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PLASMA HOMOCYSTEINE LEVELS IN PATIENTS OF CORONARY ARTERY DISEASE IN PATIENTS COMING TO RURAL MEDICAL COLLEGE OF SUBHIMALYAN REGION OF INDIA

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ABSTRACT

Homocysteine is a sulfur containing amino acid generated through the demethylation of methionine. It is largely catabolized by trans-sulfuration to cysteine but it may also be remethylated to methionine. Dubbed 'the cholesterol of 90's by the lay press, homocysteine is thought to be thrombophilic and to damage the vascular endothelium. Normal total plasma homocystine concentration ranges from 5-15 µmol/L in the fasting state. Although severe homocystinemia is rare, mildly elevated levels occur in approx. 5-7% of general population. Patients with mild homocystenemia are typically asymptomic until 3rd and 4th decade when premature CAD develops as well as recurrent arterial or venous thrombosis. **Aims and Objectives were to** determine levels of plasma homocystine in coronary artery disease, to compare the homocystine levels in patients of coronary artery disease with healthy subjects with and without the presence of other coronary risk factors and to evaluate the possible association of coronary risk factors with plasma homocystine levels. **Material and Methods** The study was conducted in Department of medicine, RPGMC, 300 patients of coronary artery disease (CAD), evidenced by acute MI beyond 8 weeks and 30 age and sex matched controls divided into two groups were included in the study. The two control groups were:- Those who had coronary risk factors for CAD like hypertension, diabetes, hyperlipidemia but not suffered any coronary event and those who had no coronary risk factors and had no clinical evidence of CAD. **Summary and conclusion** Plasma HCy levels in healthy group in our study was 12.51±5.92 µmol/It.Among subjects with CAD the plasma HCy levels were significantly higher (21.97±10.14 µmol/It).In the group of subjects free of clinical CAD but having conventional CAD risk factors like smoking, hypertension etc., plasma homocysteine level were (15.2 μmol/It+7.3µmol/It).So plasma HCy levels were significantly higher in subjects with clinical CAD and it may be independent risk factor for CAD.Further the presence of CAD risk factors is associated with elevated levels of plasma HCy irrespective of overt CAD.A crucial question is whenever plasma HCy is directly involved in the pathogenesis of vascular disease or just a marker for increased risk. Our study can not answer this question and it can not explain the mechanism of the observed relation.This intriguing series of association should incite intervention studies on the effect of HCy on CAD and influence design and analysis of future studies on plasma HCy levels and CVD.

INTRODUCTION

Homocysteine is a sulfur containing amino acid generated through the demethylation of methionine. It is largely catabolized by trans-sulfuration to cysteine but it may also be remethylated to methionine. Dubbed 'the cholesterol of 90's by the lay press, homocysteine is thought to be thrombophilic and to damage the vascular endothelium . Total plasma homocysteine (tHcy) is now established as a clinical risk factor for coronary artery disease, as well as other arterial and venous occulisive disease in adult populations. Regulation of homocysteine is dependent on nutrient intake, especially folate, vitamins B6 and B12. It is also controlled by common genetic variations (polymorphisms) in how vitamins are utilized as cofactors in the reactions controlling homocysteine metabolism. Moreover, concentrations are age-and sex-dependent and are altered by renal function, hormonal status, drug intake and a variety of common clinical factors. Molecular testing for the genetic polymorphisms is still in the research phase but the ease and reliability of molecular diagnosis will speed its introduction into clinical laboratory practice- particularly in relation to diagnosis of thrombophilic disorders. Clinical research initiatives are being driven by the benefit that should be achieved by correction with vitamin supplements, particularly folate and vitamins, but it must be recognized that prospective controlled studies to validate clinical benefit are only now being initiated.

At the moment, it is safe to say that hyperhomocysteinemia is one of the few prevalent biochemical risk factors for thromboembolic disease that might be corrected by vitamin supplements. Such possibility lies behind the growing momentum to recommend increased supplements of folate and B vitamins to at-risk population and patient groups today.

Atherosclerotic disease involving coronary, peripheral and cerebrovascular system continues to be a major health problem in adult population, both in developed and developing countries. Control of conventional risk factors (e.g. smoking, lipids etc.) have brought a decline in incidence of coronary artery disease (CAD) in developed countries. However, examination of conventional CAD risk factors not only fails to fully explain the marked difference in CAD rates among people of diverse enthicity, but also revealed several paradoxes- either low rates of CAD with high prevalence of risk factors (Asian Indian paradox). Thus, there was need to look beyond conventional risk factors for CAD and recently research is underway and many other risk factors for CAD are being identified (e.g. high Lp(a), various coagulation disorders etc.)

Homocystine and CAD Normal total plasma homocystine concentration ranges from 5-15 µmol/L in the fasting state. Although severe homocystinemia is rare, mildly elevated levels occur in approx. 5-7% of general population. Patients with mild homocystenemia are typically asymptomic until 3rd and 4th decade when premature CAD develops as well as recurrent arterial or venous thrombosis. There have been 13 reports that prospectively examined the association between homocystine and CHD. Of these, 6 indicated significantly high risk for those patients with high homocysteine levels.

Recent data from the Framingham heart study indicated an association of high homocysteine with mortality comparing the highest quartile to the lowest 3 quartile of homocysteine , adjusted RRS were 1.54 (1.31-1.82) for all cause mortality and 1.52(1.16-1.98) for CVD mortality. It was also seen that affected children who had severe hyperhomocystinemia (plasma conc.>100mmol/L) develop widespread premature atherosclerosis, although initial thickening and fibrous plaque are more common. Homocystine conc. exceeding the upper limit of normal (15 mmol/L) are common and are found in almost 30% patients with vascular disease.

Mechanism of HCy-induced vascular damage

The pathogenesis of Hcy-induced vascular damage may be multifactorial including direct Hcy damage to the endothelium. Stimulation of proliferation of smooth muscle cells, enhanced LDL peroxidation, increase of platelet aggregation and effects on the coagulation system. Besides adverse effects on the endothelium and vessel wall, Hcy exerts a toxic action on neuronal cells through the stimulation of N-methyl-D-portate (NDMA) receptors. Under these conditions, neuronal damage is a result of excessive calcium influx and reactive O generation. This mechanism may be responsible for the cognitive changes and markedly increased risk of cardiovascular disease in children and young adults with homocysenemia.

Choy PC et al have proposed another mechanism for the production of atherosclerosis by homocystine. When human hepatoma cells (Hep G2) were incubated with 4mM homocystine, enhancements in the production of cholesterol and secretion of a polipoprotein B-100 were observed. The stimulatory effect on cholesterol synthesis was mediated via the enhancement of HMG-CoA reductase, which catalyzes the rate limiting step in cholesterol biosynthesis. Cholesterol appears to play an important role in the regulation of apoB-100 secretion by hepatocytes. It is plausible that the rise in apo B secretion was caused by the elevated cholesterol level induced by homocystine. The ability of homocystine to produce a higher amount of cholesterol and promote the secretion of apo B would provide a plausible

mechanisms for the observed relationship between hyperhomocystenemia and the development of atherogenesis and CAD.

AIMS AND OBJECTIVES

To determine levels of plasma homocystine in coronary artery disease.

- To compare the homocystine levels in patients of coronary artery disease with healthy subjects with and without the presence of other coronary risk factors.
- To evaluate the possible association of coronary risk factors with plasma homocystine levels.

MATERIAL AND METHODS

Protocol:

The study was conducted in Department of medicine, RPGMC, 300 patients of coronary artery disease (CAD), evidenced by acute MI beyond 8 weeks and 30 age and sex matched controls divided into two groups were included in the study. The two control groups were:-

A) Those who had coronary risk factors for CAD like hypertension, diabetes, hyperlipidemia but not suffered any coronary event.

B) Those who had no coronary risk factors and had no clinical evidence of CAD.

Statistical methods Standard statistical methods are used. These include test, Chi square test and ANOVA. All values are expressed as mean ±SD values specified other. A p value of <0.05 is taken as statistically significant.

Observations; The subject ranged in age of 35-65 year. Cases were having mean age of 52.63 with SD of 7.74, controls with RF were having mean age of 49.07 with SD of 7.27 and controls with out RF were having mean age of 49.67 with SD of 8.07.

Most of our subjects were in the age group of 50-60 years.

In cases about 93.33% subjects were hypertensive, 50% were smokers and 53.33% were having +ve family history of CAD. More than two risk factors were present in 63.33% of cases.All the controls with RF had hypertension; 46.7% were smoker and only 13.33% subjects had +ve family history of CAD. Among cases 90% of subjects had angina on effort, 66.67% has cardiac enlargement and 100% has abnormal ECG suggestive of MI.

Table 1: Homocysteine levels in cases and controls

Parameters	Cases	Controls with RF	Control with out RF	p
n	300	150	150	a
mean	21.97	12.22	12.51	b
SD	10.14	7.3	5.92	c
Min	8.43	3.98	3.40	d
Max	51.00	26.70	23.70	
Median	20.27	16.72	11.32	

a	=	cases vs control with RF	=	0.027
b	=	cases vs control with out RF	=	0.002
c	=	control with RF vs control without RF	=	0.0278
d	=	Overall cases vs controls	=	0.002

The plasma hemocysteine levels in patients levels in patients of CAD was 21.97± 10.14 as against 15.22±7.38 in control with (p=0.027) and 12.51±6.13 in control without RF (p=0.002)[Table-1].

The male patients of CAD had plasma homocysteine ranging from (8.43 to 51.00) with mean of 23.51±11.16 while in females in this group, hemocyseine level ranged from 9.73 to 31.21 with mean of 18.91±7.27. This difference was not statistically significant. The male patients of controls with RF had plasma hemocysteine levels ranging from 3.78 to 24.82 with mean of 17.53±7.56 while female patients in this group has level ranging from 5.73 to 26.70 with mean of 13.67±7.28 with p value of >0.2. This difference is not statistically significant.

The male patients of controls with RF had plasma hemocyteine levels ranging from 3.40 to 23.70 with mean of 12.85±6.03 while female patients in this group had hemocysteine level in range of 5.60 to 18.10 with mean of 11.58 ±6.06.This difference is not statistically significant.

In smokers 52.2% of subjects were having moderate hyper homocysteine while 34.7% were having normal, 3% intermediate hyper homocysteine. In hypertension, 65.5% of subjects were having moderate hyper hamocystenemia, 27.2% were having normal and 6.8%

were having intermediate hyperhomocystine. In diabetic subjects 57.1% were having moderate, 35.7% having normal and 7.14% having intermediate hyperhomocysteine .In dyslipidemic subjects 71.4% were having moderate, 14.3% were having intermediate hyperhomocysteine.In patients having +ve family history of CAD 45.5% were having moderate, 45.5% were having normal and 9% were having intermediate hyperhomocysteine In subjects having>2 risk factors 58.3% were having moderate,29.2% having normal and 12.5% having intermediate hyperhomocysteine. In our study no person was having hemocysteine which was in severe range> 100 µmol/Lt.So, most of the subjects with other risk factors for CAD were having hemocysteine in moderate category in moderate ie 15.1- 30 µg/ml[Table-2]

Table 2: Plasma hemocysteine level in risk factor group without having CAD

Risk factor			Mean homocysteine level Mmol/lt ±SD	p value
Diabeties	Y	n =130	20.24±4	p>0.05
	N	n =20	14.44±7.54	
Smoking	Y	n =80	17.7±7.84	p>0.05
	N	n =70	12.37±6.16	p>0.05
Deranged	Y	n = 40	19.16±8.37	p>0.05
Lipids	N	n=110	13.78±6.85	
Hypertension	Y	n=150	15.22±7.39	p<0.05
	N	n= 0		
>2 risk factors	Y	n=50	16.48±9.42	p<0.05
	N	n=100	12.5±5.92	

DISCUSSION

The role of elevated plasma homocysteine levels as a risk for arteriosclerotic vascular disease has attracted growing interest. In recent years hyperhomocysteine has been found to be associated with premature peripheral vascular[1,2], cerebrovascular[1,3] and coronary artery disease[4,6] and to be a risk factor independent of hyperlipidemia, hypertension or smoking[7,8,9].

The present study was conducted to assess the relationship of plasma homocysteine level to coronary artery disease in population of subhimalyan region of India. The value of plasma HCy in the subjects without CAD and CAD risk factor in the present study was found to be 12.51±5.92 umol/L.The plasma value of HCy in study conducted by

Ridhlet et al[10] was 12.4 umol±4.18 umol/L. The normal population had plasma homocysteine value of 11.8 umol/It in study conducted by Nicholas J Wald et al. In study conducted by Meir J Stoppu et al plasma homocysteine levels in normal population were lower than our study i.e., 10.5±2.8 umol/It.

Among our subject with CAD, the plasma homocysteine levels were significantly higher than control value (21.97±10.14 vs 12.51±5.92; p=< 0.02) elevaged plasma HCy levels had been reported in CAD patients previously also. In the study conducted by Ridhlet et al[10] homocysteine levels in patients with CAD were significantly higher than the control values ($p<0.05$).Nicholas J Wald et al in their study found mean homocysteine levels in patients of CAD to be 13.1 umol/It, significantly higher than the control value of 11.2 umol/It ($p < 0.05$). Paul M. Ridkualso reported increased plasma HCy levels in subject with CAD. The reason for higher average levels of plasma HCy in our subjects with CAD as compared to the published literature are not clear. However a possible role of ethnic factors and dietary differences cannot be excluded.

The ability of HCy to produce a higher amount of cholesterol and promote the secretion of apo B would provide a plausible mechanism for the observed relationship between hyperhemocystenimia and the developopment of aterogenesis and CAD.We also studied the effect of coronary risk factor over plasma HCy levels. In the group of subjects free of clinical CAD but having conventional CAD risk factor, Plasma HCy levels were 15.22+7.3 umol/It which was significantly higher than control subjects with out CAD RFs ($p = 0.0278$).

In study conducted by Jacob, subjects without CAD but with other risk factors for CAD the level of plasma HCy was 14.8 umol/It compared to 11.2 umol/It in subjects with out CAD RF ($p<0.05$).In a study conducted the Otter Nygard et al[14] mean plasma homocysteine level were also higher in males as compared to females through overall levels were also higher in males as compared to females though overall levels were lower (12.3 vs 11.0 umol/It). Several other studies [16,17,18-23] have also showed raised levels of plasma homocysteine in males as compared to females.

Smoking has been found to be associated with increased plasm a HCy in various previous studies. In the Hordaland population study[14] plasma HCy levels increased almost proportionately to the number of cigarettes smoked per day and smoking was one of the strongest determinant of HCy level. Current smokers had a distinctly higher plasma HCy levels. This increase with combined effect of cigarette smoking, age and sex was strong with a difference of 4.8 umol/It.

In study by Otter Naygrad et al[14] heavy smoking cases had a mean HCy level 4.8 umol/It higher than never smoking cases. In our study mean plasma HCy levels in healthy smokers was 17.7±7.8 umol/It which was much higher than nonsmokers in the same group (12.37±6.16) but this difference was not statistically significant. In overall cases and controls 34.7% of smokers wee having normal homocysteine levels while 37.8% of nonsmokers were having normal HCy levels. Moderate HCy levels (15.1-30 ug/It) were found in 52.17% of smokers and 59.4% of nonsmokers. Intermediate homocysteine levels (30.1-100 ug/It) were found in 13.04% of smokers and 2.7% of nonsmokers. This difference was not statistically significant (p=0.295). In our study all the 150 subjects in the RF group were hypertensive so we could not compare this group with non hypertensive controls. However, in control subjects with out any coronary RF, the mean plasma HCy levels were 12.51±5.92 umol/It. This difference was statistically significant ($p<0.05$).

Overall (in both subject of CAD and without CAD) 27.2% of hypertensive were having normal HCy level, 65.5% of hypertensive were having moderate hyperhomoysteine and 6.88% were having intermediate homocysteine as compared to 62.5%, 31.3%, 6.3% of non-hypertensive patients having normal, moderate and intermediate hyperhomocysteine. This difference was not statistically significant ($p = 0.039$) .In European Concerted Action plan on HCy and vascular disease[15] the risk factor conferred by HCy was similar to and independent of conventional risk factor. An elevated HCy level interacted strongly with hypertension and smoking, the combined effect was more than multiplication in both studies.

In Hordaland study elevated HCy leels was associated with major components of cardiovascular risk profile i.e. hypertension and smoking. Plasma HCy level showed a positive linear association with diastolic blood pressure and systolic pressure. This relation was essentially conferred to younger age group. In our diabetic subjects without CAD, mean plasma HCy level was 20.24±4.95 umol/It as compared to non-diabetic controls (14.44±7/54 umol/It) ($p>0.05$)

Overall (both cases and controls) 35.7%, 57.1% and 7.14% of diabetics had normal, modeate and intermediate values of mean plasma homocysteine respectively. Corresponding value in non-diabetic population were 36.9%, 56.5% and 6.5% respectively ($p = .999$).Hordaland Study revealed a positive correlation between plasma homocysteine level in diabetics. Diabetics in our study were also having increased plasma HCy level.

In our study subjects with total cholesterol > 200 mg/dl had mean plasma HCy level of 19.16±8.37 umol/It as compared to 13.78±6.85 in subjects with total cholesterol < 200mg/It.

However, the difference was not statistically significant. Overall (cases and controls) among subjects with increased cholesterol 14.3% were having normal, 71.42% were having moderate and 14.3% were having intermediate hyperhomoysteine. Corresponding values in subject with normal lipids 43%, 52.1% and 4.3% respectively.

Hordaland study revealed a positive association between plasma homocysteine level and serum cholesterol levels. A weak positive relationship between scrum triglycerides and plasma HCy level were observed in younger men and older women. In our study among subjects without CAD and with > 2 risk factors the plasma hemocysteine level was 16.48±9.42 umol/It as compared to 12.51±5.92 umol/It in subjects with no CAD Rf ($p<0.05$).Overall (cases and controls) 29.2% of subjects with >2 risk factor were having normal, 58.3% were having moderate and 12.5% were having intermediate hyperhomoysteine; subjects without CAD risk factors 41.6% were having norma, 55.5% were having moderate and 2.7% were having intermediate hyperhomoysteine.

Most of our subjects without CAD but with coronary risk factors were having moderate hyperhomoysteine whereas most of subjects without CAD and without coronary risk factors had normal homocysteine. It appears that plasma HCy are positively correlated with the presence of CAD RF even in the absence of clinical CAD. In European concerted action project[15] on HCy and vascular disease the risk conferred by homocysteine was similar to and independent of conventional risk factor and elevated HCy level interacted strongly with hypertension and smoking.

We concluded that patients with CAD have significantly increase plasma HCy levels as compared to healthy controls. Further the presence of CAD risk factors is associated with elevated levels of plasma HCy irrespective of overt CAD.

SUMMARY AND CONCLUSION

- Plasma HCy is a novel and independent risk factor for CAD.
- Plasma HCy levels in healthy group in our study was 12.51±5.92 µmol/It.
- Among subjects with CAD the plasma HCy levels were significantly higher (21.97±10.14 µmol/It).
- In the group of subjects free of clinical CAD but having conventional CAD risk factors like smoking, hypertension etc., plasma homocysteine level were (15.2 μmol/It+7.3µmol/It).

So plasma HCy levels were significantly higher in subjects with clinical CAD and it may be independent risk factor for CAD.Further the presence of CAD risk factors is associated with

elevated levels of plasma HCy irrespective of overt CAD.A crucial question is whenever plasma HCy is directly involved in the pathogenesis of vascular disease or just a marker for increased risk. Our study can not answer this question and it can not explain the mechanism of the observed relation.

This intriguing series of association should incite intervention studies on the effect of HCy on CAD and influence design and analysis of future studies on plasma HCy levels and CVD.

CONFLICT OF INTEREST : None

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