RESEARCH PAPER

Serum Folate Levels in Patients with Chronic Hemolytic Anemia on Regular Folic Acid Supplementation Before and After Dose Modification

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Objective: In light of the recommendation of folic acid supplementation in chronic hemolytic anemia, with possible supratherapeutic dosing and associated side effects, we performed this study to investigate serum folate levels in children with chronic hemolytic anemia. **Methods:** Phase 1 was a cross-sectional study of 134 patients in the Pediatric Hematology service, documenting daily dosage and performing serum folate levels. In phase 2, we reduced the dose to 1 mg for 148 patients and repeated the testing after six months. **Results:** We found very high serum folate levels with Phase 1, with 93.2% above the upper level of normal. In Phase 2, values remained high with 42.5% above the acceptable upper limit. **Conclusions:** Doses of folic acid given to sickle cell and thalassemia patients exceed their actual needs. This should be re-evaluated to strike a balance between benefit and harm, with close monitoring of serum folate levels.

Keywords: *Harm, Sickle cell anemia, Supplementation, Thalassemia*.

Folic acid supplementation has been considered an integral part of the management plan for patients with chronic hemolytic anemia. It has been known for its role in DNA synthesis and normal erythropoiesis, as well as its function through its active form, tetrahydrofolate, as a coenzyme in essential metabolic reactions [1]. The rationale for folic acid supplementation in sickle cell disease (SCD) and thalassemia has mainly been compensation for increased requirements as a consequence of chronically increased red blood cell destruction [2,3]. Another proposed benefit of folic acid supplementation is the reduction of endothelial cell damage in SCD [2]. Elevated plasma homocysteine levels in patients with SCD with low folate levels despite normal vitamin B6 and B12 status have been reported which could be attributed to suboptimal folate status rendering them susceptible to increased endothelial damage [4]. However, there are insufficient follow-up studies to monitor if prescribed doses are maintaining folate levels within therapeutic levels, and whether there are any beneficial effects of this dosage in chronic hemolytic anemia. Although the recommended dose for supplementation is 1 mg/day, lack of pediatric formulations, as well as lack of compliance to guidelines, may lead to varying doses. The objectives of present study were to measure serum folate levels in patients with chronic hemolytic anemia and its correlation with prescribed doses, as well as comparison between subgroups according to disease and blood transfusion frequency.

METHODS

The initial phase of this study was a cross-sectional evaluation of patients with chronic hemolytic anemia attending the Pediatric Hematology clinic in King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia, between June 2010 and April 2012. Approval from the local research ethics board was obtained and informed consent was obtained from the parents of all patients.

A detailed drug history, including the prescribed folic acid dosage and degree of compliance was obtained. At our center, available formulating of folic acid is 5 mg tablet, and occasionally a 1 mg tablet, of which either 1 mg or 2 mg were prescribed. The details related to dosage, the disease condition, and blood transfusion regimen were recorded. Blood investigations included complete blood count, reticulocytic count, serum folate levels, and vitamin B12 levels. Fasting serum samples were collected, and serum was separated and stored at –15 to –25°C for a maximum of 28 days. Patients were instructed to avoid biotin or folate for at least 8 hours as part of their fasting. Hemolyzed samples were excluded. Serum folate assay was performed using the Elecsys Folate 111 assay on Cobas e601 chemistry analyzer with competitive test principles using natural folate binding

protein (FBP) specific for folate. The reference range of serum folate used in our study was 11.3-46.2 nmol/L [6]. As serum folate may be affected by recent dietary factors, we assessed serum folate three times (2-monthly) over 6 months, and used the average.

Following initial results, the Pediatric Hematology team standardized the dose of 1 mg to all patients and after an adjustment period of six months for serum levels to stabilize, the patients were retested for serum folate levels. This phase started from July 2012 to December 2015. An additional 14 patients were recruited after first diagnosis of sickle cell anemia or thalassemia. Serum folate levels were performed twice with a gap of 2 months and the mean of two readings was considered for this study.

Statistical analysis: All analyses were performed using the SPSS version 20 (SPSS Inc, Chicago, IL). Descriptive data are presented as mean and SD or percentages. Chi-square test was used for comparing proportions and student's t-test for comparing normally distributed continuous variables, such as age. Mann-Whitney test was used to compare continuous variables that were not normally distributed, such as mean serum folate levels. Correlation analyses were performed using Pearson correlation test. For all tests a *P* value of < 0.05 was considered statistically significant.

RESULTS

A total of 134 patients (71 girls) were included in the first phase of the study, the details of which are presented in ***Table* I**. Of these, 119 (88.8%) were patients with sickle cell disease. Of the sickle cell patients, 21 patients were on regular blood transfusions, for previous cardio vascular accident (CVA) or recurrent splenic sequestration. The patients were on different drug doses of folic acid, with 54 (40.3%) on 1 mg daily, 26 (19.4%) on 2 mg daily, and 52 (38.8%) on 5 mg daily. Only 2 (1.5%) patients were not receiving any folate supplementation due to non-compliance. All thalassemia patients were receiving 1 mg per day of folic acid. Folate levels were high (>40 nmol/L) in 125 (93.2%) patients with mean value of 77 nmol/L. There were 9 patients with folate levels in the reference range, the two patients who were not receiving supplementation and seven on 1 mg dose regimen. There was no patient with sub-therapeutic levels. There was no significant difference in folate levels between males and females. Comparison of mean serum folate levels was performed between the three groups: thalassemia major patients, sickle cell patients on regular transfusions, and sickle cell patients with transfusions on demand. The mean folate levels were highest in the group of sickle cell disease on regular transfusion (*P*=0.02). Comparison of serum folate levels between groups according to dosage showed a slightly higher level in the group on 5 mg, compared to those on 2 mg and 1 mg (P<0.05).

TABLE I DESCRIPTIVE DATA OF PATIENTS IN PHASE 1 AND PHASE 2 OF STUDY

	Phase 1 (n = 134)	*Phase 2 (n = 148)*	*P value*
Age (y)*	6.3(3)	7.2 (3.4)	0.84
Females sex	71 (53)	81 (55)	0.91
Diagnosis			
Sickle cell disease	119 (88.8)	127 (85.8)	0.88
Thalassemia major	15 (11.2)	21 (14.2)	0.75
Oral folic acid dosage			
None	2 (1.5)	31 (20.9)	<0.001
1 mg daily	54 (40.3)	117 (79.1)	<0.001
2 mg daily	26 (19.4)	0	<0.001
5 mg daily	52 (38.8)	0	<0.001

*Data expressed as n(%) except *mean (SD).*

In the second phase of this study, serum folate level was measured for 148 patients (81 girls) (***Table* II**), all of whom were prescribed 1 mg of folic acid daily. The mean (SD) age was 7.1 (3.4) years. Of these, 127 (87%) were patients with sickle cell disease and 21 (14.4%) had thalassemia major. Of the sickle cell patients, 22 were on regular blood transfusions. Because we had given feedback to parents about the initially high doses, 29 patients were non-compliant with folic acid supplementation (19.9%). Folate levels were within normal levels in 96 (64.9%) patients. The overall mean (SD) serum folate level was 42.4 (3.6) nmol/L. Mean serum

TABLE II EFFECT OF DIAGNOSIS AND BLOOD TRANSFUSION REGIMEN ON SERUM FOLATE LEVELS IN PHASES 1 AND 2

	Phase 1		*Phase 2*		*P value*
Condition	*N*	*Mean (SD)nmol/L*	*N*	*Mean (SD)nmol/L*	
Thalassemia major	15	54.1 (4.9)	21	49.2 (4.2)	0.04
Sickle cell disease, regular transfusions	21	89.8 (11.2)	22	36.8 (4.0)	<0.001
Sickle cell disease, on demand transfusions	98	56.5 (6.2)	105	41.6 (3.8)	0.01

WHAT THIS STUDY ADDS?

- Children with chronic hemolytic anemias receiving folate supplementation had high serum folate levels.
- Even minimal doses resulted in serum levels that were higher than normal ranges.

folate levels across the three groups of thalassemia, sickle cell without regular transfusions, and sickle cell on regular transfusions was 49.2 nmol/L, 36.8 nmol/L and 41.6 nmol/L, respectively (*P*=0.6). All patients who were not compliant with oral folic acid supplementation had serum folate levels within normal limits. Fifty-two (35.1%) of 148 patients had levels above normal, and the highest reported level was 67.9 nmol/L.

All serum vitamin B12 levels before and after folic acid dose modification were within normal range. Reticulocytic count was measured in both phases with mean (SD) of 3.9 (0.6)% and 3.7 (0.7)%, respectively. There was no significant difference between the two phases and no correlation with folate levels.

DISCUSSION

In this study conducted to evaluate whether daily supplementation of folic acid in patients with chronic hemolytic anemia may lead to abnormally high serum folate levels, we found that mean serum folate levels with various dose regimens (1 mg and 5 mg) were markedly increased. We also detected higher levels in patients receiving regular transfusions, possibly related to decreased requirements with transfusion, as well as presence of folate in transfused blood. Another finding was that all the patients who chose not to comply with folate supplementation after communication of Phase 1 results had normal values after a 6-month transition period.

The high folate levels in our study are concerning, especially when there is a scarcity of studies regarding dose-related side effects of folic acid. Levels in our study were considerably higher compared to studies carried out on women receiving supplements [7,8]. Toxic effects of folic acid overdose may effect multiple systems in the body, including gastrointestinal, nervous system, hypersensitivity, metabolic and cardiovascular systems [9]. For example, megaloblastic anemia caused by cobalamin deficiency may be masked by folic acid administration [10], thereby delaying diagnosis until serious, potentially irreversible, neurologic dysfunction has occurred [11]. Another finding emphasizing the necessity of serum folate assessment is a that natural killer (NK) cell cytotoxicity was found to have an inverse relationship with the plasma folic acid levels, thus affecting an important part of the immune system [12]. In observational studies in adults, higher serum folate levels have been found to be associated with risk of breast and prostate cancers [13-17].

Required amount of supplementation was investigated by Venn, *et al*. [18] with the aim of determining the minimum effective oral folic acid dose that will significantly reduce plasma total homocysteine (tHcy) and increase serum folate levels. This was a double-blinded randomized control trial on adults and found that even a daily dose of 100 µg was sufficient. The authors concluded that low-level fortification is sufficient, otherwise individuals are at risk of receiving unnecessarily high folic acid intake. Similarly, Pena, *et al*. [19] tried to assess the lowest effective dose-response of folic acid on endothelial function in children with type 1 diabetes, using a primary outcome of flow-mediated dilatation (FMD). They found that although serum and red cell folate levels significantly improved on oral folate supplementation of 0.5, 2, and 5 mg, FMD did not, thereby concluding that there is no benefit for additional folic acid above mandatory folic acid fortification. Nguyen, *et al*. [20] recently took the questionability of efficacy of folate supplementation in SCD a step further, where they stopped supplementation in 72 young children with SCD and measured red blood cell folate levels after medication cessation [20]. Surprisingly, there was no patient with low red cell folate levels. Additionally, they compared hemoglobin and reticulocyte levels before and after folic acid discontinuation, and there were no significant differences for either. A possible confounding factor in this study may have been that the majority of these patients were on hydroxyurea, possibly accounting for decreased rates of hemolysis and therefore decreased requirements for folic acid supplementation.

A major limitation of our study is that we measured serum folate and not red cell folate. In addition to this, observational design of our study could have led to confounding factors that were not taken into consideration during the design and analysis. For example, all thalassemic patients were found to have relatively lower serum folate levels, but these same patients were all adherent to the 1 mg dose regimen. Assessment of serum homocysteine was not performed, which could have added valuable insight into potential risk of thrombosis with higher folate levels.

Our results suggest that doses of folic acid regularly prescribed to patients with sickle cell disease and thalassemia exceed their actual needs, and support the need for further studies with lower doses of oral folic acid supplementation in patients with chronic hemolytic anemias. Special considerations that could decrease rates of hemolysis and therefore hypothetically decrease folate requirements should be studied in detail such as hydroxyurea intake and regular transfusions. In the light of recent observations that higher folate levels may have adverse effects, we believe that folate levels should be monitored in such patients until further studies are performed to find out most suitable dose and formulations.

Contributors: MA: designed the study, participated in collection and interpretation of data, and drafted and finalized the manuscript; SA: assisted in study design, conducted the laboratory tests for both phases, reviewed statistical analysis of the data, and drafted part of the manuscript. Both authors approved final version of the study.
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