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Correlation between homocysteine levels and stroke in patients with acute ischemic stroke

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Abstract

Ischemic strokes are mostly caused by atherosclerosis, and increased homocysteine levels are considered to play a role in atherosclerosis. The aim of this study was to investigate serum total homocysteine (tHcy) levels in the first 24 h of ischemic stroke patients and to analyze the correlation between tHcy levels and the clinical severity of ischemic stroke. The study included a total of 151 participants including a patient group of 83 patients diagnosed with acute ischemic stroke and a control group of 68 age- and gender-matched subjects with no history of ischemic vascular diseases between June 2006 and December 2007. Demographic characteristics of were recorded for each participant and stroke severity was assessed using the modified Rankin Scale (mRS) in each patient. Serum tHcy, vitamin B12, folic acid, and fibrinogen levels as well as platelet count and lipid profiles were measured for each participant. Serum tHcy levels were significantly higher and the folic acid levels were significantly lower in the patient group compared to the control group ($p<0.001$, $p=0.009$, respectively). A negative correlation was found between folic acid and serum tHcy levels and a moderately positive correlation was found between tHcy and age and total cholesterol and LDL cholesterol levels. The covariate analysis of the risk factors adjusted for tHcy indicated that the tHcy levels were significantly higher in the patient group compared to the control group. Additionally, no significant correlation was found between the homocysteine levels and the stroke severity. Serum tHcy level was increased in acute ischemic stroke. Moreover, although the increased tHcy level did not indicate stroke severity, it can be considered as a major risk factor for ischemic stroke.

Keywords: Acute ischemic stroke, modified rankin coma scale, homocysteine, vascular risk factors

Introduction

Stroke is the clinical manifestation of cerebrovascular diseases, generally described as an acute loss of neurological function because of an abnormal perfusion of brain tissue or bleeding. Strokes are mostly ischemic (87%), resulting from an arterial obstruction associated with a thrombus or embolus. However, hemorrhagic stroke (13%) results from the rupture or break of a blood vessel either within the primary brain tissue or the subarachnoid space [1]. Either ischemic or hemorrhagic, mortality from stroke accounts for 15.6% of the causes of death in the United States [2]. Stroke is the main reason of functional impairment in patients older than 65 years. Within 6 months after stroke, 26% of the patients become dependent on daily living activities and 46% of them develop cognitive impairment. Stroke changes the lives of not only the patients but also their families and other caregivers. Therefore, it is important to be aware of modifiable and non-modifiable risk factors in the primary and/or secondary prevention of stroke [3]. The modifiable risk factors of ischemic stroke include hypertension, diabetes mellitus (DM), atrial fibrillation, smoking, hyperlipidemia, atherosclerosis, lack of physical activity, and obesity [4]. In addition to these risk factors, serum total homocysteine (tHcy) level has also been shown to be an important risk factor for ischemic stroke [5-10].

Hyperhomocysteinemia is an important risk factor for atherosclerosis. The prevalence of hyperhomocysteinemia is reported to be 20-30% in atherosclerosis and 18-42% in ischemic

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stroke, with a reported prevalence of 5-7% in the general population [11-13].

Homocysteine is a sulphur containing amino acids and is the precursor of methionine and cysteine. Folate and vitamin B12 are necessary for the conversion of Hcy to methionine, whereas vitamin B6 is necessary for the conversion of Hcy to cysteine [14]. Insufficiency of folate, vitamin B12, and vitamin B6 causes accumulation of Hcy. Hyperhomocysteinemia leads to a number of changes in vessel walls, namely including endothelial dysfunction caused by the induction of TNF-α and nitric oxide synthesis with proinflammatory effects [15].

Some studies have shown a relationship between tHcy and stroke while some others suggest that there is no such relationship or it is a weak relationship even if it exists [11]. Moreover, while some studies propose that tHcy is a predisposing factor for stroke, some others advocate that it is only an outcome of stroke [12].

On the other hand, tHcy has been shown to be elevated within the first hours before and after acute ischemic stroke [8,9]. Furthermore, it has been hypothesized that tHcy elevation is an acute phase-reactant of brain ischemia [16] and this hypothesis is further supported by the fact that no reduction has been achieved in stroke risk in the studies conducted to reduce tHcy levels by using vitamin supplementation [9].

In this study, we aimed to investigate serum tHcy levels in the first 24 h of ischemic stroke patients and to analyze the correlation between tHcy levels and the clinical severity of ischemic stroke.

Material and Methods

Study population

The study included a total of 151 participants including a patient group of 83 patients diagnosed with acute ischemic stroke that were followed-up in the Neurology Clinics at Ankara Numune Education and Research Hospital between June 2006 and December 2007 and a control group of 68 age- and gender-matched subjects with no history of ischemic vascular diseases, no known cerebrovascular risk factors, and no clinical neurological deficits. None of the control subjects had a history of drug use that could affect the tHcy levels. All the participants were informed about the content of the study and gave a written consent before enrollment. The study was approved by Ankara Numune Education and Research Hospital Local Ethics Committee. Patients with subarachnoid hemorrhage, cerebral hemorrhage, intracranial tumors, thyroid and renal dysfunctions, cancer, hepatic failure and patients who had received any drugs within the last seven days before stroke that could affect the tHcy levels (e.g. multivitamins, niacin, methotrexate, tamoxifen, anticonvulsant, bile acid sequestrants, and nitric oxide anesthesia) were excluded from the study. All the participants underwent a detailed physical and neurological examination. On admission, the clinical severity of stroke was assessed in each patient using the modified Rankin Scale (mRS) [17].

Blood Sampling

Fasting blood samples of the patients were collected after admission. The samples were placed in 10 mL red-topped serum tubes (Vacutainer, BD, NJ, USA) and then were centrifuged at 4,000 rpm for 8 min. Serum levels of total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglyceride were analyzed using an enzymatic method on an Aeroset 2000 autoanalyzer (Abbott, Illinois, USA) with compatible same-brand kits. Hemogram parameters were measured routinely using a Cell-DYN 3700 fully automatic hemocytometer (Abbott, Illinois, USA). The tHcy levels were measured using the high-performance liquid chromatography (HPLC) method on a prominence device (Shimadzu, Kyoto, Japan) with compatible RECIPE brand (RECIPE Chemicals, Munich, Germany) homocysteine kit as routinely. The reference range for tHcy was accepted as 5.5-17 μmol/L.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 11.5 (SPSS Inc. Co., Chicago, IL, USA). Normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test. Descriptives were expressed as mean ± standard deviation (SD) for continuous variables with normal distribution and as median (min-max) for those with non-normal distribution. In continuous variables, statistically significant differences between groups were determined using Student's t-test for parametric variables and the Mann Whitney-U test for non-parametric variables. Correlations between groups were determined using the Pearson's and Spearman's correlation coefficients. Categorical variables were expressed as positive/negative and were compared using the Chi-Square test and Fisher's exact test as needed. For continuous variables, statistically significant differences between the mRS scores and the tHcy levels were examined using One-Way ANOVA for parametric variables and the Kruskal-Wallis test for nonparametric variables. The analysis of covariance (ANCOVA) was used for comparing the tHcy levels between the two groups based on the analysis of the risk factors adjusted for tHcy. A p value of <0.05 was considered significant.

Results

The 151 participants included a patient group of 83 (39 female and 44 male) patients and a control group of 68 (37 female and 31 male) healthy volunteers. Mean age was 65.77 ± 13.39 (range, 24-87) years in the patient group and 62.12 ± 10.25 (range, 28-82) years in the control group. No significant difference was observed between the two groups with regard to age and gender ($p>0.05$). A history of hypertension was detected in 47 (56%), DM in 12 (14%), heart disease in 30 (36%), smoking in 27 (32%), and alcohol abuse in 7 (8%) patients (Table 1).

Table 1. Demographic characteristics of the groups

	Patients (n=83)	Controls (n=68)	p
Age (years)	65.77 ± 13.39	62.11 ± 10.25	0.067
Gender (M/F)	44/39	23/45	0.364
Hypertension [n (%)]	47 (56.6)	23 (33.8)	0.005
DM [n (%)]	12 (14.5)	7 (10.3)	0.443
Heart disease [n (%)]	27 (32%)	-	
Alcohol abuse [n (%)]	7 (8%)	-	
Smoking [n (%)]	27 (32.53)	-	

Serum tHcy levels were significantly higher in the patient group compared to the control group (p<0.001). Folic acid levels were significantly lower in the patient group compared to the control group (p=0.009). No significant difference was found between the two groups with regard to total cholesterol, LDL, HDL, triglyceride, vitamin B12, platelet count, hematocrit, and fibrinogen levels No significant difference was found between the two groups with regard to total cholesterol, LDL, HDL, triglyceride, vitamin B12, platelet count, hematocrit, and fibrinogen levels (Table 2). Table 3 presents the gender-based comparison of tHcy levels.

Table 2. Laboratory parameters of the groups

	Patients (n=83)	Controls (n=68)	p
tHcy (µmol/L)	18.75 (8.30-55)*	13.85 (7.60-23)*	<0.001***
Total cholesterol (mg/dL)	208.69±51.79**	202.64±44.43**	0.461
LDL (mg/dL)	137.14±41.14**	129.98±38.50**	0.288
Triglyceride (mg/dL)	121 (47-467)*	112(59-399)*	0,965
HDL (mg/dL)	42.77±11.53**	44.75±11.45**	0.305
Platelet count (103/mL)	264 (166-711)*	260,5(156-696)*	0.703
Fibrinogen (mg/dL)	438 (209-916)*	435 (190-850)*	0.652
Hematocrit (%)	41.14±6.02**	40.93±6.13**	0.836
Vitamin B12 (pg/mL)	241 (70-1053)*	271 (84-1083)*	0.849
Folic acid (ng/mL)	4.6 (0.80-12.50)*	6.5 (3.80-12.70)*	0.009

*Median (min-max) ** Mean±SD*** tHcy (µmol/L) (Unadjusted)

Table 3. Comparison of serum tHcy levels between the genders

	Male (n=67)	Female (n=84)	p
tHcy (µmol/L)	17.30 (7.60-55)*	14.93 (8.30-53)*	0.019

*Median (min-max)

Serum tHcy levels were moderately positively correlated with age, total cholesterol, and LDL levels and were moderately negatively correlated with vitamin B12 levels. No correlation was found between the tHcy levels and serum levels of fibrinogen, platelet count, hematocrit, HDL, folic acid, and triglyceride (Table 4). On the other hand, the covariate analysis of the risk factors adjusted for tHcy indicated that the tHcy levels were significantly higher in the patient group compared to the control group (Table 5). However, no significant correlation was found between the mRS scores and the tHcy levels in the patient group (Table 6).

Table 4. Correlation between tHcy levels and demographic characteristics and laboratory parameters

Parameters	R	p
Age	0.345	<0.001
Total cholesterol (mg/dL)	0.245	0.028
LDL (mg/dL)	0.243	0.030
Triglyceride (mg/dL)	0.075	0.510
HDL (mg/dL)	0.011	0.926
Platelet count (103/mL)	-0.120	0.297
Fibrinogen (mg/dL)	-0.133	0.261
Hematocrit (%)	0.086	0.455
Vitamin B12 (pg/mL)	-0.300	0.019
Folic acid (ng/mL)	-0.146	0.221

Table 5. ANCOVA analysis

	F	P
Age (years)	8.18	0.005
Group*	40.44	<0.001
Total cholesterol (mg/dL)	5.21	0.024
Group*	42.07	<0.001
LDL (mg/dL)	5.51	0.020
Group*	41.38	<0.001
Vitamin B12 (pg/mL)	6.08	0.016
Group*	22.27	<0.001

*** tHcy (µmol/L) (Adjusted)**

Discussion

The present study found a significant difference between the patient and control groups with regard to serum tHcy levels [18.75 (8.30-55) µmol/L vs. 13.85 (7.60-23) µmol/L]. Similarly, a previous study reported that the tHcy levels were increased within the first 48 h of acute stroke [18]. Moreover, the Framingham study found a significant correlation between the tHcy levels and stroke incidence [19]. On the other hand, another study advocated that hyperhomocysteinemia is an independent risk factor for stroke and also noted that the stroke patterns in patients with hyperhomocysteinemia make the patients more prone to lesions typical of cerebral microangiopathy and to multiple infarctions compared to patients without hyperhomocysteinemia [20,21].

Table 6. Correlation between the mRS scores and tHcy levels in the patient group

mRS Score	1 (n=5)	2 (n=12)	3(n=17)	4 (n=11)	5 (n=35)	p
tHcy	17.40 (14.70-33.50)	19.25 (13.20-37)	17.60 (9-35.80)	18.30 (12.80-38)	20.50 (8.30-55)	0.674*

*Kruskal-Wallis Test

Previous studies investigating gender-based differences in tHcy levels reported that the tHcy levels were significantly increased in males compared to females and the tHcy levels in females increased and almost reached the levels assessed for males in the postmenopausal period [21,22]. Similarly, we also found that the tHcy levels were higher in males compared to females (Table 3). Previous reports also suggested that the tHcy levels increase in direct proportion to age [21,22]. Similarly, we also detected a positive correlation between age and tHcy levels (Table 4).

In a previous study, increased tHcy levels were reported as an independent risk factor for atherosclerosis in the presence of other risk factors such as hypertension, smoking, and DM [10]. However, another study revealed that there was no correlation between tHcy levels and patient characteristics including age, gender, stroke subtype and traditional stroke risk factors in patients with stroke [23]. Similarly, we also found no significant difference in the tHcy levels of patients with and without a history of hypertension, DM, heart disease, smoking, and alcohol abuse and a family history of stroke.

Literature suggests that there is a significant negative correlation between serum folate and tHcy levels in patients with acute ischemic stroke [24,25]. However, there are some other studies suggesting that the tHcy-lowering therapy including vitamin B12, vitamin B6, and folic acid supplementation was not effective on the vascular outcomes of the patients with stroke [26,27]. In our study, we found a negative correlation between the tHcy and vitamin B12 levels and a positive correlation between the tHcy levels and age and total cholesterol and LDL levels. In addition, the covariate analysis of the risk factors adjusted for tHcy indicated that the tHcy levels were significantly higher in the patient group compared to the control group (Table 5).

It has been suggested that there is no correlation between the tHcy levels and the neurological examination scores, infarct localization, and other risk factors [28,29]. Moreover, a previous study also noted that there was no significant correlation between the tHcy levels and the mRS scores [21]. Additionally, another study evaluated 113 patients with acute ischemic stroke and reported that no correlation was found between the tHcy levels and the functional outcomes obtained during rehabilitation after acute ischemic stroke [30]. In our study, we found similar results as there was no significant correlation between the tHcy levels and the clinical severity of stroke in the patient group as determined with by the mRS scores.

Our study was limited in several ways. First, the prognosis of the patients could have been followed up and their tHcy levels could have been assessed at regular intervals in later periods, which could have provided further information regarding the predictive value of tHcy in the determination of the prognosis of the patients with acute ischemic stroke. Second, regular assessment of tHcy levels during the convalescent period after stroke could have shed light on the controversy of whether elevated tHcy levels are a cause or a result of stroke.

Conclusion

In conclusion, tHcy level was increased in acute ischemic stroke. Moreover, although the increased tHcy level does not indicate the severity of stroke, it can be considered as a major risk factor for ischemic stroke.

Competing interests
The authors declare that they have no competing interest

Financial Disclosure
The financial support for this study was provided by the investigators themselves.

Ethical approval
The study was approved by Ethics Committee of Ankara Numune Education and Research Hospital.

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