ORIGINAL ARTICLE

Association of Hyperhomocysteinemia with Smoking, Hypertension, Diabetes and Age In Coronary Artery Disease Subjects

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ABSTRACT

Background: Several large-scale international studies have shown that hyperhomocysteinemia is an important risk factor for coronary artery disease (CAD). Though homocysteine levels among CAD subjects of different populations in India have been studied, the association of hyperhomocysteinemia with conventional CAD risk factors has not been thoroughly investigated. Our objective was to study the association between hyperhomocysteinemia and a few conventional risk factors among CAD subjects of Kerala.

Methods and Results: We estimated plasma homocysteine concentration among coronary artery disease subjects (n=92) and investigated the association of hyperhomocysteinemia with smoking, hypertension, diabetes and age in these subjects. Smokers had elevated homocysteine levels when compared to non-smokers (18.3 ± 8.8 vs. 12.4 ± 6.7 µmoles/L); increased homocysteine concentrations were noticed among hypertensives compared to non-hypertensives (15.3 ± 6.7 vs.12.9 ± 8.5 µmoles/L), while no significant differences were noticed between diabetics and non-diabetics and also between subjects stratified into different groups based on age. Smoking and hypertension were found to contribute to hyperhomocysteinemia independent of other variables studied.

Conclusions: It can be concluded from this study that among the variables studied, hyperhomocysteinemia was strongly associated with smoking and hypertension in CAD subjects of this population. (J Clin Prev Cardiol 2013;2(3):123-7)

Keywords: coronary artery disease (CAD); diabetes mellitus; hyperhomocysteinemia; hypertension; smoking

Introduction

Coronary artery disease (CAD) has attained epidemiological proportions in India with around 1.5 million mortalities in 2002 (1). Kerala has a high prevalence of CAD in India in spite of its better health indices compared to other states (2). This situation has made it necessary to explore the status of emerging risk factors for CAD in this population.

Homocysteine (Hcy) is a sulfur containing amino acid formed from metabolic demethylation of methionine. Impaired Hcy metabolism causes hyperhomocysteinemia, which contributes to cardiovascular events by damaging endothelium, inducing oxidative stress and increasing the thrombogenicity of endothelium (3).

Hyperhomocysteinemia has a multifactorial origin and various factors, genetic and acquired, can influence its levels. Hcy levels are higher in men than in women and the levels increase in both genders with advancing age (4-6). Deficiency of B vitamins and genetic defects, leading to improper homocysteine metabolism, contribute to hyperhomocysteinemia (7,8).

Previous studies in Western population have shown that elevated Hcy concentrations are an independent and graded risk factor for different categories of arterial occlusive diseases (9-11), though some investigators considered this association to be casual (12). Studies conducted to establish the role of Hcy as a major risk factor for CAD in different ethnical populations of India has also yielded conflicting results (13,14). An earlier study conducted in Kerala has not reported hyperhomocysteinemia among CAD subjects of this population (15). Besides, data on the correlation between conventional CAD risk factors and hyperhomocysteinemia in this population is lacking. So we conducted this preliminary study to assess the Hcy levels among CAD subjects of Kerala and investigated the association of hyperhomocysteinemia with conventional risk factors such as smoking, hypertension, diabetes and age in these subjects.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki (2000) at a tertiary care

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University hospital in Kerala. The protocol for the study was approved by the Institutional Ethics Committee and informed consent was obtained from all the subjects. Women were not considered for the study as Hcy levels are influenced by gender. A total of 143 men with clinical manifestation of CAD between 35 to 70 years of age were screened initially. Criteria for diagnosis of CAD were previously documented myocardial infarction or inducible ischemia on tread mill test (TMT) or angiographically proven CAD. Among these, subjects who were dyslipidemic or taking vitamin supplements or using non smoking tobacco were excluded. In this study, patients were considered to be dyslipidemic if one or more of the following conditions were present - a) total cholesterol level >250 mg/dL, b) HDL-cholesterol <35 mg/dL, c) LDL-cholesterol >130 mg/dL, d) triglycerides >200 mg/dL. Since earlier investigations have shown that elevated Hcy level are associated with dyslipidemia (16), subjects with this clinical condition were excluded. Vitamin B9 works closely with vitamins B6 and B12 as well as the nutrients betaine and S-adenosylmethionine (SAMe) to control blood levels of Hcy and hence subjects taking vitamin supplements were excluded. The effect of non smoking tobacco on Hcy levels is not fully established, and it was thought best to exclude patients using non smoking tobacco also from this study.

At the end, 92 subjects were included in this study (Table 1). These subjects were categorized based on age (35-45, 45-55, >55), presence/absence of hypertension and type 2 diabetes and smoking status (current smoker/non-smoker). A current smoker is defined as a person presently smoking or who had smoked cigarettes or *beedis* in the past 3 months of recruitment. All the subjects were on statin therapy and were normolipidemic. Diabetic subjects were on hypoglycemic medications and hypertensives were on antihypertensive drugs. Type 2 diabetes was diagnosed based on WHO diagnostic criteria for diabetes (fasting blood glucose levels >126mg/dL). Hypertension was defined as a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Dietary details were collected from all the subjects and all of them were on typical Kerala diet, which predominantly consists of carbohydrates.

Hcy concentrations were assayed in fasting plasma samples by ELISA using kits from Bio-Rad, USA. Hcy concentrations above 15 µmoles/L were considered high, as classified by Kang *et al*. (17).

Statistical Analysis

Statistical analysis was done using SPSS software, version 11.0. Subjects were categorized based on presence/absence of hypertension, smoking, diabetes and age (35-45, 45-55, >55). Pearson chi-square test was used for initial comparison of data between different groups. Logistic regression analysis was carried out to determine the factors independently associated with hyperhomocysteinemia. Factors that had a $p<0.25$ in univariate analysis or had clinical importance were included in the multivariate model. $P<0.05$ was considered to be statistically significant in the multivariate model.

Results

Mean age of the subjects was 54.7 ± 7 years. Out of 92 subjects, 26 were current smokers and 66 were non smokers. Forty three were hypertensive and 49 were diabetic among these subjects. Twenty two subjects were both hypertensive and diabetic and 22 subjects were neither hypertensive nor diabetic. Among smokers, 7 subjects had both diabetes and hypertension. Fifty seven subjects had unstable angina and 35 had chronic stable angina (Table 1).

Table 1

Clinical and biochemical characteristics of CAD subjects

Parameters	
Total number of CAD subjects	n = 92
Age (Mean ± SD)	54.7 ± 7 years
Subjects with CAD only	n = 22
Subjects with CAD + Hypertension	n = 43
Subjects with CAD + Diabetes	n = 49
Subjects with CAD + Hypertension + Diabetes	n = 22
Smokers	n = 26
Subjects with CAD + Hypertension + Diabetes + Smoking	n = 7
Subjects with unstable angina	n = 57
Subjects with stable angina	n = 35
Fasting plasma glucose (Mean ± SD)	101 ± 12.3 mg/dL
Total cholesterol (Mean ± SD)	149 ± 31.8 mg/dL
HDL-cholesterol (Mean ± SD)	40.4 ± 6.5 mg/dL
LDL-cholesterol (Mean ± SD)	86.4 ± 22.3 mg/dL
Triglycerides (Mean ± SD)	110.9 ± 40.6 mg/dL
Hcy (Mean ± SD)	14 ± 7.8 µmoles/L

SD: standard deviation, µmoles/L: micromoles/litre, mg/dL: milligram/decilitre

Results of univariate analysis are given in Table 2. The mean Hcy concentration among smokers was 18.3 ± 8.8 µmoles/L, while non-smoking subjects had lower Hcy levels compared to smokers, with a mean of 12.4 ± 6.7 µmoles/L. Mean Hcy concentration of smokers was significantly higher compared to non-smokers on univariate analysis (p=0.002). It was observed that the mean plasma Hcy concentration of subjects with hypertension (15.3 ± 6.7 µmoles/L) was significantly higher (p=0.026) compared to non-hypertensives (12.9 ± 8.5 µmoles/L) on univariate analysis. No significant differences were noticed between diabetic subjects (14.06 ± 9.4 µmoles/L) and non-diabetic subjects (14.1 ± 5.4 µmoles/L, p=0.161) on univariate analysis of data. Eleven subjects had age between 35 to 45 years, 36 subjects had age between 45 to 55 years and 45 subjects had age above 55 years. Univariate analysis comparing these three groups did not produce statistically significant results (p>0.25). However, age was also considered as a factor in the multivariate analysis as the relationship between advancing age and hyperhomocysteinemia has been previously established. No significant difference were found between subjects with unstable angina (14.3 ± 7.6) and stable angina (13.7 ± 8.1, p=0.49).

Table 2

Univariate analysis of risk factors in CAD subjects (n=92)

CAD risk factors	Subject Categories	No: of subjects	Hcy (µmoles/L)	*p* value
Smoking	Smokers	26	18.3 ± 8.8	0.002
	Non-smokers	66	12.4 ± 6.7	
Hypertension	Hypertensives	43	15.3 ± 6.7	0.026
	Non-hypertensives	49	12.9 ± 8.5	
Diabetes	Diabetics	49	14.06 ± 9.4	0.161
	Non-diabetics	43	14.1 ± 5.4	
Age	35-45 years	11	16.81± 8.1	> 0.25
	45-55 years	36	13.5± 7.2	
	>55 years	45	13.8± 8.1	

µmoles/L: micromoles/litre

Table 3 represents the results of multivariate analysis. On multivariate analysis, smoking was found to be associated with hyperhomocysteinemia independent of other risk factors studied (OR [odds ratio] = 4.166, 95% CI: 1.471-11.801, p=0.007). It was observed that hypertension was independently associated with hyperhomocysteinemia in these subjects even if the synergistic effects of other risk factors studied were excluded (OR = 3.384, 95% CI: 1.183-9.679, p=0.023). But, diabetes did not contribute independently to hyperhomocysteinemia in this study (OR = 2.223, 95% CI: 0.802-6.160, p=0.125). Age was also not found to contribute to hyperhomocysteinemia independent of other factors (OR = 2.879 and 1.781, 95% CI: 0.538-15.411 and 0.365-8.69, p=0.217 and 0.475).

Table 3

Multivariate analysis to show risk factors influencing hyperhomocysteinemia in CAD subjects (n=92)

CAD risk factors	No: of subjects with Hcy >15µmoles/L	Multivariate adjusted OR	95% CI	*p*-value
Smoking				
Smokers (n=26)	14 (53.8%)	4.166	1.471-11.801	0.007
Non-smokers (n=66)	14 (21.2%)			
Hypertension				
Hypertensives (n=43)	18 (41.9%)	3.384	1.183-9.679	0.023
Non-hypertensives (n=49)	10 (20.4%)			
Diabetes				
Diabetics (n=49)	18 (36.7%)	2.223	0.802-6.160	0.125
Non-diabetics (n=43)	10 (23.3%)			
Age				
35-45 (n=11)	5 (45.5%)			
45-55 (n=36)	9 (25%)	2.879	0.538-15.411	0.217
>55 (n=45)	14 (31.1%)	1.781	0.365-8.69	0.475

SD: standard deviation, OR: odds ratio, CI: confidence interval

Discussion

Several cross sectional and small prospective studies have consistently shown that CAD subjects have elevated plasma homocysteine level compared to a clinically healthy population (18,19). The prevalence of hyperhomocysteinemia among subjects with symptomatic CAD is estimated to be 13% to 47% (20). But there are concerns about the interplay of conventional risk factors in hyperhomocysteinemia observed among CAD subjects. The mean Hcy concentration among subjects with unstable angina was not significantly different from those with stable angina in this study, as has been observed by earlier studies (21).

Strong epidemiological evidence suggests that smoking is associated with elevated Hcy concentrations not only among smokers with cardiovascular diseases but also in healthy subjects (22). Smokers are likely to have reduced concentrations of folate, B6 and B12 compared to non-smokers (23) and since these factors affect Hcy metabolism, their deficiency may contribute to hyperhomocysteinemia among smokers. Among CAD subjects of this study, the mean plasma Hcy concentrations of smokers were found to be considerably higher than non-smokers. Smoking and hyperhomocysteinemia have independent adverse effects on the endothelium; smoking causes vasoconstriction while Hcy affects vaso-occlusive factors such as platelet aggregation, fibrinogen levels etc. (24,25). These multiple effects causing extensive vascular damage contribute more to the etiopathogenesis of CAD compared to hyperhomocysteinemia alone (26). Logistic regression analysis of the data from the present study indicates that smoking is an important factor contributing to hyperhomocysteinemia in this population even after excluding interaction effects of other factors considered such as hypertension, diabetes etc.

Earlier investigations have suggested that vascular risk associated with hyperhomocysteinemia is stronger in subjects with hypertension (26). It has been proposed that hyperhomocysteinemia causes the loss of elastin of arterial wall, making it less elastic, leading to hypertension (27). Elevated plasma Hcy concentrations not only confer an independent risk for CAD, but also amplify the risk of smoking and hypertension (26). There have been studies that observed higher Hcy concentrations for hypertensives compared to normotensives among CAD (19) and ischemic stroke patients (28). But, a recent large scale prospective trial in middle aged individuals concluded that the relationship of hyperhomocysteinemia with hypertension was casual, after adjustments were made for age (29). Hypertensive subjects had elevated Hcy levels compared to normotensive subjects in this study and the results obtained indicate a correlation between hypertension and hyperhomocysteinemia, even when other risk factors were excluded as covariates. Antihypertensive drugs have Hcy-elevating effects (30) and studies have reported that the use of antihypertensive medication correlate strongly with plasma homocysteine than blood pressure levels (31-33).

In diabetic patients, hyperhomocysteinemia causes endothelial dysfunction that has apparent synergistic detrimental vascular effects (34). The metabolism of Hcy is impaired in diabetic subjects due to altered transsulfuration pathway, which could be reversed by insulin treatment (35). It has been shown that diabetics, in particular those with renal dysfunction, have hyperhomocysteinemia (36). But the mean Hcy concentrations of our diabetic subjects did not show significant difference compared to non-diabetic subjects, which could be because we had excluded subjects with impaired renal function from our study. There have also been studies that did not report hyperhomocysteinemia among diabetic subjects without renal complications (37).

The increase in Hcy concentration with age has been well documented (4). But, in the present study stratification of study subjects into different age groups did not show any correlation between age and elevated Hcy level, which may be due to the small number of subjects in the groups.

Limitations

Though we have tried to exclude factors that affect hyperhomocysteinemia such as gender difference, vitamin supplementation, dyslipidemia etc. there are other factors that possibly effect Hcy concentration (level of B vitamins in diet, severity of CAD, alcohol consumption, obesity, stress etc.) that were not studied. Although this study generates some noteworthy results, prospective studies with larger sample size would be required to establish the findings of this observational study with a small number of subjects. Further, the results from this study were not compared with a healthy/control population. All the subjects were on statin therapy and this might have affected their Hcy levels, though the effect of statins on Hcy concentrations has not been fully established.

Conclusions

Our study involving CAD patients from Kerala has demonstrated that smoking and hypertension are likely to be associated with hyperhomocysteinemia in this population. Since the interactions between these risk factors could be relevant in the secondary prevention of cardiovascular events, smoking cessation and control of hypertension are particularly important in CAD subjects with hyperhomocysteinemia, as is the regulation of hyperhomocysteinemia therapeutically.

Conflict of interest: Nil

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