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Evaluation of Serum Homocysteine and Folate Levels in Patients with Ischemic Stroke: A Biochemical Correlation Study.

Obaid Ur Rahman[1], Anwar Ali[2], Ayaz Ahmed[3], Khalilullah[4], Faiza Shuaib[5], Adnan Badar[6]

[1]Associate Professor Biochemistry, Swat Medical College Swat.

[2]Associate Professor Biochemistry, Saidu Medical College Saidu Sharif Swat.

[3]Demonstrator Department of Biochemistry, Saidu College of Dentistry Saidu Sharif Swat.

[4]Assistant Professor, Department of Biochemistry, Swat Medical College Swat.

[5]Demonstrator. Department of Biochemistry, Saidu Medical College Saidu sharif Swat.

[6]Associate Professor Department of Anatomy, Saidu Medical College, Saidu Sharif Swat.

Corresponding Author:

Anwar Ali2

Email ID : dr.ali.smc@gamil.com

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ABSTRACT

Background: According to the American Heart Association, strokes are a leading cause of disability and death due to biochemical elements such as homocysteine. And folates are becoming increasingly critical given their impact on thrombosis and vascular damage. It is easy to recognize that, in rough and clinical settings, efficient early monitoring, accurate prediction, and better process management are going to be substantially determined by estimates of the elements above.

Objectives: To evaluate serum homocysteine and folate levels in patients with ischemic stroke and examine their associations with stroke characteristics, demographic variables, clinical risk factors, and relevant biochemical parameters.

Methodology: this study conducted at department of Biochemistry Saidu Medical College Saidu Sharif Swat from Jan 2025 to June 2025.100 adults who were CT/MRI-confirmed ischemic stroke patients within 72 hours of admission were included in the cross-sectional study. Serum levels of homocysteine and Folate were measured using a chemiluminescence enzyme immunoassay. The NIHSS was used to assess stroke severity. Demographic and clinical information were documented. The data were analyzed using SPSS version 24.0, including t-tests, Pearson's correlation, and Chi-square tests, with a p-value of ≤ 0.05.

Results: 100 patients mean age was 59.8 ± 10.6 years. Elevated homocysteine levels were observed in 62% of patients, while low folate levels were detected in 38%. A significant inverse correlation was found between homocysteine and folate levels ($r = –0.41$, $p = 0.002$). Higher homocysteine levels were significantly associated with moderate-to-severe stroke on NIHSS ($p = 0.01$). Folate deficiency showed a significant association with older age and hypertension ($p = 0.03$). No significant association was found with gender ($p = 0.27$).

Conclusion: The study shows there are significant correlations between ischemic strokes and an increased prevalence of hyperhomocysteinemia and folate deficiency. Additionally, there's an increased risk of vascular morbidity. The inverse biochemical relationship supports the association of folate deficiency with hyperhomocysteinemia. Regular assessment of such indicators may assist in initial risk classification and in the provision of optimal nutritional and preventive measures to support better outcomes for the cerebrovascular system.

Keywords: Homocysteine; Folate; Ischemic Stroke; Biomarkers

1. INTRODUCTION

Ischemic stroke continues to be a global health problem, representing nearly 85% of all strokes and the most common cause of death and disability among adults. There have been improvements in the treatment of strokes, thrombolysis, and Neuro-Rehabilitation, yet stroke remains a leading cause of disability and

death in adults in developing countries [1]. Older populations, limited access to primary prevention, increased treatment delays, and limited screening and access to prevention for emerging biochemical risk factors all contribute to the growing problem in lower- and middle-income countries [2]. Among these emerging risk factors, serum homocysteine and folate levels have received particular attention for their roles in vascular health, endothelial function, and thrombotic stroke. Hyperhomocysteinemia is a thrombotic stroke risk factor and is defined as elevated homocysteine levels, a sulfur-containing amino acid produced by methionine metabolism. Hyperhomocysteinemia is known to cause harm to the cerebrovascular system [3]. Elevated homocysteine, resulting from oxidative stress and lipid peroxidation, promotes endothelial dysfunction and an inflammatory response, further decreasing nitric oxide availability. These events described above ultimately result in a more austere inflammatory state that fosters further atherosclerosis or thrombus formation, ultimately resulting in a decrease in cerebral blood flow as ischemic stroke occurs. Ongoing epidemiological studies have shown that having elevated levels of homocysteine in one's blood can put one at an increased risk for having a stroke, regardless of whether they have other vascular risk factors [4, 5]. Folate, one of the B vitamins and essential for the production, methylation, and repair of DNA, is one of the key vitamins in the metabolism of homocysteine. It can regulate homocysteine levels in the blood by converting homocysteine to methionine via the demethylation pathway [6]. As a result of the statement, a deficiency in Folate can substantially increase one's risk for a vascular event. Folate also helps maintain the stability and integrity of blood vessels, making it even more important for protecting the brain's blood vessels [7]. Studies examining the relationship between homocysteine and Folate have shown a strong inverse association between the two. A low dietary intake of folate, a low nutritional status, and an elevated homocysteine level can all be observed together, particularly in South Asian populations. This is especially true in a country like Pakistan, which has a highly elevated level of nutritional deficiency, high blood pressure, diabetes, and a lack of preventive health resources [8, 9]. Although worldwide studies have shown the link between having elevated levels of homocysteine and Folate and having an ischemic stroke, studies in the area are showing little to no evidence of this, and the measurement of these substances in everyday practice is virtually nonexistent. Most medical services for stroke in Pakistan focus on traditional risk factors,.

which include hypertension, diabetes, dyslipidemia, smoking, and atrial fibrillation. Therefore, assessing the role of homocysteine and Folate may yield additional tools for risk-stratification and, thus, even enable primary preventive approaches for high-risk cases [10].

2. STUDY OBJECTIVES:

To evaluate serum homocysteine and folate levels in ischemic stroke patients and identify associations with stroke severity and demographic and vascular risk factors, as an attempt to increase risk prediction accuracy.

3. MATERIALS AND METHODS:

Study Design & Setting:

This retrospective study conducted at department of Biochemistry Saidu Medical College Saidu Sharif Swat from Jan 2025 to June 2025.

Participants:

We Retrospectively included adult patients within the age group of 30-80 years diagnosed with ischemic stroke and was CT/MRI confirmed within 72 hours of ischemic stroke onset. History included vascular risk features, and demographic data, clinical manifestations, and risk profiles were documented. Blood samples were taken before patients were supplemented with vitamins. Patients who were unable to provide consent or had incomplete imaging were excluded.

Sample Size Calculation

With a projected prevalence of hyperhomocysteinemia in ischemic stroke patients of 30%, a 95% confidence level, and 8% margin of error, a sample of 100 participants was calculated in Opine. This sample warranted sufficient power for corroboration and regression analyses.

Inclusion Criteria

Certification of CT/MRI confirmed ischemic stroke. Diagnosis of ischemic stroke confirmed within the last 72 hours. Individuals aged 30-80 years. Informed Consent was Obtained

Exclusion Criteria

Stroke due to bleeding, Chronic illnesses affecting the kidneys or liver, Active cancer, or autoimmune disease, malignantly altered, or previously supplemented with vitamin B12 or Folate

Diagnostic and Management Strategy

Every patient had neuroimaging performed, a preliminary laboratory assessment, and was scored on the NIHSS. Patients were given and treated according to standard acute stroke care principles, including the administration of antiplatelet agents, statins, management of hypertension, and intravenous fluids. To avoid affecting the biochemical analysis of the samples obtained, no vitamin supplements were started before blood collection.

Statistical Analysis

Data analysis was performed using SPSS version 24.0. Continuous variables were summarized as means ± standard deviations, while categorical variables were presented as frequencies and percentages. Group comparisons were assessed using the independent samples t-test for continuous variables and the chi-square test for categorical variables. Pearson correlation analysis was applied to examine associations between biochemical markers and clinical parameters. To identify independent predictors, multivariate regression analysis was conducted. A p-value ≤ 0.05 was considered statistically significant**.**

Ethical Approval:

The study was conducted in accordance with the ethical principles and institutional guidelines of Saidu Medical College, Saidu Sharif Swat. Approval was obtained from the Institutional Ethical Review Board before the initiation of data collection. Written informed consent was secured from all participants after explaining the study objectives, procedures, confidentiality measures, and voluntary nature of participation. No additional invasive interventions were performed beyond routine clinical assessments.

4. RESULTS:

A total of 100 patients were included with a mean age of 59.8 ± 10.6 years. Males comprised 58% of the sample. Elevated homocysteine levels were detected in 62% of patients, while 38% demonstrated low serum folate levels. A significant inverse correlation was observed between serum homocysteine and folate ($r = –0.41$, $p = 0.002$). Patients with hyperhomocysteinemia had significantly higher NIHSS scores, indicating more severe stroke ($p = 0.01$). Folate deficiency was more common in older individuals and those with hypertension ($p = 0.03$). No significant associations were found between biochemical levels and gender ($p = 0.27$) or diabetes ($p = 0.19$). The findings suggest an important biochemical contribution to ischemic stroke severity.

Intervention Outcome

shown that individuals lacking folate with elevated homocysteine levels have poorer core clinical status upon admission to treatment and show less improvement in neurological conditions in early treatment phases. Those at risk of primary cerebrovascular events might benefit from future studies targeting homocysteine with folate supplementation, given that homocysteine has shown a strong, protective effect on cerebrovascular conditions, as demonstrated in this study. Although immediate treatment has shown no changes in biochemical.

Table 1: Demographic Characteristics of Patients with Ischemic Stroke (n = 100)

Variable	Category	Frequency (n)	Percentage (%)
Age (years)	Mean ± SD	**59.8 ± 10.6**	—
Gender	Male	58	58%
	Female	42	42%
Residence	Urban	64	64%
	Rural	36	36%
Comorbidities	Hypertension	61	61%
	Diabetes Mellitus	47	47%
	Dyslipidemia	33	33%
	Smoking	29	29%

Table 1 presents the baseline demographic and vascular risk characteristics of ischemic stroke patients, including age distribution, gender, residence, and major comorbidities.

Table 2: Serum Homocysteine and Folate Levels in Ischemic Stroke Patients (n = 100)

Parameter	Mean ± SD	Normal Range	Abnormal Findings
Homocysteine (µmoll/L)	18.5 ± 6.3	5–15 µmoll/L	Elevated in 62%
Folate (ng/mL)	4.8 ± 1.9	5–20 ng/mL	Low in 38%

Table 2 summarizes the biochemical findings of serum homocysteine and folate. Hyperhomocysteinemia was observed in most patients, while more than one-third showed folate deficiency.

Table 3: Association of Homocysteine and Folate Levels with Stroke Severity (NIHSS Categories)

Variable	Mild Stroke (NIHSS ≤5)	Moderate Stroke (NIHSS 6–15)	Severe Stroke (NIHSS >15)	p-value
Hyperhomocysteinemia	18%	47%	35%	**0.01***
Low Folate Levels	12%	56%	32%	**0.03***
Mean NIHSS Score	4.1 ± 1.2	10.3 ± 2.6	18.4 ± 3.1	—

Table 3 shows the correlation between biochemical markers and stroke severity. Elevated homocysteine and low folate were significantly associated with moderate-to-severe stroke.

Table 4: Correlation of Biochemical Levels with Selected Risk Factors

Risk Factor	Hyperhomocysteinemia (n=62)	Normal Homocysteine (n=38)	p-value	Low Folate (n=38)	Normal Folate (n=62)	p-value
Age > 60 years	38 (61%)	14 (37%)	0.02*	27 (71%)	25 (40%)	0.01*
Hypertension	42 (68%)	19 (50%)	0.04*	29 (76%)	32 (52%)	0.03*
Diabetes Mellitus	32 (52%)	15 (39%)	0.19	21 (55%)	26 (42%)	0.21
Smoking	21 (34%)	8 (21%)	0.12	10 (26%)	19 (31%)	0.56

Table 4 evaluates relationships between biochemical abnormalities and common vascular risk factors. Older age and hypertension showed statistically significant associations with both elevated homocysteine and low folate levels.

5. DISCUSSION:

This study evaluating serum homocysteine and folate levels in patients with ischemic stroke demonstrated a high prevalence of hyperhomocysteinemia alongside reduced folate concentrations. These findings reinforce the growing evidence that biochemical abnormalities play a contributory role in the etiology and progression of ischemic stroke—even in non-embolic presentations—beyond the influence of traditional vascular risk factors. An inverse relationship was observed between homocysteine and folate levels, suggesting that impaired methylation pathways may contribute to both the onset and potential severity of ischemic events [11,12]. The prevalence of hyperhomocysteinemia in this cohort (62%) is comparable to recent international studies. A large multicenter study from China (2021) reported that approximately 58% of ischemic stroke patients exhibited elevated homocysteine levels and concluded that hyperhomocysteinemia is an independent predictor of greater stroke severity and early neurological deterioration [13]. Similarly, a 2020 study from India examining acute ischemic stroke documented hyperhomocysteinemia in 60–65% of cases, underscoring its significance as a modifiable biochemical risk factor in cerebrovascular disease [14]. More importantly, these studies confirm hyperhomocysteinemia is a notable and likely a preeminent risk as a result of poor nutrition, genetics, and low folate levels in many Asian populations [15]. The authors note that the absence of evidence relating to folate levels and homocysteine is valid evidence of this. Folate deficiency

affects endothelial function in stroke patients. A 2022 investigation demonstrated that low folate levels predicted elevated homocysteine levels and impaired endothelial function in stroke patients [16]. A 2019 study found that folate deficiency occurred more frequently and severely in older stroke patients and was associated with greater carotid intimal thickness [17]. Similarly, our study found folate deficiency more prevalent in older patients with hypertension. These findings suggest that age-related nutritional deficits and chronic vascular burden may lead to biochemical dysregulation. The strong correlation found between hyperhomocysteinemia and diagnostic severity (NIHSS) in stroke was demonstrated in recently published observational studies. A 2020 study found that patients with elevated homocysteine levels had larger infarcts and were more likely to have adverse outcomes early in the hospitalization [18]. A 2023 study published results that also support the negative impact of hyperhomocysteinemia, showing that patients had worse functional status at 3 months after stroke, further emphasizing the importance of evaluating homocysteine in the acute stroke setting [19]. The study showed that patients with hyperhomocysteinemia had worse functional outcomes, resulting in significantly higher NIHSS scores. Attention to the clinical consequences of folate deficiency, especially in users, is warranted, recognizing that studies have documented a growing problem of folate deficiency in stroke patients, particularly in low-income areas. A study conducted in Pakistan in 2021 found that at teatime, 42% of the cohort with ischemic stroke were folate-deficient, a figure similar to the 38% in our study [20]. The study also indicated that folate deficiency in conjunction with high levels of homocysteine was correlated with an increased risk of stroke recurrence. In 2022, a review also noted a reduction in homocysteine with folate supplementation, along with a reduced risk of stroke, which was particularly important in stroke-high-risk groups with low dietary Folate.

6. LIMITATIONS:

There was a single-center design and small sample size for this investigation; therefore, the findings may lack generalizability. There was also only one phase of biochemical data collection without long-term follow-up. Homocysteine -folate metabolism and stroke results may be altered by the diet, genetic copies, and vitamin B12 levels, none of which were captured for this study.

7. CONCLUSION:

Hyperhomocysteinemia and folate deficiency were highly prevalent among patients with ischemic stroke and showed a significant association with stroke severity. The inverse biochemical relationship between these markers suggests underlying metabolic deregulation that may contribute to cerebrovascular risk. Routine screening of homocysteine and folate levels could enhance early risk stratification and support timely, cost-effective preventive interventions particularly in resource-limited settings—to improve overall disease outcomes.

Disclaimer: Nil

Conflict of Interest: Nil

Funding Disclosure: Nil

Authors Contributions

Concept & Design of Study: **Obaid Ur Rahman[1], Anwar Ali[2]**

Drafting: **Ayaz Ahmed[3], Khalilullah[4]**

Data Collection & Data Analysis: **Faiza Shuaib[5], Adnan Badar[6]**

Critical Review: **Faiza Shuaib[5], Adnan Badar[6]**

Final Approval of version: **All Mentioned Authors Approved the Final Version.**.

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