RESEARCH PAPER

Iron Overload in Children with Leukemia Receiving Multiple Blood Transfusions

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Objective: To find out prevalence of iron overload in children with leukemia at the end of treatment, and to identify factors affecting iron overload. **Methods**: Children (age-1-14 y) treated for Leukemia of our center who completed treatment between January and August 2016 were included in the study. Serum ferritin and iron were measured at completion of treatment and total blood transfusion received throughout treatment was quantified. Serum ferritin >1000 ng/mL was considered as marker of transfusional iron overload. **Results:** Out of 66 participants, 55 (83.3%) received red cell transfusions. Average transfused volume was 48 mL/kg, and patients with high-risk leukemia received more transfusions than standard-risk patients. 16 patients (24.2%) demonstrated transfusional iron overload. Total transfused volume and treatment intensity were significant factors associated with iron overload, and total transfused volume of >100 mL/kg (approximately 10 transfusions) was the most important determinant of transfusional iron burden. **Conclusion:** One-fourth of pediatric leukemia patients demonstrated iron overload at the end of treatment. These patients need to be monitored and followed-up after treatment to assess need for later chelation therapy.

Keywords: *Acute lympoblastic leukemia, Complications, Ferritin, Transfusion therapy*.

Children with hematological malignancies receive multiple packed red cell (PRBC) transfusions throughout their treatment due to several reasons like bone marrow suppression, blood loss, repeated infections, nutritional anemia, renal insufficiency etc. This puts them at risk of iron overload and its long-term complications like thyroid dysfunction, growth retardation, diabetes, delayed puberty, cardiomyopathy and hepatic insufficiency. This contributes to morbidity and decreased life expectancy in survivors of pediatric leukemia, who are already at risk of cardiac and hepatic dysfunction due to late effects of chemotherapy. Currently there are no guidelines for monitoring iron status in pediatric leukemia patients.

METHODS

Children (age <14 y) with leukemia treated in our hospital who completed chemotherapy between January and August 2016 were included in this prospective observational study. Objective was to quantify the total volume of PRBC transfusions received throughout treatment and to determine the prevalence of transfusion-related iron overload. Approval for the study was obtained by the Institutional Ethical Committee, and informed consent was obtained from parents.

Patients with acute lymphoblastic leukemia (ALL) were stratified into standard-risk and high-risk based on age, WBC count at presentation, presence of organomegaly or central nervous system (CNS) disease, immunophenotype and cytogenetics of leukemic blasts and response to steroids. Treatment was based on BFM 95 (Berlin-Frankfurt-Munster) protocol consisting of initial phases of remission induction, consolidation, CNS preventive therapy and re-intensification using steroids, Vincristine, Daunorubicin, L-asparaginase, Cyclophosphamide, 6-mercaptopurine, Cytarabine, and intrathecal methotrexate. Chemotherapy was augmented in high-risk patients with additional doses of anthracyclines, high-dose methotrexate and presymptomatic cranial radiation. Following this, all patients received prolonged low-dose maintenance chemotherapy with Vincristine, prednisolone, 6-mercaptopurine and oral methotrexate. Total duration of treatment was two-and-a-half years. Children with acute myeloid leukemia (AML) received aggressive chemotherapy consisting of 2 induction courses with Daunorubicin and Cytarabine and 3 consolidations with high dose Cytarabine.

At the end of chemotherapy, single estimation of serum iron and ferritin was done. Age at diagnosis, type

and risk of leukemia and total transfusions received (TTV) were obtained from medical records. Patients who received any therapy or transfusions from outside institutions, patients with disease relapse, and those with incomplete transfusion records and active infection were excluded. TTV >100 mL/kg was considered as transfusional overload and serum ferritin >1000 ng/mL were taken as determinant of iron overload.

Sample size needed was 55 assuming a population proportion of 37% with iron overload [1] with sample proportion 20% with 5% alpha error and 80% power.

Statistical analysis: Chi-square test or Fisher's exact test was used for comparison of categorical variables between the same groups. Conditional logistic regression was used to estimate the odds ratios (OR) and 95% CI to measure the association between iron overload and variables analyzed. Logistic regression model was applied to identify independent predictors of iron overload. Multivariate model was constructed using variables found to be statistically significant in univariate analysis, after excluding potential confounding factors by assessing for interactions among the variables. Missing values and incomplete data were excluded from the analysis. Statistical software SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and all statistical tests were performed at the two-tailed significance level of 0.05.

RESULTS

The study group comprised of 66 children (37 boys); 31 (47%) were aged 1-6 years and 35 (53%) were aged 7-14 years at diagnosis. Fifty-eight patients (88%) had ALL and 8 patients (12%) had AML.

Fifty-five patients (83.3%) received PRBC transfusions during treatment. TTV ranged from 10-210 mL/kg, with mean TTV of 48 mL/kg. AML patients received more transfusions (mean 110 mL/kg, range 60-100 mL/kg) than ALL patients (mean 37 mL/kg, range 10-110 mL/kg), and high-risk ALL patients received more transfusions (mean 58 mL/kg, range 30-110 mL/kg) than those with standard-risk ALL (mean 30 mL/kg, range 10-60 mL/kg). Mean ferritin level was also higher for AML patients (1148 ng/mL) as compared to ALL patients (566 ng/mL).

Iron overload was found in 16 patients (24.2%), with mean ferritin levels 1228 ng/mL (range 1060-2660 ng/mL). They received higher TTV (average 71 mL/kg), with 12 out of them receiving TTV>100 mL/kg. Half of the AML patients (4 out of 8), and 20% of ALL patients (12 out of 58) demonstrated iron overload (***Table* I**).

On univariate analysis, iron overload had statistically significant association with type of leukemia, treatment intensity and TTV, and was not associated with age or gender of child (***Table* II**). On multivariate analysis, TTV >100 mL/kg (>10 transfusions) was the only significant determinant of iron overload (P=0.003).

DISCUSSION

Our study demonstrated that as high as 88% of pediatric leukemia patients received PRBC transfusions during treatment and nearly one-fourths of them developed iron overload at treatment completion, which is a significant percentage. Multiple PRBC transfusions are unavoidable in pediatric leukemia patients because of current intensive chemotherapy protocols, aggressive supportive care and liberal transfusion practices [1-3]. Transfusion-

TABLE I DESCRIPTIVE CHARACTERISTICS OF CHILDREN WITH LEUKEMIA (N=66)

Variables	*All patients (n=66)*	*Patients with Iron overload (n=16)*
Age (y); Median (range)	6.0 (1.5-14.0)	10.0 (1.5-14.0)
Male gender	37	8
Leukemia type		
ALL Standard risk	35	5
ALL high risk	23	7
AML	08	4
Total transfused volume (mL/kg); Mean (SD)		
All leukemia patients	47.82 (41.35)	71.33 (54.95)
ALL patients	37.02 (27.58)	62.22 (30.73)
AML patients	111.25 (53.03)	145 (56.86)
S. Ferritin level (ng/mL); Mean (SD)		
All leukemia patients	637.1 (631.84)	1228.9 (1060.27)
ALL patients	566.58 (592.30)	1103.6 (863.89)
AML patients	1148.63 (714.26)	1625.3 (1140.09)

ALL: *acute lymphoblastic leukemia; AML*: *acute myeloid leukemia*

TABLE II UNIVARIATE ANALYSIS OF FACTORS AFFECTING TRANSFUSION RELATED IRON OVERLOAD

Variables	*OR (95% CI)*	*P value*
Age >7y	2.6 (0.8, 8.8)	0.116
AML patients	8.3 (1.4, 48.5)	0.019
High-risk ALL patients	2.2 (0.6, 8.2)	0.78
Treatment intensity	5.6 (1.1, 28.3)	0.035
PRBC >100 mL/kg	12.2 (2.1,72.3)	0.006

ALL: *acute lymphoblastic leukemia; AML*: *acute myeloid leukemia; PRBC*: *packed red blood cell.*

WHAT THIS STUDY ADDS?

- One-fourth of pediatric leukemia patients develop iron overload at the end of chemotherapy.
- Patients who receive more than 10 transfusions, and those who receive more intensive chemotherapy should be monitored for iron burden.

related iron overload becomes a real concern because of overlapping organ toxicity with late effects of chemotherapy. It is reported in 19-40% of pediatric oncology patients in various studies [4-6]. Factors affecting iron burden in different studies are higher cumulative volume of transfusions, high-risk disease, intensity of treatment, age of the child and body surface area [2,4,7]. In our analysis, iron overload was influenced by treatment intensity and TTV. These two factors are inter-related, with patients on more aggressive therapy getting more transfusions due to prolonged marrow suppression and intercurrent infections. In a study by Eng and Fish [2], ALL patients received an average of 77 red cell transfusion mg/kg of and high-risk patients received up to 130mg/kg, suggesting the link between number of transfusions and leukemia risk type [2].

The general practice of PRBC transfusion in pediatric cancer patients on chemotherapy is to transfuse at hemoglobin levels of 8-10 g/dL, or if symptomatic like clinical pallor, fever, respiratory distress or cardiac failure [5]. The same policy is followed in our institution. Patients with transfusion dependent chronic conditions like thalassemia are reported to experience iron overload with as much as 10 transfusions [2,3]. Screening for iron overload is recommended in transplant or myelodysplastic patients [6], but no such guidelines exist for pediatric oncology patients because transfusion dependence is temporary and lifelong iron accumulation is not expected.

A major drawback of our study is that it is a cross-sectional study looking at one-point measurement of serum ferritin, and the trend over time is not available. Existing literature demonstrates difference of opinion regarding what happens to the accumulated iron over time. Some studies report that elevated serum ferritin levels eventually decline in almost all patients without any iron removal therapy by three years, probably due to iron consumption with growth [7]. In contrast, many other investigators have demonstrated that iron overload persisted in a significant number of patients beyond 1-3 years after treatment, even if no further transfusions are administered [8-10].

The small sample size and non-availability of tissue iron estimation may be considered as a limitation of our study. Serum ferritin was selected as marker of iron overload because it is easily measured, non-invasive and values >1000 ng/mL denote clinically relevant iron burden [2,9,10]. Disadvantage of using serum ferritin alone is that it may be elevated in infections and due to the malignancy itself. In our study, ferritin was estimated at the end of treatment when bone marrow had recovered and inflammatory response was not expected. Tissue iron studies being invasive and technically challenging, were not done in our patients.

Our study identified an important aspect of treatment toxicity which may have long-term implications in a significantly high number of patients who are already at risk of late sequelae of chemotherapy. Based on our present results, we suggest monitoring of iron status in pediatric leukemia patients who receive 10 transfusions or more, for early detection of iron overload. In high-risk patients who are planned for intensive chemotherapy, policy of lowering of threshold of PRBC transfusion to hemoglobin <7 g/dL or limiting transfusion only for symptomatic patients may also be considered in order to minimize iron burden.

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