RESEARCH PAPER

Effect of Pre-treatment Nutritional Status, Folate and Vitamin B12 Levels on Induction Chemotherapy in Children with Acute Lymphoblastic Leukemia

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Objective: To evaluate pre-treatment undernutrition, and folate and B12 deficiency in children with acute lymphoblastic leukemia, and their correlation with complications and outcome of induction chemotherapy.

Design: Observational study.

Setting: Tertiary care teaching hospital in Northern India.

Participants: 50 children with acute lymphoblastic leukemia.

Procedure: Children were assessed for nutritional status (Weight for age Z-score, serum albumin, folate and B12) at presentation, and were followed-up during induction for bone marrow response, counts and outcome. Folate and B12 were repeated twice at monthly intervals after induction. Univariate and multivariate analyses were done to determine the association of nutritional parameters with the outcome variables.

Results: Baseline undernutrition was observed in 66%, hypo-albuminemia in 32.6%, folate deficiency in 41.3% and B12 deficiency in 36.9% of included children. Significant decline in folate levels was noted on serial assays during chemotherapy (*P*=0.001). Folate deficient children had higher risk for delayed marrow recovery and counts on day 14 (*P*=0.007 and *P*=0.001). Hypoalbuminemia (*P*=0.04), B12 deficiency (*P*=0.001) and folate (*P*=0.03) deficiency were associated with toxic deaths during induction.

Conclusion: Baseline nutritional deficiencies negatively influence the outcome and occurrence of complications during induction chemotherapy in children with acute lymphoblastic leukemia.

Keywords: *Acute leukemia, Nutrition, Outcome, Protein energy malnutrition, Vitamin deficiency.*

Lower survival of childhood acute lymphoblastic leukemia (ALL) in the developing countries as compared to the developed ones has been related to higher risk of infection, undernutrition, lack of adequate supportive care, and poor compliance to therapy [1]. The proportion of undernourished children with ALL varies from nearly 10% in the developed nations [2,3] to more than 60% in the developing countries [4-6]. Low folate and B12 levels are common in Indian children [7], and lower levels in children with ALL has been earlier reported [8]. Folate deficiency was found to increase chemotherapy-induced toxicity in rats [9], and its repletion has been demonstrated to mitigate methotrexate toxicity in adults with rheumatological diseases [10]. Vitamin B12 deficiency has also been associated with disordered hematopoiesis and dysfunctional immune response leading to increased susceptibility to infections [11]. Most of the deaths seen in children from the developing countries undergoing treatment for ALL occur during the initial phases of treatment due to infections and intolerance to chemotherapy [12,13]. Studies investigating the nutritional factors behind this poor outcome are scarce. The present study reports the effect of nutritional parameters, serum albumin, folate and B12 levels in children with ALL on recovery of bone marrow and peripheral blood counts as well as mortality during induction phase.

Accompanying Editorials: Pages 379-81.

METHODS

This study included children with newly diagnosed ALL presenting to the pediatric hematology-oncology unit of , a tertiary care teaching and referral institute in Northern India, from August 2010 through July 2011. Written informed consent was obtained from parents/guardians of these children prior to enrolment. The study was approved by the institutional ethics committee of our Institute. Children treated prior to being referred to our hospital, and those with relapsed disease were excluded from this study.

Participants were assessed for their pre-therapy nutritional status including anthropometric parameters

(weight-for-age Z-scores), serum albumin, vitamin B12 and folate levels. Serum folate and vitamin B12 levels were repeated twice at 1 month intervals. Weight was measured using a digital weighing scale and the average of three repeated measures was recorded. Serum levels of albumin was estimated using the Bromocresol green method whereas serum folate and B12 levels were measured using Electrochemiluminescence by Cobas e411 analyser. Undernutrition was defined as Weight-for-age Z-score <-2 (WHO) and severe undernutrition as Weight-for-age Z-score <-3(WHO). Serum folate levels <4 ng/mL and B12 levels <203pg/mL were taken as deficiency [14]. Socio-economic status was assessed by the modified Kuppuswamy scale [15].

Induction chemotherapy for children with standard risk ALL (age >1yr and <9 yr and TLC <50,000/mm^3) included 4 weeks of daily prednisolone, weekly vincristine (4 doses), intrathecal methotrexate (3 doses) and 9 doses of L-Asparginase. The high risk group (all the remaining children) additionally received weekly pulses of daunomycin. Bone marrow recovery was assessed by the bone marrow cellularity (reduction of ≥25% of normal cellularity was taken as incomplete recovery) [16] at 14th and 28th day of induction. Recovery of peripheral blood counts at 14th and 28th day of induction was defined as an absolute neutrophil count (ANC) >1000/mm^3 and platelet counts >100,000/mm^3. Toxic death during induction was defined as death associated with neutropenia (ANC<1000) along with fever or other features of sepsis after initiating induction chemotherapy till post-induction recovery of peripheral blood counts.

Statistical Package for Social Sciences (SPSS) software version 16 (IL, USA) was used to analyze the data. Intergroup comparison was done using Fischer's exact or Chi-square tests in case of categorical variables, and Student's t-test for continuous variables. Mann-Whitney and Kruksal-Wallis tests were used for nonparametric data. Multivariate analysis using logistic regression was used to determine the independent effects of the various nutritional parameters and chemotherapy regimen on the complications and outcome of induction chemotherapy.

RESULTS

Of the 58 newly diagnosed children with ALL registered during the study period, 50 (39 males) were eligible and consented for participation. The mean (SD) age of these children was 7.5 (3.2) years. Immunophenotyping could be successfully done in 45 patients (B-ALL- 37, T-ALL- 8). Forty-one (82%) of these 50 children were from lower socioeconomic strata whereas 1(2%) and 8 patients were from upper and middle strata, respectively. Twenty-one (42%) children had high risk ALL. Undernutrition was observed in 33 (66%) patients; of these 10 (20%) were severely malnourished. Pre-induction folate and B12 deficiencies were observed in 19 (38%) and 17 (34%) children, respectively. Four children were excluded from subsequent analysis due to refusal of treatment by parents of three children and tumour lysis related early death in another child. Of the remaining 46 children, 17 (40%) had toxic deaths during induction. Therefore post-induction folate and B12 levels could be done in only 29 patients. A consistent and significant decline in serial folate levels during initial two months of chemotherapy (P=0.001) (***Fig* 1** and ***Table* I**) was observed.

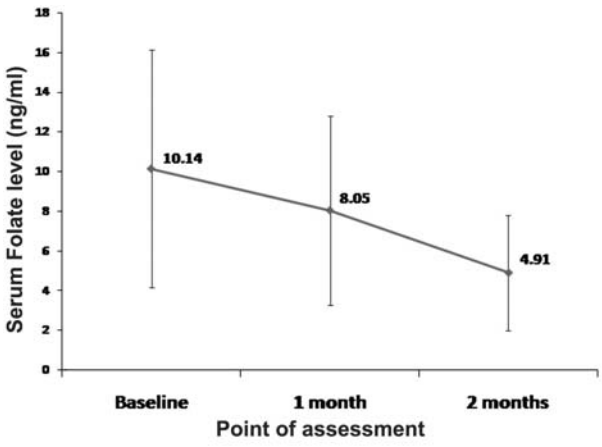

FIG. 1 *Trend of serum folate levels in patients with acute lymphoblastic leukemia on chemotherpy (n=29).*

Incomplete bone marrow recovery at days 14 and 28 of induction was documented in 33 of 46 (71%) and 3 of 29 (9%) children respectively. Recovery of peripheral blood counts beyond 14 days was seen in 27 (58 %) patients, and all of these children had incomplete bone marrow recovery at day 14. Undernutrition had no significant association with incomplete bone marrow recovery at day14, delay in recovery of peripheral blood counts or toxic induction deaths (***Table* II**).

TABLE I SEQUENTIAL CHANGE IN B12 AND FOLATE LEVELS WITH CHEMOTHERAPY (N=29)

	Baseline; Median (range)	*1 month; Median (range)*	*2 month; Median (range)*	*P-value*
Serum folate (ng/mL)	8.5 (1.28-20)	7.2 (1.28-20)	4.65 (1.28-11.8)	0.001
Serum B12 (pg/mL)	304.8 (30-1071)	250.2 (90-1218)	257.2 (102-1818)	0.58

TABLE II EFFECT OF PRE-TREATMENT VITAMIN B12 AND FOLIC ACID ON COMPLICATIONS IN CHILDREN ON CHEMOTHERAPY FOR ALL (*N*=46)

Variable	*No.*	*Bone marrow recovery*		*Toxic induction deaths (n=17)*
		Incomplete (n=33)	*Delayed (n=27)*	
Serum folate				
<4 pg/mL	19	18	17	10
≥ 4 pg/mL	27	15	10	7
Vitamin B12				
<203 ng/mL	17	10	10	12
≥203 ng/mL	29	23	17	5
Serum albumin				
<3.5 mg/dL	15	8	7	9
≥3.5 mg/dL	31	25	20	8
WAZ-score				
<-2	33	23	20	13
≥-2	13	10	7	4

Incomplete bone marrow recovery at day 14 of induction (*P*=0.007), delayed recovery of peripheral blood counts (*P*=0.001) and toxic induction deaths (*P*=0.003) were more common in folate deficient children as compared to the folate replete (***Table* II**). Toxic induction deaths were more common in B12 deficient children as compared to the B12 replete (*P*=0.001) (***Table* II**).

In multivariate analysis (logistic regression), folate deficiency continued to be a significant risk factor for incomplete bone marrow recovery (OR 16.77, 95% CI 1.62, 173.48; *P*=0.01) and delayed recovery of counts (OR 7.71, 95% CI 1.6, 36.23; *P*=0.01). This effect was even stronger than the effect of aggressive chemotherapy (***Table* III**).

DISCUSSION

In the present study, baseline folate deficiency was found to be an important determinant of recovery of blood counts and bone marrow at day 14 of induction. Hypoalbuminemia, vitamin B12 and folate deficiencies were all found to be significantly associated with toxic deaths during induction chemotherapy.

The present study had limitations of small sample size at enrolment and a high mortality rate during induction, precluding assessment of post-induction nutritional status.

The high prevalence (66%) of baseline under-nutrition in our patients was comparable to reports from other Indian centres [4,5,17] but was not found to be associated with poor survival in our patients unlike that in the previous reports [4,5], possibly due to different methods of assessment of nutritional status [5]. The association of low serum albumin levels with worse outcome in children with ALL has also been reported by a previous study [18]. A high prevalence of folate and B12 deficiency in children with ALL documented in our study is in consonance with another study from Southern India. Dysfunctional immunity (both cellular and humoral), low CD4 and CD8 counts, decreased natural killer (NK) cell function and low immunoglobulin levels have all been documented in vitamin B12 deficient individuals [11], and may explain the increased risk of toxic deaths noted in our B12-deficient children.

A progressive decline in folate levels during chemotherapy was seen in our patients irrespective of

TABLE III EFFECTS OF NUTRITIONAL PARAMETERS ON BONE MARROW RECOVERY AT DAY 14 AND TOXIC INDUCTION DEATHS (*N*=46)

Variables	*No. (%) (n=46)*	*Bone marrow recovery*				*Toxic induction deaths (n=17)*	
		Incomplete (n=33)		*Delayed (>D14) (n=22)*			
		OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
S. Folate <4 ng/mL	19 (41.3%)	16.77 (1.62 -173.48)	0.02	7.71 (1.6-36.23)	0.01	2.53 (0.56-11.49)	0.23
S. vitamin B12							
<200 pg/mL	17 (36.9%)	1.51 (0.21- 11.09)	0.68	4.49 (0.83-24.20)	0.08	7.95 (1.27-49.66).	0.03
S. albumin				.		.	.
< 3.5 g/dL	15 (32.6%)	0.27 (0.04-1.65)	0.16	1.52 (0.30-7.63)	0.61	9.74 (1.48-63.97)	0.02
WAZ score ≤-2	33 (71.7%)	0.54 (0.06-4.56)	0. 57	0.38 (0.07-2.05)	0.26	0.83 (0.16-4.41).	0. 82
High-risk chemotherapy regimen	21(45.6%)	6.73 (1.17-38.62)	0.03	3.14 (0.73-13.59)	0.13	3.03 (0.72-12.72)	0.13

D14: day 14 of induction; S: Serum.

WHAT IS ALREADY KNOWN?

- Protein energy malnutrition adversely affects the outcome of induction chemotherapy in children with acute lymphoblastic leukemia

WHAT THIS STUDY ADDS?

- Hypoalbuminemia, B12 and folate deficiency are individually associated with adverse outcomes during induction chemotherapy for childhood acute lymphoblastic leukemia.

pre-treatment folate levels. However, a study from US [19] has reported increasing levels of folates following induction for ALL and a fall in folate levels following consolidation with higher doses of methotrexate. The fall in folate levels documented in our patients occurred with intrathecal methotrexate alone, and could possibly be much higher during phases of treatment with higher doses of methotrexate and consequently lead to higher toxicity [20, 21].

Folate and B12 supplementation may be an answer to circumvent these toxicities in deficient patients. This may be more relevant in India due to a predominant vegetarian diet [22], the dietary restrictions imposed on these children during chemotherapy, and concomitant antifolate therapy. However folate supplementation during chemotherapy for cancer has been fraught with controversies due to the fear that folates may reduce chemotherapy efficacy and support neoplastic cell growth [20,21]. On the other hand, studies in adults under treatment for malignant pleural mesothelioma document better median overall survival in patients supplemented with folic acid and B12 [23,24].

The present study highlights the need for a careful consideration of folate and B12 supplementation in deficient children on treatment for ALL in order to reduce the short term mortality. The findings need to be confirmed by similar studies with larger sample size from different areas of the world, especially the resource-poor regions with higher prevalence of folate and B12 deficiencies.

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