Original Research Article

The study of plasma homocysteine level as a risk factor for ischemic strokes in young patients

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ABSTRACT

Background: According to the WHO, stroke is second leading cause of death for people above the age of 60 years & fifth leading cause in people aged 15 to 59 years old. Each year, nearly six million people worldwide die from stroke. One in six people worldwide will have a stroke in their lifetime. Approximately one-quarter of 45-year-old men will have a stroke before they reach the age of 85. Stroke is also important condition in young people and can occur at any age, including in utero, neonatal period (it is the major cause of cerebral palsy), childhood and young adult life. One-quarter of all strokes occur in people below the age of 65.
Methods: This prospective observational case control study was carried on 30 young ischemic stroke patients with 30 control match persons over a period of 20 months, from December 2011 to August 2013 at Tertiary Care Hospital Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai, India.
Results: Total plasma fasting homocysteine level in case group was 30.10±14.8 µmol/L and control group was 13±5.3 µmol/L, (p=0.001). Elevated fasting homocysteine level was found in 76.66. 0% of ischemic stroke cases and in 10% of healthy controls (p=0.001). Serum homocysteine levels were higher in subjects having risk factors such as dyslipidemia (p value <0.001), active lifestyle (p value <0.05) and smoking (p value<0.05). Serum homocysteine did not show any significant relation with age, sex, diabetes mellitus, diet pattern and defective coagulation (p value >1).
Conclusions: The present study revealed that hyperhomocysteinemia appears to be an important risk factor for young ischemic strokes. It is therefore important to use serum homocysteine level as an important tool to investigate all cases of young ischemic strokes and also in those who are at risk of developing it. Significant correlation has been found between homocysteine concentration and young ischemic strokes.

Keywords: Hyperhomocysteinemia, Ischemic Stroke, Young age

INTRODUCTION

Globally, stroke is the second commonest cause of mortality and the fourth leading cause of disease burden.[1] It makes an important contribution to morbidity, mortality, and disability in developed as well as developing countries.[2] The issue of stroke in young has been of considerable interest to neurologists in our country. The age group for stroke in young has been variable between different studies but perhaps should be restricted to 15 to 49 years as this age group tends to have unique set of causes and risk factors. Though the traditional risk factors of strokes play a significant role in young age group also, presence of higher number of cryptogenic strokes, cardio embolic and venous strokes makes diagnostic evaluation in this age group more challenging. Stroke is the most common neurological condition causing long term disability and has enormous

emotional and socioeconomic consequences in patients, their families and health services. According to the global burden of disease study 2010, stroke is the leading cause of disability adjusted life years (DALYs) in southeast Asia.[3] Age has strongest association with the incidence of stroke.[4] Age specific incidence of stroke increases progressively with increasing age. However, the impact of stroke on individual family and society is strongest when it affects a young individual. The young patients are increasingly affected by stroke, because of both the changing population exposures to risk factors and, most tragically, not being able to afford the high cost for stroke care. Majority of young stroke survivors continue to live with disabilities, and the costs of on-going rehabilitation and long term-care are largely undertaken by family members, which impoverish their families.[1] Therefore, in India as well as in other countries, there has been a large body of literature addressing the issues related to strokes in young's. Hyperhomocysteinemia is generally acknowledged as a treatable risk factor for atherothrombotic disease, but a causal relationship between both is not yet definitely established. This review tried to address the changing burden of young age stroke. The objectives of this study were to define age group for stroke in young population, to find out relationship if any, between serum homocysteine levels and risks of ischemic strokes in young patients admitted in Sir JJ Groups of Hospitals, Mumbai.

METHODS

This Prospective observational case control study was carried on 30 young ischemic stroke patients with 30 control match persons over a period of 20 months, with following criteria's.

Inclusion criteria

- First ever episodes of ischemic stroke
- Cases presenting within two weeks of the event
- Age group 15 years to 45 years
- Willing to give informed consent.

Exclusion criteria

- Non-hemorrhagic stroke
- Renal, hepatic, thyroid dysfunction
- Collagen vascular diseases
- Chronic diseases like HIV, Syphilis, TB, RHD, and cancer
- Patient on steroids
- Pregnancy state and postpartum period.

Case and controls

Total 60 subjects participated in the study. 30 clinically and radiologically confirmed (CT/MRI scans) cases of ischemic stroke in young subjects and 30 controls were selected.

Clinical information including age, sex, history of current evidence of hypertension (HTN): systolic blood pressure (SBP) - 140mmHg and diastolic BP - 90mmHg, Diabetes Mellitus (DM): fasting blood glucose 7 mmol/L/126 mg, cardiac disease, life style, diet pattern, family history of vascular diseases was recorded for all subjects. Serum total cholesterol (CH), HDL cholesterol, LDL cholesterol VLDL cholesterol and triglycerides were measured by using standard enzymatic procedures.[5]

Borderline for normal values were: total cholesterol 200-239 mg, HDL-C < 60 mg, LDL-C <130-159 mg, VLDL< 1.1 mmol/L and triglyceride < 150 mg.[5]

Serum homocysteine was estimated by Chemilunescent Immuno assay method, variant of standard ELISA.

The upper limit of the manufacturer and the laboratory was 15 µmol/L. Values above 15 µmol/L were accepted as high.[6]

Analyzers used

Immulite 1000 Chemiluminescent Technology of SIEMEN'S Company.

Normal levels of homocysteine

- Adult male: 06-15 µmol/L
- Adult female: 03-12µmol/L
- Elderly >65 years: 15-20µmol/L.

In order to compare these parameters between patients and controls, student's t-test was applied and the results were presented in tabular form.[6,7]

RESULTS

In present study of 30 patients (cases) presenting with neurological deficits due to ischemic stroke, were matched for age and sex with control group. Mean age was 39.09 years with S.D of 5.2. Mean serum homocysteine levels in males (30) were 21.52 µmol/L with S.D of 7.98. and mean serum homocysteine levels in females (30) was 20.85 µmol/L with S.D of 6.56. With p value 0.76. There was no statistically significant difference between age/sex and stroke with relation to serum homocysteine.

Most subjects were from age group between 25 to 45 years.

In previous same type of study to explore the association of homocysteine with ischemic stroke the mean age was 66 years.[8] In other study it was 66.2±11.0 years.[2]

In this study smoking was having some statistically significant co relation with stroke cases p value of <0.001. The mean value of serum homocysteine level in smokers (15) was 31.97 µmol/L with S.D. of 17.4, while

in nonsmokers (15) was 18 µmol/L. with S.D of 10.83. This difference was statistically significant with p value of <0.05.

In this study mean SBP in cases was 126.7 mm hg with S.D of 9.6. Mean DBP in cases was 80.4 mm hg with S.D. 7.6. Whereas mean SBP and DBP in controls was 117.3 and 74.9 mm hg with S.D 4.7 and 5.1 respectively. There was statistically significant correlation between stroke and hypertension with p value of 0.02 (for

hypertension SBP+DBP), p value of 0.001 (for SBP), p value of 0.002 (for DBP). However, there were 5 hypertensive subjects all cases with mean serum homocysteine level of 25.35 µmol/L with S.D 18.5. Mean serum homocysteine level in non-hypertensive 55 subjects 30 controls and 25 cases was 21.17 µmol/L with S.D.13.7 there was no statistically significant correlation between homocysteine levels and hypertensive/non-hypertensive subjects with p value of 0.58.

Table 1: Comparing S. Homocysteine level with various parameters.

Variables	Cases	Controls	S. Homocysteine level (µmol/l)	Standard deviation (s.d)	P value
Male	15	15	21.52	7.98	0.76
Female	15	15	20.85	6.56	
Smokers	15	0	31.97	17.4	<0.001
Non-smokers	15	30	18	10.83	
Hypertension	05	0	25.35	18.5	0.58
Non-hypertensive	25	30	21.17	13.7	
Vegetarians	11	06	27.67	7.98	<0.05
Mixed diet	19	24	30.41	10.09	
Normal lipid profile	16	30	19.97	14.32	<0.05
Dyslipidemia	14	0	26.60	12.02	
Active life style	22	27	32.64	12.80	<0.01
Sedentary life style	08	03	23.62	7.80	
Diabetics	02	0	-	-	0.15
Non-diabetics	28	30	-	-	
Positive family history	14	0	28.40	14.40	0.034
Negative family history	16	30	19.40	13.34	
Serum homocysteine level in cases	30	-	30.10	14.80	<0.001
Serum homocysteine level in controls	-	30	13	5.3	

There was no any statistically co relation between cases and diet pattern with p value of 0.25.

In this study 43 patients were mixed diet with mean S. homocysteine level of 30.41 µmol/L with S.D of 10.09 and 17 were vegetarian with mean value 27.67 µmol/L with S.D. of 7.98. Difference was statistically significant in mixed diet P value <0.005. There were 6 vegetarian cases with mean value of S. homocysteine level of 25.03 µmol/L with S.D. 7.87. and 24 cases were taking mixed diet with mean value (30.32 µmol/L) with S.D 9.8.

Among controls 19 were taking mixed diet with mean S. homocysteine level 30.54 µmol/L with S.D 10.1. Mean value in 11 vegetarian control persons were 29.58 µmol/L with 9.86.

S. Homocysteine level was statistically higher in mixed diet persons as compared to vegetarian persons. With p value of <0.05.

In this study 14 cases were dyslipidemic defined as (either raised LDL/HDL/VLDL/ Total cholesterol) having mean serum homocysteine value of 27.16 µmol/L with S.D of 9.89, while normal lipid profile cases 16 were having value of 33.08 µmol/L among controls mean value was 29.39 µmol/L. Among cases 6 were having raised TG triglycerides level (mean 22.27 µmol/L), each 3 were having TG+LDL and TG+CH levels mean values (22 and 22 µmol/L). Each 1 case was showing raised TG+LDL+CH with homocysteine level 34.84 µmol/L and raised HDL level with homocysteine level 56.59 µmol/L.

Overall there was significant difference in serum homocysteine level between dyslipidemic and normal lipid profile persons, serum homocysteine levels were higher in dyslipidemic subjects with mean serum homocysteine level of 26.6 µmol/L with S.D. of 12.02. While normal subjects (46) were having mean serum homocysteine level of 19.97 µmol/L with S.D of 14.32.

This difference was statistically significant with p value of < 0.05.

There was no any correlation found between serum homocysteine levels, FBS/PPBS/PT-INR values, with p value >1.

Comparison between serum homocysteine levels and diabetes mellitus

There was no any statistically significant difference between stroke incidence and DM, IHD, RHD with p value of 0.15.

Among 30 cases 16 were having negative family history with mean serum homocysteine level of 27.32 µmol/L. 14 cases were having positive family history with mean value of 27.28 µmol/L. All controls were having negative family history with mean serum homocysteine level of 38.43 µmol/L. Mean values of serum homocysteine levels with one set of homocysteine level are as shown in above table.

There were total 14 subjects with positive family history mean serum homocysteine level of 28.4 µmol/L with S.D of 14.4. While 46 subjects with negative family history were having mean serum homocysteine level of 19.4 µmol/L with S.D. 13.34. These differences were statistically significant showing higher homocysteine levels in subjects having positive family history with p value of 0.034.

Comparison between serum homocysteine level and Life style

There was very little correlation between stroke and life style pattern of subjects with p value of 0.18. There were total 11 subjects in our study with sedentary life style mean serum homocysteine level 23.62 µmol/L with S.D 7.8, while 49 subjects were having active life style with mean value of 32.64 µmol/L with S.D of 12.8. The difference with these two patterns was significant with p value <0.01. Mean serum homocysteine level in cases was 30.10 µmol/L with S.D of 14.8. Controls mean serum homocysteine level was 13 µmol/L with S.D of 5.3. Elevated fasting homocysteine level was found in 76.66.0% of ischemic stroke patient and in 10% of healthy controls (p=0.001). The incidence of hyperhomocysteinemia is higher in ischemic stroke cases than that in age-sex matched healthy control. 4 patients were exclusively not having any risk factors mean serum homocysteine level in this group was 39.10 µmol/L which was higher than the mean value of all cases (30.10 µmol/L) and controls (13 µmol/L). The incidence of hyperhomocysteinemia is higher in ischemic stroke cases than that in age-sex matched healthy control.

DISCUSSION

Many studies have showed that increased Homocysteine represents an independent risk factor for coronary, cerebrovascular and peripheral arterial disease.[9-11]

Various risk factors for cerebrovascular accidents like age, sex, food habit, hypertension, diabetes mellitus and lifestyle were studied and analyzed in relation to serum homocysteine levels.

Hyperhomocysteinemia is one of the newly recognized factors that increase risk of vascular disease.[12] Mechanisms by which hyperhomocysteinemia increases risk of cerebrovascular accidents are not clear, but several possible mechanisms have been proposed.[12] Hyperhomocysteinemia is associated with premature atherosclerosis.

Experimental studies both in vivo and in vitro shows that homocysteine causes endothelial injury and cell detachment. Hence these data suggest that homocysteine might contribute to cerebrovascular disease in patients as an additive risk factor.[13] Measurement of homocysteine may become the integral part of work up of stroke patients in near future.

Comparison of serum homocysteine with age and sex

In this study, we have taken age & sex matched controls with cases 30 males and 30 females. The difference was not statistically significant p value more than > 0.05. Our findings were consistent with study of Nigel, Narang and Modi et al.[11,14,15] However, according to findings of Longo et al and Zongte et al increase in the serum homocysteine levels were observed with increasing age.[5,16] Kang et al studies shows that young healthy women have homocysteine levels lower than healthy men.[17] This difference diminishes with ageing. An abrupt increase in serum homocysteine in women after 50 years suggests that sex difference in homocysteine disappears with increasing age.[17]

Comparison of serum homocysteine according to Smoking Habit

In this study mean serum homocysteine levels in smokers was higher (31.97µmol/L) than non-smokers (18 µmol/L). The difference was statistically significant (p <0.05). Study results were similar to findings of Modi et al, Nygard et al and Welch et al.[15,18,19]

However, Roudbari et al reported no significant relationship between smoking and serum homocysteine levels.[20] Perry et al found no evidence of an interaction with smoking.[21]

Comparison of serum homocysteine with blood pressure (BP)

In this study, it was found out that hypertension either SBP or DBP has significant co-relation with incidence of stroke in subjects with p value < 0.05.

The difference was statistically significant (p <0.05). BP is an important risk factor for stroke. However, when statistical analysis was done for serum homocysteine levels in subjects with relation to their hypertension status; wit was found out that there is no significant correlation between serum homocysteine and BP. This finding of was similar to Kittner et al who did not find definite evidence of an increased homocysteine in hypertensive patients.[22] But findings of Narang et al, Modi et al, Graham et al, Olusegun et al, Nigel et al, Nygard et al, Perry et al & Manilow et al differ with such co relation and say that definite relation between serum homocysteine level and risk of hypertension.[11,12,14,15, 18,21,23,24]

Comparison of serum homocysteine with diabetes mellitus

In this study, difference was statistically not significant (p >0.05). The findings were consistent with study of Narang et al and Modi et al. [14,15]

Comparison of serum homocysteine with lifestyle

Mean serum homocysteine levels were lower in sedentary lifestyle (23.62 µmol/L) than active lifestyle (32.64 µmol/L). Difference was statistically highly significant (p<0.05). However, Perry IJ et al differ and suggested association between hyperhomocysteinemia and established vascular risk likely to reflect lifestyle factor.[10]

Comparison of serum homocysteine with diet

In this study, found serum homocysteine level were statistically higher in mixed diet persons as compared to vegetarian persons, with p value of <0.05. Mean serum homocysteine level was higher (30.42 µmol/L) in mixed diet subjects group (43) than pure vegetarian group (17) with mean of 27.67 µmol/L. Associations between hyperhomocysteinaemia and established vascular risk factors are likely to reflect, at least in part, links with common underlying dietary and lifestyle factors, in particular, a diet high in saturated fat with inadequate folate intake from fruit and vegetables.[10] According to Jayanti kalita et al, in vegetarians homocysteine levels were higher than non-vegetarians and were related to low plasma B12 level.[25]

Comparison of Serum Homocysteine level with fasting lipid profile

In this study, 16 cases were having normal lipid profile and 14 were having abnormal lipid profile. Total 14 subjects were having dyslipidemia with mean serum homocysteine level of 26.60 µmol/L higher than normal lipid profile subjects (46) mean value 19.97 µmol/L. This difference was statistically significant (p <0.05). Among cases 6 were having raised (TG) triglycerides level (mean 22.27 µmol/L), each 3 were having TG+LDL and TG+CH levels mean values (22 and 22 µmol/L). Each 1 case was showing raised TG+LDL+CH with homocysteine level 34.84 µmol/L. and raised HDL level with homocysteine level 56.59 µmol/L. The findings were similar to study of Graham et al.[23] However Narang et al and Modi et, says that there is no such co relation between dyslipidemia and serum homocysteine.[14,15]

Comparison of Serum Homocysteine level with PT/INR

In our study, none of the cases and controls were having abnormal PT/INR levels. We found out that there is no such co relation between raised homocysteine level and deranged PT/INR similar to findings of H. Mouravas et al.[26] However Heijer MD et al in a meta-analysis of published epidemiological studies, demonstrate a modest association of homocysteine with venous thrombosis. The elevated risk associated with the MTHFR 677TT genotype provides some support for causality.[27]

Comparison of serum homocysteine level with family history

In our study, we have found out that those cases and controls having any of positive history for any cardiovascular disease have significant co relation for increased homocysteine level. Total such subjects were 14 with mean serum homocysteine level (28.4 µmol/L) higher than negative family history (46) subjects mean value 19.4 µmoll/L, p value <0.05. The finding were consistent with findings of Reis RP et al.[28]

Comparison between serum homocysteine level of cases and controls

In this study mean serum homocysteine level of cases (30.10 µmol/L) was higher than controls (13. µmol/L). Four patients were exclusively not having any risk factors (Hypertension, Diabetes Mellitus, Cardiac disease, Smoking) mean serum homocysteine level in this group was 39.10 µmol/L. Which was higher than the mean value of all cases (30.10 µmol/L) and controls (13 µmol/L). The incidence of hyperhomocysteinemia is higher in ischemic stroke cases than that in age-sex matched healthy control. This difference was statistically highly significant with p value < 0.001.

Hyperhomocystenaemia defined as elevated homocysteine concentration as one that exceeds 15.8 µmol/L (95th percentile for healthy control subjects).[29] Joosten E et al, defined an elevated homocysteine concentration as one that exceeded 13.9 m µmol/L (the mean value plus 2 S.D among healthy young controls).[30] In Framingham heart study cohort had previously

considered a homocysteine concentration of 14 μmol/L to be elevated (90th percentile for persons with apparently adequate concentrations of folate, vitamin B12, and vitamin B6).[31] In current study, serum fasting total plasma homocysteine level in case group was (30.10±4.8 μmol/L) which was significantly higher than the controls (13±5.3 μmol/L), (p=0.001).

The findings were similar to findings of Modi et al, Roudbari et al and Nigel et al who concluded that hyperhomocysteinemia as an important risk factor for ischemic stroke.[11,15,20]

Verhoef et al, in his study said that small but insignificant association between elevated homocysteine and risk of ischemic stroke.[32] Brattstrom et al findings suggested hyperhomocysteinemia might be a risk factor for atherosclerotic cerebrovascular accidents.[13] Fujishama reported that lacunar infarction is the most prevalent type of ischemic stroke in relation to elevated homocysteine in Japanese people.[35] However, Alfthal et al and Mousavi et al observed no association between hyperhomocysteinemia and cerebrovascular accidents.[33,34]

Boushey and colleagues have reported on a meta-analysis of many observational studies relating total homocysteine concentrations to atherosclerotic vascular disease, of which 11 studies addressed the association between homocysteine and risk of stroke, 9 case-control studies provided support for the hypothesis that homocysteine is an independent risk factor for stroke while 2 prospective studies did not support the study.[10,36]

CONCLUSION

The current study was aimed to explore the relationship of serum homocysteine with young ischemic stroke. Total plasma fasting homocysteine level in case group was 30.10±14.8 μmol/L and control group was 13±5.3 μmol/L, (p=0.001). Elevated fasting homocysteine level was found in 76.66.0% of ischemic stroke cases and in 10% of healthy controls (p=0.001). The incidence of hyperhomocysteinemia is higher in ischemic stroke cases than that in age-sex matched healthy controls. Hyperhomocysteinemia in ischemic stroke patients has been determined as vascular risk factor in our study. Significant correlation has been found between homocysteine concentration and ischemic stroke subjects as compared to controls. Our main observation was that serum homocysteine levels were elevated in cerebrovascular accident of young ischemic strokes patients significantly, in majority of cases.

Further serum homocysteine levels were higher in subjects having risk factors such as dyslipidemia (p value <0.001), active lifestyle (p value <0.05) and smoking (p value<0.05). Serum homocysteine did not show any significant relation with age, sex, DM, diet pattern and defective coagulation (p value >1).

People at risk for cerebrovascular diseases having risk factors such as, smoking, dyslipidemia should be screened for hyperhomocysteinemia. Our study revealed that hyperhomocysteinemia appears to be an important risk factor for cerebrovascular accidents. It is therefore important to use serum homocysteine level as an important tool to investigate all cases of young cerebrovascular accidents and also in those who are at risk of developing it. Significant correlation has been found between homocysteine concentration and ischemic stroke.

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