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ORIGINAL ARTICLE

Profile of anemia in acute lymphoblastic leukemia patients on maintenance therapy and the effect of micronutrient supplementation

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Abstract

Background Anemia is a common finding and important cause of morbidity in patients with acute lymphoblastic leukemia (ALL) at diagnosis or during the course of its protracted treatment. We studied profile of anemia in ALL patients on maintenance therapy and evaluated specific micronutrients as cause of this anemia.

Patients and methods ALL patients who were on maintenance therapy and had grade ≥ 2 anemia were recruited for the study. Serum iron studies, folate, and vitamin B12 were done to identify micronutrient deficiency and to initiate supplementation with specific components if found to be deficient. Toxicities, improvement of anemia, micronutrient levels, and disease outcome were studied after 3 months.

Results From March 2015 to September 2016, 105 ALL patients were found to be on maintenance fulfilling the inclusion criteria. Overall, the proportion of anemia was 80%($N = 84$). Majority had normocytic normochromic anemia (71%). Macrocytic anemia was seen in 18% and microcytic hypochromic in 9.5%. In patients with anemia of grade ≥ 2 ($N = 84$), 38 patients (45%) had biochemical deficiency of serum folate, and 7 (8%) had vitamin B12 deficiency. No biochemical evidence of iron deficiency was found. Supplementation of deficient micronutrients improved anemia: mean hemoglobin significantly increased from 8.06 ± 1.63 to 10.78 ± 1.53 ($p < 0.001$) at 3 months; and reduced treatment toxicities, mean number of febrile neutropenia episodes ($p = 0.007$), and treatment interruptions of > 2 weeks ($p = 0.002$) were lowered. Patients with anemia had significantly more relapses ($N = 14,64\%$) compared to patients without anemia ($N = 8,36\%$), ($p = 0.040$).

Conclusion Timely identification and correction of micronutrient deficiencies causing anemia in ALL patients on maintenance can enhance treatment outcomes.

Keywords Anemia · Micronutrient · Iron · Folate · Vitamin B12 · ALL

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Introduction

Anemia is one of the most common clinical signs of acute lymphoblastic leukemia (ALL) which can cause significant morbidity. Fatigue and other associated symptoms related to anemia impair the quality of life. Literature data suggests that 75% of children suffering from ALL present with hemoglobin that can be 10 g/dl or less accounting for anemia of grade 2 or more [1]. A number of factors have been proposed to cause anemia in ALL such as coexisting infections, overcrowding of the marrow, and nutritional deficits.

Iron, folic acid, and vitamin B12 are important components of hematopoiesis. These micronutrients have been found to be deficient in cases of acute leukemia. According to Bhakhri et al. [2] prevalence of anemia in children with

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lymphoreticular malignancy is 80%, and in majority of these children, iron deficiency was an etiological factor. Besides its role in hematopoiesis, folic acid is essential for cellular proliferation and survival. Normal cells as well as tumor cells utilize folate to synthesize or replicate their DNA. Folate, antifolate, and reduced folate transport into the cells is mediated by low-affinity receptor or reduced folate carrier (RFC) and high-affinity receptor or human folate receptor (FRα). FRα binds to folic acid with a ten-fold higher affinity than other reduced folates like folinic acid and methotrexate. RFC take up reduced folates like folinic acid and methotrexate with much higher affinity than folic acid [3]. As folate is important for methylation reactions of DNA, folate deficiency is a possible mechanism to abnormal DNA methylation and hence predispose to malignancy [4–7]. Low folate levels in pregnancy have been found to be associated with ALL. Supplementing the mother with folate reduces the risk of development of ALL in child [8]. However, studies have also found that folate supplementation may increase tumorigenesis [9]. Chemotherapeutic drugs like methotrexate used in ALL act against enzymes involved in folate metabolism (DHFR, dihydro folate reductase). Comprehensive knowledge is lacking regarding the evidence and appropriateness of the supplementation of folate during antifolate chemotherapy [10]. Vitamin B12 is needed as a cofactor of folate-metabolizing enzymes. Liu et al. [11] studied the variation of serum levels of vitamin B12, ferritin, and folate in acute leukemia (both lymphoblastic and myeloid) patients at diagnosis and at different time points of treatment. The serum levels of vitamin B12 and ferritin were significantly higher at relapse or in patients who were not in remission than in the patients in complete remission, whereas the serum folate levels were significantly lower in relapsed patients compared to patients in remission [11].

Indian and international data is lacking regarding the prevalence and type of anemia in ALL patients on maintenance therapy who receive prolong courses of antifolate drugs for almost 2 years as per various protocols. The compulsory fortification of food with folates precludes folate supplementation studies in western population [12, 13]. Hence, we undertook this study to characterize the clinical profile of anemia and to observe the clinical outcomes after supplementing the deficient nutrients.

Materials and methods

Study population

This prospective, single-center study was conducted from March 2015 to September 2016 at JIPMER (Jawaharlal Institute of Post-graduation Education and Research), Regional Cancer Centre, Puducherry, India. All adult and pediatric patients of ALL on maintenance therapy (irrespective of duration from start of maintenance) were included in the study. Patients on other phases of ALL treatment and ALL patients who relapsed before reaching maintenance phase of treatment were excluded from the study. Informed consent was obtained from all enrolled patients or parents/LAR (legally authorized representative) of patients less than 18 years, and study was approved by the Institute Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki and Indian Council of Medical Research (ICMR) guidelines.

Objectives of the study were to assess the proportion of anemia in ALL patients on maintenance therapy, to identify the clinico-demographic factors associated with anemia, to determine the clinical profile of anemia in terms of grade and type, and to evaluate the proportion of micronutrient deficiency in patients with anemia. We also aimed to assess the effect of micronutrient supplementation on improvement of grade of anemia in the study population who are having deficiency of iron, folate, or vitamin B12 and to study effect of respective supplementation on short-term treatment–related toxicity and disease outcomes in these patients.

Profiling of anemia

Patients were recruited into the study cross-sectional and prospectively as when they met the criteria for inclusion, i.e., development of anemia of grade 2 or more (CTCAE V4.0) at any time during their maintenance phase of treatment. Complete blood count (CBC) was done in every visit for all patients who were on maintenance phase. Anemia was typed as microcytic hypochromic (MCHC), macrocytic (MACRO), and normocytic normochromic (NCNC) based on peripheral smear (PS) and red cell indices [14]. At the time of study entry, 3 ml of clotted blood was collected to detect micronutrient deficiency, i.e., serum iron, ferritin, vitB12, and folic acid in all patients. The serum level of iron was determined by immunoturbidimetric analysis and serum folate, B12, and ferritin by chemiluminescence assay. The deficiency range of serum folate was kept as 0.35–5.33 ng/ml and serum levels > 5.33 ng/ml was considered normal [15, 16]. For vitamin B12, 211–911 pg/ml was considered normal range. Patients with serum levels < 211 pg/ml were considered deficient, whereas more than 911 pg/ml was considered hypervitaminosis B12. For serum iron, < 60 mcg/dl (male) and < 35 mcg/dl (female) were considered in range of deficiency and > 160 mcg/dl (male) and > 145 mcg/dl (female) were considered iron overload. Serum ferritin 0.68–34.5 ng/ml was considered in the range of deficiency and 334.6–8573 ng/ml was considered overload.

Nutritional assessment

It was done for all patients at study entry using anthropometric measurements consisting of height and weight to determine weight for age for 0–5 years, body mass index (BMI) for 5–18 years and above 18 years. *Z* scores were calculated using anthro [17] and anthro plus tools [18] by WHO to classify the nutritional status (details described in supplementary text).

Supplementation

Patients with clinical and hematological and/or biochemical evidence of micronutrient deficiency were supplemented according to the type of anemia with the respective micronutrient. Patients with clinical and hematological evidence of microcytic hypochromic or macrocytic anemia were supplemented even pending the biochemical tests. Patients with normocytic normochromic anemia (NCNC) were supplemented only when the biochemical tests for serum levels of folate, B12, ferritin, or iron were low. For iron deficiency, participants of < 2 years were supplemented 25 mg iron + 100–400 μg folic acid daily for 3 months, 2–12 years—60 mg iron + 400 μg folic acid daily for 3 month, and adolescents and adults—120 mg iron + 400 μg folic acid daily for 3 months [19]. For vitamin B12 deficiency, > 18 years were given 2000 mcg daily orally for 2 weeks, then 1000 mcg daily orally for 3 months; and < 18 years—1000 mcg daily orally for 2 weeks, then 500 mcg daily orally for 3 months [20]. For only folic acid deficiency, the recommended dietary allowance (RDA) for folic acid is 200 to 400 mcg in children and 400 to 800 mcg in adults. But due to convenience of dosing in our study, we have supplemented 5 mg one tablet to adults and half tablet to children. After 3 months of supplementation, the serum levels of deficient micronutrient were again rechecked.

Patients with anemia who did not have deficiencies were not given any micronutrient supplementation considering chemotherapy-induced myelosuppression and they were followed up for disease outcomes. Patients were followed up until the end of their maintenance and/or the final date of data analysis.

Statistical analysis

Analysis of data was done using Statistical Package for Social Sciences (SPSS) software version 19 (IL, USA). Data for continuous variables was expressed as mean with SD and/or median with range. Data for categorical variables were expressed as frequency and proportion. For independent categorical variables, chi square test was used and for non-parametric variables Fisher's exact test. McNemar's test (exact) was used for dependent categorical variables. For non-parametric data, Mann Whitney *U* test for independent continuous variables and Wilcoxon signed rank test for dependent continuous variables were used as applicable. Statistical significance was tested at $P < 0.05$.

Results

One hundred sixty-four patients were registered with diagnosis of ALL, and during the study period, 105 patients who reached maintenance phase of ALL therapy were recruited into the study. Details of the remaining 59 patients who did not reach maintenance during the study period are shown in the recruitment flow chart (Fig. 1).

Baseline clinico-demographic characteristics at the time of diagnosis ($N = 105$)

Median age for the study cohort ($N = 105$) was 10 (range 0.6–55) years. Pediatric patients (age < 18 years) were 74% ($N = 78$) and adults were 26% ($N = 27$). Diagnosis was pre B ALL in 75% ($N = 79$) and T ALL in 25% ($N = 26$) as shown in Table 1.

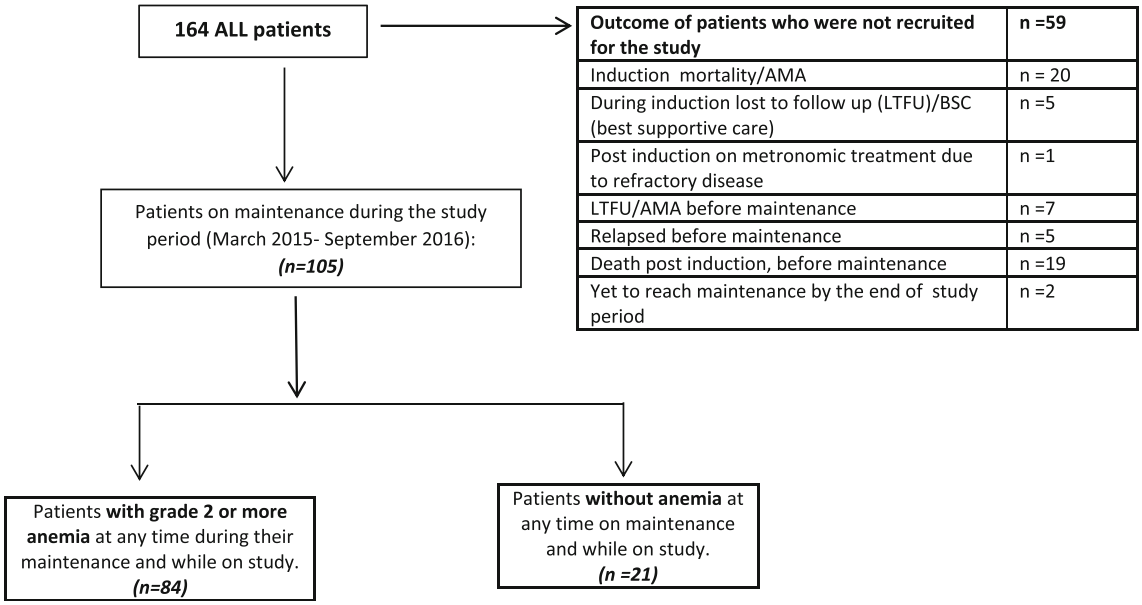
Proportion of anemia

Overall, the proportion of anemia (≥ grade 2) in our maintenance study cohort of 105 patients was 80% ($N = 84$). Twenty-one patients (20%) never developed ≥ grade 2 anemia at any time during their maintenance course while on study. Median time to study entry was 1.6 (range 0.1–20.5) months from the start of maintenance.

Factors associated with anemia during maintenance

No association was found between the baseline demographic factor (age, sex), disease-related factors (subtype of ALL), mean hemoglobin at diagnosis, and development of anemia during maintenance. Patients who had higher mean hemoglobin (11.34 + 1.34 g/dl) at the start of maintenance were significantly less likely ($P < 0.001$) to develop anemia later on during the maintenance compared to patients with low hemoglobin at start of maintenance (9.69 + 1.51 g/dl) as shown in Table 2.

Nutritional assessment of the study population showed that 83% pediatric patients of 0–5 years had normal nutrition at diagnosis, start of maintenance, and at study entry. Among the patients of age group > 18 years, majority (70% at diagnosis, 78% at start of maintenance, 76% at the study entry) had normal nutrition, among the age group of 6–18 years, 48% of the patients at the time of diagnosis, 53% at the time of start of maintenance, and 28% at the time of study entry had malnutrition. The baseline poor nutrition status and change in nutritional status during treatment were most noticeable in this age group of 6–18 years most likely due to pre-pubertal growth spurt and higher nutritional requirements. In our study, we found that in pediatric patients (age 0–5 years and 6–18 years), the presence of malnutrition did not significantly affect development of anemia ($P = 1.000$ and



Outcome of patients who were not recruited for the study	n =59
Induction mortality/AMA	n = 20
During induction lost to follow up (LTFU)/BSC (best supportive care)	n =5
Post induction on metronomic treatment due to refractory disease	n =1
LTFU/AMA before maintenance	n =7
Relapsed before maintenance	n =5
Death post induction, before maintenance	n =19
Yet to reach maintenance by the end of study period	n =2

Fig. 1 Recruitment flow chart

0.152 respectively). Among participants > 18 years, anemia was more common in patients with normal nutritional status (Supplementary Table 1 in Online Resource 2). However, this association is biologically implausible and could be because of small sample size.

Table 1 Baseline clinico-demographic factors

Study parameters at the time of diagnosis	Number	Percentage (%)
Median age in years(N = 105) = [10 years; range 0.58–55 years]		
Adults(age > 18 years)	N = 27	25.7
Pediatrics(age≤18 years)	N = 78	74.3
Gender		
Male	N = 65	61.9
Female	N = 40	38.1
Subtype of ALL		
B ALL[a]	N = 79	75.2
Philadelphia-positive ALL (N = 3, 3.7%)		
T ALL	N = 26	24.7
Risk group		
Pediatrics (NCI criteria) (N = 78)		
Standard risk	N = 33	42.3
High risk	N = 45	57.7
Adults (GMALL criteria) (N = 27)		
Low	N = 9	33.3
High	n = 18	66.7
CNS involvement		
CSF positive	N = 3	2.8
CSF negative	N = 102	97.1

[a] One patient was having biphenotypic leukemia and was treated like B ALL, so was analyzed as B ALL

Profile of anemia during maintenance

One fourth of the patients had grade 3/4 anemia (N = 23; 27%) at study entry, and three fourth (n = 61; 73%) were grade 2. At the study entry, majority of the patients had normocytic normochromic type of anemia (N = 60; 72%), 18% (N = 15) patients had macrocytic anemia, and 9.52% (N = 8) had microcytic hypochromic anemia. Combined microcytic hypochromic + macrocytic (dimorphic) type of anemia was reported in 1 patient.

Among the study population (patients with anemia of grade 2 or more, N = 84), 38 patients (45%) had biochemical deficiency of serum folate, and 7 (8%) had vitB12 deficiency. Four (5%) patients had both folate and vitB12 deficiency. Although according to the peripheral blood picture and RBC indices, 8 (9.5%) patients had microcytic hypochromic anemia; none of the patients had biochemical evidence of iron deficiency when serum iron and serum ferritin levels were analyzed. The mean folate level of patients with folate deficiency was 2.49 + 1.29 ng/ml (N = 38) and mean vitamin B12 level of patients with vitamin B12 deficiency was 155.29 + 61.17 pg/ml (N = 7). Twenty-five (30%) patients had a higher than upper limit of the normal vitB12 values, and in 74 (88%) patients, the serum ferritin levels were in the range of iron overload as shown in Table 2. Patients with anemia and micronutrient deficiency had higher grade 3/4 anemia (61%, n = 14) compared to patients without micronutrient deficiency (39%, n = 9) but it was not statistically significant (P = 0.223).

In patients with folate deficiency (N = 38), the mean folate levels in NCNC anemia group were higher than in macrocytic anemia group (2.66 ± 1.27 vs. 2.03 ± 1.37), although not statistically significant (P = 0.227), implying that a higher degree of deficiency (lower folate levels) may be required for morphological change.

Table 2 Evaluation of micronutrient deficiency for patients with anemia at study entry

Serum levels and micronutrient deficiency	Mean ± SD	Number of patients with normal range	Number of patients with deficiency	Number of patients with higher than upper limit of normal serum levels
Serum folate levels(ng/ml)	6.01 ± 4.39 (median = 5.8)	44 (52.38%)	38 (45.24%)	2 (2.38%)
Serum B12 levels (pg/ml)	840.31 ± 632.85 (median = 647)	52 (61.9%)	7 (8.33%)	25 (29.7%)
Serum iron levels (ng/ml)	422.72 ± 370.63 (median = 299.5)	14 (16.6%)	0	70 (83.3%)
Serum ferritin levels (mcg/dl)	947.04 ± 538.11 (median = 931.15)	10 (11.9%)	0	74 (88%)
Patients with iron deficiency	Yes = NIL			
	No = 84 (100%)			
Patients with vitb12 deficiency	Yes = 7 (8.33%)			
	No = 77 (91.67%)			
Patients with folate deficiency	Yes = 38 (45.24%)			
	No = 46 (54.76%)			
Combined folate and vitB12	4 (4.76%)			
Patients with all 3 micronutrient deficiency	NIL			

Effect on hematological and biochemical parameters after 3 months of supplementation ($N = 41$)

All patients with micronutrient deficiency were supplemented ($n = 41$). Out of 41 patients, 24 patients had biochemical micronutrient deficiency as well as morphological changes in peripheral smear and 17 patients had only biochemical evidence without hematological abnormality in CBC and PS.

As shown in Table 3 in patients with micronutrient deficiency, the mean hemoglobin significantly improved after supplementation of the deficient micronutrients from 8.06 + 1.63 to 10.78 + 1.53($P < 0.001$). After supplementation, the mean serum folate level of these patients improved significantly ($P < 0.001$). We found improvement in serum B12 levels, but it was not statistically significant ($P = 0.176$) possibly due to small numbers in B12-deficient group.

Effect of supplementation on toxicity parameters

After supplementation with deficient micronutrients, the mean number of febrile neutropenia (FN) episodes ($P = 0.007$), and treatment interruptions of > 2 weeks due to any cause ($P = 0.002$) were significantly reduced. There was neither significant reduction in dose modification of 6 MP and methotrexate nor change in occurrence of mucositis of any grade after supplementation (Table 4).

Disease outcomes during the study period

At time of analysis (December 31, 2016), out of 105, 22 patients, 21% had relapsed. Of these, 21 patients died and one patient was on supportive care at last follow-up. Out of total 22 deaths, 21 died due to relapse but death was reported in one

Table 3 Effect of supplementation on hematological and biochemical parameters

Parameters	Before supplementation	After supplementation at 3 months	*P* value
Mean Hb ($N = 41$)	8.06 ± 1.63	10.78 ± 1.53	< 0.001
No anemia	NIL	13 (31.71%)	< 0.001 (McNemar's exact)
Grade 1 anemia	NIL	20 (48.78%)	
Grade 2 anemia	$N = 27$ (65.9%)	$N = 6$ (14.63%)	
Grade 3 anemia	$N = 13$ (31.7%)	$N = 2$ (4.88%)	
Grade 4 anemia	$N = 1$ (2.44%)	$N = 0$	
Deficient micronutrients			
Mean folate levels (ng/ml) ($N = 38$)	2.49 ± 1.29	9.79 ± 6.57	< 0.001
Improvement in folate level to the normal range ($N = 38$)		Yes = 26 (68.4%)	
		No = 12 (31.2%)	
Mean B12 levels (pg/ml) ($N = 7$)	155.29 ± 61.17	268.14 ± 157.02	0.1763
Improvement in B12 level to the normal range ($N = 7$)		Yes 4 (57.14%)	
		No 3 (42.86%)	

Table 4 Effect of supplementation on toxicity in those with micronutrient deficiency ($N = 41$)

Parameters		Before supplementation	After supplementation	P value
Febrile neutropenia (mean number of episodes)		1.63 ± 0.74 (median 2; range 1–4)	1.04 ± 0.98 (median 1; range 0–3)	0.007 (Wilcoxon signed rank)
Dose modification 6 MP and methotrexate	Yes	$N = 12$ (29.27%)	$N = 7$ (17.07%)	0.180 (McNemar's exact)
	No	$N = 29$ (70.7%)	$N = 34$ (82.93%)	
Treatment interruptions more than 2 weeks	Yes	$N = 15$ (36.6%)	$N = 5$ (12.2%)	0.002 (McNemar's exact)
	No	$N = 26$ (63.4%)	$N = 36$ (87.8%)	
Mucositis any grade	Yes	$N = 11$ (26.8%)	$N = 8$ (19.5%)	0.549 (McNemar's exact)
	No	$N = 30$ (73.2%)	$N = 33$ (80.5%)	

patient due to febrile neutropenia. Fifty-nine percent of patients ($N = 62$) continued to be on maintenance and treatment was completed in 23% ($N = 24$).

As discussed in Table 5, patients in our study cohort with anemia had significantly more relapses ($N = 14$, 64%) compared to patients without anemia ($N = 8$, 36.3%) ($P = 0.040$). Patients with folate deficiency anemia who were supplemented with folate did not have higher rate of relapse compared to patients who had non folate–deficient anemia ($P = 0.773$).

Discussion

Anemia can be caused by cancer itself or by the cancer therapy. In a systematic review, anemia prevalence in cancer patients varied between 30 and 90%. Quality of life was positively correlated with treatment of anemia [21]. Common causes of anemia in acute leukemia are bleeding, bone marrow infiltration, excessive destruction of red blood cells (RBC) in peripheral blood, dysfunctional RBC production, and inhibition of hematopoiesis due to abnormal iron metabolism.

We found overall proportion of anemia of grade 2 or more (hemoglobin ≤ 10 g/dl) in our study cohort at any time during the maintenance therapy to be 80%. Our result is similar to the reported incidence of anemia of 75% for ALL patients at diagnosis by Steele et al. [1]. The mean hemoglobin of our study participant having grade 2 or more anemia at any time during maintenance was 8.351 ± .51 mg/dl. Most of the patients had anemia at the time of diagnosis, but those patients whose hemoglobin normalized at the start of maintenance did not develop anemia later during maintenance phase. In our study, malnutrition was more prevalent among the age group of 6–18 years but we could not find any meaningful association between malnutrition and development of anemia.

Majority of patients had NCNC anemia and a quarter of patients had grade 3/4 anemia in our study cohort. Folate deficiency was seen in 38 patients (45%) and 7 patients (8%) had vitB12 deficiency. After patients were supplemented with folic acid and vitamin B12, there was significant improvement in the mean folate levels, whereas mean vitamin B12 levels did not improve significantly. Tandon et al. [22] found in their study cohort of ALL patients on induction chemotherapy that 19 (38%) patients had folate deficiencies and 17 (34%) patients had B12 deficiency. There was significant reduction of folate and B12 levels in patients during the first 2 months of chemotherapy [22]. In another study from South India, Sadananda Adiga et al. [23] compared the folate and B12 levels of ALL patients at induction with healthy controls. They found significantly low levels of folic acid (8.56 ± 4.35 ng/ml) as compared to controls (14.04 ± 2.62), although vitamin B12 levels were not significantly different. Liu et al. [11] in their study have found 83.3% ALL patients at diagnosis, and 69% at relapse have abnormal serum folate levels (low) compared to 20% in remission. Similarly, only 16% of patients have abnormal vitamin B12 levels at diagnosis or in remission compared to 54% patients in relapse. Although the above-mentioned studies have reported folate and B12 deficiencies, none of them had done supplementation. Clinical symptoms and signs of vitamin B12 deficiencies are most of the times nonspecific and patients can be asymptomatic for a prolonged period with deranged serum levels. Low dietary intake can cause its deficiency in ALL patients. Conversely, excess serum cobalamin has also been associated

Table 5 Disease outcome in relation to anemia and folate deficiency

Disease outcomes	Relapsed ($N = 22$)	No relapse ($N = 83$)	P value
Patients with anemia ($N = 84$)	14 (63.6%)	70 (84.3%)	0.040 (Fisher's exact)
Patients without anemia ($N = 21$)	8 (36.3%)	13 (15.6%)	
Relapse in patients with anemia			
	Relapsed ($N = 14$)	No relapse ($N = 70$)	*0.*773 (Fisher's exact)
Folate deficiency anemia ($N = 38$)	7 (50%)	31 (44.2%)	
Patients with anemia without folate deficiency ($N = 46$)	7 (50%)	39 (55.8%)	

with solid tumor and malignant hematologic diseases [24–26]. In our study cohort, although 25 (30%) patients had a higher than upper limit of the normal vitB12, its clinical significance was not apparent. There have been controversies for supplementing folic acid during antifolate therapy as it is believed that folate supplementation may interfere with action of methotrexate. There have been other evidences also challenging the theory of disease progression with folate supplementation during antifolate therapy [27–34]. In our study supplementation, folate was not associated with higher relapse rates in the given short follow-up period; nonetheless, it resulted in improvement in grade of anemia and treatment-related toxicities.

Bhakhri et al. [2] have evaluated serum iron status of children with lymphoreticular malignancies (LRMs) at diagnosis and at the end of induction therapy. Among 35 children of lymphoreticular malignancy, 31 children had ALL. Compared to the normal population, 80% cases had anemia. Iron deficiency was a major causative factor of anemia. Treatment of leukemia resulted in improvement of anemia in majority of patients [2]. However, in the case of acute leukemia, the incidence of iron overload has been more commonly reported than iron deficiency. Halonen et al. [35] have reported 14% children develop long-term iron overload after treatment of ALL due to transfusions. Eng et al. [36] have quantified in their study that children with ALL receive average of 115 ml/kg of blood (77 mg/kg iron) during treatment with high-risk patients receiving significantly more transfusions and iron. In our study, we found no biochemical deficiency of iron, although 8 patients had microcytic anemia. In 92 (88%) patients, the serum ferritin levels were in the range of iron overload. The high serum iron and ferritin levels could be related to the number of transfusions these patients received during maintenance or before that. In our cohort, the mean number of packed cells transfused was 2.11 ± 1.49 (median = 1, range 1–6) during the study period. Therefore, it is prudent to keep these patients on long-term follow-up for effects of iron overload and treat if present [35, 36].

When treatment outcome was evaluated in our study population, patients with folate and B12 deficiency who received supplementation had significantly less episodes of febrile neutropenia and treatment interruptions of more than 2 weeks, without any increase in the risk of relapse. Our findings are in concordance with Tandon et al. [22] who have reported more incomplete bone marrow recovery at day 14, prolonged cytopenias, and toxic deaths in folate-deficient ALL children compared to folate replete children during induction therapy. Similarly, toxic deaths were significantly more common in children with B12 deficiency [22]. We found higher relapse rates in patients having anemia compared to patients without anemia most likely due to poor chemotherapy tolerance and more treatment breaks. The data in literature regarding association of relapse with presence of anemia is insufficient. Our limitations were a small sample size and a short follow-up which precludes definitive interpretation of long-term relapse outcomes. Also, because of the absence of modern risk stratification elements as cytogenetic, molecular markers, and minimal residual disease status for many of our patients, we did not analyze association of other baseline factors including risk group with relapse rate. Therefore, the relapse outcome has to be cautiously interpreted in the background of suboptimal risk stratification and short follow-up.

Conclusion

Apart from the disease-related factors, issues related to supportive care can improve treatment outcome of ALL. Anemia and nutritional deficiencies impair adherence to treatment protocols and patient tolerance to chemotherapy, which can cause unwarranted interruptions of maintenance therapy. Appropriate micronutrient supplementation can enhance compliance and reduce toxicities.

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Compliance with ethical standards

Informed consent was obtained from all enrolled patients or parents/LAR (legally authorized representative) of patients less than 18 years, and study was approved by the Institute Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki and Indian Council of Medical Research (ICMR) guidelines.

Conflict of interest The authors declare that they have no conflicts of interest.

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