Serum Homocysteine Level in Transfusion-Dependent Beta-Thalassemia Patients with Folic Acid Supplementation

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ABSTRACT

Background: Transfusion-dependent beta-thalassemia (TDT) patients require lifelong transfusion therapy and supportive management. Folic acid supplementation is routinely prescribed to meet increased erythropoietic demands; however, its impact on serum homocysteine levels in this population remains inadequately characterized. **Objectives:** To evaluate serum homocysteine levels in transfusion-dependent beta-thalassemia patients receiving folic acid supplementation. **Methods & Materials:** This cross-sectional study was conducted at the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, from March 2023 to February 2024. Forty TDT patients aged >5 years receiving folic acid supplementation were enrolled. Demographic, nutritional and transfusion-related data were recorded. Fasting serum homocysteine was measured using chemiluminescent immunoassay. Statistical analysis was performed using SPSS version 27.0, with $p \leq 0.05$ considered significant. **Results:** The mean age of participants was 17.33 ± 9.13 years; 60% were male. Underweight status was observed in 70% of patients. Transfusion characteristics were comparable between supplementation groups. Patients receiving regular folic acid supplementation demonstrated significantly lower mean serum homocysteine levels (5.19 ± 1.04 µmol/L) compared with irregular users (13.68 ± 5.47 µmol/L; $p<0.001$). Hyperhomocysteinemia (≥15 µmol/L) was observed in 30% of irregularly supplemented patients but in none of the regularly supplemented patients. **Conclusion:** Regular folic acid supplementation is associated with maintenance of lower serum homocysteine levels in TDT patients. Sustained supplementation may play a role in mitigating vascular risk in this high-burden population.

Keywords: *Beta-thalassemia, transfusion-dependent thalassemia, folic acid, homocysteine, hyperhomocysteinemia*

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INTRODUCTION

Beta-thalassemia is a hereditary disorder of hemoglobin synthesis characterized by reduced or absent production of β-globin chains, resulting in chronic anemia, ineffective erythropoiesis and compensatory marrow expansion [1]. The disease is highly prevalent in the Mediterranean region, the Middle East and South Asia, where carrier frequencies remain substantial and contribute significantly to pediatric morbidity and transfusion burden [2]. Patients with transfusion-dependent beta-thalassemia (TDT) require lifelong regular red cell transfusions to maintain adequate hemoglobin levels and suppress extramedullary hematopoiesis. Although transfusion therapy improves survival, it does not fully correct ineffective erythropoiesis and is associated with metabolic stress and iron overload–related complications [3].

Chronic erythroid hyperactivity in TDT increases the demand for micronutrients essential for DNA synthesis and cellular proliferation. Folate is a critical cofactor in one-carbon metabolism and is indispensable for thymidylate and purine synthesis, processes fundamental to erythropoiesis [4]. In conditions of sustained red cell turnover, folate stores may be rapidly depleted, predisposing patients to subclinical or overt deficiency. Previous hematological investigations have demonstrated altered folate kinetics in chronically transfused thalassemia patients, suggesting that routine supplementation is physiologically justified [5]. For this reason, folic acid supplementation is widely recommended in transfusion-dependent individuals as part of standard supportive management [6].

In addition to its hematologic significance, folate is central to homocysteine metabolism. Homocysteine is an intermediary amino acid generated during methionine metabolism and is metabolized through folate- and vitamin B12-dependent remethylation pathways [7]. Disruption of these pathways results in accumulation of plasma homocysteine. Hyperhomocysteinemia has been independently associated with endothelial dysfunction, impaired nitric oxide bioavailability, vascular smooth muscle proliferation and increased oxidative stress [8]. These mechanisms collectively contribute to atherogenesis and thromboembolic risk.

Patients with β-thalassemia represent a population already predisposed to vascular complications due to chronic hemolysis, platelet activation, endothelial injury and iron-induced oxidative stress [9].

Hypercoagulability has been increasingly recognized as a significant contributor to morbidity in thalassemia syndromes, particularly in adolescents and adults [10]. The presence of elevated homocysteine may exacerbate this prothrombotic tendency, potentially compounding cardiovascular and cerebrovascular risk. Moreover, nutritional variability, transfusion intensity and iron overload may further modulate homocysteine metabolism in this population [11].

Despite the recognized biochemical relationship between folate and homocysteine, limited data are available regarding the distribution of serum homocysteine levels among transfusion-dependent beta-thalassemia patients who are routinely receiving folic acid supplementation in real-world clinical settings. Most available evidence has focused on genetic polymorphisms or interventional supplementation studies rather than descriptive metabolic profiling in transfusion-dependent cohorts [12]. In resource-limited contexts, where adherence to supplementation may vary and nutritional monitoring is inconsistent, characterization of homocysteine status assumes additional clinical importance.

Given the growing recognition of vascular complications in thalassemia and the modifiable nature of folate-dependent homocysteine metabolism, evaluating serum homocysteine levels in transfusion-dependent beta-thalassemia patients receiving folic acid supplementation is both clinically and epidemiologically relevant. A clearer understanding of homocysteine distribution in this population may inform strategies aimed at optimizing supportive therapy and mitigating long-term vascular risk.

Accordingly, the present study was designed to determine serum homocysteine levels in transfusion-dependent beta-thalassemia patients receiving folic acid supplementation and to examine their demographic and clinical characteristics in relation to homocysteine status.

OBJECTIVES

The objective of this study was to evaluate serum homocysteine levels in transfusion-dependent beta-thalassemia patients receiving folic acid supplementation.

METHODS & MATERIALS

This cross-sectional comparative study was conducted in the Department of Transfusion Medicine at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from March 2023 to February 2024. The study population comprised transfusion-dependent beta-thalassemia patients attending the Day Care Transfusion Unit during the study period. A total of 40 patients receiving folic acid supplementation were included.

Sample Selection

Inclusion Criteria

- Diagnosed cases of transfusion-dependent beta-thalassemia confirmed by hemoglobin electrophoresis
- Age >5 years
- Both sexes
- History of folic acid supplementation for at least 6 months
- Received ≥10 lifetime blood transfusions

Exclusion Criteria

- Pregnancy or lactation
- History of chronic kidney disease or malignancy
- Use of medications affecting homocysteine metabolism (e.g., metformin, methotrexate, anticonvulsants, theophylline)
- Refusal to provide informed consent

Data Collection Procedure

Eligible participants were recruited consecutively from the transfusion unit after written informed consent was obtained. Demographic information, clinical history, transfusion details, nutritional status (BMI) and hemoglobin electrophoresis findings were recorded using a structured questionnaire and review of medical records. Anthropometric measurements were performed following standard procedures. After overnight fasting, 3 mL of venous blood was collected under aseptic conditions. Serum homocysteine was measured in the Department of Biochemistry and Molecular Biology, BSMMU, using chemiluminescent immunoassay (CLIA) technology on the automated Atellica analyzer (Siemens, Germany). All samples were processed according to the manufacturer's instructions to ensure analytical accuracy and reproducibility. Data were checked for completeness and entered into a master database for analysis.

Ethical Consideration

Ethical approval was obtained from the Institutional Review Board of BSMMU before study initiation. Written informed consent was obtained from all adult participants and from guardians in the case of minors. Confidentiality was maintained through coded data entry and no personal identifiers were disclosed. Participation was voluntary and patients retained the right to withdraw at any stage without affecting their clinical care.

Statistical Analysis

Data were analyzed using SPSS version 27.0 (IBM Corp., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were presented as frequencies and percentages. Comparisons between groups were performed using the independent sample t-test for continuous variables and the Chi-square test for categorical variables. A two-sided p-value ≤0.05 was considered statistically significant.

RESULTS

Table I shows that the baseline demographic, socioeconomic and nutritional characteristics of the study participants were comparable between the regular and irregular folic acid supplementation groups. The mean age was 15.40 ± 9.10 years in the regular supplementation group and 19.25 ± 8.93 years in the irregular group, without a statistically significant difference (p = 0.154). Most participants were adolescents. A male predominance was observed overall, although the sex distribution between groups did not differ significantly (p = 0.197). Residence, educational background and socioeconomic status were also similar across groups. A high prevalence of underweight nutritional status was observed in both groups (65.0% and 75.0%, respectively), with no statistically significant difference (p = 0.490).

Table I
Baseline Characteristics of the Study Participants (*n* = 40).

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

Sex	Male	10 (50.0%)	14 (70.0%)	0.197
	Female	10 (50.0%)	6 (30.0%)	
Residence	Urban	10 (50.0%)	12 (60.0%)	0.525
	Rural	10 (50.0%)	8 (40.0%)	
Socioeconomic Status	Poor	6 (30.0%)	11 (55.0%)	0.063
	Middle	10 (50.0%)	9 (45.0%)	
	Upper	4 (20.0%)	0 (0.0%)	
BMI Category	Underweight	13 (65.0%)	15 (75.0%)	0.49
	Normal	7 (35.0%)	5 (25.0%)	

Table II shows that transfusion-related characteristics were comparable between the two groups. The mean age at first transfusion was 1.69 ± 1.78 years in the regular supplementation group and 2.43 ± 2.24 years in the irregular group (p = 0.253). The mean duration of transfusion therapy was 13.75 ± 8.42 years and 16.75 ± 8.33 years, respectively (p = 0.264). The interval between transfusions and the approximate total number of transfusions did not differ significantly between groups, indicating similar transfusion burden and disease severity.

Table II
Transfusion Characteristics of the Study Participants (*n* = 40).

Transfusion characteristics	Group A (n=20)	Group B (n=20)	p-value
Mean age at first transfusion (years)	1.69±1.78	2.43±2.24	0.253
Duration of blood transfusion (years)	13.75±8.42	16.75±8.33	0.264
Interval between two transfusions			
< 15 days	5(25.0%)	6(30.0%)	
15-30 days	14(70.0%)	13(65.0%)	
>30 days	1(5.0%)	1(5.0%)	
Mean±SD	27.75±10.06	25.75±10.9	0.55
Total no of transfusion (approximate)			
10-50 unit	1(5.0%)	1(5.0%)	0.405
51-100 unit	9(45.0%)	5(25.0%)	
>100 unit	10(50.0%)	14(70.0%)	

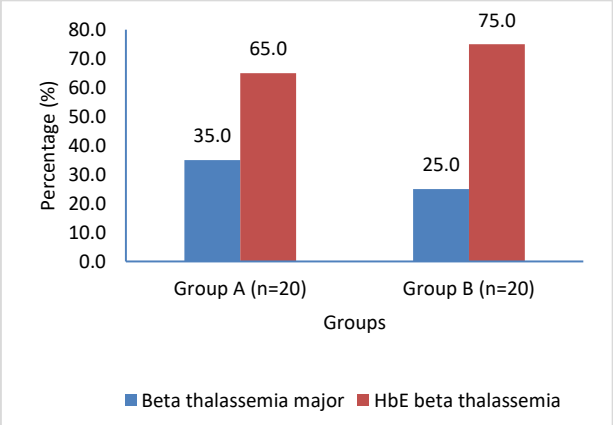

Figure 2 Hemoglobin Electrophoresis Distribution.

Figure 2 shows that HbE β-thalassemia was the predominant subtype in both groups, accounting for 70% of the overall cohort, while β-thalassemia major constituted 30%.

Table III shows that serum homocysteine levels differed markedly between the two groups. All patients receiving regular folic acid supplementation had homocysteine levels within 3–7 µmol/L, with a mean of 5.19 ± 1.04 µmol/L. In contrast, 70% of irregularly supplemented patients had levels between 8–14 µmol/L and 30% had levels ≥15 µmol/L. The mean homocysteine concentration in the irregular group was 13.68 ± 5.47 µmol/L and the difference between groups was statistically significant (p < 0.001).

Table III
Serum Homocysteine Distribution (*n* = 40).

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 evaluated serum homocysteine levels in transfusion-dependent beta-thalassemia patients receiving folic acid supplementation and examined their demographic, nutritional and transfusion-related profiles. The principal finding was that patients who maintained regular folic acid supplementation demonstrated significantly lower serum homocysteine concentrations compared with those who had irregular intake. This observation reinforces the biochemical relevance of folate in homocysteine metabolism and highlights its potential protective role against hyperhomocysteinemia in this vulnerable population.

The baseline characteristics of the study population revealed no statistically significant differences between groups in age, sex, residence, socioeconomic background, or nutritional status. The absence of demographic imbalance strengthens the internal validity of the homocysteine comparison. The predominance of adolescents and young adults in this cohort reflects the improved survival of transfusion-dependent patients in recent years with regular transfusion and supportive therapy, consistent with global epidemiological trends in beta-thalassemia [1].

Nutritional assessment demonstrated that underweight status was common in both groups. Growth retardation and low body mass index are well-documented features of transfusion-dependent thalassemia due to chronic anemia, endocrine dysfunction and increased metabolic demand [13]. Sherief et al. reported similar nutritional challenges among children and adolescents with β-thalassemia major, emphasizing the multifactorial etiology of growth impairment in this population [14]. Although nutritional status did not differ significantly between groups, the high prevalence of underweight patients underscores the importance of comprehensive metabolic support alongside transfusion therapy.

Transfusion characteristics were comparable between regularly and irregularly supplemented patients. The mean age at first transfusion and duration of transfusion therapy were similar to findings reported by Baghersalimi et al., who also described long-standing transfusion dependency in their cohort [15]. The similarity in transfusion burden suggests that differences in homocysteine levels were unlikely attributable to disease severity or transfusion exposure, thereby strengthening the association with supplementation status.

The most striking finding of this study was the marked difference in serum homocysteine distribution between groups. All regularly supplemented patients had homocysteine concentrations within the lower physiological range (3–7 µmol/L), whereas a substantial proportion of irregularly supplemented patients exhibited elevated levels, with 30% meeting criteria for hyperhomocysteinemia (≥15 µmol/L). This finding aligns with the biochemical role of folate as a cofactor in the remethylation of homocysteine to methionine [16]. Disruption of this pathway due to inadequate folate availability results in accumulation of circulating homocysteine.

Baghersalimi et al. demonstrated that cessation of folic acid supplementation in thalassemia major patients led to a significant rise in serum homocysteine levels, supporting a direct metabolic relationship [15]. Similarly, Mojtahedzadeh et al. showed that folic acid supplementation effectively corrected folate deficiency in beta-thalassemia patients, although they did not primarily focus on homocysteine as an outcome measure [17]. The present findings extend this evidence by characterizing the homocysteine profile in routinely supplemented transfusion-dependent patients and highlighting the biochemical consequence of irregular intake.

Hyperhomocysteinemia has been recognized as an independent risk factor for endothelial dysfunction, atherosclerosis and thromboembolic disease [18]. Patients with beta-thalassemia are already predisposed to vascular complications due to chronic hemolysis, iron overload and oxidative stress. Abd-Elmawla et al. further emphasized that genetic polymorphisms affecting homocysteine metabolism may exacerbate this risk in the context of folate deficiency [19]. Therefore, maintenance of optimal folate status may be particularly important in minimizing additive vascular risk in transfusion-dependent individuals.

The predominance of HbE β-thalassemia in this cohort mirrors national epidemiological data from Bangladesh and neighboring regions [1]. Rahman and Jamal previously reported a similar distribution pattern in Dhaka-based populations, reinforcing the representativeness of the present sample [20]. Although thalassemia subtype distribution was comparable between groups, the high overall frequency of HbE β-thalassemia emphasizes the regional genetic burden and the need for tailored management strategies.

Taken together, the findings of this study suggest that regular folic acid supplementation is associated with maintenance of physiologically lower homocysteine levels in transfusion-dependent beta-thalassemia patients. While this study does not establish causality due to its cross-sectional design, the biochemical plausibility and consistency with prior interventional data strengthen the observed association. These results support the continued integration of routine folic acid supplementation into standard supportive therapy for TDT patients.

CONCLUSION

Regular folic acid supplementation in transfusion-dependent beta-thalassemia patients is associated with significantly lower serum homocysteine levels compared with irregular intake. Given the established role of hyperhomocysteinemia in vascular pathology, sustained adherence to folic acid supplementation may contribute to reducing long-term thrombotic and atherosclerotic risk in this population. Routine biochemical monitoring and reinforcement of supplementation adherence should be considered integral components of comprehensive TDT management.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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