Hyperhomocysteinaemia among Acute Ischaemic Stroke Patients in a Tertiary Care Centre in Thiruvananthapuram, Kerala – A Cross-Sectional Study

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ABSTRACT

BACKGROUND

Over the last three decades, prevalence of stroke has been high in India, while the Western countries have witnessed a downward slide. Epidemiological studies suggest that hyperhomocysteinaemia might be a potentially modifiable nonlipid risk factor associated with stroke, in addition to the long-recognized factors like hypertension, diabetes mellitus, hyperlipidaemia and smoking. Hyperhomocysteinaemia occurs due to deviation in the metabolic pathway of methionine, attributed by deficiency of vitamins, enzymes and other factors. The present study was undertaken to assess the proportion of hyperhomocysteinaemia in patients with acute ischemic stroke. We also compared the risk factors associated with stroke and serum levels of homocysteine.

METHODS

This is a cross sectional observational study conducted in a tertiary care hospital. The sample size was 140. Both male and female consecutive patients of age more than 18 years, with first attack of acute ischaemic stroke admitted in the Department of Neurology were selected. Baseline fasting serum samples were obtained for testing serum homocysteine levels. Statistical tests used were proportion, chi square and logistic regression.

RESULTS

Among 140 acute ischaemic stroke patients, total homocysteine level was raised in 83.6 % cases. The prevalence of moderate hyperhomocysteinaemia in our study was 65.4 % and intermediate hyperhomocysteinaemia was 17.9 % among stroke patients. The mean (± SD) homocysteine level was 22.75 (± 8.19).

CONCLUSIONS

A strong association was found between hyperhomocysteinaemia and acute ischaemic stroke. We could not find any significant correlation between total homocysteine level and most risk factors of stroke.

KEYWORDS

Stroke, Homocysteine, Risk Factors, Endothelial Dysfunction, Atherosclerosis, B Vitamins

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BACKGROUND

Stroke is the second common cause of mortality world over, and the leading cause of acquired disability and dementia and also the third leading cause of death in adults.[1] Altered lifestyles and food habits due to changes in the socioeconomic status and work-related stress have led to a rise in the occurrence of stroke disorders in India.[2] There are many conventional risk factors for stroke viz; age, sex, family history of stroke, hypertension, diabetes mellitus, obesity, hyperlipidaemia and atrial fibrillation. Even though numerous risk factors have been identified, hyperhomocysteinaemia is rapidly emerging as an essential risk factor for atherosclerotic disorders including stroke. Hyperhomocysteinaemia accelerates endothelial damage by causing oxidative stress and hence atherosclerosis and this increases the risk of stroke.[3]

Owing to its high prevalence, high burden of illness, economic cost, well-defined modifiable risk factors, and effective preventive measures, stroke is well suited for prevention.

Homocysteine is a sulphurous amino acid that is not usually obtained from the diet. Instead, it is biosynthesized from methionine by a series of steps. It is formed as an intermediary product during the conversion of the amino acid methionine to cysteine. Normal serum homocysteine levels in adults vary between 5 - 15µmol / L.[4] Homocysteine is produced from S-adenosyl-L-homocysteine (SAH) by S-adenosyl-homocysteine hydrolase. SAH is a transmethylation product from S-adenosylmethionine (SAM) that is made from methionine and adenosine triphosphate (ATP).

In the metabolic cycle, homocysteine is either remethylated to methionine through methionine synthase or degraded to cysteine through cystathionine beta-synthase. Methionine is an essential amino acid acquired mostly from the methionine recycle system and partly from the diet. It combines with adenosine triphosphate to yield S-adenosylmethionine, which is the most important donor to methyl group in our body.[3]

Intracellular homocysteine is also released into blood and urine. Vitamin B_{12} folate and B_6 are required as co factors for the homocysteine re-methylation pathway and trans-sulphuration pathway.[5] The rate of re-methylation and trans-sulphuration processes maintains the plasma level of homocysteine within the normal range.

Plasma homocysteine exists in different forms. 70 to 80% of total homocysteine exist as protein bound form (mainly to albumin), which is the major form. The other forms include mixed disulfides of homocysteine which account for about 5 – 10% of total homocysteine. Free homocysteine, or reduced homocysteine, is the least existing form, less than 1 %. Therefore, total serum homocysteine includes the sum of protein bound, oxidized and reduced forms.[5]

Total homocysteine testing is done by most clinical laboratories. Hyperhomocysteinaemia arises from an interruption in the pathways of methionine-homocysteine metabolism: hereditary abnormalities that cause disturbances of enzymes related to the homocysteine metabolism, vitamin deficiencies (B_6, B_{12}, and folic acid), age related changes, chronic renal insufficiency, hypothyroidism, pernicious anaemia, systemic lupus erythematosus (SLE), end stage diabetes, malignancies and use of various medication. Patients who are on drugs like methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate may have higher levels of homocysteine due to metabolic interference with homocysteine metabolism. Homocystinuria and severe hyperhomocysteinaemia are caused by rare inborn errors of metabolism resulting in marked elevation of plasma and urine homocysteine concentrations. Cystathionine synthase deficiency is the most common genetic cause of severe hyperhomocysteinaemia.

Hyperhomocysteinaemia is classified into three, according to the plasma levels in the fasting state: moderate (between 16 to 30µmols/dL), intermediate (between 31 to 100µmols/dL) and severe (more than 100µmols/dL).[6]

Moderate hyperhomocysteinaemia usually reflects impaired pathway of re-methylation. The possible causes include deficiency of folic acid, vitamin B_{12} or dysfunction of methyl tetra hydro folate reductase enzyme. Severe hyperhomocysteinaemia may be caused by deficiency of cystathionin beta synthase enzyme, or any enzymes catalysing vitamin B_{12} metabolism.[3]

Increased homocysteine can cause atherosclerosis, probably by the following mechanisms:

1. Direct toxic effects which damages the intima of arteries.
2. Causes endothelial damage and thereby promotes vascular inflammation.
3. Accelerates oxidation of low-density lipoprotein (LDL).
4. Increases activation of platelets and thrombotic tendency.
5. Synergetic effects with other risk factors.

Nitric oxide is normally formed from L-arginine in a reaction catalysed by nitric oxide synthase and has various antithrombotic properties. Homocysteine may directly inhibit nitric oxide synthase enzyme, impairing the synthesis of nitric oxide (NO) by endothelial cells. It also increases oxidative degradation of nitric oxide.[7] This leads to endothelial injury.

The endothelium possesses many properties that facilitate vascular homeostasis and maintain tissue perfusion. The response to injury hypothesis proposes that the primary event in endothelial damage and dysfunction disrupts these properties, resulting in adhesion and aggregation of platelets and leukocytes, thrombosis, proliferation of smooth muscle cells, vasospasm, lipid accumulation and ultimately atheroma formation.

Immune system activation is hypothesized as another cause of hyperhomocysteinaemia. In several conditions like cerebrovascular and neurodegenerative disorders where hyperhomocysteinaemia has been described, immune activation is deeply involved in the pathogenesis. Chronic activation of the immune system and enhanced oxidative stress are main pathogenetic mechanisms contributing to the development and progression of these diseases. Homocysteine mediated interaction between leukocytes and endothelial cells is one potential mechanism by which

inflammatory mediated atherosclerosis occurs. There is increased production of reactive oxygen species (ROS) in response to immune cell proliferation. They can oxidize antioxidants and oxidation-sensitive B-vitamins. There will be enhanced demand for antioxidants as well as for folate and vitamin B_{12}, despite sufficient dietary intake.[8] Homocysteine accumulates in the stimulated peripheral blood mononuclear cells. In patients with coronary heart disease, with rheumatoid arthritis and in patients with dementia, an association between cellular immune activation and homocysteine metabolism is found.

Hyperhomocysteinaemia causes thrombotic tendency. Elevation of thromboxane A_2 formation, reduction of anti-thrombin III activity and activation of Factor V and Factor XII have been shown as the causes of increased coagulability in hyperhomocysteinemia.[9]

Homocysteine also inhibits the expression of thrombomodulin, induces the expression of tissue factor, and suppresses the expression of heparan sulphate by the endothelium. All of these effects ultimately facilitate the formation of thrombin and create a prothrombotic environment.

Homocysteine auto-oxidation has been shown to enhance the oxidation of low-density lipoprotein through the generation of the superoxide anion radical, accelerating the process of atherosclerosis.[9] It has been suggested that if enhanced oxidative stress is a result of increased homocysteine levels on the endothelial cells, dietary antioxidants such as vitamin E may have a definite role in reducing the risk of vascular disease associated with hyperhomocysteinaemia.

Concentrations of total homocysteine may vary with time in patients with acute vascular events. This may explain the possible reason for elevated total homocysteine observed in many case control studies as hyperhomocysteinaemia may be a consequence, rather than a possible cause of an acute vascular event.

The role of hyperhomocysteinaemia in patients with pre-existing atherosclerotic vascular disease is not clearly explained, and some studies claim that homocysteine serves as a mere marker of tissue damage and repair.[10]

A reduction in homocysteine level by about nearly 3μmol/L was associated with 19% lower stroke risk, shown by the VISP trial by Spence et al. They also suggested that elevated homocysteine levels may be reduced by vitamin B supplementation (vitamin $B_{,}^{6}$ vitamin B^{12} and folic acid), which may be an indispensable asset in future stroke prevention.[11]

HOPE–2 trial, which is one of the largest prospective, randomized trials, evaluated the effects of homocysteine lowering the use of folic acid and vitamins B_6 and B_{12} on major cardiovascular events. A daily folic acid intake of 0.5 to 5 mg may decrease the plasma homocysteine level by about 25%. Daily vitamin B12 intake of at least 0.4 mg decreases the level of homocysteine further by 7%. Vitamin B_6 seems to decrease the plasma homocysteine levels after methionine loading.[12]

In Indian population, apart from low dietary folic acid intake, the intake of vitamin B2, B6 and B12 are also low as compared to that of Western population. So, consumption of green leafy vegetables, legumes, eggs and fruits should be encouraged in order to increase vitamin B intake in them.

The present study was undertaken to assess the proportion of hyperhomocysteinaemia in patients with acute ischemic stroke. We also compared the risk factors associated with stroke and serum levels of homocysteine.

METHODS

This is a cross-sectional study that was recommended by the human ethical committee of Government Medical College, Thiruvananthapuram and was financed by the State Board of Medical Research (SBMR), Kerala. The study was carried out in the Neurology Department of Government Medical College, Thiruvananthapuram. The sample size was 140. Categorical and quantitative variables were expressed as frequency (percentage) and mean ± SD respectively.
The duration of our study was two years – from January 2015 to January 2017.

Inclusion Criteria

Both male and female consecutive patients aged more than 18 years, with the first attack of acute ischaemic stroke admitted in the Department of Neurology were selected. Each case was diagnosed by a thorough clinical examination and brain CT scan.

Exclusion Criteria

We excluded patients with renal, hepatic and thyroid disorders, malignancies, psychiatric illness, chronic treatment with drugs – methotrexate, tamoxifen, L-DOPA, phenytoin and corticosteroids.

The sample size was calculated based on the formula,

$$n = \frac{Z^2_{1-\alpha/2} p\,(1-p)}{d^2}$$

Where,

$Z_{1-\alpha/2}$ = 1.96 for α (significance level) = 5%,

p (proportion of hyperhomocysteinaemia among acute ischaemic stroke patients) =0.419 [13]

d (precision) = 20% of p = 0.084

N= 134 rounded to 140

Data Collection

Informed consent was obtained from the patients or their caregivers. The collected data included age, sex, blood pressure, present history, duration of onset, past history and family of diabetes mellitus, hypertension, coronary heart disease, smoking, alcohol consumption and any co existing illness. The antecubital vein of either arm was used for drawing blood samples. Two millilitres of blood samples were taken after fasting. They were centrifuged as soon as possible and thereafter stored at - 20C. Serum homocysteine level in blood samples was estimated using "Homocysteine

2 - Reagent Enzymatic Assay kit" by Diazyme Laboratories. The Diazyme Homocysteine 2 Reagent Enzymatic assay is based on a novel assay principle that assesses the co-substrate conversion product instead of assessing co-substrate. Results were printed out in µmol/L. Normal value of homocysteine was taken as 5 to 15µmol/L.

Statistical Analysis

Chi-square test was used to find association of serum homocysteine levels with stroke risk factors. For all statistical interpretations, $P < 0.05$ was considered the threshold for statistical significance. Statistical analysis was performed by using a statistical software package SPSS, version 20.0.

RESULTS

Out of 140 acute ischaemic stroke patients, 27 (19.3 %) were in the age group 51-60, 39 (27.9 %) in the age group 61 - 70, 47 (33.6 %) in the age group 71 - 80 and 27 (19.3 %) were of more than 80 years. Of these, majority were males (62.1 %) and females were 37.9 %. 56 % of patients were from rural area and 43 % from urban area.

Serum Homocysteine	Count	Percent
Normal	23	16.4
Moderate	92	65.7
Intermediate	25	17.9
Mean ± SD	22.75 ± 8.19	

Table 1. Percentage Distribution of the Sample According to Serum Homocysteine

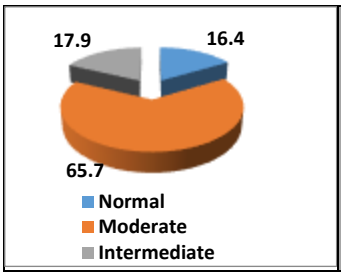

Figure 1. Percentage Distribution of Serum Homocysteine among Acute Ischaemic Stroke Patients

Out of 140 cases, serum homocysteine level was elevated in 117 (83.6 %) cases. 65.7 % of the patients had moderate hyperhomocysteinaemia, while 17.9 % had intermediate hyperhomocysteinaemia. The mean (± SD) homocysteine level was 22.75 (± 8.19) (Table -1 and Figure -1). Association of stroke risk factors between cases with hyperhomocysteinaemia were studied. Among acute ischaemic stroke patients, 47 (33.6 %) had past history of diabetes mellitus, 103 (73.6 %) had history of hypertension and 19 (13.6 %) had history of coronary heart disease (CHD). Association of stroke risk factors (hypertension, diabetes mellitus and coronary heart disease) between cases with different levels of hyperhomocysteinaemia and those with normal homocysteine levels were studied (Table – 2 & 3 and Figure - 2).

There was no significant association between different levels of hyperhomocysteinaemia (moderate, intermediate and severe) and the conventional stroke risk factors.

In table 2, for three variables such as age, history of coronary heart disease (CHD) and family history of CHD, expected cell frequency for few cells were found less than 5. Hence, rows and columns were regrouped so as to form 2 x 2 tables.

Then Fischer exact test was carried out to find association of presence of hyperhomocysteinaemia among patients with acute ischaemic stroke with these three variables. The P values were still more than 0.05, hence these three variables - age, history of CHD and family history of CHD were not significantly associated with hyperhomocysteinaemia.

		Normal		Abnormal		P
		Count	Percent	Count	Percent	
Age	< = 70	14	21.2	52	78.8	0.149
	>70	9	12.2	65	87.8	
History of CHD	Absent	21	17.4	100	82.6	0.739 #
	Present	2	10.5	17	89.5	
Family history of CHD	Absent	18	15.5	98	84.5	0.548 #
	Present	5	20.8	19	79.2	

Table 2. Comparison of Factors Associated with Hyperhomocysteinaemia among Patients with Acute Ischaemic Stroke

CHD – Coronary heart disease. # is Fisher Exact test

		Normal Homocysteine		Moderate Hyper Homocystinaemia		Intermediate Hyperhomocysteinaemia		χ^2	P
		Count	Percent	Count	Percent	Count	Percent		
Gender	Male	15	17.2	58	66.7	14	16.1	0.53	0.769
	Female	8	15.1	34	64.2	11	20.8		
Residence	Urban	10	16.4	41	67.2	10	16.4	0.17	0.920
	Rural	13	16.5	51	64.6	15	19.0		
History of DM	Absent	11	11.8	67	72.0	15	16.1	5.72	0.057
	Present	12	25.5	25	53.2	10	21.3		
History of HTN	Absent	6	16.2	27	73.0	4	10.8	1.8	0.406
	Present	17	16.5	65	63.1	21	20.4		
Family history of DM	Absent	18	16.5	71	65.1	20	18.3	0.09	0.954
	Present	5	16.1	21	67.7	5	16.1		
Family history of HTN	Absent	14	16.9	58	69.9	11	13.3	2.98	0.225
	Present	9	15.8	34	59.6	14	24.6		

Table 3. Comparison of Factors Associated with Hyperhomocysteinaemia among Patients with Acute Ischaemic Stroke

DM = Diabetes mellitus, HTN = Hypertension, CHD = Coronary heart disease) χ^2 – Chi square test

DISCUSSION

In this cross-sectional study, serum homocysteine levels in acute phase of ischaemic stroke (within 24 hours) were significantly higher than normal limits (83.6 %). Hyperhomocysteinaemia was seen in all age groups among ischaemic stroke patients. The results of this study are consistent with many case control studies in acute ischaemic stroke patients in India, which showed hyperhomocysteinaemia among them ranging from 60 to 92 %.[14,15] Hyperhomocysteinaemia was found in half the patients with ischemic stroke in Northern India.[14] No definite

threshold level for homocysteine that correlates with a sudden increase in the risk of vascular events could be found out by these studies. The case control study by Prabhakar S et al. suggested to consider homocysteine levels of above 10 µmol / l as significant in patients with stroke for secondary prevention and supplementation with folate and vitamin B_{12}.[15] Majority of the studies conducted in other countries also showed convincing relation between elevation of serum homocysteine and ischaemic stroke.[9,16] But in a study by Verhoef et al. there was a small but insignificant relationship between high levels of plasma homocysteine and ischemic stroke. It may be probably due to the small sample size of that study.[17]

The relationship between stroke risk factors (hypertension, diabetes mellitus and coronary heart disease) and total homocysteine was evaluated in our study. Like Haapaniemi et al. this study also showed no significant correlation between total homocysteine level and most risk factors of stroke.[18] Some studies suggest that elevated total homocysteine levels may be an acute phase reactant that rises after the stroke or other similar vascular event in response to tissue damage or tissue repair.[19] The observations of Framingham Heart Study in 2003 does not support the hypothesis that plasma homocysteine is causally related to elevated blood pressure.[20] Our study also strengthens the assumption that hyperhomocysteinaemia is a risk factor that does not necessarily accompany other stroke-related risk factors, such as hypertension, coronary heart disease or diabetes mellitus.

One aetiology of hyperhomocysteinaemia is insufficient dietary intake of vitamins B_6, B_{12} and folic acid, therefore an effective treatment to correct the deficiency of these vitamins may be beneficial. But some randomized controlled trials have yielded mixed findings regarding the effect of therapeutic homocysteine lowering on stroke prevention. A meta-analysis by Meng Lee et al. identified 13 randomized controlled trials which found a trend toward mild benefit of folic acid supplementation on the risk of stroke among persons at high cardiovascular risk, but did not demonstrate a significant effect in averting stroke.[21] However, they found that potential mild benefits in primary stroke prevention were present, especially when folate is combined with B vitamins and in male patients. They also found a mild benefit of folic acid supplementation in trials in which stroke was not a qualifying event, but not in secondary prevention trials. They concluded that homocysteine lowering is beneficial at early stages of vascular diseases but is less effective in the case of established, advanced disease.

In the meta-analysis by Xiaobin Wang et al. a greater beneficial effect was seen in those trials with a treatment duration of more than 36 months by folic acid supplementation.[22] They found that folic acid supplementation significantly reduced the risk of stroke by 18%. Because of the low cost and safety of the therapy, such supplementations may be considered to treat patients with a stroke and hyperhomocysteinaemia.

Further studies are needed to test the effect of such treatments.

CONCLUSIONS

In our cross-sectional study serum homocysteine levels were found to be elevated significantly in acute ischaemic stroke patients. Elevated total homocysteine values may easily be reduced by vitamin supplementation, which may be an important asset in future secondary stroke prevention. Our findings add uncertainty to conclusions derived from several studies that hyperhomocysteinaemia has major, independent, causative association with increased blood pressure, diabetes mellitus and coronary heart disease. Elevated plasma homocysteine level may be an independent risk factor for stroke.

Limitations

A case control study would have given a better confirmatory result. Also, we could not measure the levels of vitamin B_{12} and folate in our study group, which have been documented to be strong correlates by many researchers, as they are co-factors in homocysteine metabolism. Further research is needed for using homocysteine as screening test and for initiation of preventive therapy of stroke.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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