

## Effect of folate supplementation on hematological profile of sickled children: A longitudinal study

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### ABSTRACT

**Background:** Folic acid supplementation in sickle cell (SC) children has been a topic of interest for the past two decades. **Objective:** The objective of the study was to determine the changes in the hematological profile of cohorts of SC children after receiving folic acid tablets 1 mg/day. **Materials and Methods:** A total of 425 homozygous SC were enrolled in this present prospective study as per predefined inclusion and exclusion criteria, after receiving written informed consent from the parents/guardians. Patients were followed up at four subsequent visits (0, 6, 12, and 24 weeks). All the relevant statistics were done by SPSS v25.0. **Results:** Out of 425 subjects, 246 (57.9%) were male and 179 (42.1%) were female with a mean age of the study population being 7.09 (3.58) years. There was no significant change in the mean sickle hemoglobin, fetal hemoglobin, hemoglobin, corrected reticulocyte count, total leukocyte count, total platelet count, absolute neutrophil and lymphocyte count, mean corpuscular volume, mean corpuscular hemoglobin (MCH), and MCH concentration over a period of 24 weeks ( $p>0.05$ ); however, there was a significant increase in the mean serum folate level ( $p<0.001$ ). **Conclusion:** Folic acid supplementation has no significant effect on the hematological profile of the children with SC anemia.

**Key words:** Hemoglobin, Longitudinal analysis, Mean corpuscular volume, Other hematological parameters, Serum folic acid level, Sickle cell anemia

Sickle cell disease (SCD) is a single gene defect disorder with autosomal recessive inheritance [1]. There is deficiency of sufficient healthy red blood cells (RBCs) to carry adequate oxygen throughout the body [2]. Normally, RBCs are flexible, round, and move easily through blood vessels. However, in sickle cell (SC) anemia, they become rigid, sticky, and sickle or crescent moon shaped [3]. These irregularly shaped cells get stuck in small blood vessels, causing slowing or blocking of the blood flow and oxygen to different parts of the body causing pain (SC crisis) [4]. Furthermore, the sickled cells are short-lived due to hemolysis, leaving a shortage of healthy RBCs in SC anemia (SCA) [5]. There is increased erythropoiesis in SC patients to survive this crisis [6]. To further facilitate this increased erythropoiesis, folate supplements are often routinely prescribed.

However, folic acid supplementation in SC children is still controversial. The studies in support of folic acid supplementation have cited low serum and erythrocyte folate levels and high incidence of megaloblastic anemia in pediatric patients with SCD and the positive effects of supplementation including reversal of growth retardation, reduced dactylitis, and reduction of homocysteine levels, leading to reduced cardiovascular, stroke, and venous thrombosis risk [7].

The studies against folic acid supplementation conversely found that megaloblastic change is uncommon in SCD. They have also shown that folic acid does not lead to improvements in hemoglobin, growth characteristics, infections, splenic sequestration, and dactylitis [7]. As per the recent studies, the possible dangerous effects of supplementation are increased priapism, increased twin pregnancy rates, the risk of masking cobalamin deficiency with consequent neuropsychiatric manifestations, and most importantly the risk of colorectal, lung, pancreatic, esophageal, stomach, cervical, ovarian, breast, bladder, and other cancers [8-11]. Hence, the present study was conducted to evaluate the role of folate supplementation in treating SC anemia.

### MATERIALS AND METHODS

This was a longitudinal study conducted over a period of 2 years (November 2017 to October 2019) in the outpatient department and inpatient department of the department of pediatrics and SC institute of a tertiary care teaching hospital of Western Odisha after approval from the Institutional Ethics Committee. All the high-performance liquid chromatography (HPLC) confirmed

cases of homozygous SCA, of either gender between age groups of 1–14 years who started taking folic acid tablets within the past 1 month, were included in the study. The critically ill children, patients who received anti-folate drugs or regular goat milk during the past 6 months, patients with chronic liver or bowel diseases, and splenectomized patients were excluded from the study.

The sample size estimation was done by nMaster v 2.0 (BRTC, Vellore) applying method one sample proportion – confidence interval estimating single proportion – absolute precision method. As there was no previous study, as per rule of assumption, we took 50% prevalence of folic acid deficiency in SCA. Taking CI to be 95%, absolute precision of 5%, minimal sample size was calculated to be 384. Minimum 422 samples were required for the study after correction for attrition. Out of 6348 SCD patients who presented to our study site, 468 cases were enrolled in our study as per the predefined inclusion and exclusion criteria. Among these 48 cases were dropped out, 425 cases were included in the study after receiving written informed consent from their parents/guardians.

The serum folate was estimated by enzyme immune assay (UNION Immune Analyzer, YHLO, China). The range of normal serum folate value was taken from 11.3 to 46.2 nmol/L [12]. Patients who had serum folate level <11.3 nmol/L were considered as folate deficient. About 2 ml of serum was collected using standard sampling tubes by the principal investigator and was sent to laboratory for analysis. Grossly, hemolyzed, lipemic, microbial contaminated, and heat-inactivated samples were avoided. Repetitive freezing and thawing of samples for more than 3 times were avoided [13,14]. Specimens and reagents were made sure to be kept at 18–25°C before measurement. Two ethylenediaminetetraacetic acid venous samples 2 ml each were collected under aseptic precautions and were sent for HPLC and complete blood count (CBC). Mean sickle hemoglobin (HbS) and fetal hemoglobin (HbF) were estimated by HPLC (BioRad Laboratory, California). The CBC estimation was done by autoanalyzer (Sysmex XN1000, Japan).

All the relevant data were recorded in a predesigned case report format. Data validation was done manually by two separate persons not involved in the study. Continuous data were expressed in mean±standard deviation; categorical data were expressed in proportions. Data normalcy testing of continuous data was done by Shapiro–Wilk’s test and no transformation was required. All the relevant statistics were done by SPSS v25.0 (IBM, New York).  $p < 0.01$  was considered statistically significant.

## RESULTS

A total of 425 subjects were included in the study group. The mean age of the study participants was 7.09 (3.58). Of these, 246 (57.9%) were male and 179 (42.1%) were female, giving M:F ratio of 1.37:1. Table 1 shows the baseline characteristics of the study participants.

Assuming unequal variances among all groups (Mauchly’s test of sphericity  $p < 0.001$ ) and Greenhouse–Geisser correction

estimation, there was overall significant increase in mean serum folate level in ng/ml over a period of 24 weeks, no significant change in mean hemoglobin level, mean corpuscular volume (MCV), and mean corrected reticulocyte count. There was no significant change in mean of other hematological parameters over the period of 24 weeks (Table 2).

## DISCUSSION

The study revealed that there was a significant increase in mean serum folate level with folate supplementation over a period of 24 weeks. There was no significant change in the HbS, HbF, corrected reticulocyte count, total leukocyte count (TLC), total platelet count (TPC), absolute neutrophil (ANC) and absolute lymphocyte count (ALC), MCV, mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC).

The study done by Kennedy *et al.* in 2001 found the prevalence of folate deficiency in SCA patients to be around 15% [15]. The prevalence of folate deficiency among homozygous sickle children in our study was found to be 20.7%. In our study, after supplementation, there was a significant increase in the level of serum folate over a period of 24 weeks among both folate deficient and sufficient groups. Rabb *et al.* found that there was a significant increase in serum folate after folate supplementation [16]. Nguyun *et al.* in their study conducted at Northern Virginia in 2017 reported that there was no significant decrease in serum folate after discontinuation of folate supplementation in sickled children [17].

In our study, we found that there was no significant change in hemoglobin level after folate supplementation. This was in accordance to the results of previous studies [16,17]. In the present study, there was no significant change in MCV, MCH, MCHC, ANC, and ALC levels after folate supplementation. This was in accordance to the study done by Dixit *et al.* [18].

In our study, we found no significant change in corrected reticulocyte count, TLC, and TPC levels after folate

**Table 1: Baseline variables of the study subjects**

Baseline characteristics	Mean±SD
Weight (kg)	20.13±10.03
HbS (%)	75.56±6.69
HbF (%)	1.930±0.99
Serum folate (nmol/l)	15.45±4.30
Folic acid deficiency (number, %)	88±20.7
Hemoglobin (gm/dl)	8.48±0.98
Total leukocyte count (/mm <sup>3</sup> )	9607.46±3737.050
Absolute neutrophil count (/mm <sup>3</sup> )	6801.31±2676.207
Absolute lymphocyte count (/mm <sup>3</sup> )	2359.61±1118.724
Total platelet count (lakhs/mm <sup>3</sup> )	2.65±1.03
Mean corpuscular volume (fl)	71.03±9.67
Mean corpuscular hemoglobin (pg)	25.450±3.045
Mean corpuscular hemoglobin concentration (g/dl)	33.375±2.596
Corrected reticulocyte count (%)	2.16±0.84

SD: Standard deviation

Table 2: Longitudinal analysis of variables after correction for Mauchly's test of sphericity

Variables, mean (SD)	0 weeks	6 weeks	12 weeks	24 weeks	Epsilon (€)	G.G. correction	p value*
HbS <sup>@</sup> (%)	75.56 (6.69)	75.55 (6.93)	75.54 (6.67)	75.54 (6.94)	0.000	0.130	0.741
HbF <sup>#</sup> (%)	1.93 (0.99)	1.94 (0.98)	1.96 (0.97)	1.95 (0.99)	0.010	4.311	0.035
TLC <sup>^</sup> (/mm <sup>3</sup> )	9607.46 (3737.050)	9608.00 (3757.72)	9606.54 (3757.58)	9607.28 (3814.88)	0.000	0.000	0.994
ANC* (/mm <sup>3</sup> )	6801.31 (2676.21)	6803.25 (2987.72)	6800.71 (3058.36)	6806.34 (3074.22)	0.000	0.000	1.000
ALC <sup>%</sup> (/mm <sup>3</sup> )	2359.61 (1118.72)	2360.70 (1146.291)	2373.68 (1217.010)	2390.35 (1301.040)	0.003	1.484	0.228
TPC <sup>†</sup> (lakh/mm <sup>3</sup> )	2.65 (1.03)	2.66 (1.04)	2.65(1.06)	2.64 (1.09)	0.000	0.001	0.991
MCH <sup>‡</sup> (pg)	25.45 (3.05)	25.440 (3.166)	25.421 (3.620)	25.406 (4.173)	0.000	0.000	0.997
MCHC <sup>€</sup> (gm/dl)	33.38 (2.60)	33.289 (2.697)	33.389 (2.915)	33.351 (3.101)	0.000	0.000	1.000

<sup>@</sup>Hemoglobin S, <sup>#</sup>hemoglobin F, <sup>^</sup>total leukocyte count, <sup>\*</sup>absolute neutrophil count, <sup>%</sup>absolute lymphocyte count, <sup>†</sup>total platelet count, <sup>‡</sup>mean corpuscular hemoglobin, and <sup>€</sup>mean corpuscular hemoglobin concentration, SD: Standard deviation, G.G. correction: Greenhouse - Geisser correction

supplementation. This was in accordance to the results obtained by Rabb *et al.* [16]. At present, there is an undergoing randomized control trial in Canada [19] and the results are awaited.

The study had a few limitations. It was an observational study where causal relation could not be established. It was a hospital-based study which could have included some patients with acute illnesses; inclusion of only steady-state sickle patients could have yielded more reliable results. There are increased chances of involvement of bias in the study such as recall bias and reporting bias, a form of information bias. Here non-random sampling was used, but random sampling technique is a better option, as selection bias could not be prevented in non-random sampling. Confounder and effect modifier could not be adjusted at the stage of design and analysis level.

## CONCLUSION

Folic acid supplementation has no effect on hematological profile of sickled children. However, its detrimental effects on health are worrisome. Hence, there should be judicious use of folic acid supplementation among SC children and larger multicentric trials with longer duration of follow-up are required to weigh the risk versus benefits of folic acid supplementation for better level of evidence.

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