



ORIGINAL ARTICLE

HOMOCYSTEINE LEVELS IN STROKE PATIENTS PRESENTING AT A TERTIARY CARE HOSPITAL OF PESHAWAR

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Background: Cerebrovascular disease, is a serious, disabling illness that has a high social burden and a substantial risk of morbidity and death for medical systems. Objective of this study was to determine frequency of elevated homocysteine levels in stroke patients. **Methods:** This cross-sectional study was conducted in Department of Chemical Pathology, Combined Military Hospital, Peshawar from Nov 2023 to Apr 2024. Total 131 ischemic stroke patients were included. Their plasma homocysteine sample was collected in EDTA tube and analysed on Roche Cobas 501 enzymatic assay. Data were analysed on SPSS-26. **Results:** Mean age of the patients was 43.23 ± 9.82 years, and mean homocysteine was 21.25 ± 9.18 $\mu\text{mol/L}$. The frequency and percentage of elevated homocysteine (>15 $\mu\text{mol/L}$) was 69 (52.7%). There was significant differences between group having homocysteine >15 $\mu\text{mol/L}$ and ≤ 15 $\mu\text{mol/L}$, ($p < 0.001$). The elevated homocysteine level was discriminately higher in age subgroup 51–60 Years as compared to age subgroup 31–40 Years while insignificant differences were observed on multiple subgroup comparison. The gender distribution remained insignificant between groups ($p = 0.217$) in terms of the number of participants. There were insignificant differences between males and females in levels of homocysteine in elevated homocysteine group ($p = 0.217$). **Conclusion:** Elevated homocysteine was frequently observed among patients with ischemic stroke, with higher levels observed in the 51–60 year age subgroup.

Keywords: Cardiovascular disease, Frequency, Homocysteine, Ischemic stroke, Risk equivalent

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INTRODUCTION

Cerebrovascular disease, another name for stroke, is a serious, disabling illness that has a high social burden and a substantial risk of morbidity and death for medical systems. The American Heart Association and the American Stroke Association report that stroke is the second leading cause of mortality in the country, behind cardiovascular disease.^{1,2} In Pakistan (Khyber Pakhtunkhwa, or KP), the prevalence of stroke was reported to be 1.2% (1,200 per 100,000 people).³ The most frequent type of stroke is an ischemic stroke, which is characterised by persistent focused neurological signs or symptoms lasting longer than twenty-four hours. The obstruction in the blood vessels supplying the brain is assumed to be the origin of an ischemic stroke.^{4,5} Impaired cerebral blood flow results in ischemia and the death of brain tissue. This disruption results in ischemia and injury to the brain parenchyma by preventing oxygen and other nutrients from reaching the brain cells.⁶ Stroke risk factors include diabetes, smoking, high blood pressure, dyslipidemia, abnormal cholesterol levels, abnormal heart rhythms (often atrial fibrillation), being overweight, and leading a sedentary lifestyle.⁷

An oxidative stressor that can lead to plaque development and vascular inflammation is homocysteine. Methionine serves as the main substrate

for the synthesis of homocysteine. The main enzyme deficiencies that cause homocysteinemia are cystathionine-beta synthase (CBS), 5, 10-methylenetetrahydrofolate reductase (MTHFR). Moreover, homocysteine metabolism depends heavily on folic acid and vitamin B12 (methylcobalamin), and deficiencies in these nutrients can result in elevated plasma homocysteine levels.⁸ Increased homocysteine levels generate pro-coagulant conditions, induce thrombogenicity, and encourage smooth muscle cell growth.^{9,10} Elevated levels of homocysteine are linked to endothelial dysfunction, vascular smooth muscle cell trans-differentiation, and vascular tissue death. This trans-differentiation and cell death cause hydroxyapatite to build up in the blood vessel walls, which causes atherosclerotic constriction of the vessels and stroke.¹¹

Although it is thought to be a separate risk factor for myocardial infarction, its connection to ischemic stroke is rarely studied. Although studies have been conducted in the past to ascertain the prevalence of elevated homocysteine levels in stroke patients, the outcomes have been highly inconsistent. It is crucial to carry out more research in this area because the frequency of elevated homocysteine levels in stroke patients varies so much (32–56%).^{12,13} This study was, therefore, being conducted with the aim to find out frequency of elevated homocysteine levels in stroke patients.

METHODOLOGY

It was a cross-sectional study carried out at Department of Chemical Pathology, Combined Military Hospital, Peshawar for 6 months fulfilling the STROBE statement for reporting observational studies. Sample size ($n=131$) was calculated using WHO sample size calculator software with following parameters: confidence level ($1-\alpha$) with 95% absolute precision (d) 8% and anticipated population proportion (P) 32%.¹² Sample was obtained by non-probability consecutive sampling on data collection proforma. Patients of both gender with age 20–60 years and diagnosed with ischemic stroke (on the basis of presence of focal neurological deficit and hypodensity on CT brain plain) were included in the study and divided into four groups. Pregnant and lactating mothers and patients with hemorrhagic stroke were excluded from the study.

After approval of the study proposal from ethical committee, CPSP, the informed consent of the participants was taken in written form. Once included, blood sample of patients was taken as per standard protocol for assessing blood for plasma homocysteine levels. Patients having history of nil by mouth for at least 8 hours were included in this study. A fresh sample of 4 cc was drawn, put in purple EDTA containing vial and that vial was taken immediately to lab with ice pack for centrifugation to obtain plasma. Homocysteine levels were measured in plasma by an automated analyzer Roche Cobas 501 using enzymatic assay. Homocysteine levels of $>15 \mu\text{mol/L}$ was considered as elevated.¹⁴ Homocysteine level was documented in the standard proforma.

Data analysis was performed using Statistical Package of Social Sciences (SPSS) version 26.0 (IBM Corp. Armonk, NY, USA). The numeric variables (age, homocysteine levels) was presented as Mean $\pm$ SD, while frequency and percentages was used

for categorical variables (gender, presence/absence of elevated levels of homocysteine). Data was stratified by age and gender to deal with effect modifiers. For inferential statistics, independent t -test, Chi-square test and one way analysis of variance (ANOVA) and post hoc Tukey's were used. A $p \leq 0.05$ was considered significant.

RESULTS

Among 131 ischemic stroke patients, mean age was 43.23 ± 9.82 years. Their mean homocysteine was $21.25 \pm 9.18 \mu\text{mol/L}$, in 69 (52.7%) it was $>15 \mu\text{mol/L}$, and in 62 (47.3%) cases it was $\leq 15 \mu\text{mol/L}$. Stratification on age showed significant ($p < 0.001$) differences between high and low homocysteine groups. The gender distribution remained insignificant between groups (homocysteine $>15 \mu\text{mol/L}$ vs $\leq 15 \mu\text{mol/L}$) ($p = 0.217$) in terms of the number of patients. Comparison of the two groups is shown in Table-1.

Table-1: Comparison of homocysteine level in study participants ($n=131$)

Parameter	Homocysteine >15 μmol/L (n=69)	Homocysteine ≤15 μmol/L (n=62)	p
Age (Year, Mean±SD)	41.68±11.99	44.95±6.28	0.057*
Age groups [n (%)]			
20–30 Years	15 (22.0)	3 (5.0)	<0.001 [†]
31–40 Years	18 (26.0)	9 (15.0)	
41–50 Years	13 (19.0)	40 (64.0)	
51–60 Years	23 (33.0)	10 (16.0)	
Gender [n (%)]			
Male	56 (81.0)	44 (71.0)	0.217*
Female	13 (19.0)	18 (29.0)	
Past Medical History			
Type 2 DM	41 (60.0)	36 (57.0)	0.672*
Hypertension	21 (30.0)	22 (36.0)	
CVDs	7 (10.0)	4 (7.0)	
Homocysteine Level (μmol/L, Mean±SD)			
Homocysteine	29.43±3.85	12.16±1.90	<0.001 [†]

*Chi-square test, †Independent t -test

Comparison of age subgroups did not reveal any significant differences except between age group 31–40 and 51–60 years ($p < 0.05$) (Table-2).

Table-2: Age stratified comparison of homocysteine levels in elevated homocysteine group ($n=69$)

Age group (Years)	N	Homocysteine ($\mu\text{mol/L}$, Mean $\pm$ SD)	p -value (ANOVA)	Multiple comparison by post hoc Tukey's		
				Compared Age subgroup	Mean difference (95% CI)	p
20–30	15	29.31 $\pm$ 4.37	0.194	31–40 Years	1.17 (-1.48—3.8)	0.381
				41–50 Years	0.23 (-2.6—3.11)	0.870
				51–60 Years	-1.40 (-3.93—1.11)	0.269
31–40	18	28.13 $\pm$ 3.37		20–30 Years	-1.17 (-3.83—1.48)	0.381
				41–50 Years	-0.93 (-3.70—1.82)	0.501
				51–60 Years	-2.58 (-4.97—-0.19)	0.035
41–50	13	29.07 $\pm$ 4.51		20–30 Years	0.23 (-3.11—2.64)	0.870
				31–40 Years	-0.93 (-1.82—3.70)	0.501
				51–60 Years	-1.64 (-4.28—0.99)	0.217
51–60	23	30.72 $\pm$ 3.27		20–30 Years	1.40 (-1.11—3.93)	0.269
				31–40 Years	2.58 (0.19—4.97)	0.035
				41–50 Years	1.64 (-0.99—4.28)	0.217

DISCUSSION

This study showed that elevated homocysteine level was 52.7% prevalent in ischemic stroke patients, residing in Khyber Pukhtunkhwa province of Pakistan. The elevated homocysteine level was significantly

remarkable in middle-age subgroup (51–60 years), while insignificant differences were observed between male and female patients having ischemic stroke.

Consistent with our findings, a study by Wang JX *et al*¹⁵ observed that 50.7% of stroke patients had elevated homocysteine levels. Another study by Ahmad

S *et al*¹² found that 56% of stroke patients had elevated homocysteine levels, which is essentially identical to this study.

In contrast to these investigations, studies by Ali S *et al*¹¹ and Ahmad S *et al*¹² indicated a substantially lower frequency of elevated homocysteine levels in stroke patients (36%), with nearly identical results seen in another study (32%). Friedman showed elevated homocysteine levels in 40 cases of older patients aged ≥ 65 years who had atrial fibrillation and stroke.¹⁶

Prior studies found that patients with hypertension, diabetes, or hyperlipidemia had a higher risk of having an elevated homocysteine level than those without these conditions or those with a normal lipid profile. In our study cardiovascular disease was observed in 10% of participants in the elevated homocysteine group compared to 7% reported by other studies in non-elevated group.^{12,17,18}

LIMITATIONS

It was a single-centered study with small sample size and only included ischemic stroke patients. Inclusion of non-stroke control patients could have been exhibited a better comparison. Recent cardiovascular status, biochemical and related cardio-metabolic assessment during their hospital stay and time of onset of stroke and delay till sample collection for homocysteine were not included.

CONCLUSION

Elevated homocysteine was frequently observed among patients with ischemic stroke, with higher levels observed in the 51–60 year age group. Further studies are recommended to determine the independent association of elevated homocysteine with ischemic stroke.

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MBK: Study design, acquisition data and manuscript drafting

SS: Analysis and interpretation of data

ZHH: Concept, study design and analysis

SF: Critical review

MF: Concept and study design

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