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Original Research Article

Comparison of serum homocysteine levels in transfusion-dependent β-thalassemia patients with regular and irregular folic acid supplementation

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ABSTRACT

Background: Transfusion-dependent β-thalassemia (TDT) patients have increased folate requirements due to chronic erythropoietic stress. Inadequate folate intake may result in elevated serum homocysteine, a recognized risk factor for vascular complications. This study aimed to compare serum homocysteine levels between TDT patients with regular and irregular folic acid supplementation and to assess correlations with duration of supplementation and age.

Methods: This cross-sectional comparative study was conducted at the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, between March 2023 and February 2024. Forty TDT patients were categorized into regular (n=20) and irregular (n=20) folic acid supplementation groups. Fasting serum homocysteine levels were measured using chemiluminescent immunoassay. Data were analyzed using SPSS version 27.0.

Results: Mean serum homocysteine was significantly lower in the regular supplementation group (5.19±1.04 µmol/l) compared with the irregular group (13.68±5.47 µmol/l; $p<0.001$). Hyperhomocysteinemia (≥15 µmol/l) was observed exclusively among irregularly supplemented patients (30%). A negative correlation was identified between duration of supplementation and homocysteine level, while age showed a modest positive trend with homocysteine among irregular users.

Conclusions: Irregular folic acid supplementation is associated with significantly elevated serum homocysteine levels in transfusion-dependent β-thalassemia patients. Sustained adherence to supplementation may reduce metabolic risk and should be emphasized in routine clinical management.

Keywords: β-thalassemia, Folic acid adherence, Transfusion-dependent thalassemia

INTRODUCTION

TDT is a severe hereditary hemoglobinopathy marked by defective β-globin synthesis, chronic anemia and lifelong reliance on regular blood transfusions.[1] While advances in transfusion and iron chelation have improved survival, long-term metabolic and vascular complications remain prominent concerns in these patients.[2,3]

Among biochemical abnormalities, disruptions in homocysteine metabolism have garnered attention due to potential cardiovascular risks. Homocysteine, a sulfur-containing amino acid derived from methionine metabolism, is normally regulated through remethylation and transsulfuration pathways requiring folate, vitamin B12 and B6 as cofactors.

Folate deficiency impairs homocysteine remethylation, causing its accumulation, which independently increases the risk of endothelial dysfunction, thrombosis and premature atherosclerosis.[4] Patients with TDT face increased folate demands owing to chronic hemolysis and persistent ineffective erythropoiesis despite regular transfusions, sustaining a high folate requirement for nucleic acid synthesis. Consequently, routine folic acid supplementation forms standard supportive care, however, adherence is variable, especially in low-resource settings where socioeconomic and educational factors impede compliance.[5]

Research has shown folate deficiency correlates with elevated homocysteine levels across populations. For instance, Baghersalimi et al documented serum homocysteine rising significantly upon folic acid cessation in β-thalassemia major, indicating a direct metabolic link.[6] Similarly, Abd-Elmawla et al highlighted altered homocysteine metabolism contributing to vascular risk in thalassemia, particularly with genetic polymorphisms affecting folate pathways.[7,8] Yet, direct comparative studies on homocysteine levels in TDT patients with regular versus irregular folic acid supplementation remain scarce.

Bangladesh, located within the "thalassemia belt," faces a high prevalence of HbE β-thalassemia and β-thalassemia major. Expanding transfusion services in tertiary centers are not always accompanied by biochemical monitoring or adherence assessment of supportive therapies, thereby limiting insight into how irregular folic acid intake might affect homocysteine levels and clinical outcomes.[9]

Furthermore, age and duration of folic acid supplementation may influence homocysteine metabolism, as age-related variations have been reported in general populations, potentially reflecting cumulative metabolic stress and micronutrient status.

Whether similar patterns occur in TDT patients under varying supplementation regimens is unexplored and warrants investigation.[10] This study was therefore undertaken to compare serum homocysteine levels between transfusion-dependent β-thalassemia patients with regular and irregular folic acid supplementation and to explore the correlation of homocysteine with duration of supplementation and age.

METHODS

This cross-sectional comparative study was conducted at the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from March 2023 to February 2024. The study included 40 transfusion-dependent β-thalassemia patients attending the Day Care Transfusion Unit during the study period. Participants were categorized into two groups based on folic acid supplementation status: regular supplementation (n=20) and irregular supplementation (n=20).

Inclusion criteria

Confirmed diagnosis of transfusion-dependent β-thalassemia by hemoglobin electrophoresis. Age>5 years. History of regular transfusion therapy. Receiving folic acid supplementation for at least 6 months.

Exclusion criteria

Pregnancy or lactation. Chronic kidney disease or malignancy. Use of medications influencing homocysteine metabolism (e.g., methotrexate, metformin, anticonvulsants).

Data collection

Participants were recruited consecutively after obtaining written informed consent. A structured questionnaire was used to collect demographic information and details regarding folic acid supplementation, including regularity and duration. Regular supplementation was defined as daily intake without interruption, whereas irregular supplementation referred to inconsistent or interrupted intake as reported by the patient or guardian.

Medical records were reviewed to confirm diagnosis and transfusion history. After overnight fasting, 3 ml of venous blood was collected under aseptic precautions. Serum homocysteine levels were measured using chemiluminescent immunoassay (CLIA) technology on the Atellica automated analyzer (Siemens, Germany) in the Department of Biochemistry and Molecular Biology, BSMMU. Standard laboratory protocols were followed to ensure analytical precision and reliability. Data were verified for completeness before statistical analysis.

Ethical consideration

Ethical approval was obtained from the Institutional Review Board of BSMMU. Written informed consent was secured from adult participants and from guardians of

minors. Confidentiality was maintained through anonymized data coding. Participation was voluntary and withdrawal was permitted at any stage without affecting medical care.

Statistical analysis

Data were analyzed using SPSS version 27.0. Continuous variables were expressed as mean±standard deviation (SD) and compared using independent sample t-tests. Categorical variables were presented as frequency and percentage and analyzed using Chi-square tests. Pearson's correlation coefficient was used to assess relationships between serum homocysteine and duration of supplementation and age. A two-tailed p-value≤0.05 was considered statistically significant.

RESULTS

The majority of the participants, 35% (7 out of 20) in Group A were in the age group 11-15 years and in Group B majority of participants, 40% (8 out of 20), were in the age group 16-20 years. No significant differences in mean age between the two groups (p>0.05). Among the participants, 24 were male and 16 were female. Group A included 10 (50.0%) males while Group B included 14 (70%).

The rest were female, 50% and 30% respectively in Group A and Group B. The majority of patients in Group A had consumed folic acid for >10 years (13 out of 20), followed by 6-10 years (7 out of 20).

In contrast, the majority of patients in Group B had consumed folic acid for 1-5 years (15 out of 20), followed by 6-10 years (5 out of 20). None of the patients in Group B had consumed folic acid for more than 10 years.

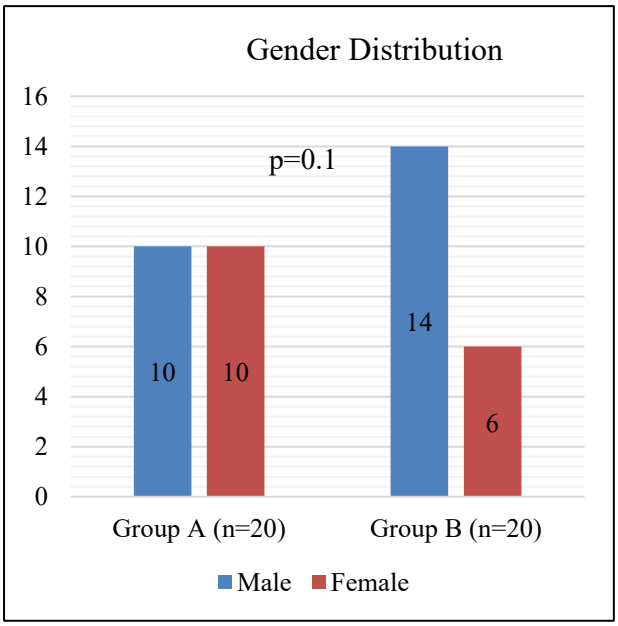

Figure 1: Sex distribution among the patients.

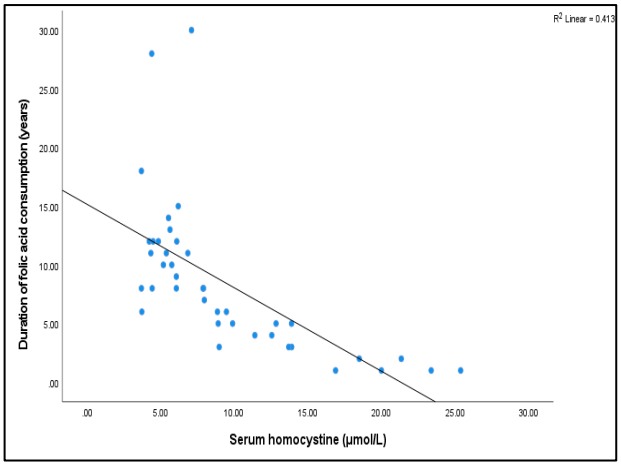

Figure 2: Correlation between serum homocysteine and duration of folic acid supplementation.

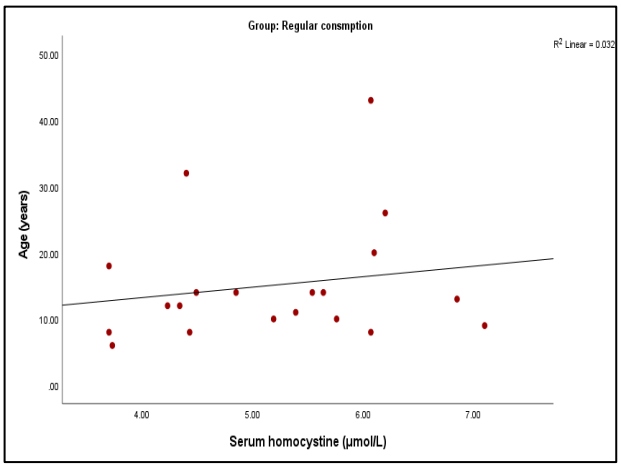

Figure 3: Correlation between serum homocysteine and age in regular supplementation group.

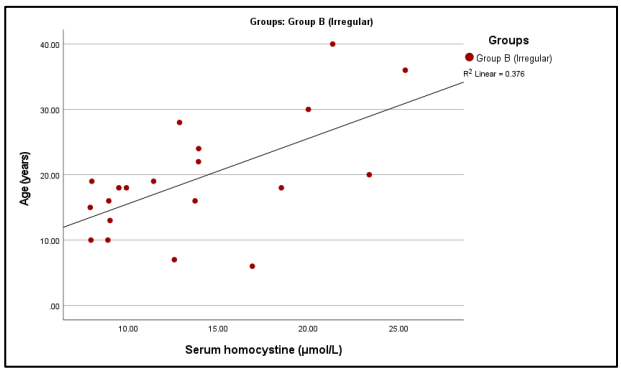

Figure 4: Correlation between serum homocysteine and age in irregular supplementation group.

Table 3 shows that patients with regular folic acid supplementation had significantly lower serum homocysteine levels compared with those with irregular intake (p<0.001). Hyperhomocysteinemia (≥15 µmol/l) was observed exclusively among irregularly supplemented patients. A strong negative correlation between the serum

homocysteine level and the duration of folic acid consumption. This means that a longer duration of folic acid consumption correlates with a lower serum homocysteine level. The p-value provided ($p<0.001$) indicates that the observed correlation is highly statistically significant. The correlation between serum homocysteine levels and the age of patients in Group A suggests a non-significant weak positive correlation ($r=0.180$, $p=0.449$). Non-significant weak positive correlation indicates that serum homocysteine level may increase with the increase of age but that is not significant, in patients with regular consumption of folic acid supplements.

The correlation coefficient ($r=0.613$, $p= 0.004$) suggests a strongly significant positive correlation between serum homocysteine level and age of patients in Group B. It indicates that with an increase in age, serum homocysteine level increases significantly in patients with irregular consumption of folic acid supplements. Here, irregular consumption is more responsible for the elevation of serum homocysteine levels.

Table 1: Age distribution of study participants.

Age (in years)	Group A (n=20)	Group B (n=20)	P value
5–10	5 (25.0%)	3 (15.0%)	
11–15	7 (35.0%)	5 (25.0%)	
16–20	5 (25.0%)	8 (40.0%)	
>20	3 (15.0%)	4 (20.0%)	
Mean±SD	15.40 ± 9.10	19.25 ± 8.93	0.154

Table 2: Duration of folic acid supplementation.

Duration of folic acid consumption (in years)	Group A (n=20)	Group B (n=20)
1-5	0 (0.0%)	15 (75.0%)
6-10	7 (35.0%)	5 (25.0%)
>10	13 (65.0%)	0 (0.0%)
Mean±SD	12.90±6.15	4.0±2.29

Table 3: Comparison of serum homocysteine levels.

Serum homocysteine (µmol/l)	Group A (n=20)	Group B (n=20)	P value
3-7	20 (100.0%)	0 (0.0%)	
8-14	0 (0.0%)	14 (70.0%)	
≥15	0 (0.0%)	6 (30.0%)	
Total	20 (100.0%)	20 (100.0%)	
Mean±SD	5.19±1.04	13.68±5.47	<0.001

DISCUSSION

The present study compared serum homocysteine levels between transfusion-dependent β-thalassemia patients with regular and irregular folic acid supplementation and explored their relationship with duration of supplementation and age. The findings demonstrate a marked and statistically significant elevation of serum homocysteine among patients with irregular folic acid intake compared with those adhering to regular supplementation. In addition, a negative correlation between duration of supplementation and homocysteine concentration was observed, while age-related trends differed between groups. The central observation of this study was the substantial difference in mean serum homocysteine levels between regularly supplemented patients (5.19±1.04 µmol/l) and irregularly supplemented patients (13.68±5.47 µmol/l), with hyperhomocysteinemia (≥15 µmol/l) occurring exclusively in the latter group. This finding is biologically plausible, as folate is an essential cofactor in the remethylation of homocysteine to methionine. Impaired folate availability disrupts this pathway, resulting in homocysteine accumulation.[9] Finkelstein described the central role of folate-dependent enzymatic reactions in maintaining homocysteine homeostasis, emphasizing the sensitivity of circulating homocysteine to changes in folate status.[9]

The magnitude of difference observed in this study aligns with the findings of Baghersalimi et al who reported a significant rise in serum homocysteine following discontinuation of folic acid supplementation in thalassemia major patients.[6] Their work suggested that folate withdrawal directly influences homocysteine metabolism in this population, supporting a causal association. Similarly, Abd-Elmawla et al highlighted the interplay between folate metabolism and hyperhomocysteinemia in β-thalassemia patients,

particularly when additional metabolic stressors are present.[7] The present findings reinforce these observations by demonstrating that even irregular intake, rather than complete cessation, is associated with significantly elevated homocysteine levels. The absence of significant differences in age and sex distribution between the two groups strengthens the validity of the comparison, suggesting that the observed biochemical disparity is unlikely to be explained by demographic imbalance. Selhub et al reported that homocysteine levels tend to increase with advancing age in the general population, potentially due to cumulative nutritional deficiencies and metabolic changes.[12] In the current study, age did not correlate significantly with homocysteine levels in the regularly supplemented group, implying that adequate folate intake may mitigate age-related increases in homocysteine. In contrast, a modest positive trend between age and homocysteine was observed among irregularly supplemented patients, suggesting that inadequate supplementation may permit the expression of age-associated metabolic vulnerability.

The negative correlation between duration of folic acid supplementation and serum homocysteine further supports a protective effect of sustained supplementation. Longer duration of consistent intake was associated with lower homocysteine concentrations, underscoring the importance of adherence over time. Although the cross-sectional design precludes definitive causal inference, the observed association is consistent with established biochemical mechanisms and prior interventional evidence.[6] Hyperhomocysteinemia has been implicated in endothelial dysfunction, oxidative stress and prothrombotic states.[13] Taher et al emphasized that patients with thalassemia exhibit a hypercoagulable profile related to chronic hemolysis, platelet activation and endothelial injury.[14] The addition of elevated homocysteine may potentiate this prothrombotic milieu, increasing the risk of vascular complications. Hankey and Eikelboom described homocysteine as an independent risk factor for vascular disease in broader populations and it is plausible that this risk is magnified in transfusion-dependent patients already burdened by iron overload and chronic inflammation.[13]

In resource-limited settings such as Bangladesh, where HbE β-thalassemia constitutes a major proportion of cases, adherence to supportive therapy may be influenced by socioeconomic constraints and limited health literacy.[15] While transfusion services have expanded in tertiary centers, routine biochemical monitoring of micronutrient status is not uniformly practiced. The present findings suggest that reinforcing adherence to folic acid supplementation could represent a simple, low-cost strategy to reduce biochemical risk markers associated with vascular pathology. Collectively, the results highlight that regular folic acid supplementation is associated with maintenance of lower serum homocysteine levels in transfusion-dependent β-thalassemia patients. The consistent difference between groups, the correlation with duration of supplementation and the age-related trends observed among irregular users provide converging evidence that adherence plays a critical role in metabolic homeostasis. These findings underscore the importance of patient education, counseling and routine follow-up to ensure sustained compliance with supplementation as part of comprehensive thalassemia care.

Limitations

There are several limitations to consider in this study all samples were collected from a single center. Long term follow up was beyond the scope of the study. The study did not explore other potential factors, such as genetic variations and undiagnosed comorbidities which could influence serum homocysteine level.

CONCLUSION

Irregular folic acid supplementation in transfusion-dependent β-thalassemia patients is associated with significantly elevated serum homocysteine levels compared with regular intake. Longer duration of supplementation correlates with lower homocysteine concentrations, suggesting a protective metabolic effect. Strengthening adherence to routine folic acid supplementation may represent a practical and cost-effective strategy to mitigate biochemical risk factors associated with vascular complications in this high-risk population.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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