ORIGINAL ARTICLE

AHI EVRAN MEDICAL JOURNAL
E-ISSN: 2619 - 9203

Homocysteine Levels in Patients with Hemorrhagic Stroke

Hemorajik İnmeli Hastalarda Homosistein Düzeyleri

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ÖZ

Amaç: Biriken veriler, artan homosistein iskemik inme için bir risk faktörü olduğunu göstermiştir. Ancak, yükselmiş homosistein seviyesinin, inme hastalarının yaklaşık beşte birini oluşturan hemorajik inme için de bir risk faktörü olup olmadığı belirsizdir. Amacımız, olası bir bağlantıyı araştırmak için hemorajik inmeli hastalarda plazma homosistein düzeylerini ölçmekti.
Araçlar ve Yöntem: Çalışmaya otuz iskemik inmeli hasta, otuz hemorajik inmeli hasta ve otuz sağlıklı gönüllü aldık. Bütün hastalar, konvansiyonel risk faktörleri ve homosistein, vitamin B12 ve folik asit düzeyleri açısından incelendi.
Bulgular: Hipertansiyon, hem iskemik inme hem de hemorajik inme hastalarında sağlıklı katılımcılara göre daha yüksekti (p=0.029). Homosistein düzeyleri, hem iskemik inme hem de hemorajik inme gruplarında kontrol grubuyla karşılaştırıldığında istatistiki olarak yüksek bulundu (p=0.001) ve aralarında istatistiki olarak anlamlı bir farklılık yoktu (p>0.05). B12 vitamini düzeyleri hem iskemik inme hem de hemorajik inme hastalarında kontrollere göre anlamlı olarak daha düşüktü (p=0.001) ve aralarında istatistiksel olarak fark yoktu (p>0.05). Folik asit seviyeleri açısından gruplar arasında istatistiki olarak anlamlı bir farklılık bulunmadı (p>0.05). Homosistein, vitamin B12 ve folik asit düzeyleri arasında korelasyon bulunmadı (p>0.05).
Sonuç: Çalışmamız, hemorajik inme hastalarında homosistein düzeylerinin yüksek olduğunu göstermiştir. Hemorajik inmede homosisteinin rolünü netleştirmek için daha büyük kohortlara ihtiyaç vardır.

Anahtar Kelimeler: B12 vitamini; folik asit; hemorajik inme; homosistein

ABSTRACT

Purpose: Accumulating data demonstrated that raised homocysteine is a risk factor for ischemic stroke. However, it remains unclear whether high homocysteine level is also a risk factor for hemorrhagic stroke, which accounts for about one-fifth of stroke patients. Our aim was to measure the plasma homocysteine levels in patients with hemorrhagic stroke to explore a possible link.
Materıals and Methods: We included thirty patients with ischemic stroke, thirty patients with hemorrhagic stroke, and thirty healthy volunteers. All participants were examined for traditional risk factors and levels of folic acid, homocysteine, and vitamin B12.
Results: Hypertension was higher in both ischemic stroke and hemorrhagic stroke patients than in healthy participants (p=0.029). Homocysteine levels were significantly higher in both ischemic stroke and hemorrhagic stroke groups than in the control group (p=0.001), with no statistically difference between each other (p>0.05). Vitamin B12 levels were significantly lower in both ischemic stroke and hemorrhagic stroke patients than in the controls (p=0.001), with no statistically difference between each other (p>0.05). Folic acid levels did not significantly differ between the groups (p>0.05). We did not find correlation between homocysteine, vitamin B12 and folic acid levels (p>0.05).
Conclusion: Our study indicated that the homocysteine levels were high in patients with hemorrhagic stroke. Larger cohorts are needed to clarify the role of homocysteine in hemorrhagic stroke.

Keywords: folic acid; hemorrhagic stroke; homocysteine; vitamin B12

Received: 08.02.2022; Accepted: 10.10.2022

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How to cite: Yetiş A. Çelikbilek A, Kaya Tekesin A, Yağız O. Homocysteine Levels ın Patients with Hemorrhagic Stroke. Ahi Evran Med J. 2023;7(1):101-105. DOI: 10.46332/aemj.1069981

INTRODUCTION

Homocysteine (Hcy) is obtained from metabolic demethylation of dietary methionine.[1] High Hcy levels may be occured by many factors, such as inadequate intake, absorption, or several drugs deficiency of vitamin B12, folate, and/or vitamin B6,[2] Fasting Hcy levels widespreadly range from 5 to 15 μmol/L and high values above 15 μmol/L is referred as hyper homocysteinemia (HHcy).[3]

Several epidemiologic investigates have indicated that HHcy is a qualifable risk factor for ischemic stroke (IS), and Hcy-lowering therapy widely used in patients with IS.[2,4] HHcy causes atherogenesisthrough endothelial damage and vascular smooth muscle proliferation.[5-7] However, data are limited regarding the relationship between Hcy and hemorrhagic stroke (HS).[7] HS accounts for about 20% of stroke patients.[8] It is more severe and disabling than IS.[9,10] Previous study by Hiroyasu et al. did not show an association between Hcy level and risk of HS.[11] Some reports revealed that patients with HS had commonly high Hcy levels when compared to healthy volunteers.[12,13] However, it is unclear whether high plasma Hcy level is a risk factor for HS. Our aim was to evaluate the plasma Hcy levels in patients with HS to explore a possible link.

MATERIALS and METHODS

Study Design and Participants

A total of 90 consecutive in-patients, including thirty IS, thirty HS, and thirty healthy subjects were registered in this prospective cross-sectional study. All patients aged between 50 and 80 years old. This study was carried out at Istanbul Training and Research Hospital between June and October 2009. Istanbul Training and Research Hospital Ethics Committee Presidency approved the study (approval date-number: 04/06/2009-74).

IS was refered as a focal neurological insufficient of immediate onset lasting longer than 24 hr with diffusion-weighted magnetic resonance imaging showing hyperintensity while no signal change on the apparent diffusion coefficient mapping.[14] HS was defined as hyperdensities scanned by cerebral computerized tomography.[15] The controls were selected among patients admitted with radiculopathy-related symptoms at our neurology outpatients clinic.

The participants with malignant diseases; chronic renal, hepatic, or cardiovascular disease; and those who were taking drugs such as methotrexate, antilipidaemics which may affect Hcy metabolism were excluded.

Data Collection

Demographic data (age and gender) and systemic comorbidities, smoking/alcohol use and bleeding localization for each subject were recorded. Fasting serum samples were taken in the morning. Laboratory analyses were performed using standard methods. Hcy was measured with a fluorescent polarization immunoassay technique on an Abbott AxSYM analyzer. All tests were calibrated using commercial standards set by the manufacturers. Serum Hcy above 15 μmol/L was considered as HHcy. Vitamin B12 (reference range: 197-866 pg/mL) and folate (reference range: 2-9.1 ng/mL) levels were measured with a chemiluminescent microparticle intrinsic factor assay and a chemiluminescent microparticle folate-binding protein assay, respectively (Abbott Laboratories)

Statistical Analysis

SPSS 11.5 for Windows was used for analysis.[16] Values are expressed as n(%) or median(25th-75th percentiles). Oneway ANOVA was used for the comparison of the parameters between groups. Baseline differences between patients and controls were analysed by using the chi-squared test for categorical data. The correlation between parameters was determined by Pearson correlation test. Multiple comparisons were carried out using Tamhane's and Tukey tests. Results were considered to be significant at $p<0.05$.

RESULTS

Baseline characteristics of patients were shown in Table 1. The groups were similar in terms of age (p=0.428) and gender (p=0.094). The groups were also similar regarding smoking and alcohol use (p=0.287). Diabetes mellitus did not significantly differ between the groups (p=0.053) whereas hypertension was higher in both IS and HS groups

than in healthy group (p=0.029). Bleeding localization in HS patients was 11(36.6%) for lobar, 9(30%) for basal ganglions, 6(20%) for thalamus, 2(0.66%) for cerebellum and 2(0.66%) for brainstem.

Table 1. Demographic data of ischemic and hemorrhagic stroke and controls

Variables	Ischemic stroke (n=30)	Hemorrhagic stroke (n=30)	Control (n=30)	p
Age (years)	66.53(51-80)	66.80(51-80)	61.23(50-79)	0.428
Gender (male)	17(56.7)	18(60)	16(53.3)	0.094
Hypertension (present)	14(46.6)	23(76.6)	4(13.3)	0.029
Diabetes mellitus (present)	3(10)	1(0.33)	0(0)	0.053
Smoking	9(30)	7(2.33)	6(20)	0.287
Alcohol	5(16.6)	4(13.3)	3(10)	0.287
Bleeding localization				
Lobar		11(36.6)		
Basal ganglia		9(30)		
Thalamus		6(20)		
Cerebellum		2(0.66)		
Brainstem		2(0.66)		

Values are expressed as n(%) or median(25th-75th percentiles).

Laboratory data of participants were shown in Table 2. Hcy levels were significantly higher in bothof stroke groups than in the controls (p=0.001). However, Hcy levels did not significantly differ between IS and HS groups (p=0.356). Low vitamin B12 levels were significantly more common in stroke groups when compared to the controls (p=0.001), with lack of statistically difference between each other (p=0.217). Folic acid levels seemed to be higher in stroke groups than in the controls without statistical significance (p=0.077). As well, folate levels were did not significantly differ between IS and HS groups (p=0.580). No correlation was found between Hcy, vitamin B12 and folic acid levels (p=0.336). With respect to lipid levels, no significant difference was obtained between the groups (p=0.292).

Table 2. Laboratory data of ischemic and hemorrhagic stroke and controls

Variables	Ischemic stroke (n=30)	Hemorrhagic stroke (n=30)	Control (n=30)	p
Homocysteine (µmol/L)	9.107(7.1-10.9)	9.123(7.3-11.8)	7.727(5.1-9.4)	0.001
B12 vitamin (pg/mL)	398.11(72.3-2000)	405.30(150.1-2000)	568.10(158.3-2000)	0.001
Folic acid (ng/mL)	8.63(3.11-20)	8.32(3.87-20)	6.81(1.96-15.89)	0.077
TC (mg/dL)	200.67(119-361)	222.50(136-299)	198.57(112-383)	0.292
TG (mg/dL)	161.10(61-611)	117.57(65-196)	117.60(46-303)	0.787
HDL-C (mg/dL)	38.53(24-54)	43.17(23-79)	44.27(24-66)	0.059
LDL-C (mg/dL)	128.37(51-193)	141.80(82-204)	131.13(64-294)	0.287

Values are expressed as median(25^{th}-75^{th} percentiles). TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

DISCUSSION

Considering our study results; hypertension was higher in both stroke patients compared to the control group, Hcy levels were significantly higher in stroke groups compared to controls, vitamin B12 levels were significantly lower in both stroke groups than in the controls, folate levels did not differ significantly between the groups and there was no relationship between Hcy, vitamin B12 and folate levels.

Hcy is involved in the metabolism of methionine.[1,5] This process mainly requires the contributions of vitamin B12, folic acid and vitamin B6.[5] Accumulating data demonstrated that HHcy is a risk factor for vascular diseases.[17,18] This risk increases notably when the Hcy level is above 15 µmol/L, indicating the possible association between Hcy and IS.[3,19] A recent study reported a 59% raised risk of stroke in response to an increase of Hcy about 5 µmol/L.[20] However, data are limited on the relationship between

HHcy and HS.[7] Cerebral parenchymal hemorrhage accounts for the vast majority of HS.[6] The most underlying etiology is hypertension,[21] which is also high in our HS patients. HHcy causes endothelial damage leading to rupture of vascular elastic membrane which increase interstitial collagen fibers.[22] This cause vascular wall hardening that might create a ground for increased blood pressure.[5] On the other hand, fibronecrosis probably due to the chronic hypertension occurs in the capillary artery wall, thus the wall elasticity is weakened, causing a propensity to rupture and ultimately to bleed.[5,22] Animal models have already demonstrated that Hcy may have potential to destruct the blood brain barrier and exacerbate intracerebral hemorrhagea by activating matrix metalloproteinase-9.[23,24] Clinically, a recent meta-analysis found that Hcy levels in patients with HS were significantly higher than in control subjects.[7] However, a few studies demonstrated the contrary results demonstrating the HHcy as a predicting factor for the favorable outcomes following HS.[25,26] In our study, we found significantly higher Hcy levels in both stroke groups than in the controls. However, Hcy levels did not significantly differ between IS and HS group. This is line with the recent published systematic review by Zhou et al.,[7] suggesting that plasma Hcy level might be an triggering factor in atherosclerosis, which also positively associated with high risk of ICH, too.[7,27] We did not find an association between plasma Hcy levels and hypertension that could be explained because of the small sample size.

Vitamin B12, vitamin B6 and folate were well-known determinant factors on plasma Hcy levels.[14] Supplementation with these vitamins were shown to reduce Hcy levels.[28] We found lower vitamin B12 levels in both stroke groups than in the controls. Also, folic acid levels were found to be higher in both stroke groups than in the controls but did not reach statistical significance. As well, folate levels were did not significantly differ between IS and HS groups. According to the correlation analysis, no significant correlation was obtained between Hcy, vitamin B12 and folate levels in either IS or HS group. This requires futher research in larger population to make a definite result.

Several limitations need to be mentioned. First, this is a single-centre study of relatively small sample size. Second, it is unrealistic to exclude additional nutritional and genetic factors which may influence Hcy levels. Third, the clinical data was derived in 2009, though we discussed the association between Hcy and stroke subgroups with the current literature, which may also have certain clinical significance.

In conclusion, the present study indicated that the Hcy levels were high in patients with stroke regardless of stroke subgroups. Besides, we lacked any correlation between Hcy, vitamin B12 and folate levels in the subgroups. Larger cohorts are needed to clarify the role of Hcy in HS.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

Ethics Committee Permission

Istanbul Training and Research Hospital Ethics Committee Presidency approved the study (approval date-number: 04/06/2009-74).

Authors' Contributions

Concept/Design: AY, AÇ, AKT, OY. Data Collection and/or Processing: AY, AÇ, AKT, OY. Data analysis and interpretation: AY, AÇ, AKT, OY. Literature Search: AY, AÇ. Drafting manuscript: AY, AÇ. Critical revision of manuscript: AY, AÇ. Supervision: AÇ, OY.

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