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To Study the Prevalence of Plasma Homocysteine Levels in Cases of Stroke: A Case- Control Study

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ABSTRACT:

Background: we have planned to explore an association between homocysteine levels in Indian patients with stroke (esp. acute ischemic stroke patients) so that some practical recommendations for screening and treatment of this modifiable risk factor can be provided.

Materials & Methods: This is a case control study done on 50 patients of stroke admitted in indoor and emergency wards of PG Department of Medicine, SN Medical College, Agra. 20 healthy, age and sex matched, controls were also taken for valid comparison. Diagnosis of stroke was confirmed by CT and MRI. Serum or plasma may be used for homocysteine assay.

Results: Our study showed that maximum no. of cases was in the age group of 60-69 yrs. Mean age in males was 56.55 yrs and females was 53.00 yrs. Total no. of control was 20. Among controls 85% had normal homocysteine levels while only 15 % controls had mild hyperhomocystienemia. Among cases 44 % cases had moderate hyperhomocystienemia and 38 %had mild hyperhomocyetenemia.18%cases had normal homocysteine levels. On statistically analyzing the data of homocysteine levels in controls and cases t value was found to be 10.37 and p value was <0.03 which is statistically significant.

Conclusion: We herewith conclude from the present study that homocysteine is one of the risk factors for cerebrovascular disease in approximately 82% cases. Whether homocysteine is the cause or effect of cerebrovascular disease has to be worked out.

Keywords: Homocysteine, Stroke, Hyperhomocystienemia, Risk Factor.

INTRODUCTION

Stroke is an etiologically heterogeneous disease, but atherosclerosis contributes to a large proportion of cases either directly via aortic, cervical, or intracranial large-artery atherosclerosis or indirectly by cardioembolism, e.g., as a result of cardiac arrhythmias caused by coronary heart disease (CHD) or emboli after myocardial infarction.[1]

Recently, there has been much interest in homocysteine, a sulfur containing amino acid as an important risk factor for vascular disease including stroke; independent of the long-recognized factors like hyperlipidemia, hypertension, diabetes mellitus and smoking; although its association was described many decades ago. During the last decade, numerous studies observed a strong positive correlation between hyperhomocysteinemia and stroke; while others could not establish the same.

Homocysteine is a thiol-containing amino acid derived from the metabolism of methionine that circulates in plasma in 3 forms: as a single free amino acid (1%), as homocysteine or cysteine-homocysteine disulfides (20% to 30%), or bound to plasma proteins (70% to 80%). Together, these account for total plasma homocysteine (tHcy). Inborn errors of metabolism arising from a deficiency of Homocysteine metabolizing enzymes result in extremely high tHcy concentrations (severe hyperhomocystienemia) and are associated with premature thrombosis, possibly as a result of oxidative damage mediated by the sulfhydryl group of free single-chain homocysteine Mild hyperhomocystienemia results from both nutritional and more subtle genetic influences. Subclinical deficiencies of folate, vitamins B12 and B6, and inheritance of the thermolabile variant of methylenetetrahydrofolate reductase3 are associated with modest elevations above the 90th percentile of the normal range. Other conditions, including renal impairment, hypothyroidism, and drug therapy (e.g., folate antimetabolites, theophylline, smoking, or oral contraceptives), are also associated with mild hyperhomocystienemia.[2]

Retrospective case-control studies have associated raised tHcy with both arterial and venous thrombosis. In contrast, results from prospective studies have been inconsistent, both supporting raised tHcy levels as a risk factor for myocardial infarction (MI).[2]

Consideration of reports of plasma tHcy and stroke (cerebrovascular accident, CVA) identifies similar difficulties. Case-control studies have reported stronger associations than prospective studies, in which some but not others claim that hyperhomocystienemia is a risk factor for future stroke development. In the case of CVA, 2 further issues are relevant: first, strokes arise from numerous pathophysiological processes, including intracranial hemorrhage, cardiac embolization, atherothrombosis (rupture of either large-vessel atheroma with cerebral embolism or of small-vessel atheroma with occlusion), and vasculitis. Most studies have failed to distinguish between these diverse stroke types, and any individual risk factor might influence only one of these processes. Recent studies in humans have shown that acute hyperhomocystienemia causes endothelial dysfunction, which might promote atheroma development. Furthermore, raised homocysteine concentrations are associated with asymptomatic carotid artery wall thickening and stenosis.[2]

Numerous case control studies have shown an association between hyperhomocystienemia and stroke. Based on a change of 5 μmol/L in homocysteine levels, a met analysis found risk for cerebrovascular disease by 1.5[3] from the NHANES III data, for comparing the top quartile with the bottom quantities is 2.25.[4] In the Framingham study the relative risk for stroke comparing the lowest quartile with the highest was 1.82.[3] Folic acid, together with vitamin B_6 and B_{12} have been shown to be effective in reducing elevated plasma homocysteine levels.[5] Case control and prospective studies have suggested an association between moderate hyperhomocystienemia and risk of ischaemic

stroke: with a meta-analysis of the data indicating that hyperhomocystienemia confers at least 2.5 increased risk of stroke.[6]

No authentic work has been done in India and other Asian countries to study the relationship between homocysteine and stroke. Keeping in mind the above evidences, we have planned to explore an association between homocysteine levels in Indian patients with stroke (esp. acute ischemic stroke patients) so that some practical recommendations for screening and treatment of this modifiable risk factor can be provided.

MATERIALS & METHODS

This is a case control study done on 50 patients of stroke admitted in indoor and emergency wards of PG Department of Medicine, SN Medical College, Agra. 20 healthy, age and sex matched, controls were also taken for valid comparison. Diagnosis of stroke was confirmed by CT and MRI.

Inclusion Criteria

All diagnosed cases of stroke more than 35 yrs. of Age.

Exclusion Criteria

1. All patients below 35 years of Age.

2. Patients not willing for study.

3. Patients suspected to have stroke on clinical grounds without correlation with Brain Imaging studies.

4. Patients in which plasma homocysteine levels may be increased due to psoriasis, systemic lupus erythematous, severe hepatic impairment, pernicious anemia, malignancies of breast, ovary, pancreas, drugs.

Methods

Estimation of Serum Homocysteine

Principle

Homocysteine assay is based on the principle of Fluorescence Polarization Immunoassay (FPIA) technology. Bound HCY (oxidized form) is reduced to free HCY that is enzymatically converted to S-adenosyl-L-homocysteine (SAH) as follows:

Reduction

Homocysteine, mixed disulfide and protein bound forms of homocysteine in the sample are reduced to form free homocysteine by the use of dithiothreitol (DTT).

Enzymatic Conversion

Free HCY is converted to S-adenosyl-L-homocysteine (SAH) by the use of hydrolase and excess adenosine.

HCY+Adenosine--------- SAH

Under physiological conditions, SAH hydrolase converts SAH to homocysteine. Excess adenosine in the pretreatment solution drives the conversion of HCY to SAH by the bovine SAH Hydrolase.

Specimen Collection

Serum or plasma may be used for homocysteine assay. To minimize increases in Homocysteine concentration from synthesis by red blood cells, sample should be processed as follows;

❖ Place the sample on ice after collection and prior to processing.

❖ sample can be kept on ice for up to 6 hrs prior to separation by centrifugation.

❖ Inspect all samples for bubble, remove bubbles prior to analysis.

❖ For optimal results sample should be free of fibrin red blood cells, or other particulate matter.

Limitations of The Procedure

The following drugs may elevate levels of homocysteine, methotrexate, carbamazepine, phenytoin, nitrous oxide.

S-adenosyl-methionine is an antidepressant whose molecular form is similar to S-adenosyl-homocysteine.

RESULTS

Sixty-eight patients of ischemic stroke admitted to Postgraduate Department of Medicine, SN Medical College & Hospital, Agra, were enrolled in this study. Out of sixty-eight, eighteen patients had been excluded because of presence of factors interfering with serum homocysteine levels and presence of predisposing factors responsible for embolic stroke as shown in Table-1.

Our study showed that maximum no. of cases was in the age group of 60-69 yrs. Mean age in males was 56.55 yrs and females was 53.00 yrs. Total no. of control was 20. The minimum age was 38 yrs. and maximum age was 76 yrs (table 2).

Among controls 85% had normal homocysteine levels while only 15 % controls had mild hyperhomocystienemia. Among cases 44 % cases had moderate hyperhomocystienemia and 38 %had mild hyperhomocyetenemia.18%cases had normal homocysteine levels (table 3). On statistically analyzing the data of homocysteine levels in controls and cases t value was found to be 10.37 and p value was <0.03 which is statistically significant (table 4).

Table 1: Subjects excluded from study due to various reason.

No. of subjects	Exclusion criteria
8	Rheumatic heart disease
6	Deranged renal function
3	Old ischemic infarct in brain
1	Regular user of multi-vitamin
18	Total

Table 2: Age and Sex Distribution of Cases & Controls Group

Age (Yrs)	Cases			Control		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
30-39	6	2	8	2	3	5
40-49	5	1	6	5	1	6
50-59	10	2	12	2	2	4
60-69	14	6	20	1	2	3
70-79	3	1	4	1	1	2
Total	38	12	50	11	9	20
Mean Age	56.55	53.00	54.77	50.45	52.77	51.5

Table 3: Showing Distribution of Homocysteine Levels in Controls and Cases

Homocysteine Levels	Control (n=20)	%	Cases (n=50)	%
Normal **(<15 µmol/L)**	17	85	9	18
Mildly Elevated **(>15-<30 µ mol /L)**	3	15	19	38
Moderately Elevated **(>30-<100 µmol/L)**	0	0	22	44
Severely Elevated **(>100 µmol/L)**	0	0	0	0

Table 4: Mean Homocysteine Levels In Controls And Cases

Groups	Total (n=70)	Homocysteine level (Mean $\pm$ SD)	t-value	P-value
Controls	20	11.87 $\pm$ 3.19	10.37	<0.03**
Cases	50	29.65 $\pm$ 11.02		

DISCUSSION

Stroke puts tremendous burden on health budget of a country and puts the patient and the family in great difficulty. Preventing the stroke is the biggest challenge for medical fraternity and various researches are going on for prevention of stroke.[7]

The objective of the present study was to evaluate the prevalence of serum homocysteine levels in acute ischemic stroke patients. 15 % controls had mild hyperhomocystienemia and in cases 85% had normal homocysteine level.

Among cases 38% had mild hyperhomocystienemia while 44% had moderate hyperhomocystienemia. Only 18% had normal homocysteine levels. 18% cases had normal homocysteine levels as against 85 % controls. The t test performed on serum homocysteine values in controls and cases showed a significant difference (t-value = 10.37, p-value = <0.03) in the mean normal levels which was significantly higher in case group. Perry IJ et al.[8] suggested that the association with serum total homocysteine concentration in stroke victims was a strong, independent and graded increase in the relative risk for stroke. Another study done by Coull et al. reported that increased homocysteine levels were an independent risk factor for stroke.[9]

Brattstrom et al.[10] 1992 subsequently studied the relationship between plasma homocysteine and different stroke types in 147 survivors of strokes. They found that 40% of stroke patients had hyperhomocystienemia versus 6% of control subjects; this finding was independent of stroke type, being as true for hemorrhagic and lacunar stroke as for carotid stroke patients. Much of the variation in plasma homocysteine levels was accounted for by serum cobalamin, folate and creatinine levels.

A.P.S. Narang et al[11] was undertaken to compare the homocysteine levels in patients of ischemic stroke with controls. Our study included 117 patients of ischemic stroke and 101 controls. The mean homocysteine levels in patients with ischemic stroke were 16.80±6.71µmol/L while in controls it was 12.30±4.68 µmol/L, the difference being statistically significant (P<0.01). An association between elevated homocysteine and stroke has been postulated which may be due to acute vascular events themselves.

David Tanne et al[12] 2003 suggested that an increase of 1 natural log unit in homocysteine concentration was associated with a >3-fold increase in relative odds of incident ischemic stroke (3.3; 95% CI, 1.2 to 10.2). Homocysteine concentrations at the highest quartile (>17.4 µmol/L) were associated with significantly higher odds of ischemic stroke compared with the lowest quartile in matched-pair analysis (3.1; 95% CI, 1.1 to 9.8) and after multivariable adjustments (4.6; 95% CI, 1.3 to 18.9). The linear trends across the quartiles were significant for all models (P<0.05). Serum total homocysteine concentration is a strong predictor for incident ischemic stroke among patients at increased risk because of chronic CHD. The graded association observed is independent of traditional risk factors or inflammatory markers and indicates the importance of serum homocysteine levels in patients with pre-existing vascular disease.

John W. Eikelboom et al[13] 2003 suggested that increasing homocysteine was a strong and independent risk factor for ischemic stroke (adjusted OR 2.7, 95% CI 1.4 to 5.1 for a 5-mmol/L increase in fasting plasma homocysteine from 10 to 15 mmol/L). Compared with the lowest quartile, the highest quartile of homocysteine was associated with an adjusted OR of ischemic stroke of 2.2 (95% CI 1.1 to 4.2). Mean plasma homocysteine was significantly higher in cases of ischemic stroke due to large-artery disease (14.1 mmol/L, 95% CI 12.5 to 15.9, P,0.001) and small-artery disease (12.7 mmol/L, 95% CI 11.4 to 14.1, P50.004) compared with control subjects (10.5 mmol/L; 95% CI 10.0 to 11.0) but not in cardioembolic or other etiologic subtypes of ischemic stroke. Compared with the lowest quartile of homocysteine, the upper 3 quartiles were associated with an adjusted OR of ischemic stroke due to large-artery disease of 3.0 (95% CI 0.8 to 10.8) for the second quartile, 5.6 (95% CI 1.6 to 20) for the third quartile, and 8.7 (95% CI 2.4 to 32) for the fourth quartile (P for trend50.0005). There is a strong, graded association between

increasing plasma homocysteine and ischemic stroke caused by large-artery atherosclerosis and, to a much lesser extent, small-artery disease, but not cardioembolic or other etiologic subtypes of ischemic stroke.

CONCLUSION

We herewith conclude from the present study that homocysteine is one of the risk factors for cerebrovascular disease in approximately 82% cases. Whether homocysteine is the cause or effect of cerebrovascular disease has to be worked out. A larger sample size is needed to elucidate this enigma.

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