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Hyperhomocysteinemia, folateo and B12 vitamin in Iranian patients with acute ischemic stroke

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Abstract

BACKGROUND: The objective of this study was to evaluate the association of some factors such as serum levels of homocysteine, folate and B12 vitamin with stroke in acute ischemic stroke patients.

METHODS: In this case control study, serum levels of homocysteine, folate and B12 vitamin in 93 patients with acute ischemic stroke admitted to Imam Khomeini Hospital between September 2008 and January 2010, and 93 healthy controls were measured. Cerebrovascular risk factors including age, sex, hypertension, hyperlipidemia, smoking, diabetes mellitus, alcohol consumption, coronary artery disease and obesity were recorded. The results were compared between the case and control groups.

RESULTS: The mean ± standard deviation (SD) of fasting total homocysteine (tHcy) level in acute ischemic stroke patients was 20.58 ± 19.6 µmol/l, which was significantly higher than that of control group being 14.11 ± 9.5 µmol/l (P = 0.002). 39 (41.9%) stroke cases and 25 (26.8%) controls had hyperhomocysteinemia. There were no significant relationships between tHcy, folate and B12 vitamin levels with the above mentioned cerebrovascular risk factors except for smoking (p> 0.05). No significant difference in B12 vitamin and folate levels between patients and healthy controls were detected (P> 0.05).

CONCLUSION: Hyperhomocysteinemia is common in Iranian patients with acute ischemic stroke and might play a role as an independent risk factor in stroke.

Keywords: Stroke, Homocysteine, B12 Vitamin, Folate.

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Introduction

Stroke is one of the leading causes of death in any population, and its prevention is a key strategy in reducing the rate of mortality and morbidity.

Several risk factors for stroke have been identified, which are the target of both primary and secondary preventive strategies. In 1969, McCully showed that elevated total Homocysteine (tHcy) levels were the cause of vascular diseases in children with inborn error of B12 vitamin metabolism. Since then, many studies have been performed to understand the effect of hyperhomocysteinemia in cerebrovascular diseases. While some studies indicated that tHcy is an independent risk factor, others didn't confirm it.[1]

Hyperhomocysteinemia may cause endothelial dysfunction through oxidative stress, resulting in local thrombosis and subsequent ischemia. Another possible mechanism is the direct toxicity of homocysteine to blood vessels but there is no definite evidence to support either of these mechanisms.[2]

Several factors can influence tHcy level, among which the most important are the concentrations of B vitamins especially B12 and folate. Decreased serum levels of these factors result in high plasma tHcy levels.[3]

Since 1998, fortification of cereal grain flour products with folic acid was mandated in the United States to reduce the risk of neural tube defects in newborns. Supplementation with folate resulted in about 20-25% reduction in tHcy levels and cerebrovascular diseases. But some other trials such as Vitamin Intervention for Stroke Prevention (VISP) trial did not confirm it.[3-4]

The two strong determinants of fasting tHcy concentrations are age and sex, so that concentrations are higher in the elderly and greater in men than in women. In addition, there is a strong negative

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correlation between estradiol levels and tHcy levels in postmenopausal women. Therefore, hormone therapy may lower tHcy levels. Some studies have shown that smokers have higher levels of tHcy than non-smokers, independent of age or sex. Heavy coffee consumption is among the strongest lifestyle determinants of tHcy level. Also, both exercise and alcohol consumption are weak but significant determinants of homocysteine. Results of cross-sectional and population-based studies provided evidence that changes in several of these lifestyle factors, such as higher intake of B vitamins, cessation of smoking and abstention from coffee consumption, may lead to a reduction in tHcy concentration over time.[5]

The objective of this study was to determine whether there was a significant difference in tHcy, folate and B12 vitamin levels between stroke patients and healthy controls or not.

Materials and Methods

The approval of this study was obtained from the Ethnic Committee of Tehran University of Medical Sciences. In this case-control study, 93 consecutive ischemic stroke patients admitted to Imam Khomeini hospital between September 2008 and January 2010 were matched for age and sex with 93 patients from the same hospital who were not affected with acute cerebrovascular diseases and did not have a history of stroke.

Informed consent was obtained from all stroke patients or their proxies and all healthy controls. The data (collected from case and control groups) included age, sex, weight, blood pressure and/or history of hypertension, diabetes mellitus, smoking, alcohol consumption, previous thrombotic episodes, and history of oral contraceptive products consumption in women. Patients who had conditions (or took certain medications) affecting tHcy levels, including renal insufficiency requiring dialysis, use of methotrexate, tamoxifen, L- DOPA, phenytoin or bile acid sequestrants were excluded

The antecubital site of either arm was used as the

Table 1. Homocysteine, folate and B12 vitamin plasma values in cases*

	N	Homocysteine	Folate	B12 vitamin
Sex				
male	52	24.0538± 3.33	9.3942± 0.79	356.0192±32.7
female	41	16.1951± 1.68	12.2537± 0.98	419.4146± 42.54
Hypertension				
positive	74	21.3757± .33	10.6730± 0.72	366.0811± 26.54
negative	19	17.5263± 2.56	10.5842± 1.34	453.6316± 94.14
Diabetes				
Yes	26	23.6923± 5.13	12.2538± 1.40	237.09005± 46.49
No	67	19.3851± 2.02	10.0343± 0.68	290.11512± 35.44
Smoking				
Yes	25	28.3520± 6.33	10.6680± 1.31	217.30564± 43.46
No	68	17.7353± 1.44	10.6500± 0.72	294.91953± 35.76
Body mass index				
<27	24	19.3333± 4.96	11.3875± 1.43	402.2083± 46.13
≥27	69	21.0261± 2.16	10.4000± 0.69	377.6232± 35.10
Coronary artery disease				
Yes	30	25.6267± 4.60	9.4733± 1.15	197.43614± 36.04
No	63	18.1905± 2.02	11.2175± 0.75	305.33575± 38.46
OCP				
Yes	11	12.9545± 0.75	14.9091± 2.42	367.7273± 78.75
No	30	21.6134± 2.28	10.0841± 0.62	386.1463± 30.75
Alcohol				
Yes	5	31.0000± 10.02	10.6800± 3.75	486.0000± 37.39
No	88	19.9977± 2.07	10.6534± 0.63	378.1705± 28.71

*Patients were divided into subgroups characterized by different stroke risk factors

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first choice for venipuncture and (after 12 hours of fasting) blood samples were obtained. The blood sample was centrifuged as soon as possible but could be kept on wet ice for up to 6 hours. Aliquots were prepared from the plasma, frozen for 12 hours and sent on dry ice the next shipping day to the central laboratory. tHcy, B12 vitamin and folate levels were determined by Elisa method. Hyperhomocysteinemia was defined as plasma tHcy levels above 14 μmol/l.[2]

The statistics in this study were done by SPSS (version 16.0) software. The comparison of serum levels of the main three factors between cases and controls was done using t test.

Results

A total of 93 patients with acute ischemic stroke (42 women and 51men) and 93 healthy controls (42 women and 51 men) were evaluated in this study. The mean age ± SD of the patients and controls were 62.2 ± 9.8 years and 61.8 ± 9.9 years, respectively (T = 0.32, P = 0.75). There was no significant difference between the mean age ± SD of male (62.0 ± 8.9) and female (62.7 ± 11.0) stroke patients (T = 0.69, P = 0.49).

Homocysteine, folate and B12 vitamin values in patient groups characterized by different stroke risk factors are shown in Table 1. Table 2 shows mean and standard deviation values of tHcy, B12 vitamin and folate serum levels. The mean ± SD level of fasting tHcy in acute ischemic stroke patients was 20.58 ± 19.6 μmol/L, which was significantly higher than its level in controls

(14.11 ± 9.5 μmol/L) (P = 0.002). The median tHcy value in the case group was 14 μmol/l, while that of controls was 12.5 μmol/L. Thirty nine (41.9%) of 93 patients and 25 (26.8%) of 93 controls had hyperhomocysteinemia. Therefore, the relationship between tHcy levels and various subgroup characteristics was explored. There were no significant relationships between tHcy levels and most cerebrovascular risk factors including hypertension, hyperlipidemia, diabetes mellitus, alcohol consumption, coronary artery disease and obesity (P> 0.05). Neither was there any relationship between B12 vitamin and folate serum levels and the above mentioned risk factors. However, increased tHcy levels were found in smoking stroke patients (28.3 μmol/l) compared to non-smokers (17.7 μmol/l) (P= 0.02), (Table 3)

But, no significant relationships were detected between smokers and non-smokers in folate or B12 vitamin serum levels.

T test analysis of the tHcy, B12 vitamin and folate serum levels showed significant difference in tHcy levels between case and control groups. No significant differences in B12 vitamin and folate levels were found (P> 0.05).

Discussion

In this study 41% of patients had hyperhomocysteinemia. The main findings were as follows: (i) tHcy plasma levels in the acute phase of ischemic stroke (within 24 hours) were significantly higher than normal limits. (ii) No correlation between tHcy levels and stroke risk factors such as hypertension, hyperlipidemia,diabetes mellitus, alcohol consumption, coronary artery disease and obesity was observed. (iii) tHcy levels were significantly higher in smoker patients than in non-smokers. (iv) There was no relationship between B12 vitamin and folate serum levels with risk factors. (v) No significant differences in B12 vitamin and folate serum levels were observed between cases and controls.

Table 2. Mean and standard deviation values of tHcy, B12 vitamin and folate serum levels in the two groups.

		Homocysteine	B12 vitamin	Folate
Cases	Mean	20.5892	383.9677	10.6548
	SD	19.67253	275.22420	6.10491
Controls	Mean	14.1183	407.8495	9.4720
	SD	9.52514	228.95030	5.14231

Table 3. Distribution of homocysteine levels in smoker and non-smoker stroke patients.

		Mean	N	SD	Paired Differences
Homocysteine	Cases	20.5892	93	19.6725	P=0.002
	Controls	14.1183	93	9.5251	
Folate	Cases	10.6548	93	6.1049	P=0.164
	Controls	9.4720	93	5.1423	
B12 vitamin	Cases	383.9677	93	275.2242	P=0.498
	Controls	407.8495	93	228.9503	

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Parnetti et al. studied 161 consecutive patients with first-ever ischemic stroke classified using TOAST criteria and 152 neurologically healthy controls to assess the association between risk of stroke and increasing values of plasma homocysteine and the interaction between the mild hyperhomocysteinemia and conventional vascular risk factors. tHcy was elevated in all stroke subtypes: 13.0±2.5 μmol/l in patients with cardioembolic disease, 13.9±5.4 μmol/l in those with small vessel diseases, 15.5±6.8 μmol/l in cases of undetermined stroke, and 17.8±13.5 μmol/l in patients with large vessel disease. Mean homocysteine level was 8.10 μmol/l (SD=2.5) in controls. They suggested that mild hyperhomocysteinemia is confirmed to have a significant role as risk factor for all etiological subtypes of stroke.[6]

Although we didn't sort our cases by their subtypes, we reached the same result as Parnetti et al. We both found that homocysteine is significantly higher in stroke patients than in controls, and it could be a risk factor for stroke.

In a prospective study, Perini et al. measured homocysteine plasma levels in stroke patients in order to investigate possible correlations of homocysteine with stroke severity and clinical outcome. The plasma level of Hcy was neither an independent determinant for stroke severity nor for patient's outcome by the Barthel index. Mean plasma homocysteine of both ischemic and hemorrhagic stroke was significantly higher than in controls ($P<0.05$). Homocysteine in the acute phase of stroke was not associated with stroke severity or outcome. Elevated plasma homocysteine in the acute phase of stroke was associated with both ischemic and hemorrhagic stroke. Higher levels were associated with higher risk of small artery disease subtype of stroke. They failed to demonstrate that patients with high tHcy levels in the acute phase of ischemic stroke have a worse outcome. According to their study, hyperhomocysteinemia had no prognostic value.[7]

We *did* assess tHcy plasma levels in the *acute* phase of ischemic stroke, but we did not evaluate the severity or outcome of stroke in this study. In spite of that, Perini et al.'s main finding was the same as that of our study; i.e., elevated plasma homocysteine in the acute phase of stroke.

In 2005, Haapaniemi et al.[8] measured plasma tHcy levels in 102 consecutive stroke patients on admission and at 1 week, 1 month, and 3 months after stroke and only once in 102 control subjects. Compared with controls, plasma tHcy levels in patients were significantly lower on admission but not at later time points, with levels increasing by week and remaining at this level for 3 months. tHcy levels showed a positive correlation with age and a negative correlation with Mini-Mental State Examination (MMSE) scores. Plasma tHcy levels inversely correlated with plasminogen activator inhibitor. No correlation between tHcy levels and stroke severity, outcome, etiology, recurrence, infarct volume, CRP, or risk factors was observed. They proposed that this phenomenon was due to acute phase response reflecting increased synthesis of acute-phase proteins. However, they were unable to identify any correlation between CRP concentration in the acute stage of stroke and tHcy levels that corroborates earlier results.[8]

In this study, the relationship between stroke risk factors and mentioned serum factors were evaluated. Like Haapaniemi et al.,[8] our research showed no correlation between tHcy level and most cerebrovascular risk factors. But, there was one difference: we found tHcy levels were significantly higher in smoker patients than in non-smokers.

All in all, we can conclude that elevated plasma homocysteine level might be an independent risk factor for ischemic stroke. But, because this study was a case control one, we could not rule out the possibility of acute phase response being responsible for the elevation of serum tHcy level in acute stroke patients. More prospective and population based studies are needed to define whether elevated plasma homocysteine level is an independent risk factor for cerebrovascular diseases or stroke by itself is the cause for hyperhomocysteinemia.

Conflict of Interests

Authors have no conflict of interests.

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