Importance of Raised Serum Homocysteine Levels in Ischemic Stroke Patients

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Abstract

Background: Around 5.5 million people die from stroke each year, making it the second leading cause of mortality worldwide. Increased homocysteine levels lead to early neurological deteriorations in ischemic stroke and may have an association with other risk factors of stroke.

Objective: To determine how closely homocysteine levels are associated with other risk factors for ischemic stroke.

Methods: This is a cross-sectional study, which was performed in the Department of Neurology, Liaquat National Hospital, Karachi from January to December 2021. All ischemic stroke patients of either gender above the age of 16 were included. Ischemic stroke was identified by neuroimaging MRI in patients presenting with focal neurological deficits lasting more than 24 hours. For statistical analysis, data was entered into SPSS version 21.

Results: A total of 100 patients were included in the study with a mean age of 56.8 ± 15.6 years. Most of the patients were males (65%). Hypertension and diabetes were present in 76% and 42% of patients respectively. 45% of patients had homocysteine levels <15 µmol/l while 55 % had raised homocysteine levels. Amongst them, 42% had levels of homocysteine in the range of 15-30 µmol/l (mild) whereas 13% had >30 µmol/l homocysteine levels (intermediate). None of them had a level >100 µmol (severe). On univariate analysis, the odds of increasing homocysteine levels were higher in males than females. Increasing B12 levels were associated with decreased odds of intermediate homocysteine levels. On the multivariable model after adjusting the model with other covariates, increasing B12 levels remained associated with homocysteine levels with a lower likelihood of intermediate homocysteine levels.

Conclusion: According to the results of our study, there is a strong correlation between high homocysteine levels and low B12 levels, making homocysteine a substantial risk factor for ischemic stroke. Vitamin B12 has a major role in homocysteine pathomechanisms and its deficiency predisposes to hyperhomocysteinemia and hence stroke. Larger multicenter studies may be done to evaluate the role of B12 as a homocysteine-lowering agent both for the treatment and prevention of ischemic stroke.

Keywords: *Homocysteine; ischemic stroke; vitamin B12, hypertension, diabetes.*

INTRODUCTION

Around 5.5 million people die from stroke each year, making it the second leading cause of mortality worldwide. It is also linked to increased morbidity, with half of survivors becoming chronically handicapped [1].

Homocysteine, a sulfur-containing amino acid, is produced as a result of the metabolic demethylation of dietary methionine. Homocysteinemia is associated with well-recognized occlusive thrombotic event consequences [2]. Increased homocysteine levels have been shown in several epidemiological studies to be an independent risk factor for vascular disorders, including stroke [3-8]. Increased homocysteine levels also lead to early neurological deteriorations in ischemic stroke and have a predictive value in the outcome of stroke. A study conducted in South Korea showed that patients with acute stroke with high blood homocysteine levels are more likely to develop END (early neurological deterioration) [9]. One study conducted in the Chinese population showed that higher homocysteine levels have a predictive value for the risk of death or new vascular events in first-onset stroke patients during the long-term follow-up period [10].

A review of the literature revealed that there are limited international and regional studies on the association between homocysteine levels and the other major ischemic stroke risk factors. As a result, the current study's objective is to ascertain how closely homocysteine levels are associated with other ischemic stroke risk variables.

METHODOLOGY

This cross-sectional study was conducted in the neurology department of Liaquat National Hospital Karachi from January 2021 to December 2021. The study enrolled a total of 100 patients. Each patient provided informed consent. The hospital's ethical committee approved the trial.

Previously conducted research reported a frequency of 6.8% hyperhomocysteinemia among ischemic stroke patients [11]. At a 95% confidence interval and bound of error of 5%, a calculated sample size was 98. We rounded it off to 100 and enrolled 100 patients in this study.

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Inclusion Criteria: All ischemic stroke patients of either gender above the age of 16 were included. Ischemic stroke was identified by neuroimaging MRI in patients presenting with localized neurological impairment that lasts longer than 24 hours

Exclusion Criteria: Patients with venous sinus thrombosis and hemorrhagic stroke.

The patient's smoking habits, as well as their comorbidities such as hypertension, diabetes mellitus, thyroid, and autoimmune disorders, were documented. Homocysteine levels, HbA1c, lipid profile, immunoblotted ANA profile, Vitamin B12, TSH, ESR, and CRP were all checked. The frequency of raised homocysteine levels was determined. As per the American Heart Association (AHA), we divided hyperhomocysteinemia into three categories: mild, intermediate, and severe. The (AHA) states that the normal range for homocysteine levels is 5 to 15 µmol/L. There are three levels of hyperhomocysteinemia: mild (15–30 µmol/L), intermediate (30–100 µmol/L), and severe (>100 µmol/L) [12, 13]. Analysis was done on the association between raised homocysteine levels and stroke variables.

Data Analysis: For statistical analysis, data was entered into SPSS version 21. Categorical variables were presented as frequency and percentage. Numerical variables were tested for the assumption of normality with the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation. Non-normal variables were presented as median with inter-quartile range (IQR). Categorical variables were compared among three groups of homocysteine levels using the Chi-square/Fisher exact test. Non-normal variables were compared among three homocysteine groups using the Kruskal-Wallis test. Ordinal logistic regression was applied to determine the association of other variables with homocysteine levels. Variables with p-values<0.25 in univariate analysis were put up in the final multivariable model. A p-value less than or equal to 0.05 was taken as statistically significant for the final regression model.

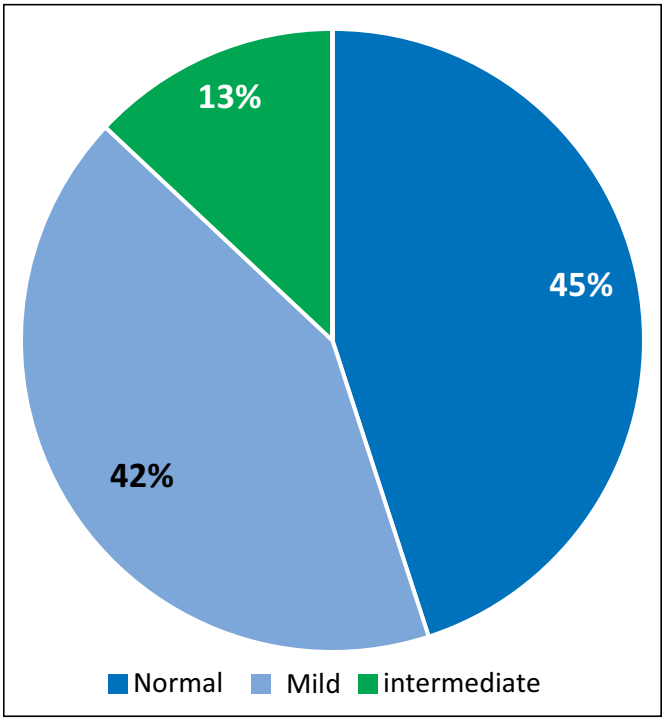

Fig. (1): Frequency of normal, mild, and intermediate homocysteine levels.

RESULTS

The study included a total of 100 patients with a mean age of 56.8 ± 15.6 years. The age range was 20-92 years. Most of the patients were males (65%).

Homocysteine levels in 45% of the patients were within the normal range, or <15 µmol/. While 55% of people had elevated homocysteine levels. Amongst them, 42% had levels of homocysteine levels within the range of 15-30 µmol/L (mild) whereas 13% had >30 µmol/L homocysteine levels (intermediate). None of them had a level >100 µmol/L (severe). Therefore, we categorized homocysteine levels into normal, mild, and intermediate groups (**Fig. 1**).

Table **1** shows the frequency of various risk factors for stroke (age, gender, smoking history, chronic medical condition (HTN, DM, autoimmune diseases, thyroid disorders, and dyslipidemia) homocysteine levels, and

Table 1: Socio-demographic and clinical profile of patients.

Study Variables	Frequency (%)
Demographics	
Age (in years)#	56.8 ± 15.6
Gender	
Male	65 (65)
Female	35 (35)
Smoking history	48 (48)
Medical conditions	
Hypertension	76 (76)
Diabetes	42 (42)
Ischemic heart disease	15 (15)
Positive autoimmune profile	10 (10)
Hypothyroidism (TSH levels)ł	1.9 (0.9-3)
Inflammatory markers	
CRPł	2.1 (0.7-8.2)
ESRł	13 (8-30.3)
Biochemical parameters	
Total lipid	696 (648.3-766.5)
Cholesterol	166 (136-202.5)
Triglycerides	111 (74-158.8)
HDLł	39.5 (34-47)
LDL#	107.3 ± 46.3
VLDLł	22 (16.3-32)
B12ł	616 (298.3-781)
Homocysteine levels	16.7 (12-22.6)

#: Normally distributed variable was presented as mean ± standard deviation, ł: Non-normally distributed variable was presented as median with inter-quartile range

Table 2: Comparison of patients' demographic variables among three homocysteine groups.

Patients' variables	Homocysteine levels			p-value
	Normal n (%)	Mild n (%)	Intermediate n (%)	
Age (in years)ł	62 (50-70)	54.5 (46.5-65.2)	48 (41-69.5)	0.249
Gender				
Male	25(55.6)	29(69)	11(84.6)	0.119
Female	20(44.4)	33(94.3)	2(5.7)	
Smoking history	19(42.2)	20(47.6)	9(69.2)	0.229

ł: Non-normally distributed variable was presented as median with inter-quartile range.

Table 3: Comparison of patients' medical conditions among three homocysteine groups.

Medical conditions	Homocysteine levels			p-value
	Normal n (%)	Mild n (%)	Intermediate n (%)	
Hypertension	36(80)	32(76.2)	8(61.5)	0.389
Diabetes	20(44.4)	19(45.2)	3(23.1)	0.333
Ischemic heart disease	9(20)	5(11.9)	1(7.7)	0.418
Hypothyroidism (TSH levels)ł	2 (1-3.3)	2 (1.1-3.1)	0.8 (0.5-2.1)	0.110
Positive autoimmune profile	7(15.6)	3(7.1)	0(0)	ǂ0.294

TSH: Thyroid-stimulating hormone, ł: Non-normally distributed variable was presented as median with inter-quartile range, ǂ: Fisher-exact test is reported.

Table 4: Comparison of inflammatory markers among three homocysteine groups.

Inflammatory markers	Homocysteine levels			p-value
	Normal Median (IQR)	Mild Median (IQR)	Intermediate Median (IQR)	
CRP	2.4 (0.70-8.2)	2.1 (0.6-8.2)	1.3 (0.8-6.4)	0.870
ESR	17 (7.8-46)	14 (8-32.5)	8 (6.5-14.4)	0.120

CRP: C-reactive protein, ESR: Erythrocyte Sedimentation rate.

Table 5: Comparison of biochemical parameters among three homocysteine groups.

Biochemical parameters	Homocysteine levels			p-value
	Normal Median (IQR)	Mild Median (IQR)	Intermediate Median (IQR)	
Total lipid	721 (642-761)	706 (648-768)	680 (653-697)	0.349
Cholesterol	148 (134-200)	172 (136-204)	149 (134.5-186.5)	0.307
Triglycerides	113 (86-162.5)	114 (82-159)	83 (37.5-146.5)	0.189
HDL	40 (34.5-46.5)	40 (34-47)	38 (26.5-48)	0.842
LDL	100 (65.5-150)	107.5 ± 48.1	105.8 ± 32.7	0.874
VLDL	21 (17.5-33)	22 (16-32)	18 (15-28)	0.620
Vitamin B12	781 (707-885)	375 (280-411)	117 (114-127.5)	*<0.001

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low density lipoprotein, *Significant at p<0.05.

Table 6: Association of patients' variables with homocysteine levels.

Study variables	OR (95% CI)	p-value	aOR (95% CI)	p-value
Age (in years)	1.02 (0.99-1.04)	0.143	0.95(0.86-1.04)	0.274
Gender				
Male	0.44 (0.2-1)	0.045	0.7(0.06-7.54)	0.766
Female	Reference category		Reference category	
Hypertension	1.69 (0.71-4.03)	0.237	-	-
Diabetes	1.4 (0.66-3)	0.380	-	-
IHD	2.07 (0.69-6.18)	0.192	0.05(0-11.2)	0.279
Total lipid	1 (1-1)	0.936	-	-
Triglycerides	1 (1-1)	0.381	-	-
HDL	1 (0.99-1.01)	0.796	-	-
LDL	1 (0.99-1.01)	0.746	-	-
VLDL	1 (0.98-1.03)	0.725	-	-
TSH	0.97 (0.87-1.08)	0.596	-	-
CRP	0.99 (0.96-1.03)	0.605	-	-
ESR	1.01 (1-1.03)	0.123	1.12(0.97-1.29)	0.133
Vitamin B12	1.05 (1.03-1.08)	*<0.001	1.08(1.02-1.15)	*0.007

aOR: Adjusted Odds ratio, CI: Confidence interval, IHD: Ischemic heart disease, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low density lipoprotein, TSH: Thyroid-stimulating hormone, CRP: C-reactive protein, ESR: Erythrocyte Sedimentation rate, OR: Odds ratio, *Significant at p<0.05.

B12 levels. Hypertension and diabetes were present in 76% and 42% of patients respectively (**Table 1**).

A comparison between three groups of homocysteine was done with the above-mentioned risk factors. The following tables illustrate demographic variables and smoking history (**Table 2**), medical condition. (**Table 3**), inflammatory markers (**Table 4**) and biochemical (**Table 5**).

Age, gender, smoking history, hypertension, diabetes, IHD, hypothyroidism, autoimmune profile, CRP, ESR, total lipids, cholesterol, triglycerides, HDL, LDL, and VDL were not significantly different among the three homocysteine groups.

The only factor that was significantly different among the three homocysteine groups was serum B12 level which showed a decreasing trend from normal levels to intermediate levels (**Tables 2-5**).

Table **6** displays the association of other covariates with homocysteine levels. On univariate analysis, the odds of increasing homocysteine levels were higher in males than females. Increasing B12 levels were associated with decreased odds of intermediate homocysteine levels.

On multivariable analysis after adjusting the model with other covariates, increasing B12 levels were also associated with a lower likelihood of intermediate homocysteine levels (**Table 6**).

DISCUSSION

Our findings significantly imply a high degree of association between hyperhomocysteinemia and ischemic stroke. Raised homocysteine levels were found to be present

in 55% of our patients. This incidence is comparable to research that found elevated homocysteine levels in 48% of ischemic stroke patients [14]. An Indian study revealed that 50% of stroke patients had elevated homocysteine levels [15]. Our study revealed 42% had homocysteine levels between 15-30 µmol/L (mild) whereas 13% had >30 µmol/L homocysteine levels (intermediate). None of them had a level >100 µmol/L (severe).

Homocysteine is an extremely reactive amino acid that is harmful to vascular endothelial cells. It enhances LDL autoxidation and increases arterial and venous thrombosis, increasing the risk of cerebrovascular accident, coronary arteries, and peripheral vascular disease.

Our study looked at the relationship between common risk factors for ischemic stroke and homocysteine levels. These included age, gender, smoking status, hypertension, diabetes, IHD, autoimmune illnesses, thyroid problems (hypo/hyperthyroidism), inflammatory markers (CRP, ESR), total lipids, and Vitamin B12 levels.

As reported previously [16, 17], our study showed that males have higher homocysteine levels in comparison to females. One reason could be that males create more creatinine due to high muscle mass and hence have higher amounts of methionine demethylation, contributing to raised homocysteine levels [18]. In our studies, there was no association between age and hyperhomocysteinemia, while other studies have demonstrated that homocysteine levels rise with age [16, 19].

Among the major risk factors, our study illustrated that B12 levels were significantly lower among the three homocysteine groups, with a downward trend from normal to intermediate levels. These findings are consistent with those of earlier investigations [20, 21]. According to one study, inadequate vitamin B12 concentrations were responsible for 28% of hyperhomocysteinemia [22]. A case report found that homocysteine level significantly declined with vitamin B12 in an ischemic stroke patient. According to one study, hyperhomocysteinemia increased the risk of arterial thrombosis in patients with acquired vitamin b12 deficiency when compared to deficiency alone [23]. The patho-mechanism is that a deficiency of vitamin B12 in the blood slows homocysteine conversion to methionine, raising serum homocysteine levels. Thus, homocysteine levels are inversely related to plasma vitamin B12 levels. Several therapeutic trials on vitamin B12 replacement therapy have been conducted to see whether it lowers the incidence of ischemic stroke and its associated disability. HOPE-2 (Heart Outcomes Prevention Evaluator 2) study claims that people under the age of 70 with untreated dyslipidemia, hyperhomocysteinemia, or vitamin B12 or folate deficiency and who are not getting anti-platelets, could benefit from homocysteine-reducing therapies, which have been proven to reduce the incidence of stroke by roughly 25% after a reasonable period of treatment duration of three years [24, 25]. A study showed intake of vitamin B12 was inversely related to the risk of ischemic stroke [26]. In one prospective research that lasted more than four years, plaque formation in the carotid artery was significantly reduced as a result of B-vitamin intake [27].

Our findings revealed that there was no statistically significant disparity between the three homocysteine groups in terms of smoking status, hypertension, diabetes, IHD, autoimmune diseases, thyroid issues (hypo/hyperthyroidism), inflammatory markers (CRP, ESR), or total lipids. This is comparable to the study that found no association between homocysteine levels and the occurrence of hypertension, smoking, hypercholesterolemia, blood glucose, or glycosylated hemoglobin [28]. Another study found no statistically significant difference in homocysteine levels between groups of people who had hypertension, smoked, and had high cholesterol and those who did not [21]. In contrast, one study found that hyperhomocysteinemia was inversely associated with greater HbA1c and an abnormal lipid profile [29]. Another study found a link between high homocysteine levels with DM and HTN [30].

There is consistent evidence that persons with hypothyroidism have greater total homocysteine levels in their blood and that homocysteine levels are reduced after T4 therapy [31]. Hyperhomocysteinemia combined with lipid abnormalities in hypothyroidism may constitute a dynamic atherogenic illness, predisposing to stroke risk. Several studies have found low homocysteine levels in hyperthyroidism, with the conclusion that rapid creatinine clearance contributes to the low levels of homocysteine [32]. Both homocysteine and CRP have been linked to vascular inflammation, and this link has been demonstrated in cardiovascular disease and ischemic stroke [33].

CONCLUSION

According to our analysis, homocysteine is a substantial risk factor for ischemic stroke. There was a strong association between low vitamin B12 levels and high homocysteine levels in our study. Vitamin B has an important role in homocysteine pathomechanisms and its deficiency predisposes to hyperhomocysteinemia and hence stroke. Larger multicenter studies may be done to evaluate the role of B12 as a homocysteine-lowering agent both for the treatment and prevention of ischemic stroke.

ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Review Committee of Liaquat National Hospital, Karachi (REF letter No. App # 0738-2021 LNH – ERC). All procedures performed in studies involving human participants were by the ethical standards of the

institutional and/ or national research committee and with the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written informed consent was taken from the participants.

AVAILABILITY OF DATA

The data set may be acquired from the corresponding author upon a reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

All the authors contributed equally to the publication of this article.

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