stable IHD		
	r	Slope	p	r	Slope	p
Hcy Vs TC	-0.055	4.055	0.76	-0.083	4.583	0.66
Hcy Vs HDL-chol	0.221	0.982	0.23	0.0196	1.269	0.917
Hcy Vs TG	-0.009	0.0002	0.96	0.053	4.425	0.77

Hcy = Homocysteine; r = Coefficient of correlation.

the Hcy metabolism. Such enzyme deficiencies are very rare, occurring 1 in 1,00,000 live births. Hence, the hyperhomocysteinemia in these patients is not due to enzyme deficiency.

The third possibility of increased plasma Hcy could be due to increased production of Hcy (Fig). This increased production of Hcy may occur due to increased breakdown of methionine. The dietary intake of methionine in these patients could have been in excess of daily requirement along with proportionately lower folic acid and vitamin B_{12}. As a result, the reutilization of Hcy to methionine might have been impaired due to relative deficiency of vitamin coenzymes. Folic acid and vitamin B_{12} are involved as coenzymes for Hcy utilization. Since serum folic acid and vitamin B_{12} levels in these patients were almost identical to the controls, any abnormality in the recycling of Hcy due to vitamin deficiency is ruled out.

It appears most likely that the cause of hyperhomocysteinemia may be due to abnormal functioning of vitamin coenzymes. It may be impaired conversion of folic acid vitamin to its biological active form (N^5 methyl tetrahydrofolate coenzyme). There could be increased body demand of folic acid, vitamin B_{12}, B_6, B_2 either alone or in combination to regulate Hcy metabolism in the face of excessive dietary intake of methionine. This possibility seems to be more logical.

Increased plasma Hcy leads to the formation of atheromatous changes, which ultimately cause MI. Hcy contains reactive sulfhydryl group, which undergoes oxidation to the disulfide at physiological pH. The reaction is catalysed by transition metals and a variety of reactive oxygen species are produced which initiate lipid peroxidation responsible for endothelial cytotoxicity.

The Hcy on oxidation forms Hcy thiolactone. It is complexed with LDL-particles. This LDL-Hcy-thiolactone complex aggregates and deposits in the form of thioco and then thioretinamide. These intermediates promote proliferation and fibrosis of smooth muscles. During conversion of thioco to thioretinamide highly reactive oxygen species are generated, which cause several changes in the intima of the blood vessels and endothelial dysfunction. Thus resulting in atherosclerotic plaque (17).

In the present study, there was no significant correlation between plasma Hcy and TC, HDL-cholesterol and TG (Table 3). This confirms that elevated plasma Hcy is an independent risk factor for Indian IHD patients also, like western population, in addition to other risk factors. However, the question remains as to what extent hyperhomocycteinemia determines the risk of IHD. Further studies are warranted to elucidate the precise role of hyperhomocycteinemia in IHD.

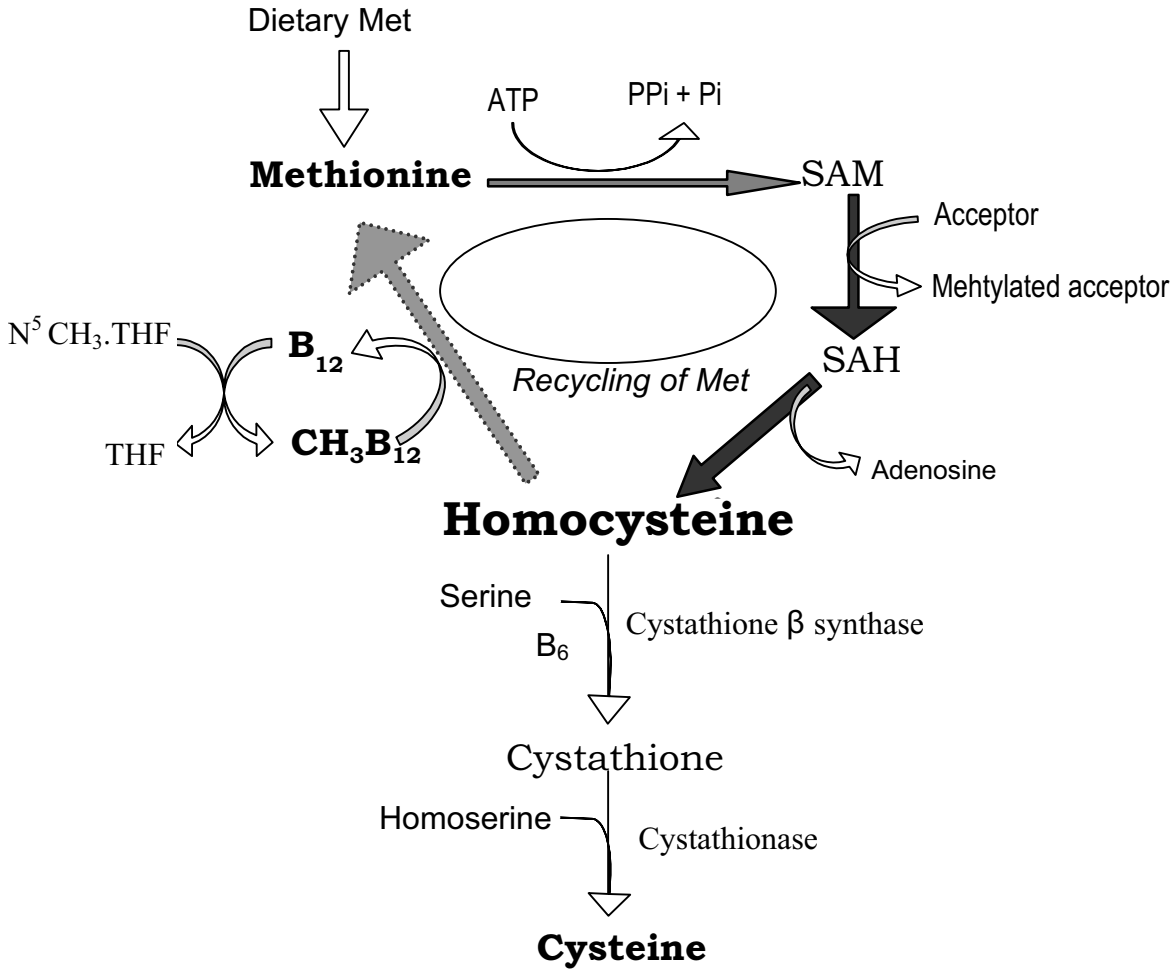

Fig. Metabolic pathway of methionine showing formation and fate of homocystein in IHD (SAM = S-Adenosyl Methionine; SAH = S-Adenosine Homocystein; THF = Tetrahydrofolic acid)

It is suggested that the simple preventive measures may reduce the risk of MI in individuals prone to IHD such as dietary supplementation of adequate vitamins from natural sources (fruits and green leafy vegetables), substitution of animal proteins by plant proteins in the diet, as animal proteins have higher methionine content than the plant proteins. This may, in part, decrease the body burden of Hcy and regularize the deranged Hcy metabolism in most cases.

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