Original Research Article

STUDY ON CORRELATION OF HOMOCYSTEINE LEVEL AND ISCHEMIC STROKE

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Abstract:

Background: Epidemiologic studies have identified hyper-homocysteinemia as a possible riskfactor for atherosclerosis. The aim of the study was based on evaluation of relationship between homocysteinemia with ischemic stroke patients.

Methods and materials: It was a prospective observational study conducted in the Departmentof Biochemistry, Darbhanga Medical College , Laheriasarai, Darbhanga from January 2017 to March 2018.Thirty six consecutive patients with ischemic stroke were analyzed by serum total homocysteine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and Equal number of of controls same ages were compared with the case group.

Result: Mean Fasting blood sugar, serum fasting total cholesterol (TC), serum fasting Low densitylipoprotein (LDL) were significantly higher in case group (p=0.001). Serum TC and LDL had a positive correlation with serum homocystine a (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 homocystein level was found in 75.0% of ischemic stroke patient and in 16.67% of healthy controls (p=0.001). The incidence of hyperhomocysteinemia is higher in ischaemic stroke cases than that in age-sex matched healthy controls. Hyperhomocysteinemia in ischaemic stroke patients has as been determined as vascular risk factor in our study. Significant correlation has been found between homocysteine concentration and ischaemic stroke.

Introduction:

Stroke is a worldwide health problem. It is a major cause of morbidity, mortality and disability in developedas well as developing countries. Hyperhomo-cysteinemia has been associated with premature peripheral vascular, cerebrovascular, and coronary artery disease. Hyperhomocysteinemia, has been identified as being associated with vascular disease,including cerebrovascular disease [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 and ischemic stroke. The current study was aimed to explore the relationship of serum homocysteine with 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 of Darbahanga Medical College & Hospital Laheriasarai, Darbhanga with the diagnosis of acute ischemic stroke was included in this study and they were comparedwith 36 control age-matched volunteer subjects of outpatient department. The study was conducted at department of Biochemistry, Darbhanga Medical College, Laheriasarai , Darbhanga from January 2017 to March 2018. Criteria for entry into the study were 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.

(5)

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].

All patients were examined by a neurologist and they had Cranial Tomography (CT) or Magnetic Resonance Imaging (MRI). Clinical information including age, sex, history or current evidence of Hypertension (HT) [systolic blood pressure (SBP)150mmHg and diastolic BP 90mmHg], Diabetes Mellitus (DM) [fasting blood glucose 3.5- 5.5 mmol/L], cardiac disease, were recorded for all subjects. In case, venous blood samples were obtained after their admission and in control subjects from 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 mmol/L. Values above 15 mmol/ L were acceptably high.

Statistical analyses related with this study were performed by use of SPSS 13 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 (Table1)

Table-I Distribution of age by group

Age (in year)	Group		p value*
	Case	Control	
<30	3 (8.3) #	4 (11.1)	
30-50	17 (47.2)	15 (41.7)	
50-70	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 Case 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) (mmol/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.

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%. 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-IV)

Table-IV 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	7 (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 5)

Table-V 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, $r = 0.151$, $p = 0.205$

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 ischemic stroke. 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].However, most of these findings were derived from white populations, and whether such findings also apply for Asians remains to be determined. 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 previous same type of study to explore the association of homocysteine with ischemic stroke the mean age was 66 years[15], and in other study it was 66.2 ± 11.0 years[16]. Diabetes mellitus, hypertension and ischemic heart disease, family history of hypertension and diabetes mellitus, smoking are considered as significant risk factors of stroke. In some of the studies that are limited in number, any association with known risk factors has not been determined [17]. In numerous studies, elevated homocysteine levels were found to be significantly correlated and associated with smoking, male gender, hyperlipidemia and hypertension [18.19].

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 [20].

Hyperhomocystenaemia defined an elevated homocysteine concentration as one that exceeds 15.8mmol per liter (95th percentile for healthy control subjects) [21]. 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) [22]. 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) [23]. 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 [24]. Concentrations of total homocysteine were higher in two thirds of the matched pairs in the case subjects [25].

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% [26]. 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.

Fujishama reported that as a manifestation lacunar infarction is the most prevalent type of ischemic stroke in relation to elevated homocysteine Japanese people [27]. Another 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 atherosclerosis.

Although Mousavi et al, failed to demonstrate any meaningful difference in homocysteine related atherosclerosis in the Asian population [28].

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

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