Bangladesh Journal of Neuroscience 2012; Vol. 28 (1) : 1-9

ORIGINAL ARTICLES

Relationship Between Homocysteine and Carotid Artery Stenosis in Ischemic Stroke

AMINUR RAHMAN[1], FIROZ AHMED QURAISHI[2], MD NURUL AMIN MIAH[3], MALIHA HAKIM[4], UTTAM KUMAR SAHA[5], MD AKTERUZZAMAN[6], ZAHED ALI[7], NARAYAN CHANDRA KUNDU[8]

Abstract:

Background: *Epidemiologic studies have identified hyper-homocysteinemia as a possible risk factor for atherosclerosis. The aim of my study was based on evaluation of relationship between homocysteinemia with carotid artery stenosis in ischemic stroke patients.* ***Methods and materials:*** *It was a prospective observational study conducted in the Department of Neurology, Sir Salimullah Medical College & Mitford hospital, Dhaka. Thirty six consecutive patients with ischemic stroke were analyzed by serum total homocysteine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and extracranial Doppler ultrasonography and vascular risk factors were recorded. Equal number of controls of same ages were compared with the case group.* ***Result:*** *Mean fasting blood sugar, serum fasting total cholesterol (TC), serum fasting Low density lipoprotein (LDL) were significantly higher in case group (p=0.001). Serum TC and LDL had a positive correlation with serum homocystine (p=0.001). Serum High density lipoprotein (HDL) had a negative correlation (p=0.718) and serum triglyceride (TG) had a negative correlation (p = 0.182). Total plasma fasting homocysteine level in case group was 21.89 ± 9.38 ìmol/l and control group was 12.31 ± 3.27 ìmol/l, (p=0.001). Elevated fasting homocysteine level was found in 75.0% of the ischemic stroke patients and in 16.67% of healthy controls (p=0.001).On the basis of clinical evaluation and results of imaging studies, etiological classification of the ischemic stroke patients were made, where 36.1% cases were small artery disease, 38.9% large artery disease, 8.3% cases cardioembolic and in 16.7 % other causes. Among the cases, carotid duplex study was found normal in seven cases (19.4%), Group 1 findings in seven cases (19.4%), group 2 findings in eight cases (22.2%), group 3 findings in thirteen cases (36.1%) and group 4 findings in one case (2.8%). All abnormal carotid duplex findings were significantly higher among cases with elevated level of homocysteine (p=0.001, 0.001, 0.001) .* ***Conclusion:*** *The incidence of hyperhomo-cysteinemia is higher in ischaemic stroke cases than that in age-sex matched healthy controls. Hyperhomocysteinemia in ischaemic stroke patients has been determined as vascular risk factor in our study. Significant correlation has been found between homocysteine concentration and intraluminal thickness and carotid artery stenosis.*

Key words: *Homocysteine, carotid artery stenosis, ischaemic stroke*

Introduction:

Hyperhomocysteinemia has been associated with premature peripheral vascular, cerebrovascular, and coronary artery disease. Hyperhomo-cysteinemia, has been identified as being associated with vascular disease, including cerebrovascular disease in

1. Registrar, Department of Neurology, Sir Salimullah Medical College and Mitford Hospital, Dhaka
2. Professor, Department of Neurology, National Institute of Neurosciences& Hospital, Dhaka.
3. Assistant Professor, Department of Medicine, Sir Salimullah Medical College, Dhaka
4. Professor, Department of Neurology, Shaheed Suhrawardy Medical College, Dhaka
5. Assistant Professor, Department of Neurology, Sir Salimullah Medical College, Dhaka.
6. Assistant Registrar, Department of Cardiology, Sir Salimullah Medical College and Mitford Hospital, Dhaka.
7. Assistant Professor, Department of Neurology, Sir Salimullah Medical College, Dhaka.
8. Associate Professor, Department of Neurology, Sir Salimullah Medical College, Dhaka

general, particularly in subjects with significant carotid stenosis [1, 2]. 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[3, 4, 5].

In this study, we undertook a prospective case-control study of consecutive patients hospitalized with a first-ever ischemic stroke and examined specifically whether there may be an association between homocysteine, serum lipid profile, the degree of stenosis of carotid arteries and the specific etiologic subtypes of ischemic stroke. The current study was aimed to explore the relationship of serum homocysteine with carotid stenosis in ischemic stroke.

Methods:

This prospective, case-control study of serum total homocysteine as a potential risk factor for acute ischemic stroke. 36 consecutive male and female patients admitted in the department of Medicine and Neurology of Sir Salimullah Medical College & Mitford Hospital (Dhaka, Bangladesh) from January 2008 - June 2009 with the diagnosis of acute ischemic stroke was included in this study and they were compared with 36 control age-matched volunteer subjects of outpatient department. Criteria for entry into the study were as follows: (1) with neurological examination and neuroimaging (CT/ MRI) methods, diagnosis of ischemic stroke was strictly verified within 48 hours, (2) no disorders related to hepatic, renal and endocrinologic functions, (3) no systemic malignancy, (4) The subjects that do not use any preparations including vitamin B12 and folic acid or any medications having antimetabolite effects such as methotrexate or phenytoin, etc.

Stroke was defined as a clinical syndrome characterized by rapidly developing clinical symptoms and/or signs focal and at times global loss of brain function, with symptoms lasting >24 hours or leading to earlier death, and with no apparent cause other than that of vascular origin [6].

On the basis of clinical evaluation and results of imaging studies, the neurologist classified all strokes into 4 major etiologic subtypes according to the following criteria[7].

1. Large-artery disease (LAD): ischemic stroke with
   (a) evidence of extracranial or intracranial occlusive large-artery disease and
   (b) no cardioembolic source, and
   (c) clinical opinion that the most likely cause of brain infarction was atherothrombosis involving the aortic arch, carotid arteries or major branches, or vertebral, basilar, and posterior cerebral arteries;
2. Small-artery disease (SAD, lacunar): ischemic stroke with
   (a) Consciousness and higher cerebral function maintained plus
   (b) One of the classic lacunar syndromes or nonlacunar small-artery syndromes and
   (c) CT or MRI brain scan, performed within 3 weeks of symptom onset that is either normal or shows a small deep infarct in the basal ganglia, internal capsule, or brain stem;
3. Cardioembolic (CE) disease: ischemic stroke with
   (a) A major cardioembolic source plus
   (b) No definite evidence of occlusive large-artery disease, and
   (c) Clinical opinion that the most likely cause of brain infarction was embolism from the heart;
4. Other causes: ischemic stroke that did not meet criteria for one of the categories outlined above or where there was more than one likely explanation. All patients were examined by a neurologist and they had Cranial Tomography (CT) or Magnetic Resonance Imaging (MRI), Electrocardiography, Echocardiography, and high resolution B-mode Doppler Ultrasonography (DUSG) (made by a radiologist blinded to results of homocysteine levels). Clinical information including age, sex, history or current evidence of Hypertension (HT) [systolic blood pressure (SBP) $\geq$150mmHg and diastolic BP $\geq$90mmHg],

Diabetes Mellitus (DM) and cardiac disease, were recorded for all subjects. In case, venous blood samples were obtained after their admission and control subjects were both admission and outpatient department in the morning after an overnight fast of at least 12 hours into EDTA tubes. Serum total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), VLDL cholesterol (VLDL-C) and triglycerides were measured by using standard enzymatic procedures. Borderline for normal values were total cholesterol < 5.2 mmol/L, HDL-C > 1.0 mmol/L, LDL-C < 3.0 mmol/L, VLDL < 1.1 mmol/L and triglyceride < 2.3 mmol/L. Total plasma homocysteine level was measured in subjects within the first 48 hours after stroke onset. Plasma homocysteine levels were determined with FPIA (fluorescence polarization immunoassay) on the Abbott AxSYM system. Kit supplied from AxSYM Germany. The upper limit of the manufacturer and the laboratory was 15 µmol/L. Values above 15 µmol/L were acceptably high. Extracranial vessels were examined with the use of high resolution DUSG in a non-invasive manner. Real time scanner equipped with a 7.5 MHz imaging transducer. DUSG was done by ultrasonix, model-sonix SP from Germany. DUSG was done in Nuclear Medicine and Ultrasound, Mitford, Dhaka, Bangladesh.

The degree of stenosis of carotid arteries was recorded by the following criteria;

0. Normal,
1. Atherosclerotic lesions on one side <20% stenosis or nonstenotic plaque (Group 1),
2. 20-50% stenosis on one side or atherosclerotic lesions on both sides (Group2);
3. 50-70% stenosis on one side or 20-50 % stenosis on both sides (Group3);
4. Stenosis >70% on one side or 50-70 % on both sides and occlusion of one carotid artery on one side (Group 4).

Intima-Media Thickness (IMT) was defined as the distance between the characteristic echoes from the lumen intima and media-adventitia interfaces. Vertebral artery flow volume and mean systolic flow velocity were determined by using B-mode Doppler ultrasonography. The vertebral artery flow volume less than 200 ml/min were evaluated as vertebrobasilar insufficiency (VBI). Statistical analyses related with this study were performed by use of SPSS 9.0 package program. In the course of the evaluation of the data gathered, descriptive statistical methods (average, standard deviation) were used; in addition to these methods, free t test was used for the comparison between the paired groups through the use of the non-parametric tests taking into consideration the number of patients in the groups composed with the classification of the patient group by etiologies. The comparisons between the groups were evaluated with the chi-square test was used for the comparisons between the qualitative data. Correlations between numeric variables, like cholesterol, triglyceride and homocysteine were investigated by t test. The results of these tests were considered at the significance level of $p<0.05$ and the confidence interval 95%.

Results and observations

36 patients with ischemic stroke (18 female and 18 male) and 36 control subjects (18 male and 18 female) were included in the study. The mean age of the patient group was within the range of 50.28 ± 14.29 and the mean age of the control group was within the range of 51.08 ± 14.50 (TableI-I)

Table I

Distribution of age by group

Age (in year)	Group		p value*
	Case	Control	
≤30	3 (8.3) #	4 (11.1)	
31-50	17 (47.2)	15 (41.7)	
51-70	14 (38.9)	15 (41.7)	
>70	2 (5.6)	2 (5.6)	
Total	36 (100.0)	36 (100.0)	
Mean ± SD	50.28 ± 14.29	51.08 ± 14.50	0.813

*t test was done to measure the level of significance.
#Figure within parentheses indicates in percentage.

In respect of the risk factors, HT, Ischemic Heart Disease (IHD), DM displayed significantly higher

rates of prevalence in the patient population. In the patient group, only HT was found to be significantly higher in CE group in respect of the distribution of the risk factors in the etiological subgroups (p=0.0.230); any significant difference has not been determined (Table II).

Table-II

Distribution of history of risk factors by group

Risk factors	Group		p value*
	Case	Control	
Hypertension(HT)	17 (47.2) #	12 (33.3)	0.230
Ischemic heart disease(IHD)	7 (19.4) #	6 (16.7)	0.759
Valvular heart disease	2 (5.6) #	0 (0.0)	0.151
Diabetes mellitus(DM)	9 (25.7) #	8 (22.9)	0.780

*Chi square test was done to measure the level of significance.
#Figure within parentheses indicates in percentage.

Mean, standard deviation of fasting total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C in patients and control group summarized in Table III. There was no statistical difference between the two groups.

Table III

Fasting blood sugar, serum fasting lipid profile and fasting total plasma homocysteine level by group

Parameter	Group		p value*
	Case(n=36)	Control(n=36)	
Fasting blood sugar (mmol/l)	8.30 ± 3.82	5.87 ± 1.77	0.001
Total Cholesterol (TC) (mg/dl)	207.14 ± 56.52	166.69 ± 28.55	0.001
HDL (mg/dl)	35.94 ± 15.95	39.14 ± 18.27	0.432
LDL (mg/dl)	137.06 ± 57.21	93.13 ± 26.44	0.001
Triglyceride (TG) (mg/dl)	205.19 ± 77.03	183.19 ± 60.60	0.182
Total plasma homocysteine level(fasting) (ìmol/l)	21.89 ± 9.38	12.31 ± 3.27	0.001

*t test was done to measure the level of significance.
#Figure within parentheses indicates in percentage.

In respect of the distribution of the patient group by etiology, the rates were determined as 38.9% for LAD, 36.1% for SAD, 8.3% for CE and 16.7% for Others (Table IV).

Table IV

Distribution of etiological subgroups in case group

Etiological subgroups	Frequency	Percent
Small artery disease(SAD)	13	36.1
Large artery disease(LAD)	14	38.9
Cardioembolic(CE)	3	8.3
Others	6	16.7
Total	36	100.0

#Figure within parentheses indicates in percentage.

Compared to the control subjects, the mean fasting plasma homocysteine level was found to be significantly higher (p<0, 01). While the homocysteine concentration was found within the normal levels in 25% of the patients, it was found to be elevated in 75% cases. The homocysteine level in the control group was found to be within normal levels for 83.33% of the control subjects; and only 16.67% of the control subjects displayed elevated levels of homocysteine. (Table V)

Table-V

Distribution of respondents according to level of homocysteine by group

Homocysteine	Group		Total	p value*
	Case (%)	Control (%)		
Normal	9(25.0) #	30 (83.33)	46 (63.89)	0.0.001
Elevated	27 (75.0)	6 (16.67)	26 (36.11)	
Total	36 (100.0)	36 (100.0)	72(100.00)	

*Chi square test was done to measure the level of significance.
#Figure within parentheses indicates in percentage.

In the determination of correlation between the homocysteine level and lipid levels in the patient group Serum TC and LDL had a positive correlation with serum homocystine with a p value 0.001. Serum HDL had a negative correlation with p value 0.718 and TG had a positive with a p value 0.205. (Table VI).

Table VI

Correlation between serum fasting lipid profiles with total plasma homocysteine level

Serum fasting lipid profiles	r value	p value
TC(mg/dl)	0.388	0.001
HDL(mg/dl)	-0.043	0.718
LDL(mg/dl)	0.416	0.001
TG(mg/dl)	0.151	0.205

Pearson's correlation was done to find the significance value.
Pearson's correlation, r = 0.151, p = 0.205

Evaluation of the Doppler parameters in the patients is shown in Table IV. IMT was found in 29 (80.4%) patients. There were 7 (19.4%) patients in the Group 1, 8 (22.2%) patients in the Group 2, 13 (36.1%) patients in the Group 3,1 (2.8%) patients in the Group 4 .(Table VII)

A higher tHcy level was found to be associated with plaque score independently. Family history of hypertension & DM. fasting blood sugar, serum fasting TC& LDL which were atherosclerotic risk factors.(Table VIII)

Significant correlation (p-0.001) has been determined between IMT and first 3 groups (1, 2, 3) in the patients that displayed elevated levels of homocysteine. (Table IX)

Table-VII

Distribution of carotid duplex by groups

Carotid duplex	Group		p value*
	Case	Control	
Normal	7 (19.4)#	27 (75.0)	0.001
Group 1	7 (19.4)	6 (16.7)	
Group 2	8 (22.2)	0 (.0)	
Group 3	13 (36.1)	2 (5.6)	
Group 4	1 (2.8)	1 (2.8)	
Total	36 (100.0)	36 (100.0)	

*Chi square test was done to measure the level of significance.
#Figure within parentheses indicates in percentage.

Table-VIII

Binary logistic regression analysis

					95.0% C.I. for EXP(B)	
Risk factors	B	Wald	p value	Exp(B)	Lower	Upper
Family history of hypertension	2.191	4.261	0.039	8.943	1.117	71.610
Family history of diabetes mellitus	-2.463	2.022	0.155	0.085	0.003	2.539
Fasting blood sugar	0.263	3.078	0.079	1.300	0.970	1.744
Serum fasting lipid profile TC	-0.011	.283	0.594	0.989	0.949	1.030
Serum fasting lipid profile LDL	0.035	1.977	0.160	1.035	0.986	1.087
Fasting total plasma homocysteine level	0.220	6.085	0.014	1.246	1.046	1.485

Table-IX

Correlation between homocysteine level and subgroups with Doppler study

Doppler findings	Normal homocysteine (n=9)	Elevated homocysteine (n=27)	p value
Normal	5	2	0.056
Group 1	1	6	0.001
Group 2	1	7	0.001
Group 3	2	11	0.001
Group 4	-	1	-
Total (%)	9	27	

*Chi square test was done to measure the level of significance.
#Figure within parentheses indicates in percentage.

Discussion:

In this study, we have aimed to determine the correlation between the plasma homocysteine level and stroke and to discover the association between the elevated plasma levels and the lipid levels and carotid atherosclerosis and stenosis. Elevated plasma total homocysteine (tHcy) levels have been indicated as a risk factor for coronary heart disease [8,9,10], ischemic stroke[11, 12], and peripheral artery disease [13, 14]. Also, studies have related tHcy levels to carotid atherosclerosis as a surrogate end point for cardiovascular diseases [15, 16]. However, most of these findings were derived from white populations, and whether such findings also apply for Asians remains to be determined. Current study was conducted to evaluate the association of serum Homocysteine with carotid stenosis in patients of ischemic stroke. In this study thirty six patients of ischemic stroke was enrolled as case and equal number of age-sex matched normal healthy adult were taken as controls. Mean age of the cases were 50.28 ± 14.29 years and controls were 51.08 ± 14.50 years, with no significant difference between two groups. Most of the subjects were from age group between above twenty to eighty years. In two previous same type of study was done to explore the association of homocysteine with carotid stenosis , the mean age of one study was 66 years[15],and in other study it was 66.2 ± 11.0 years[17].

Diabetes mellitus, hypertension and ischemic heart disease, family history of hypertension and diabetes mellitus, smoking are considered as significant risk factors of stroke and carotid atherosclerosis. In some of the studies that are limited in number, any association with known risk factors has not been determined [18]. In numerous studies, elevated homocysteine levels were found to be significantly correlated and associated with smoking, male gender, hyperlipidemia and hypertension [19.20].

In present study mean serum fasting sugar, serum fasting lipid profile (TC) and serum LDL was significantly higher among cases, but serum HDL and triglyceride (TG) had no such difference. In a series mean (± standard deviation) of total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C in patients and control group summarized had no statistical difference [21].

Hyperhomocystenaemia defined an elevated homocysteine concentration as one that exceeds 15.8 mmol per liter (95th percentile for healthy control subjects) [22]. Other defined an elevated homocysteine concentration as one that exceeded 13.9 mmol per liter (the mean value plus 2 SD among healthy young controls) [23]. In the Framingham Heart Study cohort had previously considered a homocysteine concentration of 14 mmol per liter to be elevated (90th percentile for persons with apparently adequate concentrations of folate, vitamin B12, and vitamin B6) [24]. In current study, serum fasting total plasma homocysteine level in case group was 21.89 ± 9.38 ìmol/l which was significantly higher than the controls (12.31 ± 3.27 ìmol/l), (p=0.001).

In a series the median concentration of total homocysteine was 16.4 mmol/L among cases versus 14.3 mmol/L among controls [25]. Concentrations of total homocysteine were higher in two thirds of the matched pairs in the case subjects [26].

In current study 25.0% of cases serum total homocysteine level was normal and in 75.0% of cases it was elevated. In control group in 83.33% respondents it was within normal level and 16.67% elevated. Homocysteine level was significantly higher among cases (p=0.001). In a recent study the homocysteine concentration was found within the normal levels in 35.4% of the patients, it was found to be lightly elevated in 56.3% of the patients and moderately elevated in 8.3%. The homocysteine level in the control group was found to be within normal levels for 90% of the control subjects; and only 10% of the control subjects displayed lightly elevated levels of homocysteine ($p<0.01$) [21]. In a study, total homocysteine level was normal in 71.5% of stroke cases and elevated in 28.5% [27]. Serum total cholesterol (TC), LDL had a positive correlation with serum homocysteine with a p value 0.001. Serum HDL had a negative correlation and TG had a positive with no statistical significance.

In present series we have determined 36.1% cases had small artery disease, 38.9% large artery disease, 8.3% cases cardioembolic and in 16.7 %

other causes. Fujishama reported that as a manifestation of cerebral small-artery diseases, lacunar infarction is the most prevalent type of ischemic stroke in Japanese people[28]. In the studies carried out in relation with etiological correlations, Eikelboom and colleagues have found in a case-control study that hyperhomocysteinemia is associated in particular with stroke due to large-vessel atherosclerosis. However, it was found to be less associated with small-artery disease and any correlation with CE or other etiologic subgroups has not been determined which was consistent with our current study[29].

To further examine the link between carotid atherosclerosis and tHcy levels, multiple regression analysis was performed. When traditional atherosclerotic risk factors were controlled for, tHcy was found to be significantly associated with plaque score, suggesting a potential effect of higher tHcy in the evolution of carotid atherosclerosis. This finding is consistent with a previous study showing associations between tHcy and carotid plaque area [17].

According to extracranial carotid duplex study current series shows that normal finding was found in seven cases(19.4%), Group 1 finding was found in seven cases(19.4%), group 2 findings in eight cases(22.2%), group 3 findings in thirteen cases(36.1%) and group 4 findings in one case(2.8%).In present study we have found five cases with normal level of homocysteine and two with elevated homocysteine had normal Doppler findings (p=0.056), one case with normohomocysteinemic cases and six of hyperhomocysteine cases had group 1 findings (0.001), one case with normo-homocysteinemic cases and seven with hyperhomocysteinemic cases had group 2 findings (p=0.001), two with normal homocysteine and eleven with higher homocysteine had group 3 findings (p=0.001) and one of hyper homocysteinemic patients had group 4 findings. All abnormal Doppler findings were significantly higher among cases with elevated level of homocysteine (p=0.001, 0.001, 0.001). Elevated total homocysteine concentrations were found to be associated with carotid artery wall thickening and stenosis; and hyperhomocysteinemia and the ischemic events with manifestations of significant carotid stenosis were considered as independent risk factors [15]. Malinow et al reported that fasting plasma levels of homocysteine were significantly higher in 287 subjects with thickened intimal-medial carotid walls than in control subjects[15,30] performed a cross-sectional study of 1041 elderly subjects (418 men and 623 women; age range, 67 to 96 years) from the Framingham Heart Study, found that plasma homocysteine concentrations are associated with extracranial carotid-artery stenosis in a population-based cohort of elderly people [15]. Another study demonstrated that persons with carotid-artery walls whose thickness exceeded the 90th percentile for the study cohort had significantly higher fasting plasma homocysteine concentrations than persons with carotid- artery walls whose thickness was below the 75th percentile[16].

Although Mousavi et al, failed to demonstrate any meaningful difference in carotid stenosis between patients with normal and elevated tHcy levels is probably due to the low frequency of extracranial disease in the Asian population and homocysteine related atherosclerosis [31].

The current study demonstrated that higher level of homocysteine is significantly associated with carotid stenosis in patients of stroke and hyperhomocysteinemia is an independent risk factor for carotid stenosis in patients of ischemic stroke.

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