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Original Research Article

Clinical profile and serum homocysteine level in young patients with stroke: a prospective, observational study

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ABSTRACT

Background: According to the WHO, stroke is the second most important cause of death in elderly people with age >60 years and fifth leading cause in the age group of 15 to 59 years. Hyperhomocysteinemia has been linked to increased incidence of ischemic strokes. Thus, the aim of the present study was to assess serum homocysteine levels as an individual risk factor of stroke in young patients.
Methods: This was a prospective, cross-sectional, single center study performed in 50 patients admitted in the Department of Medicine, Thanjavur Medical College and Hospital, Thanjavur, over a period of 7 months (i.e., from December 2013 to June 2014). Young patients, aged 1545 years, and diagnosed with stroke were included in the study. Serum homocysteine was measured by fluorescein polarization immunoassay (FPIA). Significant difference between the patients with normal and elevated mean serum homocysteine levels was identified by using unpaired t-test. P value ≤0.05 was considered as statistically significant.
Results: Majority of the stroke patients were male (78%). Similarly, male patients dominated the total number of patients with elevated serum homocysteine levels (75%). Thirty-two (64%) patients had an elevated serum homocysteine level. There was a significant difference between the patients with increased homocysteine levels as compared to patients with normal homocysteine levels (p value <0.05). Out of 32 patients with hyperhomocysteinemia, 27 (84.38%) patients had ischemic stroke, 4 (12.50%) had cortical vein thrombosis and 1 (3.12%) had hemorrhagic stroke.
Conclusions: Findings of the present study confirm that hyperhomocysteinemia is associated with an increased incidence of stroke in young patients. As healthcare providers, we must stress on prevention of stroke, especially by identifying treatable risk factors.

Keywords: Cortical vein thrombosis, Fluorescein polarization immunoassay, Hyperhomocysteinemia, Ischemic stroke, Serum homocysteine, Young stroke

INTRODUCTION

According to the latest data, stroke is the second most important cause of death and the third leading cause of disability worldwide.[1,2] Stroke is identified as an abrupt onset of a neurological deficit which can be attributed to a focal vascular cause.[3] Currently, many risk factors have been identified for stroke and modification of these factors is of prime importance for its primary and secondary prevention.[4] Stroke in young patients is a major health problem. The age of young patients suffering from stroke is variable, but possibly, it should be between 15-49 years. Age has a definite relationship with incidence of stroke. The incidence of stroke

increases with increasing age.[5] Risk factors for stroke in young patients are cardio-embolic (35%), large vessel atherosclerosis (12%), lacunar infarcts (7%), collagen vascular diseases (3%), and indeterminate origin (43%). Stroke resulting due to hyperhomocysteinemia, secondary to vitamin B12 deficiency, is very rare and preventable cause of ischemic stroke.[6]

Homocysteine is a sulfur containing amino acid which is released as an intermediate in methionine metabolism and is essential for intravascular metabolism.[7,8] Hyperhomocysteinemia is defined as an elevated levels of total homocysteine concentration (>10 µM/l) in plasma.[9] Each increase in plasma homocysteine levels by 2.5 µM/l leads to an increased risk of stroke by about 20%. Moreover, as compared to concentrations below 9µM/l, there is nine-fold increase in the risk of myocardial infarction and stroke with total plasma homocysteine levels above 20 µM/l.[3] It has been cited in the literature that elevated serum homocysteine is an independent risk factor for the progress of atherosclerotic vascular disease processes, which is responsible for cardiovascular disease (CVD) and stroke, but a causal relationship between both is not yet definitely established.[7,8,10] Raised levels of total serum homocysteine are responsible for impairment of endothelial function and intensification of thrombosis. Animal and human studies have reported a significant association between elevated total serum homocysteine and carotid atherosclerosis, lacunar infarction, and increased risk of stroke in atrial fibrillation.[11] Homocysteine possesses both prothrombotic and atherogenic properties.[12] However, very few studies have elaborated the role of homocysteine in young Indian patients with stroke. Hence, the present study was performed with an aim to assess serum homocysteine levels as an individual risk factor of stroke in young patients.

METHODS

This was a prospective, cross-sectional, single center study conducted in 50 patients admitted in Department of Medicine, Thanjavur Medical College and Hospital, Thanjavur, over a period of 7 months (i.e., from December 2013 to June 2014).

Young patients, aged 15-45 years, and diagnosed with stroke were included in the study. While, patients with stroke having risk factors such as diabetes mellitus, hypertension, smoking, alcoholism, and hyperlipidemia were excluded. Study commenced only after approval of the study protocol by Institutional Ethics Committee. All the patients (caregivers of comatose patients) were explained the nature of study and a written informed consent was taken before enrollment.

Diagnosis of stroke was based on history, clinical features, CT brain and MRI brain, if needed. The following investigations were done on admission: complete blood count, random blood sugar, serum cholesterol, serum triglyceride, blood urea, serum creatinine, fasting serum homocysteine level, HIV, VDRL, ECG, Carotid Doppler and Echocardiogram.

Specimen collection and storage: 4 ml blood was collected in EDTA coated tubes. Samples were stored at 2-8 degrees, in case of delay in testing. The serum was separated by centrifugation. Serum homocysteine was measured by fluorescein polarization immunoassay (FPIA).

Principle of the procedure

At first, the bound homocysteine present in serum was reduced by the use of dithiothreitol (DTT). Then, the free homocysteine was converted to S-adenosyl homocysteine (SAH), by using S-adenosyl homocysteine hydrolase and adenosine. This mixture containing SAH, antibody, FPIA diluents, buffer, and a tracer tagged with a fluorescent chromophore were added to the cuvette. In cuvette, SAH from the serum sample competed with the fluorescent tagged tracer to bind the antibody. Then, the intensity of the polarized light was measured using FPIA optical assembly.

Statistical analysis

Categorical data was expressed as absolute numbers and percentages and the continuous data was expressed as mean ± standard deviation (SD). Significant difference between the patients with normal and elevated mean serum homocysteine levels was identified by using unpaired t-test. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

RESULTS

Figure 1 depicts the number of patients with elevated homocysteine levels. Majority of the patients i.e., 32 (64%) had an elevated homocysteine levels. Age and gender distribution according to the number of stroke patients as well as according to the patients with hyperhomocysteinemia are depicted Table 1.

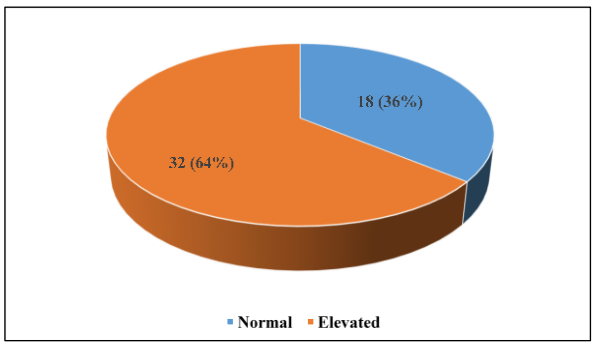

Figure 1: Distribution of patients with an elevated serum homocysteine levels (µM/l).

Table 1: Age and gender distribution of the study patients.

Characteristics	Stroke	Hyperhomocysteinemia
	Number of patients (%)	Number of patients (%)
Age group (Years)		
15-20	2 (4)	2 (6.25)
20-25	5 (10)	4 (12.5)
25-30	15 (30)	7 (21.85)
30-35	19 (38)	12 (37.5)
35-40	5 (10)	3 (9.37)
40-45	4 (8)	4 (12.5)
Total	50 (100)	32 (100)
Gender		
Male	39 (78)	24 (75)
Female	11 (22)	8 (25)
Total	50 (100)	32 (100)

There was a male preponderance in both the number of stroke patients (78%) as well as the number of patients with elevated homocysteine levels (75%).

The mean age of the study population was 30±15 years and range of age was 16-42 years. Comparison of mean homocysteine levels was done between two groups i.e., <15 and ≥15 and is depicted in Table 2.

Table 2: Comparison of mean serum homocysteine levels (µM/l).

Homocysteine levels (µM/l)	Mean±SD	*p value
<15 (n=18)	7.92±3.61	< 0.05
≥15 (n=32)	32.00±14.22	

Data expressed as mean±SD, p value <0.05 was considered as statistically significant; * - unpaired t-test.

There was a statistically significant difference between the groups (p value <0.05). Table 3 depicts the types of strokes and their distribution in patients with hyperhomocysteinemia.

Majority of patients i.e., 84% had ischemic stroke. All the 4 patients with cortical vein thrombosis (CVT), in the elevated homocysteine group, were female.

Table 3. Types of stroke in patients with hyperhomocysteinemia.

Types of stroke	Number (%)
Ischemic	27 (84.38)
Hemorrhagic	1 (3.12)
Cortical venous thrombosis	4 (12.50)

DISCUSSION

Globally, stroke is one of the most common causes of morbidity and mortality. Stroke is an event resulting due to interruption of blood supply to the brain, either because of blood vessel bursts or blockage by a clot. This results in cut off of the oxygen and nutrient supply causing damage to brain tissue. The commonest symptom of stroke is sudden weakness or numbness of face, arm or leg, often on one side of the body, in 90% of stroke patients. Other symptoms are confusion, difficulty in speaking or understanding speech, difficulty while seeing with one or both eyes, difficulty in walking or dizziness, severe headache without known cause, and unconsciousness. A very severe stroke can cause sudden death.[13]

Epidemiological research has shown that even small rise in total homocysteine levels is associated with an increased risk of thromboembolic disease.[14] Levels of homocysteine depend on gender, age, smoking, and intake of coffee, alcohol and folate.[15] Total plasma homocysteine levels is comprised of about 70% of the homocysteine bound to albumin, 30% oxidized to disulphides and approximately 1% is present as free homocysteine.[3] Folic acid and B vitamins are needed for homocysteine metabolism as a substrate. Low serum folic acid and B vitamin levels may lead to rise of homocysteine levels in blood and vice versa. When homocysteine is produced in excess, it is excreted out of the tightly regulated cell environment into the blood.[7] Homocysteine is an independent predictive biomarker for cardiovascular disease, probably due to an increased oxygen stress and endothelial dysfunction leading to thrombotic events.[15] In 1995, Boushey et al. confirmed the results of their meta-analysis of 27 observational studies on homocysteine and atherosclerotic vascular disease, out of which 11 studies reported an association between homocysteine and risk of stroke. Nine case-control studies supported the hypothesis that homocysteine is an independent risk factor for stroke, while 2 prospective studies provided negative results. Thus, in future, measurement of homocysteine can be an important part of workup of young stroke patients.

Stroke in young patients, less than 45 years of age, accounts for 10% of all strokes. In India, about one-fifth of patients with first episode of stroke admitted to hospital are shown to have age less than 40 years. It justifies an extensive etiologic work-up needed for the confirmation of exact cause responsible for an episode of stroke, as the conventional risk factors such as hypertension, hyperlipidaemia, and smoking do not account for all these cases.[6]

In the present study, majority of the patients had hyperhomocysteinemia. Moreover, these patients had an increased incidence of stroke. This was in accordance with the findings of the study by Desai et al. in which plasma homocysteine levels was raised in 56.67% patients with stroke.[13] Overall, mean plasma homocysteine level was also raised.

In the present study, all the included patients were in the age range of 15 to 45 years.

Majority of the patients i.e., 19 (38%) were in the age group of 30 to 35 years, followed by 15 (30%) patients in the age group of 25 to 30 years. Out of 32 patients with elevated serum homocysteine levels, majority of the patients i.e., 12 (37.5%) were in the age group of 30 to 35 years, followed by 7 (21.85%) patients in the age group 25 to 30 years. Similarly, in a study by Desai et al., 50% patients were aged between 41 to 45 years, followed by 30% patients between 31 to 40 years. However, 20% of the patients were in the age group of 18 to 30 years. Mean age was 38.53 ± 6.92 years.13 Also, in a study by Gajbhare et al., most of the patients with stroke were in the age group of 25 to 45 years, with a mean age of 39.09±5.2 years.[10]

Out of 50 patients studied, 39 (78%) patients were males, showing male preponderance. Similar male preponderance was also observed in patients with an elevated serum homocysteine levels, where 24 (75%) male patients had serum homocysteine levels > 15 µM/l. Similarly, study by Zongate et al, demonstrated that male gender is linked with higher total serum homocysteine concentration.[8] The difference between the genders could be attributed to large muscle mass in males, as the formation of muscle accompanies simultaneous formation of homocysteine in connection with creatine/creatinine synthesis.

The increased serum homocysteine levels may also be a result of influence of sex hormones in males.[8] This was in consistency with the findings of Desai et al, who reported that out of the 30 patients of stroke, 73% were male, with male to female ratio of 2.7:1.13 Also, study by Modi et al, and Bogdan et al, supported author findings.[1,4,16] Murmu et al. in their study stated that young healthy women have homocysteine levels lower than healthy men. This difference diminishes with ageing. An abrupt increase in serum homocysteine in women after 50 years suggests that sex difference in serum homocysteine levels disappears with increasing age.[3]

In the present study, mean homocysteine level was significantly higher in hyperhomocysteinemia group (32±14.22) compared to normal homocysteine group (7.92±3.61) ($p<0.05$). Out of 32 patients with hyperhomocysteinemia, patients with ischemic stroke were 27 (84%) and that with CVT and hemorrhagic stroke were 4 (13%) and 1 (3%), respectively. Author findings were consistent with the studies performed by Datta et al. and Boysen et al. who reported significantly raised serum homocysteine levels in ischemic stroke as compared with hemorrhagic stroke.[17,18] Narang et al. carried out a similar study in 100 patients of ischemic stroke and reported higher serum homocysteine level in them.[19] Furthermore, Gajbhare et al, confirmed that serum homocysteine levels were significantly raised in young patients with ischemic stroke.[10] In a study by Raheem SA, mean serum homocysteine levels were higher in patients with infarct.[20] Various studies have demonstrated hyperhomocysteinemia as a risk factor of ischemic stroke, but others have demonstrated contradictory findings. Modi et al, in their study concluded that hyperhomocysteinemia is an important risk factor for ischemic stroke.[4] Similarly, Brattstrom et al. reported that hyperhomocysteinemia might be a risk factor for atherosclerotic cerebrovascular accidents.[21] However, Alfthan et al, and Mousavi et al, concluded that there was no association between hyperhomocysteinemia and strokes.[22,23]

Stroke in young patients requires a comprehensive investigations looking into all potential etiologies. As healthcare providers, we must stress on prevention of stroke, especially by identifying treatable risk factors. Since, it has been concluded that hyperhomocysteinemia is associated with an increased incidence of stroke, large group of organized studies needs to be conducted to find out whether homocysteine lowering therapy with high dose folic acid, pyridoxine and vitamin B12 actually reduces an incidence of stroke in young patients. The preventive measures for stroke could help greatly in bringing down economical as well emotional burden on the family. Due to small sample size, findings of this study needs further evaluation in large population, to further enlighten the precise role of homocysteine in young patients with stroke. Lack of follow up data, due to cross sectional study design.

CONCLUSION

In the present study, author excluded patients with conventional risk factors and evaluated whether hyperhomocysteinemia has a role as an independent risk factor for stroke. Auhor found that significant number of young patients with stroke had hyperhomocysteinemia. Hence, hyperhomocysteinemia can be considered as an emerging independent risk factor of stroke.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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