Serum homocysteine as a risk factor for carotid intimal thickening in acute stroke: A cross sectional observational study

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Abstract

Introduction: The present study aimed to analyse if there is a correlation between carotid intima medial thickening (CIMT) and Hcy in stroke patients. **Methodology:** We studied 100 consecutive cases of acute anterior circulation strokes at St. John's Medical College, Bangalore, India. Fasting serum samples for homocysteine were sent within 24 hours of admission and all patients underwent a carotid Doppler scan and carotid intima-medial thickness (CIMT) was estimated on both sides. **Results:** There was significant correlation between serum homocysteine levels and carotid intima-medial thickness (r = 0.409, p = 0.000). Also after controlling for other possible risk factors it was found that elevations in serum homocysteine levels would cause a variation of 60% in the carotid intima-medial thickening. **Conclusion:** Serum Hcy levels correlate well with CIMT and hence may predict atherothrombotic events.

Key Words

Homocysteine, carotid intima-medial thickness, atherosclerosis, stroke

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Introduction

Identification of modifiable risk factors for stroke may lead to more effective prevention of cerebrovascular diseases. Elevated serum homocysteine (Hcy) has been associated with an increased risk of atherothrombotic disease.[1-3] Whether this association is causal or not remains uncertain. Experimental evidence suggests that the atherogenic propensity associated with hyperhomocysteinemia results from endothelial dysfunction and injury followed by platelet activation and thrombus formation.[4,5] Carotid Doppler is a non-invasive technique in evaluating the patency of carotid artery and carotid intima-medial thickness (CIMT) is commonly used as a surrogate marker for atherosclerosis. Atherosclerotic plaque is initially revealed sonographically by an increase in the combined thickness of the intima and media layers. In the general population, a weak association has been reported between Hcy and CIMT.[6] One Japanese study however found an association between plasma Hcy levels and carotid intimal thickening in normal individuals.[7] However in patients with atherothrombotic stroke, no significant correlation was found between these two variables.[8] This study was undertaken to investigate the correlation between hyperhomocysteinemia and the degree of CIMT in stroke patients.

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Materials and Methods

After getting approval from the institutional review board, we undertook a prospective cross-sectional analysis of 100 consecutive patients who were newly diagnosed to have acute anterior circulation stroke at St. John's Medical College, Bangalore, India from September 2008 to September 2010. Major exclusion criteria included factors that are known to influence Hcy levels like renal failure, thyroid disease, pregnancy, hepatic failure, and treatment with drugs like antiepileptics, fibrates, hormone replacement therapy, folate, vitamin B12, and vitamin B6. All patients underwent a detailed evaluation, which included a detailed history and physical examination. In all cases, the diagnosis of ischemic stroke was confirmed by neuroimaging of the brain. Fasting serum samples for Hcy were sent within 24 hours of admission. Serum Hcy level was estimated by the microplate enzyme immune

assay technique. All patients underwent a carotid Doppler scan and CIMT was estimated on both sides at the free wall of the common carotid artery, internal carotid artery or the carotid bulb as described. On a longitudinal two-dimensional ultrasonographic image, the far wall of the carotid artery is displayed as two bright white lines separated by a hypoechoic space. The distance from the leading edge of the first bright line (lumen-intima interface) to the leading edge of the second bright line (media-adventitia interface) was identified as the CIMT. The average of the right and left side was calculated and was taken as mean CIMT for analysis. A serum Hcy level of >15 μ mol/L and a CIMT of > 0.8 mm were taken as abnormal. Statistical analysis was done using the statistical software SPSS 16.0. A probability value of less than 0.05 was considered as significant.

Results

Hundred (65 males and 35 females) patients were included in the study with a median age of 59.6 years. Table 1 shows the baseline characteristics of all patients. Type 2 diabetes mellitus (T2DM) was found in 38% patients (26 males and 12 females) and hypertension was found in 61% patients (38 males and 23 females). Twenty-two patients (14 males and 8 females) were previously detected to have dyslipidemia and were on treatment. Twenty-five patients were smokers. Three patients gave a history of a prior coronary event.

Serum Hcy was found to be elevated in 55 patients (34 males and 21 females) with a mean level of 26.80 μmol/L. The mean serum Hcy in males was 19.30μ mol/L and in females was 17.64 μmol/L. The main factors associated with increased Hcy levels in our study population were serum cholesterol (correlation coefficient r = 0.324, *P* = 0.001), serum LDL (r = 0.261, *P* = 0.004), serum triglyceride levels (r = 0.253, *P* = 0.006) and presence of diabetes (r = 0.185 *P* = 0.035) [Table 2]. Eighty-six patients (54 males and 32 females) had CIMT > 0.8 mm (mean of all patients –1.022 mm, females –1.0286 mm, males –1.0185 mm). Out of the 55 patients who had an elevated Hcy levels, 51 patients (92.7%) had carotid intima medial thickening. On doing a bivariate correlation, we found that there is a significant correlation between elevated serum Hcy levels and CIMT (r = 0.409, R^2 = 0.362, *P* = < 0.01) [Figure 1].

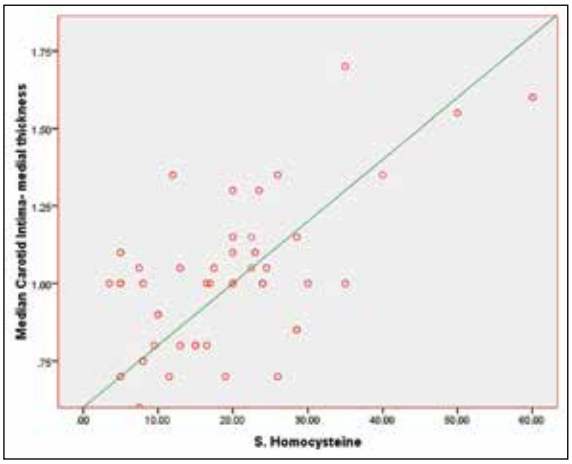

Figure 1: Scatter plot with the best fit regression line depicting the correlation b/w Homocysteine levels and median carotid intima-medial thickness. R^2 = 0.362, *P* = < 0.001

Univariate analysis with the various other atherothrombotic risk factors found that the presence of diabetes statistically affected serum Hcy levels in our study population [Table 2]

Discussion

The role of Hcy as a risk factor for stroke has not been extensively studied in India. Due to dietary and other ethnic differences, data generated in the West need not necessarily be applicable to our population. Recent reports on Hcy suggest that it is an independent predictor of vascular disease, including stroke and CAD.[1,2]

The present study aimed to analyze whether there is a correlation between elevated serum Hcy levels and CIMT and to see if Hcy levels independently influence CIMT, which is an established early indicator of systemic atherosclerosis preceding stroke and myocardial infarction.

Table 1: Baseline characteristics of all patients

Characteristic	*N* (range)
Total no of patients	100
Male: Female	65:35
Age	
Median age of all patients (yrs)	59.6 (23-88)
Median age of male patients	58 (23-88)
Median age of female patients	62 (30-83)
Dietary habits	
Veg: Mixed (Male)	17:48
Veg: Mixed (Female)	6:29
Serum Hcy > 15 μ mol/L	55
Male: Female	34:21
Mean Hcy levels in all patients (μ mol/L)	
Male	19.30
Female	17.64
Mean Hcy levels in patients with elevated serum level (μ mol/L)	26.80
Mean Hcy level in patients with normal serum level	8.84

Table 2: Correlation coefficient of various risk factors with serum homocysteine

Risk factors	Correlation coefficient (r) with serum homocysteine	*P*
Age > 55 yrs	0.005	0.481
Male gender	0.065	0.261
T2DM	0.185	0.033*
Hypertension	0.136	0.089
Dietary habits	0.123	0.111
Smoking	0.108	0.143
Alcohol	0.095	0.174
Systolic BP	0.110	0.138
Diastolic BP	0.060	0.278
ESR	0.086	0.196
S. Creatinine	0.088	0.193
S. Cholesterol	0.324	0.001*
S. LDL	0.261	0.004*
S. HDL	0.140	0.082
S. Triglycerides	0.253	0.006*

The majority of the patients in our study were males. The mean age of males in the study population was 58 years and that of female patients was 62. In our cohort, the mean age of patients was about one decade less than a Greek study[8] but is comparable to a German study.[9] Indians are known to be at high risk of developing atherosclerotic heart disease and cerebrovascular diseases and it occurs one decade earlier than in the West.[10]

A low prevalence of hyperhomocysteinemia has been recorded in South India (10.7% in patients with coronary heart disease and 5.7% in healthy controls).[11] The prevalence rate of hyperhomocysteinemia in another Asian population group, Bangladeshis, residing in East London was 33% as compared to 15% in Caucasians.[12]

The mean serum Hcy level in our patient cohort was 18.72 μmol/L. The mean serum Hcy levels found in the Framingham study was 11.9 μ mol/L,[13] whereas that of other two western studies –17.4 μmol/L in Greek population[8] and 10.9 μmol/L in Japanese population[7] were also less than our mean value. From this it is clear that our study group has elevated serum levels of Hcy compared to other ethnic groups. The highest recorded mean level of serum Hcy in Indians was 23.9 μ mol/L observed among residents of urban Delhi.[14] In general, high mean levels of Hcy appear to be a common observation in native Asian Indians. Similar results were obtained in Western India (19.7 μmol/L in healthy controls and 20.0 μmol/L in CHD patients),[15] and in South-east India (19.7 μmol/L in healthy controls and 21.5 μmol/L in CHD patients).[16]

One of the reasons for the discrepancy between Indians and other ethnic groups may be due to the differences in their dietary habits. In India, due to cultural and socio-economic factors, there are many vegetarians who consume a Vitamin B12 deficient diet, thus causing elevated serum Hcy levels. Moreover, among people consuming a mixed diet, the frequency of consumption of non-vegetarian food items is low.

In our study, serum cholesterol (P = 0.001), serum LDL (P = 0.004), serum triglyceride levels (P = 0.006) and diabetes (P = 0.035) were found to have statistically significant correlation with raised Hcy levels. A Japanese study[17] showed that age and sex were the main determinants of serum Hcy levels in the Japanese population and serum creatinine was an independent determinant of serum Hcy levels. Our study failed to show any such correlation. However, the size of the study population is small and the age band is narrow. The mean CIMT of all patients in our group was 1.022 mm, which was similar to that reported by Ntaios *et al*.[8]

Overall the association of Hcy with CHD and stroke is still unclear and the American Heart Association has not recommended testing for Hcy as part of the screening for cardiovascular risk in population based studies. The present study found that serum Hcy levels correlate with the median CIMT (r = 0.409, P < 0.0001 two-tailed). Other variables that were found to be associated with increased CIMT in our study cohort were serum cholesterol (P = 0.040) and serum triglycerides (P = 0.002). CIMT was high in all subjects with T2DM (N = 38). A Greek study found that there was no correlation between Hcy levels and CIMT in stroke patients.[6] Similar observations were also seen in two Japanese studies.[7,18] The latter study by Inamoto *et al*.,[18] concluded that Hcy levels were significantly correlated with maximum CIMT with a correlation coefficient (r) = 0.17 in the general population. Other variables that significantly affect CIMT according to Inamoto *et al.,* were current smoking (r = 0.12), current drinking of alcohol (r = 0.11), SBP (r = 0.17), HDL-cholesterol (r = 0.16), and creatinine (r = 0.31). Also serum creatinine and systolic blood pressure were found to be independent determinants of CIMT.[18]

On multiple linear regression analysis, with CIMT more than 0.8 mm as the dependent variable, after adjusting for all the other risk factors, serum Hcy levels, ESR and consumption of alcohol were found to be significantly correlated with increased CIMT. Also it was found that variance caused by Hcy level alone on the CIMT is 60.2% (R = 0.602, P = 0.000). Alcohol and serum Hcy levels both combines together to cause a variance of 64.2% in the mean CIMT (R = 0.642, P = 0.000). No studies so far has reported the variance caused in CIMT by Hcy alone or in combination with other risk factors.

The finding of elevated Hcy levels in patients with atherothrombotic stroke and a significant association of elevated Hcy levels to CIMT brings to fore the importance of framing strategies for the prevention of atherosclerosis and its complications. Estimation of serum Hcy level holds promise for slowing the progression of atherosclerosis and subsequent morbidity and mortality due to coronary and cerebrovascular diseases by early identification of at risk people.

This study is limited by the small sample size and the lack of a control group. Larger population-based studies are needed to further verify the fact of elevated Hcy levels and its causation.

Conclusion

This single center study has found out positive correlation between elevated serum Hcy levels and CIMT in stroke patients. Elevations in serum levels of Hcy causes variation of 60% in CIMT when other risk factors are controlled, and so serum Hcy may be considered as an independent risk factor for cerebrovascular disease.

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