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SERIAL CHANGES IN PLASMA HOMOCYSTEINE IN ACUTE CLINICAL STROKE

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Abstract

PURPOSE. The relationship between plasma homocysteine and stroke is controversial in many studies. There are only a few serial-sample studies which have looked at changes in stroke homocysteine during acute stroke. None of these studies investigated the changes in homocysteine in the first 3 days after stroke onset. Therefore, we designed this serial-sample prospective study to elucidate patterns of homocysteine concentration fluctuation at 0, 24 and 48 hours post stroke.

MATERIAL AND METHODS. Thirty one (22 ischemic and 9 hemorrhagic) patients with stroke and thirty three controls were selected. Three homocysteine levels were obtained from all stroke patients, while only one sample was taken from controls.

RESULTS. Plasma homocysteine concentration was higher in males and in hemorrhagic stroke patients at all time points. Paired sample testing revealed significant differences in the mean values of homocysteine taken at 48 hours (p=0.047, 95% CI: -1.467 to -0.011) for all cases. For patients with hemorrhagic stroke, significant values were again obtained at 48 hours only (p=0.024, 95% CI:-8.266 to -0.763). After gender stratification, we found significantly higher mean homocysteine concentrations at all time points in male patients (at 0 hour: p=0.043, 95%CI: -5.197 to -0.908; after 24 hours: p=0.002, 95%CI: -7.899 to -2.279; after 48 hours: p=0.032, 95%CI: -4.644 to -0.246).

CONCLUSIONS. In this pilot study, we found that, on average, homocysteine levels initially decrease and then gradually rise in stroke patients, especially patients with hemorrhagic stroke. Also, there are significant gender-based differences in plasma homocysteine levels in our study population. In addition to increased levels of homocysteine after 48 hours in stroke patients, we found moderate hyperhomocysteinemia in our healthy controls, consistent with previous data from Pakistan.

Keywords

• Plasma homocysteine • Serial samples • Ischemic stroke • Haemorrhagic stroke • Stroke

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1. Introduction

Stroke is a major cause of morbidity and mortality worldwide [1]. The past three decades have seen the burden of stroke to rise in South Asian countries (including Pakistan) [2]. One of the main foci for stroke related research is preventable risk factors. Conventional risk factors for stroke are hypertension, smoking, diabetes mellitus, obesity, and family history). Increased blood homocysteine, hyperhomocysteinemia, has been cited as a possible new risk factor for various neurodegenerative conditions, including stroke [3]. Various experiments have shown homocysteine to cause vascular injury, which may contribute to the development of cardio- and cerebrovascular diseases via various mechanisms [4,5].

However, the correlation between homocysteine levels and risk of stroke is still controversial [6-11]. The risk of ischemic stroke associated with an elevated homocysteine level has been found to be significantly weaker in prospective studies than in retrospective studies, which may reflect difficulties in selecting controls or the effect of changes in treatment, or other factors after stroke onset [12]. Furthermore, results of many large prospective trials have shown no benefit of the prophylactic use of folic acid and vitamin B12 in patients with established vascular disease and mild hyperhomocysteinemia [12-14].

In this pilot study, we wanted to investigate how homocysteine levels change during the acute phase of stroke. We therefore designed this study to compare serial homocysteine levels in acute stroke along a timeline of 0, 24 and 48 hours from onset of stroke.

2. Materials and methods

From a consecutive series of 38 patients with stroke admitted to Services Institute of Medical Sciences (SIMS) and Shaikh Zayed Hospital, Lahore (Northern Pakistan) between August 2010 to December 2010, 31 (82%) were selected for this study. Inclusion criteria were admission to the hospital within 12 hours of stroke onset and the last meal taken within the period of 6 hours prior to admission. All included participants were diagnosed as having stroke, ischemic or haemorrhagic based on clinical history, physical examination and computed tomography (CT) brain scan by the same physician and were diagnosed according to National Institute for Health and Clinical Excellence (NICE) criteria [15]. Both ischemic and hemorrhagic stroke

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patients were included, while those with transient ischemic attack (TIA) diagnoses were excluded. Five participants (13%) left the hospital against medical advice before a second sample could be acquired, while two participants (5%) died within 24 hours from admission. Thirty-three gender-matched, non-stroke, healthy controls were recruited from the same social and ethnic background as the controls. All patients received standard medical care including aspirin for the first 48 hours along with palliative therapy. The study was approved by the relevant institution review board and ethical committees of the abovementioned hospitals.

Blood samples were collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA). The first sample ('0 hour') was collected within mean 8.6 hours±5.6 after reported stroke symptoms began, and after informed consent was taken. The second and third samples were taken after 24 and 48 hours, respectively. This time period of sampling has been shown to provide the most precise homocysteine determinations for serial studies [16]. Only one sample was collected from control subjects. Plasma was separated after centrifugation at 3000 rpm for 10 minutes and stored at –20°C until analysis.

Plasma homocysteine levels were estimated using standard automated procedures with commercial enzyme-linked immunosorbent assay (ELISA) kits (Axis Shield Dundee, Scotland, cat. no. FHET100), performed in the same laboratory, by the same technician. Sample handling and temperature conditions were strictly monitored for all procedures according to manufacturer's protocol. The analytical sensitivity of the kit was 1.0 µmol/L.

3. Statistical analysis

All analyses were performed using SPSS for Windows, version 17. Descriptive statistics included mean and standard deviation (ST DEV) for quantitative variables and percent prevalence for categorical variables. The distributions of the parameters were tested with Shapiro-Wilk's test. A t-test was used to compare plasma homocysteine concentrations of patient and control groups. Differences between patients and control subjects for nominal scale variables were assessed with the χ^2 test. The paired sample t-test was used for comparison of '0-hour', '24-hour'and '48-hour' plasma homocysteine concentrations. The ANOVA test was used for comparison of plasma homocysteine concentration in more than two groups. Linear regression model was used to investigate the effect of various risk factors on plasma homocysteine levels. A value of $P<0.05$ was considered significant.

4. Results

4.1 Clinical characteristics of the study

Refer to Table 1.

4.2 General homocysteine concentration results

Mean homocysteine values in all stroke and control participants were found to be in moderate hyperhomocysteinemic range i.e. between 15-30 micromoles/liter. Plasma homocysteine concentration was higher in males than females, and higher in hemorrhagic than ischemic stroke participants at all time points (Table 2). Differences in mean homocysteine concentrations at each time point compared with mean homocysteine concentration in controls (20.73±8.59) were not insignificant. Figure 1 shows mean homocysteine concentrations of cases at different time intervals and controls as a bar chart. It was noted that mean homocysteine concentration in stroke patients at admission (0-hour sample) was lower than the mean homocysteine concentration in controls.

4.3 Serial homocysteine concentration results

Paired sample testing revealed significant differences in mean values of homocysteine between 24 and 48 hours (p=0.047, see Table 3 for details) for all cases. Table 4 shows the same parameters for hemorrhagic stroke only, with significant values again between 24 and 48 hours (p =0.024). Ischemic stroke *per se* did not reveal significant differences.

4.4 Gender stratification results

After data stratification for gender, we found significant differences between mean homocysteine concentrations at all time points in male stroke cases (Table 5). Homocysteine data for females did not show any significant results. There were no gender

Table 1. Clinical characteristics of study group cases.

	Cases n (%)
Gender	Males 14 (45) Females 17 (55)
Age (±STD DEV)	Males 52.64±14.65 Females 61±11.73
Presence of Hypertension	Males 10 (71) Females 13 (76)
Presence of Heart Disease	Males 5 (35) Females 6 (35)
Presence of Diabetes	Males 5 (35) Females 7 (41)
Number of smokers	Males 7 (41) Females 1 (5)
Stroke subtype	Ischemic: Males 10 (45), Females 12 (55) Hemorrhagic: Males 4 (45), Females 5 (55)

Table 2. Mean homocysteine concentration in micromoles/liter (±STD DEV) by timeline, for all subjects, and by gender, stroke subtype (no. of subjects).

	At '0 hour'	At '24 hours'	At '48 hours'
All (n=31)	20.52±9.12	21.19±10.72	22.71±10.19
Gender			
Male (n=14)	19.78±10.01	23.67±12.12	26.12±12.66
Female (n=17)	21.09±8.46	19.14±9.82	19.91±6.78
Stroke Subtype			
Ischemic (n=22)	18.47±9.30	20.45±9.47	20.45±7.94
Hemorrhagic (n=9)	24.77±10.02	23.73±13.61	28.24±13.24

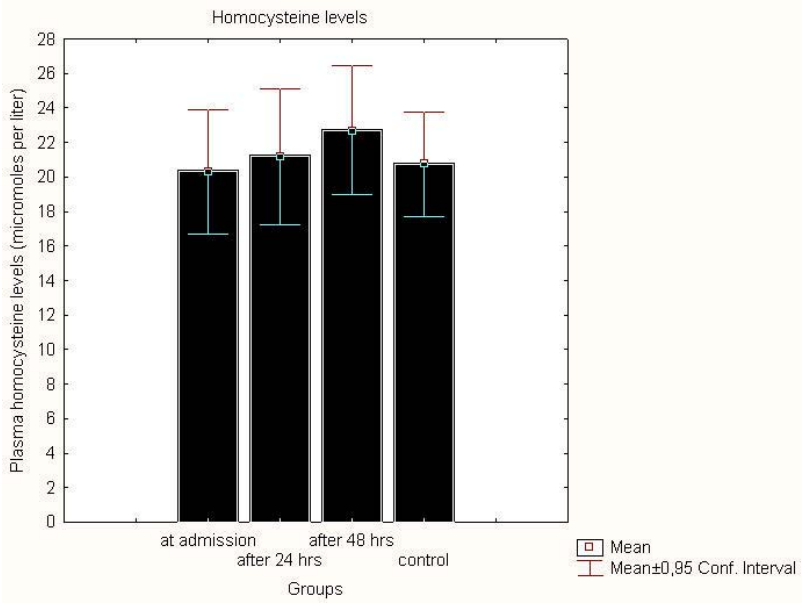

Figure 1. Homocysteine levels shown as mean ± 0.95 CI at 0, 24 and 48 hours in stroke patients, and controls.

Table 3. Paired samples test results for serial homocysteine concentrations in micromoles/liter (±STD DEV) by timeline for all subjects.

	Paired mean ± STD DEV	p value (95% CI)
HC at 0 and 24 hours	-0.89±7.55	0.517 (-3.661 to 1.880)
HC at 0 and 48 hours	-2.42±7.49	0.082 (-5.164 to 0.331)
HC at 24 and 48 hours	-1.53±4.08	0.046* (-3.021 to -0.031)

HC: Homocysteine concentration, STD DEV= Standard Deviation, 95% CI= confidence interval, * significant at p<0.05

Table 4. Paired samples test results for serial homocysteine concentrations (±STD DEV) by timeline for hemorrhagic stroke

	Paired mean ±STD DEV	p value (95%CI)
HC at 0 and 24 hours	1.05±7.08	0.669 (-4.392 to 6.486)
HC at 0 and 48 hours	-3.47±8.19	0.240 (-9.763 to 2.827)
HC at 24 and 48 hours	-4.51±4.88	0.024* (-8.266 to -0.763)

HC: Homocysteine concentration, STD DEV= Standard Deviation, 95% CI= confidence interval, * significant at p<0.05

Table 5. Paired samples test results for serial homocysteine concentrations (±STD DEV) by timeline for males only.

	Paired mean ± STD DEV	p value (95%CI)
HC at 0 and 24 hours	-2.64±4.42	0.043* (-5.198 to -0.908)
HC at 0 and 48 hours	-5.09±4.87	0.002* (-7.899 to -2.279)
HC at 24 and 48 hours	-2.45±3.81	0.032* (-4.644 to -0.246)

HC: Homocysteine concentration, STD DEV = Standard Deviation, 95% CI= confidence interval, * significant at p<0.05

differences when mean homocysteine concentrations were compared between cases and controls.

4.5 Linear regression results

Regression analysis showed that age and traditional stroke risk factors such as gender, hypertension, heart disease, diabetes, and smoking did not affect homocysteine levels.

5. Discussion

Our key findings in this study are: 1. Mean plasma homocysteine concentration rises significantly between 24 to 48 hours after stroke onset, especially in hemorrhagic stroke, and 2. In males as opposed to females, serial plasma homocysteine concentrations are raised incrementally at all time points i.e. at admission, after 24 and 48 hours.

The linkage between stroke and homocysteine is inconclusive. Only few prospective studies have looked at homocysteine levels in acute stroke [17-19]. The time duration in most of these studies spans across days, and some lack control homocysteine levels for comparison. To the best of our knowledge, this is the first serial-sample study to refine the existing knowledge on homocysteine in acute and convalescent phase of stroke by providing homocysteine levels in the initial 48 hours after stroke onset, along with control comparison. We confirm the incremental pattern of homocysteine levels in acute phase of stroke as described in other studies, and further demonstrate that this pattern in fact starts within the initial 48 hours (Figure 1), indicating the acuteness of the homocysteine response.

Our unstratified data shows a slight decrease in mean plasma homocysteine levels in stroke participants at admission, in comparison to mean homocysteine levels in controls (20.30 pg/ml vs. 20.73 pg/ml respectively, difference not statistically significant), followed by a rise in the same levels at 24 and 48 hours (significant difference, p=0.047). Haapaniemi et al. [17] reported a similar initial decrease that was statistically significant. This decrease in homocysteine, early on post-stroke onset, is of interest from a pathophysiological viewpoint

since it may represent a unique (immune?) reaction limited to the first few hours post-stroke onset, and may be protective in nature. Other possible explanation could be increased production of acute phase proteins [18]. Moreover, male stroke participants showed significant differences in mean homocysteine levels at all time points, indicating the importance of gender in stroke-homocysteine relationship. We cannot elaborate on this point further because of our small sample size, limited homocysteine related data available from this part of Asia, and the scarcity of published data on serial homocysteine levels in stroke (especially prospective studies).

Hyperhomocysteinemia has been found to be common in normal populations of South Asia including Pakistan (Southern parts mostly) [19-21]. In addition to stroke participants, our healthy control participants also showed moderate hyperhomocysteinemia. Our study population was in Northern Pakistan while the published literature on hyperhomocysteinemic controls is related to Southern parts of the country (the two parts differ ethnically and culturally). A recent meta-analysis also describes a genetic variant conferring higher plasma homocysteine levels in Asian communities than in other parts of the world [22]. Therefore, the initial decrease in plasma homocysteine in stroke cases described in our results also becomes clinically important in this context.

There is conflicting evidence about the relationship of homocysteine and hemorrhagic stroke with some studies finding raised homocysteine levels in hemorrhagic stroke [23,24], others reporting the same in ischemic stroke [25], and still others finding raised homocysteine in both stroke subtypes [26]. Our finding of raised homocysteine concentrations in hemorrhagic stroke cases adds to the homocysteine-hemorrhagic stroke linkage. This may represent specific pathophysiological, especially genetic, differences between the two subtypes.

In addition to the sample size, the most serious limitation of this study is the lack of data on immune activation. Concurrent detailed leucocyte differential data would have helped us to understand the seemingly linked roles of immune activation and homocysteine. Concurrent plasma vitamin B12 and folate levels could not be attained due to financial constraints, though they would have helped to clarify the results further. It is for these reasons that these results may not be extrapolated more generically.

In conclusion, further work is needed to elucidate the importance of homocysteine changes in stroke patients, as well as to reveal reliable normative homocysteine reference data in the Pakistani population.

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