Original Article

Is Hyperhomocysteinemia a Risk Factor for Ischemic Stroke? A Case Control Study

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Objective: The results of the previous studies on the correlation between plasma levels of Homocysteine (Hcy) and ischemic stroke have been controversial. The aim of the present study is to investigate this correlation by meticulously observing risk factors for the ischemic stroke as well as factors affecting the plasma levels of Hcy.
Method: A case-control study was conducted between 2005-2006 with 30 cases (55-84 years of age) who had never had a Transient Ischemic Attack (TIA) all within one week of their first-ever ischemic stroke. The 30 control subjects who had never had a stroke or TIA episode were selected from the orthopedics ward of the same hospital. The cases and controls were paired based on age, sex, cardiac disease, hypertension and smoking status and were adjusted for other risk factors. Their total non-fasting plasma Hcy level was determined using the High-Performance Liquid Chromatography (HPLC) method.
Results: The mean plasma levels of Hcy was significantly higher in cases than controls (mean= 20.79 ± 11.938 versus 14.45 ± 8.028 mol/L; P<0.017). After using the multivariate logistic regression model, a significant correlation was found between plasma levels of Hcy and stroke (OR=1.149 with 95% CI of 1.032-1.280 for each 1 mol/L increment).
Conclusion: The results indicate that there is a correlation between increasing plasma Hcy level and ischemic stroke ; however, future research is needed to prove the causal relationship between the two.

Key Words:
Brain ischemia, Cerebrovascular disorders, Homocysteine, Risk factors

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Ischemic stroke is a major problem in health care and is also one of the most common causes of death from neurological disorders in many countries. This disorder which causes more morbidity than mortality is regarded as the most debilitating neurological disorder and, imposes exorbitant expenses on the society from both views of treatment costs as well as the caregiver burden.

There are two types of risk factors for the ischemic stroke: unchangeable risk factors such as age, sex, positive family history and ethnicity; and changeable ones such as high blood pressure, cardiac disease (especially atrial fibrillation), diabetes mellitus, hypercholesterolemia, sedentary lifestyle, cigarette smoking, alcohol consumption, asymptomatic carotid stenosis and prior history of TIA. Other factors have been proposed to be associated with strokes. However, the evidence supporting the definite correlation is absent at this time. The examples of such probable risk factors are migraine headaches, the use of oral contraceptives, snoring, drug abuse, polycythemia, sickle cell disease, elevated white blood cell count, hyperuricemia, hyperhomocysteinemia, protein C and S deficiency, Lupus anticoagulants and anticardiolipin antibodies (1).

The importance of Hcy (a sulphur-containing amino acid) as a risk factor for ischemic events in both heart and brain has been a matter of debate for many years. A number of studies have pointed out a positive linear correlation between the plasma levels of Hcy and the occurrence of cardiac diseases. Nevertheless, the relationship between Hcy and cerebrovascular disease was first postulated when observed that people with low levels of Cysthionine b-synthase had high plasma levels of Hcy, many of which suffered atherosclerosis and early stroke.

Initial studies suggested that in non-homocysteinuric population the plasma levels of Hcy in those diagnosed with stroke was higher than the normal population. However, the later studies did not confirm the previous results (2-12). The aim of this study was to assess the correlation between non-fasting plasma levels of Hcy and ischemic stroke in patients admitted in two teaching hospitals through meticulous observation of the different factors affecting both ischemic stroke and plasma levels of Hcy.

Materials and Method

Participants

The case group consisted of 30 patients aged 55-84 with the diagnosis of ischemic stroke who were admitted to Sina and Dr. Shariati Hospitals, Tehran, Iran between April 2005 and April 2006. The criteria by which patients were labelled by a neurologist were the occurrence of sudden focal neurological deficit compatible with a hypodense lesion on the brain CT scan without contrast. If these patients had no prior history of either stroke or TIA and were in their first week of admission, after filling out a written informed consent they were included in the study. For each case, a control was chosen from the orthopaedics wards of the same hospitals. The age range for the control group was also 55-84 and they should have been in their first week of admission as well with a no history of stroke or TIA. Each case was paired with its compatible control based on four factors: sex (male or female), blood pressure (Less than and equal to 140/90 or more than 140/90), smoking (yes or no), and cardiac disease (yes or no). All controls also filled out a written consent form prior to entering the study. For each case as well as the controls a questionnaire was filled out according to patients' statements which included prior history of any diseases, family history of stroke, physical activity (during both leisure and work time), cigarette smoking, the use of oral contraceptives, aspirin or other anti-platelet agents and alcohol consumption. Moreover, patient's CBC and FBS were extracted from the clinical profiles. The history of stroke in the patients was assessed by both asking the patient and performing a neurological exam.

Procedure

Blood sampling was done for each case and control two hours post-prandial (after breakfast) and after a rest in supine position for 30 minutes. The blood samples were held in ice-containing containers and in less than 30 minutes time each sample was centrifuged with the Maximum speed of 5000 rpm for 5 minutes. Then the plasma was poured into a different test tube and was kept in -80 .

The plasma levels of Hcy were determined using HPLC method and were added to the patients' files together with the results of Urea and Creatinine (Cr.), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Triglyceride (TG), Cholesterol and Uric Acid.

Statistical analysis

Chi-square test was used to compare case and control groups at the level of the following qualitative variables: diabetes mellitus (yes/no), level of physical activity during leisure time (low/intermediate/high), physical activity during work time (low/intermediate/high), alcohol consumption (yes/no), family history of stroke (yes/no), the use of anti-platelet drugs (aspirin/dipyridamole/both/none) and the use of oral contraceptives in women (yes/no).

T-test was used to evaluate the equality of variances between the case and control groups at the level of the quantitative variables (Hcy, uric acid, cholesterol, LDL, HDL, TG, Fasting Blood Sugar (FBS), urea, Cr., White Blood Cell (WBC), Hematocrite (Hct) and Platelet) using the results of Levene's test.

In all of the tests, $P < 0.05$ was considered as statistically significant. All the variables with $P < 0.01$ were chosen and entered the second phase of the test, using multivariate logistic regression.

Results

When comparing each variable in the case and the control groups, only some of them generated statistically significant results. These included physical activity during leisure time, plasma HDL level, plasma TG level, Plasma urea level, plasma Cr level and plasma Hcy level (Table 1).

Table 1. Variables Found to a Significant p-value in the Analysis

Variable	p-value
Physical Activity During Leisure Time	.018
Plasma HDL¥ Level	.040
Plasma TG† Level	.034
Plasma Urea Level	.022
Plasma Cr‡ Level	.019
Plasma Hcy§ Level	.017
Other variables	> .05

¥High Density Lipoprotein; †Triglyceride; ‡Creatinine; §Homocysteine

Table 2.Variables with Significant Means and Standard Deviation

Variable	Standard Deviation		Mean	
	Case	Control	Case	Control
Hcy¥	11.94	8.03	20.79	14.46
HDL†	6.38	14.58	35.10	41.23
Urea	33.00	9.20	44.68	29.90
Cr‡	0.28	0.14	1.11	.97
TG§	115.48	61.27	170.81	119.35

¥ Homocysteine; †High Density Lipoprotein; ‡Creatinine; §Triglyceride

Table 3. Number of Patients Involved in Physical Activity during Leisure Time

Amount of Activity	Case	Control
Low	28	19
Intermediate	2	10
High	0	1
Total	30	30

Table 4. Factors Increasing Plasma Levels of Homocysteine

Old Age
Male Sex
Smoking
Hypertension
Cardiac Diseases
Inactivity
Hypercholesterolemia
Renal Failure
High LDL
Low Folic Acid/ B12/ B6
Coffee Consumption

The mean and standard deviation for the significantly different quantitative variables between cases and controls are demonstrated in Table 2 .
Among the non-quantitative variables, only physical activity during leisure time was different between the case and control groups (Table 3).

Discussion

The epidemiologic evidence has suggested hyperhomocysteinemia as a risk factor for cardiovascular, cerebrovascular and peripheral vessels disease (3, 5, 11). Since hyperhomocysteinemia is a common finding, study of its causes and effects and an efficient way to reduce those unfavourable effects could lead to greater success in handling the untoward phenomena such as stroke. Previous studies concerning the correlation between hyperhomocysteinemia- defined differently in various studies- and strokes have generated controversial results and this difference is mainly due to the different study designs (13, 14).
In many of the studies reporting a significant correlation between Hyperhomocysteinemia and stroke (7, 12), the effects of confounding variables and other independent variables affecting stroke are overlooked. Some of the reasons for generating contradicting results are as follows: difference between age and sex groups assigned to the study, diverse situations of blood sampling (the plasma levels of Hcy in standing position is 10% greater than that of the supine position.) (15), and also the difference in the time of the blood sampling. In the present study, the sampling was done during the first week of admission, mainly due to the alteration of plasma levels of Hcy in the acute phase (8). Other risk factors such as hyperlipidemia, could undergo changes after the first week. By choosing the early period for sampling, this possible confounding effect was avoided in this study.
In addition, each case was paired with a compatible control based on age, sex, blood pressure status, smoking and cardiovascular diseases ;consequently, there is no correlation between these strong independent variables and ischemic stroke in our study . Paired match design, compared to adjustment, increases the statistical validity of our design; further to the point choosing these specific risk factors as variables to be matched eliminates the bias in the results due to their dual effect on stroke and plasma levels of Hcy.The factors affecting the plasma levels of Hcy are shown in Table 4 and the factors involved in increasing the risk of stroke (1) are presented in Table 5.In our study, in the univariable analysis, the following variables were found to have significant correlation with stroke: Physical activity in leisure time, Plasma levels of Hcy, Plasma HDL levels, Plasma TG levels, Plasma Urea levels, and Plasma Cr Levels.
Nevertheless in the second stage of analysis , multivariate logistic regression, all of these variables except plasma levels of Hcy were not significantly correlated with ischemic stroke. The only independent variable correlating with ischemic stroke was determined to be plasma levels of Hcy which is justifiable considering our sample size based on out main variable- plasma levels of Hcy. Therefore, the lack of correlation between other risks and stroke in our study does not contradict with the results of previous studies. Odds ratio for levels of Hcy was 1.149 (with 95% confidence interval=1.032-1.280). This means for each 1 mol/L increment of the plasma levels of Hcy a 1.149 increment in the occurrence of ischemic stroke exists. Such low confidence interval confirms the sufficiency of the sample size .
This increase was determined to be 1.5 for each 5 mol/L increase in plasma levels of Hcy (with confidence interval= 1.3-1.9) in the previous studies (11, 16). Since the mean plasma levels of Hcy in our case and control groups were significantly different from that of other studies the higher odds ratio of this study may suggest higher susceptibility to stroke related Hcy levels in our population. Nutritional factors affecting both plasma levels of Hcy and stroke- not included in our study as well as others- could have also played a role in confounding the results. Moreover, it is possible that the plasma levels of anti-oxidants of our population were lower compared to other populations which facilitated the effects of Hcy in this study.
The significant difference between the mean plasma levels of Hcy in our sample and others could mean that the mean plasma levels of Hcy in the Iranian population is higher than other nations. This hypothesis, if true, urges further studies to determine the causes.
All the samplings in the current studies were performed by only two people, which reduce the experimenter's bias. We included many variables related to stroke in the current study which was rare in similar studies.
In this study, we have found evidence showing a positive correlation between plasma levels of Hcy and the risk of ischemic stroke. The conclusions we have drawn based on our results should be considered tentative. However, our study had some limitations. For one thing, the range of cardiac diseases posing different risks to cause stroke has been considered a single risk factor (for example atrial fibrillation poses more risk than congestive hear failure). The same bias is seen in the classification of "smoking" and "blood pressure" due to practical obstacles. Moreover, most of our patients belonged to the low economic status. Therefore, considering the difference between the dietary habits of the low and high income earners, the results of this study may not be generalizable to all the economic classes of the society. Furthermore, blood sampling was done during the first week in this study (mostly the first three days of the admission). Some studies have shown that the plasma levels of Hcy raises progressively during the first week which could in turn change the outcome. Finally, to determine the amount of physical activity, alcohol consumption, cigarette smoking and to obtain a family history of stroke no objective measures were used; patients' assertions were

considered to be reliable.
Nonetheless, our results clearly show a significant correlation between plasma levels of Hcy and the risk of ischemic stroke. Continued research should further elucidate the generalizability of the results and even a causal effect.

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