SERUM HOMOCYSTEINE AS A RISK FACTOR IN ISCHAEMIC STROKE- A CROSS-SECTIONAL OBSERVATIONAL STUDY IN A TERTIARY CARE TEACHING HOSPITAL IN NORTHERN KERALA

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ABSTRACT

BACKGROUND

Stroke is a clinically-defined syndrome of rapidly developing symptoms or signs of focal loss of cerebral function with no apparent cause other than that of vascular origin. A previously unrecognised risk factor for stroke, which is prevalent and modifiable and may be causal, is elevated plasma homocysteine. To establish a correlation whether homocysteinaemia has any effect on incidence of young with stroke on our wards, we decided to undertake a cross-sectional observational study of patients admitted in the medical wards with first ever-ischaemic stroke.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Government Medical College, Calicut. 50 patients of age group 20-60 years, admitted to General Medicine Department with diagnosis of ischaemic stroke either clinically or proven by investigations were selected for the study. 50 age matched normal individuals also included. The period of study was one year. Homocysteine enzyme immunoassay was done by ELISA. The results were analysed statistically using Pearson chi-square test.

RESULTS

Serum homocysteine showed significant increase in patients than in the normal individuals. Mean fasting plasma homocysteine also showed statistically significant difference in both groups. Statistically significant positive correlation was present between plasma Homocysteine and MCV values collected from the subjects.

CONCLUSIONS

Our study supports that elevated levels of homocysteine might be an independent and modifiable risk factor for stroke in young in our country. Young patients presenting with ischaemic stroke need to be investigated for homocysteine level and treatment modalities to reduce homocysteine levels may help in preventing recurrence of ischaemic stroke.

KEYWORDS

Homocysteine, Homocysteinaemia, Ischaemic Stroke.

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BACKGROUND

Stroke is a clinically defined syndrome of rapidly developing symptoms or signs of focal loss of cerebral function with no apparent cause other than that of vascular origin. The syndrome varies in severity from recovery in a day, through incomplete recovery, to severe disability, to death.[1] Estimates from Indian Council of Medical Research (ICMR) indicate that there were 9,30,985 cases of stroke in India with 6,39,455 deaths and 6.4 million Disability Adjusted Life Years (DALY) lost in 2004.[2] By the year 2020, stroke and coronary artery disease together are expected to be the leading causes of lost healthy life years.[3]

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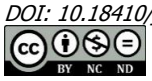

Age and hypertension are the most important risk factors for stroke. Other factors such as diabetes mellitus, smoking, raised blood lipids and obesity seem to be less important in stroke than in heart disease.[4,5] Reduction of the worldwide burden of stroke by effective prevention depends on the recognition and control of all causal and modifiable risk factors.[6] A previously unrecognised risk factor for stroke, which is prevalent and modifiable and maybe causal is elevated plasma homocysteine (tHcy).[7]

Classic homocysteinaemia is due to deficiency of the enzyme cystathionine β-synthase. Other causes of elevated levels of homocysteine are N^5, N^{10}-methylene THFA reductase (MTFR) deficiency and deficiency of cobalamide (vitamin B_{12}) coenzyme synthesis. Severe hyperhomocysteinaemia (levels >100 µmol/L) has been related to early onset of arteriosclerosis and thromboembolic events including stroke[8] and moderate hyperhomocysteinaemia (20-100 µmol/L) has been suggested to be a vascular risk factor. Many case control and cohort studies have identified a strong, independent and dose-related association between moderately elevated

homocysteine and atherosclerotic vascular disease including stroke.[9-11] The possible mechanism maybe the increasing homocysteine level caused neurotoxicity, endothelial dysfunction and other associated prothrombotic factors, which is reported to be an independent predictor of poor outcome in patients with ischaemic stroke.[12-14] However, not all reports have been consistent; several other studies failed to establish such an association.[15-16] Prospective cohort studies also have failed to demonstrate a positive association between elevated homocysteine and stroke.[17,18]

In several studies of stroke patients, the analysis were performed at least one month after stroke onset or the time of measurement was not stated. Little has been published about possible variations of plasma concentrations in the acute phase and there are only limited data on the prevalence of homocysteinaemia in selected Indian population. In a developing country like India, where food habits and malnutrition coupled together increase dietary deficiency in vitamins and minerals, homocysteine level could really be high and hence increased risk.

To establish a correlation whether homocysteinaemia has any effect on incidence of young with stroke on our wards, we decided to undertake a cross-sectional observational study of patients admitted in the medical wards with first ever ischaemic stroke.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Government Medical College, Calicut. 50 patients, males and females in the age group of 20-60 yrs admitted to General Medicine Department with diagnosis of ischaemic stroke either clinically or proven by investigations were selected for the study. 50 age-matched normal individuals are also included in the study. The period of study was one year. The Institutional Ethics Committee of Calicut Medical College Hospital approved the study. Stroke patients with aetiology other than thrombotic episodes confirmed either by clinical examination or by investigations and patients with onset of illness more than 24-hour duration excluded from the study. EDTA samples were collected and transported in ice packs. Samples centrifuged immediately, refrigerated and properly thawed before estimation. Homocysteine enzyme immunoassay was done by ELISA (Transasia). Measuring range of calibration is from 2-50 µmol/L. Statistical comparison of demographic and biochemical features between the two groups was done using Pearson correlation. The results were analysed statistically by chi-square test using Epi info. A 'p' value of <0.05 was taken as statistically significant.

RESULTS

Of the total 50 patients, 33 (66%) were between the age group 30-50 years. Of the remaining, 11 (22%) were between the age group 50-60 years and 6 (12%) between 20-30 years. In the normal group studied, 37 (74%) were between the age group 40-60 years, 4 (8%) were between 20-30 years and 9 (12%) were between 30-40 years. Of the 100 subjects studied, males constitute 74% of patients and 56% of normal group. 80% of the total study group (45 patients and 35 normal individuals) were belonged to poor socioeconomic group.

The main parameter estimated was serum homocysteine, which showed significant increase in patients than in the normal individuals. Normal value of serum homocysteine is 4.4-10.8 µmol/L. Of the total 10 subjects who had homocysteine value above 20 µmol/L, 9 were suffering from stroke. 45.9% of the cases (23) had homocysteine value between 15-20 µmol/L. In the normal group, 68.7% (37) had normal homocysteine value. 2 subjects had homocysteine value below 5 µmol/L and 7 had value between 15-20 µmol/L. Mean fasting plasma homocysteine also showed statistically significant difference in cases and controls. Mean values of homocysteine in cases was 16.2900 and that of control group was 12.1500. Statistically significant positive correlation was present between plasma homocysteine and MCV values collected from the subjects.

Homocysteine Level (µmol/L)		Group		Total
		Patients	Normal	
<5	Count	3	2	5
	% within HCY	60.0%	40.0%	100.0%
	% within group	6.0%	4.0%	5.0%
5-10	Count	14	23	37
	% within HCY	37.8%	62.2%	100.0%
	% within group	28.0%	46.0%	37.0%
10-15	Count	11	17	28
	% within HCY	39.3%	60.7%	100.0%
	% within group	22.0%	34.0%	28.0%
15-20	Count	13	7	20
	% within HCY	65.0%	35.0%	100.0%
	% within group	26.0%	14.0%	20.0%
>20	Count	9	1	10
	% within HCY	90.0%	10.0%	100.0%
	% within group	18.0%	2.0%	10.0%

Total	Count	50	50	100
	% within HCY	50.0%	50.0%	100.0%
	% within group	100.0%	100.0%	100.0%

Table 1. Homocysteine Values in Patients and Normal Individuals

Group	Mean	Std. Deviation	Std. Error Mean
HS1			
Patients	16.2900	10.5115	1.4865
Normal group	12.1500	6.4192	.9078
MCV1			
Patients	87.4720	7.2374	1.0235
Normal group	90.0380	4.4988	.6362

Table 2. Mean Values of Homocysteine and MCV in Patients and Normal Individuals

DISCUSSION

In this cross-sectional observational study, we found a strong correlation of hyperhomocysteinaemia with ischaemic stroke (P value-0.019). The results of the present study are consistent with many case-control and prospective studies.[19,20] But, a few prospective studies have failed to establish any association between hyperhomocysteinaemia and stroke.[21]

Homocysteine is a sulfhydryl amino acid formed by the demethylation of methionine. Homocysteine is formed during the course of formation of Active Methionine S-Adenosyl Methionine (SAM). The homocysteine thus formed is metabolised by one of the two pathways- In remethylation cycle, homocysteine is salvaged by getting methyl group in a reaction catalysed by methionine synthase; N^5-methyl-tetrahydrofolate is the methyl donor in this reaction and N^5, N^{10}-methylenetetrahydrofolate reductase is the catalyst. In another transsulfuration pathway, homocysteine condenses with serine to form cystathionine in a reaction catalysed by vitamin B_6 dependent enzyme, cystathionine beta synthase.

Hyperhomocysteinaemia has a multifactorial origin incorporating genetic, nutritional, pharmacological and pathological factors. Homocysteine has been linked in numerous in vitro studies with a diversity of mechanisms that could potentiate atherothrombosis including disrupted endothelial function, impaired protein C activation, increased thrombin generation and platelet aggregation.[22] High homocysteine levels have been linked to increased risk for coronary heart disease events, but there are limited data on its association with stroke risk. Investigators from the Framingham study followed 787 men and 1,158 women for 10 years to evaluate the association between total plasma homocysteine level and incident stroke in the elderly.[23] They found a strong, independent association between total homocysteine level and risk for stroke and suggested randomised trials to evaluate the effects of lowering homocysteine levels on stroke risk.

In 1999, a prospective study done by Bots et al[24] in a community with 7983 cases of atherosclerotic diseases, including strokes have found a positive association with high homocysteine values. These patients were observed under four years of follow up.

Although, severe hyperhomocysteinaemia is clearly related to atherosclerosis, it is less clear whether mild-to-moderate elevation in plasma homocysteine level is a risk factor for cardiovascular disease and death. Kark and colleagues[25] followed a cohort of 1,788 men and women aged 50 years or older for a decade. Compared with the lowest levels of plasma total homocysteine (less than 8.5 micromole/l), there was a significant increasing risk for death from any cause for patients with high homocysteine levels.

An extensive and statistically rigorous meta-analysis by Barbara Voetsch, et al[26] in 2002 found that hyperhomocysteinaemia is associated with atherothrombotic disease and venous thrombosis. This study also showed that even mild elevations of homocysteine increase ischaemic stroke risk.

Even though many studies are in favour of hyperhomocysteinaemia as an independent risk factor in ischaemic stroke, few studies suggest that elevation of homocysteine after ischaemic stroke is due to the disease process itself. Study done by D. J. Meikeljohn et al in 2001 showed that homocysteine concentrations are not elevated after recent atherothrombotic stroke, but rise in the convalescent period[21] and suggested that an increase in methylation reactions after tissue injury results in conversion of methionine to S-Adenosyl homocysteine, which leads to generation of homocysteine.

In our study, MCV values collected from the subjects showed a positive correlation with serum homocysteine levels. But, there was no significant relationship between the frequencies of vegetables and fruits intake with the level of serum homocysteine. This may be due to "Indian dietary paradox" like prolonged heating of food during cooking and frying. About 90% of folate maybe destroyed in cooking as it is heat sensitive and 10-50% of vitamin B_6 can be lost during processing and storage of foods.[27,28] Folic acid together with vitamins B_6 and B_{12} has been shown to be effective in reducing elevated plasma homocysteine levels,[29] but no randomised trials have as yet been completed to determine whether, lowering of elevated homocysteine levels will subsequently reduce stroke.

Even though controversies abound regarding homocysteine being a causal or casual factor in the presence of stroke, it has generally been accepted as one of less well-documented independent risk factors in stroke. But, recent studies go even further and hypothesise that elevated homocysteine levels may lead on to hypertension and subsequent stroke.

At present, there is insufficient data to recommend routine screening and treatment of high tissue homocysteine with B-vitamins to prevent atherosclerotic vascular disease in our country. A recent study in North India shows that 46.9% of normal subjects studied had subnormal level of

vitamin B_{12} or folate. However, keeping in view the prevalence of malnutrition, trials like VISP (Vitamin In Stroke Prevention) have not shown much benefit in supplementing vitamins in the prevention of stroke. A possibility could be that vitamin supplementation should begin early in life to prevent the subsequent occurrence of stroke.

CONCLUSION

Our study supports the hypothesis that elevated levels of homocysteine might be an independent and modifiable risk factor for stroke in young in our country. Young patients presenting with ischaemic stroke need to be investigated for homocysteine level and treatment modalities to reduce homocysteine levels may help in preventing recurrence of ischaemic stroke. Insignificant relation between frequency of vegetable and fruit intake with the level of homocysteine in the present study maybe due to the Indian practice of cooking vegetables and increasing Westernisation of our culture. Further, large trials are required to determine whether reducing homocysteine will reduce the risk of ischaemic stroke.

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