Association of Serum Homocysteine Level with Acute Ischaemic Stroke among Young Adult Patients: Experience at a Largest Medical College Hospital in Bangladesh

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Abstract

Background: Hyperhomocysteinemia is emerging as a possible risk factor for stroke, possibly because of accelerated atherosclerosis. **Objectives:** This study was conducted to evaluate the association between the serum homocysteine level and ischemic stroke in young Bangladeshi patients. **Methodology:** This cross-sectional study was carried out among patients 18 to 40 years of either gender with sign and symptoms of acute focal neurological deficit, in the Department of Medicine of Dhaka Medical College Hospital, Dhaka, from January 2015 to December 2015. History taking and physical examination were done in a predesigned data collection sheet. Then required investigations like CT-scan of brain, fasting serum homocysteine, fasting blood sugar, 2 hours ABF blood glucose, HbA1c and fasting lipid profile were done. **Results:** Out of 96 patients after completing brain imaging 61(65.0%) patients were found suffering from ischemic stroke. Among the patients 74 had normal homocysteine level, 17 patients had moderate hyperhomocysteinemia and 5 patients had intermediate hyperhomocysteinemia. Mean levels of fasting serum homocysteine were significantly higher in patients those had ischemic stroke (16.02 µmol/L, 95% CI: 12.87 to 19.87) compared with those who did not have ischemic stroke (10.47 µmol/L, 95% CI: 9.16 to 11.95, P<0.001). Logistic regression showed fasting homocysteine as an important independent risk factor, with an adjusted OR of 1.1 (95% CI: 1.1 to 1.2; p=0.04966) for every 1 µmol/L increase in homocysteine. **Conclusion:** The relationship between increasing homocysteine and ischemic stroke risk is strong, graded, and significant. *[Journal of National Institute of Neurosciences Bangladesh, January 2023;9(1):24-29]*

Keywords: Homocysteine; ischemic stroke; atherosclerosis

Introduction

Stroke remains a leading cause of death and disability in the world. Ischemic stroke can be caused by large artery atherosclerotic disease, small vessel or penetrating artery disease (lacunes), cardiogenic or artery-to-artery embolism, nonatherosclerotic vasculopathies, hypercoagulable disorders, or infarcts of undetermined causes[1]. The risk factors for ishaemic stroke reflect the risk factors for underlying vascular disease[2,3]. Homocysteine (Hcy) is a four-carbon amino acid with a free thiol group, which is formed by demethylation of methionine, an essential amino acid derived from diet[4]. It is metabolized with folate as a co-substrate, vitamin B12 as a co-factor and the help of several other enzymes[5]. There is evidence that homocysteine may cause endothelial dysfunction through formation of reactive oxygen species that has an important role in atherogenesis. It also interferes with the vasodilation and antithrombotic function. Oxidative damage to the vascular endothelium and the proliferation of the

vascular smooth muscle create a prothrombotic condition, which contributes to the development of premature atherosclerosis[5,6]. Normal total Homocysteine (tHcy) concentrations range from 5-15 µmol/L in the fasting state. Hyperhomocysteinemia refers to increased total serum concentration of homocysteine with or without an increase in free homocysteine (disulfide form). Hyperhomocysteinemia (Hcy) has been classified into moderate (serum tHcy concentrations of 15-30 µmol/L), intermediate (serum tHcy concentrations of 31-100 µmol/L), and severe (serum tHcy concentrations 100 µmol/L)[7].

An increase in total serum homocysteine represents an independent risk factor for coronary, cerebrovascular, and peripheral arterial disease as well as for deep venous thrombosis. Homocysteine is synergistic with hypertension and smoking and is additive with other risk factors that predispose to vascular disease. Vitamin B12, vitamin B6, and folate, all of which have dietary origins, are three main cofactors in Hcy metabolism[8]. Deficiencies in these supplements are more prevalent in the most developing countries and may account for many cases of moderate hyperhomocysteinemia and increased risk of stroke[9]. Decreased plasma levels of folate, vitamin B12, and vitamin B6 as well as male gender and living in urban areas were significantly related to Several studies have postulated that elevated tHcy is a strong and independent risk factor for vascular diseases including ischemic cerebral stroke. Some studies have shown that even mildly increased plasma tHcy can also be a significant risk factor for stroke, more specifically ischemic stroke in young adult[6,10]. The aim of this study was to evaluate hyperhomocysteinemia as a risk factor for ischemic stroke in young adult in Bangladeshi population.

Methodology

Study Settings and Population: This cross sectional observational study was carried out among 96 patients 18 to 40 years of either sex with sign and symptoms of acute focal neurological deficit, in the Department of Medicine of Dhaka Medical College Hospital, Dhaka, from January 2015 to December 2015. Inclusion criteria were - Patients admitted with sign and symptoms of focal neurological deficit, within 7 days of presentation, young adult (below 40 years of age) both sexes. Exclusion criteria were patient suffering from valvular heart disease, atrial fibrillation (AF), pregnancy & patient need ICU, patients taking drugs causing changes in serum homocysteine level.

Study Procedure: After meeting inclusion and exclusion criteria informed written consent was taken. Demographic information was prospectively recorded and substantiated by means of inspection of medical record. Information included was the subject's age, gender, medical history, clinical history of DM. History taking and physical examination were done according to a predesigned data collection sheet. Investigation was done to ascertain presence of ischemic stroke by CT scan of brain. Homocysteine assays were performed within the first 7 days after the acute stroke event in our study. After collection, blood samples were sent to Department of Biochemistry. Sera were separated by centrifugation, then homocysteine level were measured by chemiluminescence immunoassay by Architect plus c4000, Abbott, USA. Other relevant investigation was done in Department of pathology & Biochemistry, Dhaka Medical College.

Statistical Analysis: Statistical analysis was carried out by using the statistical package for social sciences version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). A descriptive analysis was performed for all data. The mean values were calculated for continuous variables. The quantitative and qualitative observations were indicated by frequencies, percentage. Chi-square test with 95% CI was used to analyze the categorical variables and was shown with cross tabulation. Independent t-test was used to analyze the continuous variable. A p-value will have considered to be statistically non-significant if >0.05 and significant if <0.05.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system.

Results

A total of 96 subjects with symptoms and signs of focal neurological deficits admitted in the medicine department, DMCH were studied. Among them 55.0% cases were male and 41.0% cases were female. Majority of the patients were belonged to age group 34 to 40 years (Table 1).

After completing brain imaging (CT scan of brain) 61 (65%) patients were found suffering from ischemic stroke. The prevalence of conventional vascular risk

factors such as diabetes mellitus (p=0.002), hypertension (p=0.002), hyperlipidemia (p=0.049) and heart disease (p=0.022) were significantly higher in ischemic stroke group compared with the group other than ischemic stroke. There was no significant difference in smoking status (p =0.645) and sex (p =0.801) between these two groups (Table 2).

Table 1: Distribution of Study Population by Age with Gender (n=96)

Age Group	Male	Female	Total
18 to 25 Years	9	6	15(15.7%)
26 to 33 Years	20	16	36(37.5%)
34 to 40 Years	26	19	45(46.8%)
Total	**55**	**41**	**96(100.0%)**
Range (min, max)	(23-40)	(20-40)	(20-40)

Table 2: Association of Co-Morbidities with Ischemic Stroke

Co-Morbidities	Ischaemic Stroke		P value
	Yes	**No**	
DM			
Absent	33(54.1%)	30(85.7%)	0.002s
Present	28(45.9%)	5(14.3%)	
Hypertension			
Absent	35(57.4%)	31(88.6%)	0.002s
Present	26(42.6%)	4(11.4%)	
Hyperlipidemia			
Absent	31(50.8%)	25(71.4%)	0.049s
Present	30(49.2%)	10(28.6%)	
Heart Disease			
Absent	39(63.9%)	30(85.7%)	0.022s
Present	22(36.1%)	51(4.3%)	

S=significant; P-value reached from Chi square test. Result was considered as significant when it was less than 0.05

Among the patients 74 patients had normal homocysteine level 17 patients had moderate hyperhomocysteinemia and 5 patients had intermediate hyperhomocysteinemia (Figure I).

Mean levels of fasting serum homocysteine were significantly higher in patients those had ischemic stroke (16.02 µmol/L, 95% CI: 12.87 to 19.87) compared with those who did not have ischemic stroke (10.47 µmol/L, 95% CI: 9.16 to 11.95, P<0.001 (Table 3).

Fasting homocysteine shows an independent relationship to risk of ischemic stroke. The highest quartile of homocysteine was associated with crude odds ratio (OR) of 6.0 compared with the lowest quartile (Figure II).
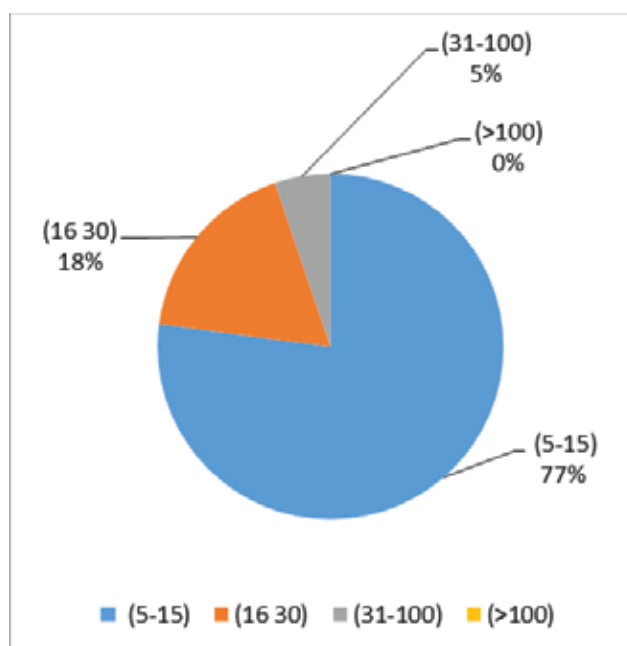

Figure I: Distribution of Study Population by Level of Homocysteine (n=96)

Table 3: Correlation of Mean Homocysteine with Acute Ischemic Stroke

Homocysteine (µmol/L)	Ischemic Stroke	Other Stroke	P value
Mean	16.02	10.47	
(range)	(4.1-59.0)	(4.5-29.2)	0.015s
Median	12.5	10.25	

s=significant; P value reached from independent sample t test. Result was considered as significant when it was less than 0.05
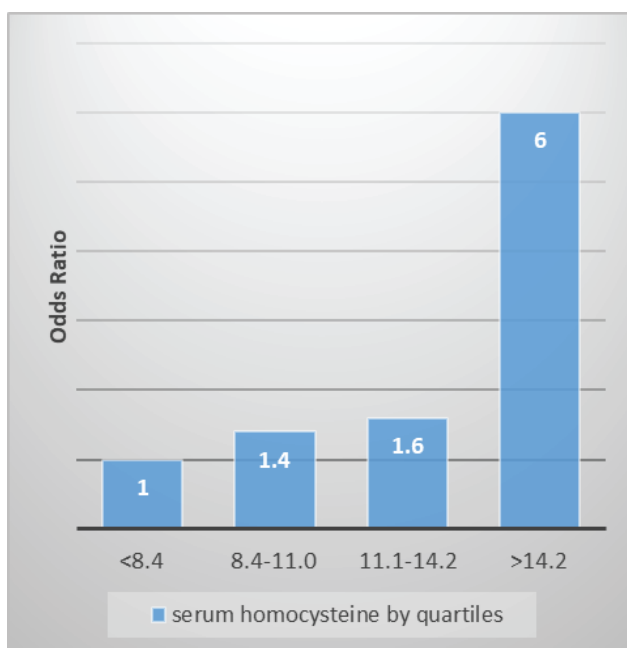

Figure II: Association between Serum Homocysteine Quartiles and Risk of Ischemic Stroke

Logistic regression was performed with ischemic stroke as the dependent variable with continuous variables homocysteine and categorical variables (diabetes mellitus, hypertension, hyperlipidemia and heart disease) as independent variables. The results are summarized in Table 3.6. Fasting homocysteine was seen to be an important independent risk factor, with an adjusted OR of 1.1 (95% CI: 1.1 to 1.2; p=0.04966) for every 1 µmol/L increase in homocysteine. Presence of heart disease was the most important independent risk factor among the conventional vascular risk factors.

Table 4: Risk Factors of Ischemic Stroke Modeled with Logistic Regression

Risk Factor	Adjusted OR	95% CI	P value
Homocysteine	1.10	1.0-1.2	0.048
Hypertension	5.81	1.5-21.9	0.009
DM	3.53	1.0-12.3	0.049
Hyperlipidemia	3.86	1.2-12.03	0.020
Heart disease	6.53	1.7-24.1	0.005

Discussion

In the present study, among 96 enrolled patients with symptoms and signs of acute focal neurological deficit and age less than 40 years 65% patients were found to have ischemic stroke after doing CT scan. This finding is almost similar with the study of Alam et al done in Dhaka Medical College Hospital[11]. A study in Mymensingh Medical College showed that 61% patients had ischemic stroke[12]. But this study contradicted with the study of Hayee et al. which was also done in Dhaka where the incidence of ischemic stroke was higher (83.89%)[13].

Regarding conventional risk factors DM, Hypertension, Hyperlipidemia and presence of heart disease showed significant association with ischemic stroke. These findings are similar to others. AM Hossain et al showed that 63% of the stroke patients were suffering from hypertension[12]. Hayee et al found that 52.11% were hypertensive, which was 58% by Alamgir et al[13,14]. In BIRDEM a study on 165 cases of diabetic patients, all of them developed stroke in less than 10 years duration[15].

This study was done to examine homocysteine levels in young Bangladeshi patients with ischemic stroke. This study showed that there is an independent association (p= 0.015) between elevated homocysteine levels and ischemic stroke. Results from previous studies have been conflicting, ranging from no association or weak associations to moderate to strong associations between homocysteine and stroke risk[16,17,18]. This might be because of methodological differences. Non fasting homocysteine was sampled for some studies, whereas fasting specimens were used for other studies which is the recommended method[19,20].

The higher level of homocysteine at 16.02 µmol/l is consistent with several major European studies and the results of a recent case-control study in Taiwan on Chinese ischemic stroke[21,22,23]. The latter study involved 92 patients. The mean homocysteine level for the patients was 14.9µmol/l as compared to 11.78 µmol/l among the control subjects.

It is observed that there was a graded increase in the risk of stroke with higher level of homocysteine. Whereas the odd ratio of stroke in the top quarter compared to the lower quarters of the homocysteine distribution was 6.0. Comparison of ischemic stroke risk with each quartile of homocysteine showed a clear and consistent trend toward increasing risk with each higher quartile. These results are similar to the Australian study except that the odds ratios are higher in this study[20]. This graded association was in keeping with other published literature in Western populations with ischaemic stroke[23,24].

This study also showed that on multivariate analysis, homocysteine remained a significant risk factor for ischaemic stroke with an adjusted OR of 1.1 (95% CI: 1.1 -1.2; p=0.049) for every 1 µmol/L increase in homocysteine. Hyperhomocysteinemia is thus an important risk factor of ischaemic stroke in Bangladesh. In a study in Japan it was found that fasting homocysteine was seen to be an important independent risk factor, with an adjusted OR of 5.17 (95% CI: 1.96 to 13.63) for every 1 µmol/L increase in log homocysteine[7]. Homocysteine assays were performed in the first 7 days after the acute stroke event in our study during which time homocysteine may be lower than usual[25]. This study may thus underestimate the importance of homocysteine in the development of stroke.

The mean homocysteine levels in patients other than ischemic stroke was 10.5 µmol/l. This was in the range of several reported studies where the levels ranged from 9.73[3] to 10.64[23,26]. On the other hand, homocysteine levels in this study was considerably lower in comparison to a Singapore study with a mean level of 16.3 µmol/l among healthy subjects[27.] As Singapore's ethnic composition and lifestyle is not similar to that of Bangladesh, the difference can be for that reason.

Limitation of the study

Although sample size was calculated statistically, sample size was relatively small in relation to huge number of population. Confounding variables couldn't be fully eliminated. Methylene tetrahydrofolate reductase genotype was not assessed in our study. Physical activity and lifestyle factors were also not assessed in this study.

Conclusion

This study suggests that in young adults with ischemic stroke, elevated fasting homocysteine is an important independent risk factor. It is recommended to screen homocysteine level of those ischemic stroke patients who present no clue for vascular disease and thrombosis, with an ischemic stroke at a young age because of the low cost and safety of the combined therapy of vitamin. Multicenter long term case control studies can be done to consolidate the findings of the study.

Conflict of interest: There is no conflict of interest relevant to this paper to disclose.

Contribution to authors: MFIC was guide of this study. MNU, TZ, Al-Amin, MARD were involved in protocol preparation, data & sample collection and MSI & SKR were involved in literature search and manuscript writing, MH, MAY, MH were involved in manuscript preparation and revision. All the authors have read and approved the final version of the manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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free thiol group, which is formed by demethylation of methionine, an essential amino acid derived from diet[4]. is metabolized with folate as a co-substrate, vitamin B12 as a co-factor and the help of several other enzymes[5]. There is evidence that homocysteine may cause endothelial dysfunction through formation of reactive oxygen species that has an important role in atherogenesis. It also interferes with the vasodilation and antithrombotic function. Oxidative damage to the vascular endothelium and the proliferation of the