Plasma Homocysteine Levels in Patients with Acute Ischemic Stroke: A Cross-Sectional Study

Akut İskemik İnmeli Hastalarda Plazma Homosistein Seviyeleri: Kesitsel Bir Çalışma

Abdurrahman SÖNMEZLER,[a]
Turgay ULAŞ,[b]
Mehmet Sinan DAL,[c]
Mehmet Emin DEMİR,[b]
İbrahim Fatih KARABABA,[d]
Hakan BÜYÜKHATİPOĞLU[b]

Departments of
[a]Neurology,
[b]Internal Medicine,
[d]Psychiatry
Harran University Faculty of Medicine, Şanlıurfa
[c]Department of Internal Medicine, Dicle University Faculty of Medicine, Diyarbakır

Geliş Tarihi/*Received:* 09.03.2012
Kabul Tarihi/*Accepted:* 16.09.2012

Yazışma Adresi/*Correspondence:*
Turgay ULAŞ
Harran University Faculty of Medicine, Department of Internal Medicine, Şanlıurfa, TÜRKİYE/TURKEY
turgayulas@yahoo.com

ABSTRACT Objective: In this study, we aimed to investigate plasma homocysteine (Hcy) changes in patients within the acute period of ischemic stroke. **Material and Methods:** In this cross-sectional study, a total of 110 patients with acute ischemic stroke presenting within 48 hours of onset and 45 age and gender-matched healthy subjects were evaluated. Hcy was measured on 2nd, 4th, 7th, 10th and 14th days of the hospitalization. Control samples were also collected after a 12-hour fasting period. **Results:** Statistically significantly lower Hcy levels were found in acute ischemic stroke patients on the 2nd day compared to the control group ($p<0.001$). No significant difference was found in the 4th day between the groups ($p=0.478$). In all other days on which test was performed, Hcy levels were statistically significantly higher in acute ischemic stroke group compared to the control group ($p<0.001$ for all). **Conclusion:** This study suggests that Hcy levels are not significantly elevated after recent ischemic stroke, but rise in the 2nd week of admission, and our findings do not support the hypothesis that raised plasma Hcy levels predate ischemic stroke. Therefore, increased plasma Hcy level is a consequence of disease process, and has no value as a predictor of ischemic stroke.

Key Words: Homocysteine; stroke; risk factors

ÖZET Amaç: Biz bu çalışmada, iskemik inmenin akut dönemindeki hastalarda plazma homosistein değişikliklerini incelemeyi amaçladık. **Gereç ve Yöntemler:** Bu kesitsel çalışmada, akut iskemik inmenin ilk 48 saatinde başvuran 110 hasta ile yaş ve cinsiyet uyumlu 45 sağlıklı kontrol değerlendirildi. Homosistein düzeyleri hastaneye yatışın 2., 4., 7., 10. ve 14. günlerinde ölçüldü. Kontrol örnekleri 12 saatlik açlık sonrası bakıldı. **Bulgular:** Homosistein düzeyleri 2. günde kontrol grubuna göre istatiksel olarak anlamlı düşük bulundu ($p<0.001$). Dördüncü günde anlamlı farklılık gözlenmedi ($p=0,478$). Kalan diğer günlerde ise kontrol grubuna göre istatiksel olarak anlamlı yüksek bulundu (hepsi için $p<0,001$). **Sonuç:** Bu çalışma, homosistein düzeylerinin akut iskemik inmede anlamlı olarak yüksek olmadığını, akut inmenin 2. haftasından sonra yükseldiğini göstermiş ve yüksek homosistein seviyelerinin iskemik inme gelişiminde rol oynadığını söyleyen hipotezleri desteklememiştir. Bu yüzden, plazma homosistein düzeyleri iskemik inme göstergesi değildir ve hastalığın sonucu olarak ortaya çıkmaktadır.

Anahtar Kelimeler: Homosistein; inme, felç; risk faktörleri

Turkiye Klinikleri J Med Sci 2013;33(2):384-8

Homocysteine (Hcy) is believed to cause atherogenesis and thrombogenesis via endothelial damage, vascular smooth muscle proliferation and coagulation abnormalities.[1-3] Several epidemiological studies have demonstrated that elevated plasma homocysteine is a risk factor for arteriosclerosis of the coronary, cerebral and peripheral vessel. In some prospective studies, increased plasma Hcy level has also been shown to be an

doi: 10.5336/medsci.2012-29486

independent risk factor for secondary vascular events in stroke patients and for a new atherothrombotic brain ischemic stroke.[4-8] However, numerous case-control studies have shown an association between elevated Hcy level and stroke, but the results of the these prospective studies are inconsistent, with most showing only a small or no association.[9-15] According to these studies, as a result, hyperhomocysteinemia is associated with increased risk of cardiovascular and cerebrovascular disease, though some studies raise doubts about its role.[16-21]

The crucial question remains whether plasma Hcy is directly involved in the pathogenesis of vascular disease or elevated Hcy is a consequence rather than a cause of the disease process. In the elucidation of this situation in Turkey, there are no a long-term studies performed to evaluate and demonstrate the Hcy changes in patients within the acute period of ischemic stroke. Therefore, in the present study we aimed to evaluate the patterns of acute Hcy changes after cerebral infarction, to find whether these levels are directly responsible for increased risk of ischemic stroke or acute phase response in the 2-week period after the stroke in patients with acute ischemic stroke.

MATERIAL AND METHODS

The study was approved by the local Ethics Committee, and each patient or their proxies provided written informed consent for participation. A series of 110 consecutive patients were admitted to the Stroke Unit of the Department of Neurology in Cukurova University Medical School, and all patients with clinically and radiologically proven acute stroke were included in this cross-sectional study. The control group composed of 45 healthy volunteers matched for age and gender.

Patients with liver and kidney failure, hyperthyroidism and venous sinus thrombosis were excluded. Pregnant patients and those on drugs which could affect Hcy were also excluded from the study. Brain computerized tomography (CT) scans were conducted to exclude hemorrhagic stroke and other intracranial structural causes of focal neurological deficits. All CT scans were reviewed by the study consultant radiologists to confirm the diagnosis of ischemic stroke. All patients underwent medical history taking; physical and neurological examinations conducted by a neurologist; routine blood biochemistry and blood count, 12-lead electrocardiography, chest X-ray, brain magnetic resonance imaging (MRI), anda cerebral angiography was performed when indicated. Total plasma Hcy was consecutively determined on fasting (after 12 hours) samples within 2nd, 4th, 7th, 10th and 14th day of hospitalization. Control samples were also collected after a 12-hour fasting period. Total L-Hcy was assayed by the Axis enzyme immunoassay method.

All statistical analyses were performed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). Tests were used to test the normality of data distribution. The data were expressed as arithmetic means and standard deviations. The C was used to compare the categorical variables between groups. was used for comparison of continuous variables between two groups. Two-sided p value <0.05 was considered statistically significant.

RESULTS

The ages and genders of the patients showed no significant differences between the patients and the healthy controls (p=0.110, p=0.741, respectively). Number of the patients with ischemic stroke risk factors such as smoking history, hypertension, hearth disease were significantly different compared to the control group (p=0.013, p<0.001,

TABLE 1: Comparison of demographic characteristics of the study groups.

	Patients (n=110)	Controls (n=45)	p
Gender, male/female	66/44	25/20	0.741
Age, years	65.9±11.3	63.4±7.44	0.110
Smoking, %/n	54.5/60	31.1/14	0.013
Hypertension, %/n	75.4/83	15.5/7	<0.001
Hearth disease, %/n	60.9/67	4.4/2	<0.001
Diabetes mellitus, %/n	29.1/32	13.3/6	0.062

All measurable values were given as mean±standard deviation, and number (percentage).

p<0.001, respectively), whereas the number of the diabetic patients were not significantly different (p=0.062) (Table 1). Statistically significantly lower Hcy levels were found in acute ischemic stroke patients on the 2nd day compared to the control group (p<0.001) whereas no significant difference was found between the groups in the 4th day (p=0.478). In all other days on which test was performed, Hcy levels were statistically significantly higher in acute ischemic stroke group compared to the control group (p<0.001 for all) (Table 2, Figure 1).

DISCUSSION

This study suggests that Hcy levels are not elevated after recent ischemic stroke, and a high Hcy level is an acute phase response of the disease process.

The precise mechanisms underlying the apparent adverse effect of hyperhomocysteinemia on the risk of ischemic stroke are not clear at present. Several prospective studies showed a significant association between Hcy and the risk of total and ischemic strokes, whereas others showed no association.[17,22-26] On the basis of studies, it has been postulated that an elevated Hcy level before stroke acts as a risk factor for the stroke. It could be, as some suggest, that elevated Hcy is an acute-phase reactant and a consequence rather than a cause of the disease process.[11,27]

Studies that performed Hcy measurement in the acute phase gave conflicting results. Eikelboom et al. found higher Hcy in 219 patients with respect to 205 controls within 7 days after the event.[28] Lindgren et al. and Meiklejohn et al. compared plasma Hcy levels in the acute and convalescent periods following stroke, and found that, in contrast to several earlier studies, the concentration of plasma Hcy did not differ between patients and controls in the acute phase, and plasma Hcy levels were in fact higher in the convalescent period following stroke.[29,30] They hypothesised that elevated Hcy plasma levels could be a consequence, rather than a causal factor. In a cross-sectional study, Okubadejo et al. also reported that Hcy levels in 69 acute ischemic stroke patients were not significantly different from 86 Nigerian controls.[31] Three Italian studies also gave conflicting results: the first showed higher Hcy in 113 patients within 3 days compared to 135 controls, the second studied 161 stroke patients and have found increased Hcy levels compared to 152 controls but the time of samples was not stated, and the third found no differences.[32-34] In our study, we found significantly lower Hcy levels in patients in the acute stage of stroke, with levels increasing over the next 7 days and remaining stable thereafter.

As an affirming finding regarding to lower Hcy levels in the acute period of ischemic stroke in literature, Howard et al. demonstrated a significant elevation in non-fasting Hcy levels in 76 ischemic stroke patients, from 24-48th hours to 3rd, 5th, 7th and 10-14th days.[35] They have found that baseline Hcy levels were lower than the following days' values.

TABLE 2: Comparison of laboratory and clinical characteristics of the groups.

	Patients	Controls	p
Total cholesterol, n/mg/dL	110/187.85±38.20	45/187.10±41.33	0.913
LDL cholesterol, n/mg/dL	110/119.79±27.31	45/121.23±31.42	0.823
Triglyceride, n/mg/dL	110/120.14±33.43	45/127.15±29.51	0.495
Vitamin B12, n/pg/mL	110/326.97±88.34	45/335.58±75.47	0.763
Folic acid, n/ng/ml	110/6.28±2.31	45/9.09±3.58	0.001
Homocysteine 2nd day, n/μmol/L	110/10.85±3.46	45/16.75±5.54	<0.001
Homocysteine 4th day, n/μmol/L	96/17.50±6.23	45/16.75±5.54	0.478
Homocysteine 7th day, n/μmol/L	93/24.69±6.66	45/16.75±5.54	<0.001
Homocysteine 10th day, n/μmol/L	75/27.89±6.54	45/16.75±5.54	<0.001
Homocysteine 14th day, n/μmol/L	53/27.45±6.09	45/16.75±5.54	<0.001

All measurable values were given as mean±standard deviation. LDL: Low density lipoprotein.

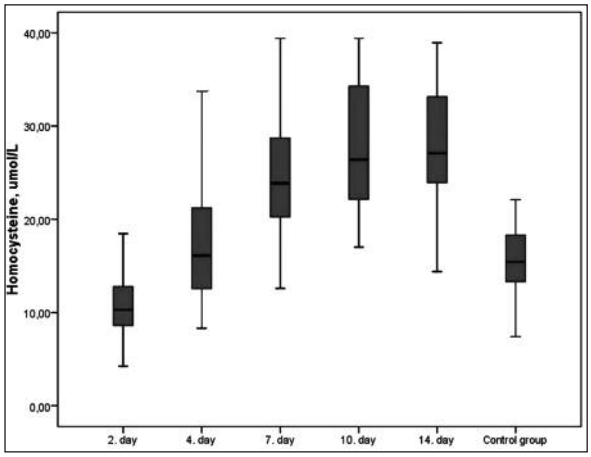

FIGURE 1: Graphic demonstrating homocysteine levels in controls and patients with acute ischemic stroke.

Haapaniemi et al. evaluated 102 acute ischemic stroke patients and 102 controls, and have found significantly lower Hcy levels at admission, with Hcy levels increasing over the next 7 days and remaining stable for 3 months.[36] The authors have not clearly speculated these results. They have tried to explain the results as a protective reaction of the human body to limit brain injury or solely as a nonspecific acute-phase reaction, and speculated that reduced level of Hcy could be directly related to the strength of acute-phase response, which is in turn directly linked to the severity of stroke, or other yet unknown factors.[36]

In Turkey, a limited number of studies have performed to investigate the plasma Hcy levels in patients with acute ischemic stroke.[37-40] Delikan et al. performed a cross-sectional study and evaluated 81 patients and 41 healthy subjects, and have found similar Hcy levels.[37] Kavaklı et al. evaluated 41 patients and 20 healthy subjects, Yılmaz et al. evaluated 83 patients and 41 healthy subjects, and have found higher Hcy levels in acute ischemic stroke patients.[38,39] In addition, another cross-sectional study which was performed by Somay et al. has shown that Hcy levels are significantly higher in 48 patients with acute ischemic stroke compared to 38 healthy subjects.[40] Our study differed from these previous studies and was able to provide important new insights and data because it assessed Hcy levels in consecutive days.

Certain limitations of the present study should be considered. First, it is a single-center study, the sample size is relatively small, and the study design is cross-sectional. Second, more detailed information would be gained by assesing high-sensitive C-reactive protein levels along with the Hcy levels, and the investigation would perhaps provide deeper insight into the changes of the Hcy levels in patients with acute ischemic stroke and might add to the value of our manuscript.

CONCLUSION

In conclusion, most of the previous studies concerning Hcy report that it is an acute-phase reactant and a consequence rather than a cause of the disease process. In our study, compared to healthy subjects, we found decreased plasma Hcy levels in acute period of the ischemic stroke, and these levels increased in the 2nd week of admission. Therefore, we cautiously assert that plasma Hcy level has no value as a predictor of stroke. Nevertheless, further multicenter studies with large-scale cohorts should be conducted in order to clarify this issue.

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