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Original Article

Study of transfusion-related iron overload (trio) in pediatric patients with hematological malignancy and bone marrow failure syndromes

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Abstract: Background: Pediatric patients with hematological malignancy and bone marrow failure syndrome receive multiple transfusions before diagnosis and treatment. Iron overload leads to damage to vital organs like the heart, liver, thyroid, Gonads, Pancreas. Materials and methods: A prospective study was done from June 2017-December 2019 in a tertiary care pediatric hematology oncology unit in northern India on children diagnosed with hematological malignancy and bone marrow failure syndromes receiving packed cell transfusion. After due ethical considerations and patient consent, the details were documented in predesigned proforma. All cases were planned to be investigated with Liver function test, Thyroid function test, Serum ferritin level, 2 D Echocardiography, Ultrasonography of abdomen, and MRI of the abdomen at admission and six months of enrollment. Results: Out of 58 cases enrolled, ferritin levels were high in 65% of subjects at the start of treatment and 76% at the endpoint. Mean ferritin level was 725 ng/ml at baseline and 1268 ng/ml end of 6 month follow up period. Fifty-seven percent had a ferritin level above 1000 ng/ml, which correlated to basal ferritin level (*P*-value 0.005). The final ferritin level correlated strongly with the final number of packed cell transfusions (*P*-value 0.0002). Functional derangement of the liver was evident biochemically in 13.7% before starting treatment and 31.8% at six months follow-up period. Echocardiography detected diastolic dysfunction in 2% of patients at baseline before starting treatment and increased to 22% in 6 months follow-up period. The percentage of subclinical hypothyroidism increased from 22.8% to 48.8% during treatment. Conclusion: Like transfusion-dependent anemias, children with hematological malignancy and bone marrow failure syndrome on chronic transfusion are at risk of transfusion-related iron overload and organ damage.

Keywords: Blood transfusion, iron overload, leukemia, pediatric patients, ferritin levels, BM failure

Introduction

Iron overload or hemochromatosis has various etiologies, of which the most common is transfusional iron overload [1]. The most common cause of the iron overload is Hemolytic anemia [2]. Both iron overload and chemotherapy-related organ toxicity have overlapping features [3]. In hematological malignancies, blast cells proliferate in the bone marrow leading to the inadequacy of other required cell lineages, causing severe anemia. There is also autoimmune destruction of RBCs due to autoantibody production, leading to ineffective erythropoiesis [4]. Bleeding, nutritional deficiency [5] contributes to anemia. In Bone marrow failure syndromes, there is gross failure to produce cells of all lineages in the bone marrow, which may result from a drug, aromatic compounds, viral infections (2% of acute viral hepatitis [6], parvovirusB19 infection), ionizing radiations. The Iron taken orally is absorbed by reducing ferric to ferrous by a ferrireductase and taken up by DMT-1 to intestinal cells and subsequently to

blood by Ferroprotein and Hephastin, and the absorption of oral Iron is regulated at this stage by increasing or decreasing the number of DMT-1 receptors [7]. The transfused Iron, however, doesn't have such a regulatory mechanism of absorption. Transferrin becomes saturated after the administration of 10-15 units of packed red cell [8]. The circulating Iron, when in excess, is toxic to various tissues of the body.

Iron accumulates in the liver, heart, and endocrine organs (thyroid, pancreas), leading to heart failure, liver failure, diabetes, and hypothyroidism. Ferritin and hemosiderin by Fenton's reaction form free radicals [9] that lead to oxidative damage of the cells. Other than transfusion Hemolysis [10], nutritional supplementation and ineffective erythropoiesis also lead to iron overload in these groups of patients. The majority of cardiovascular problems in hematological malignancy patients are chemotherapy-related toxicities produced by anthracyclines [11] and cyclophosphamide, isocyanide, and high dose cytarabine [12]. Anthracycline chemotherapy is associated with acute effects (occurring during and shortly after administration), e.g., electrocardiographic alterations including prolongation of QT interval, development of late ventricular potentials, and various arrhythmias [13]. This complication can occur at lower doses in susceptible individuals such as the elderly, and children [14]. As per Altena, diastolic measurements are probably the most sensitive to early changes in cardiac function [15]. The clinical patterns of liver injury are defined as hepatocellular; elevation in serum enzyme levels is taken as an indicator of liver injury, whereas increases in bilirubin levels, albumin concentration, and the prothrombin time are measures of overall liver function [16]. This toxicity is manifested in a variety of patterns. In addition to hepatitis and cholestasis, we find steatosis, ductal injury fibrosis, cirrhosis, veno-occlusion, peliosis hepatis, and nodular regenerative hyperplasia [17]. In a contemporary study of 5-year survivors in the Childhood Cancer Survival Study, the cumulative proportion affected with hypothyroidism was the highest (32.3%) in survivors of Hodgkin lymphoma, and hypothyroidism was attributed especially to bleomycin and cyclophosphamide [18]. Thyroid dysfunction can occur dramatically, or it may not be diagnosed until decades into survivorship after previous therapy because childhood cancer survivors experience a steadily increasing cumulative incidence for all thyroid disorders [19]. Diabetes-related autoantibodies are seen in 20% of ALL patients [20].

Iron overload can be treated with chelation therapy by deferoxamine and other iron chelators like Deferiprone [21] if detected early. Still, there is no distinct guideline for starting chelation in these patients according to biochemical or radiological evidence.

The most standard method for screening iron overload is Serum ferritin [22]. The normal level for males is 25-300 ng/ml and females 10-300 ng/ml. When the level is above 300 ng/ml, it may indicate iron overload [23] though a level >1000 ng/ml is definitive of iron overload. Cardiac hemochromatosis is checked by Echocardiography in supine or left lateral decubitus position and the liver is evaluated by Ultrasound of the abdomen. MRI is the radiological imaging modality of choice for, the assessment of organ damage due to iron overload [24].

Material and methods

Our prospective cohort study aimed to find the proportion of pediatric patients admitted with hematological malignancy and bone marrow failure syndromes who developed iron overload. The prevalence and pattern of organ damage in hematological malignancy and bone marrow failure syndromes were also documented. We tried to see a correlation between severities of iron overload with disease status. All children diagnosed with hematological malignancy and bone marrow failure syndromes at our center and receiving packed cell transfusion were enrolled in the study over 18 months. Children were treated as a part of the observational cohort, and the control group was excluded for ethical considerations, as the child cannot be denied transfusion if clinically indicated. The clinical and pathological parameters were correlated pre- and post-transfusion. The study was undertaken to highlight the effect of chronic transfusion on children with hematological malignancy and BM failure syndromes. Their systemic effects of iron overload were studied and summated, as shown in results. Children already receiving treatment were excluded from the study.

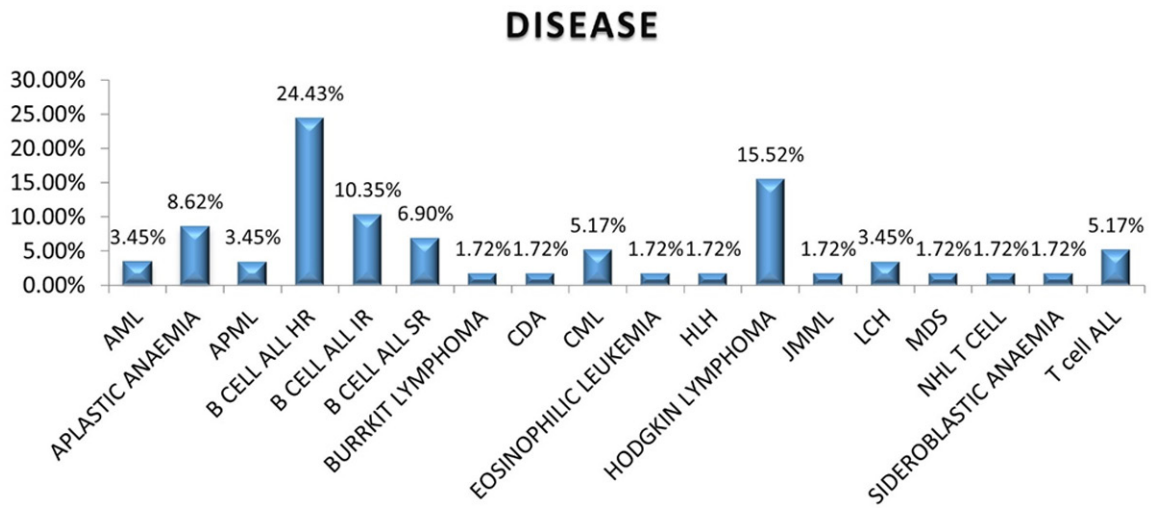

Figure 1. A frequency bar chart showing the distribution of diseases in the study subjects.

Inclusion criteria: (1) Children aged 1-12 years with an underlying diagnosis of BM failure syndrome and transfusion requirement. (2) Children aged 1-12 years who were diagnosed with malignancy at our centre and having transfusion requirement due to underlying disease or due to chemotherapy-induced bone marrow suppression.

Exclusion criteria: (1) Patients who were partially treated outside before admission in the center. (2) Patients with co-existing infectious diseases like-HIV, HCV, and HBV infection at admission. (3) Patients with underlying organ dysfunction (cardiac, hepatic) at time of presentation.

Results

In this study, 58 patients were enrolled with different hematological malignancies and bone marrow failure syndromes. **Figure 1** shows the underlying diagnosis in the study cohort.

In our study, the hematological malignancy was the most common malignancy associated with chronic blood transfusion. High risk acute lymphoblastic leukemia comprised 23.43% of the study population. There were five patients (8.62%) with idiopathic bone marrow failure syndrome and one patient each with sideroblastic anemia and congenital dyserythropoietic anemia.

Demography

In the demographic profile, 34.5% of the subjects were below five years of age, and the rest were above five years, and 79.3% of the subjects were male. The study showed a higher percentage of the over five population because of acquired deficiency and also the presentation of the disease is commoner at this age group. Also, the male predominance highlights the gender disparity in the population seeking tertiary care medical help.

Laboratory investigations

The volume of packed cell transfusion received before starting treatment varied from zero to 120 ml/kg packed cells (**Figure 2**). Of 58 study subjects, 65% had high ferritin levels (>300 ng/ml) before starting treatment, and 35% had ferritin levels above 1000 ng/ml. Twenty-two percent of study subjects had subclinical hypothyroidism with a serum TSH level above 5 IU/ml with normal T4 and normal T3 levels. Of all study subjects, 13.8% had a deranged Liver function test.

Radiology investigations

However, at baseline, USG abdomen and MRI abdomen did not show any significant abnormality in hepatic architecture. Fasting Blood Sugar level of all study subjects was normal at baseline. 2 D Echocardiography could be done for 50 subjects before starting treatment, which revealed 2% of patients with diastolic dysfunction.

Follow up results

Children enrolled were followed up for six months over the 24 months of study duration.

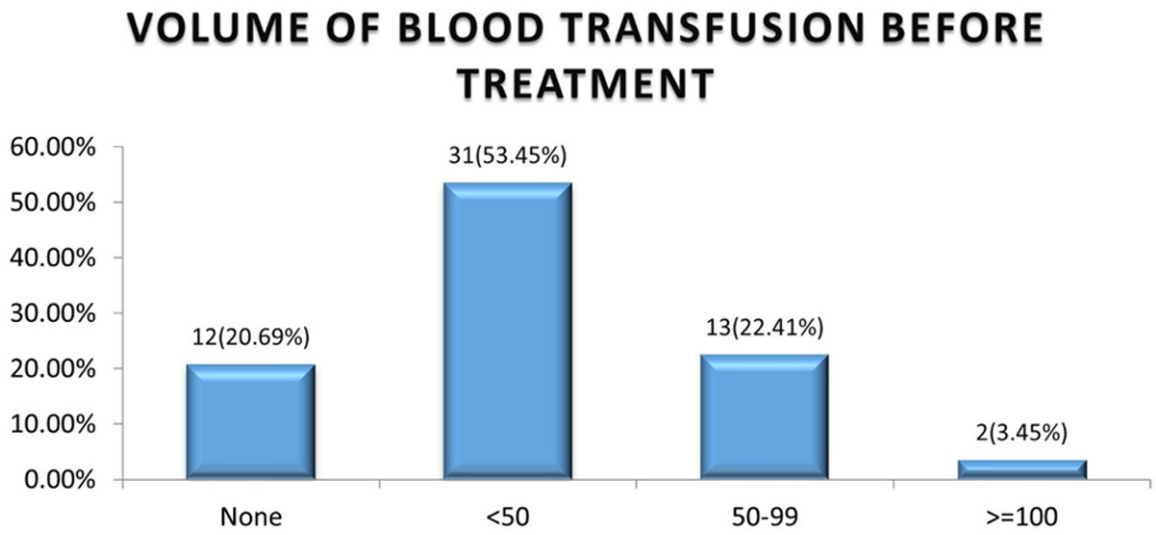

Figure 2. Depicting the distribution of study subjects receiving packed cell trnasfusion (ml/kg) before treatment.

Sixty-three percent of patients received more than five transfusions, whereas 16% received more than ten packed cell transfusions overall by the time of 6 months follow-up period.

At six months follow up of enrolled subject, 57.8% of patients had ferritin level above 1000 ng/ml, and only 24% of patients had a ferritin level in the normal range (13 patients lost to follow up). Of all subjects tested for Serum TSH level at the end of 6 months, 48.8% had high Serum TSH level. Despite a significant increase in the number of subjects with high serum TSH levels, no patient had abnormal T3 and T4 levels.

Compared to 13.7% of patients having deranged LFT, it was found that by the end of 6 months, 31.8% had significantly deranged liver function test with transaminases above three times the normal range with another (13 subjects lost due to death or loss of follow up, data of one subject could not be collected).

USG whole abdomen was done at the end of 6 months. It did not reveal any significant difference in the echotexture of the liver despite significantly deranged liver function in many patients as evident biochemically. MRI abdomen was done at the end of 6 months follow up period, and no difference was spotted in the architecture of the liver as compared to the initial study.

Fasting blood sugar level of all subjects followed up till six months did not reveal any abnormalities abdomen also didn't find any significant abnormality in the echo-texture of the pancreas of the subjects. The percentage of patients with diastolic dysfunction increased from an initial value of 2% to 22.8% at the final six months follow-up time.

In our experience, all patients inadvertently had some degree of transfusion overload, which was reflected by high serum ferritin levels. Baseline ferritin was a strong predictor of final ferritin level as 57 percent of subjects having ferritin level above 1000 ng/ml at the end of 6 months follow up period had a high initial ferritin level of 1000 ng/ml, which was statistically significant (*P*-value 0.005). Retrospectively basal ferritin level was found to be a predictive factor for the final number of packed cell transfusion with strong statistical association (*P*-value 0.018).

As iron overload was determined by ferritin level, it was strongly correlated with the initial number of packed cell transfusions received before treatment, statistically significant (*P*-value 0.041). Baseline ferritin level was a strong predictor of final ferritin level (*P*-value 0.005).

Eighty-three percent of subjects with baseline ferritin between 500 and 1000 ng/ml had final ferritin level above 1000 ng/ml, and only in 11% of patients with baseline ferritin level above 1000 ng/ml, the final ferritin level came down below 1000 ng/ml which was found to be

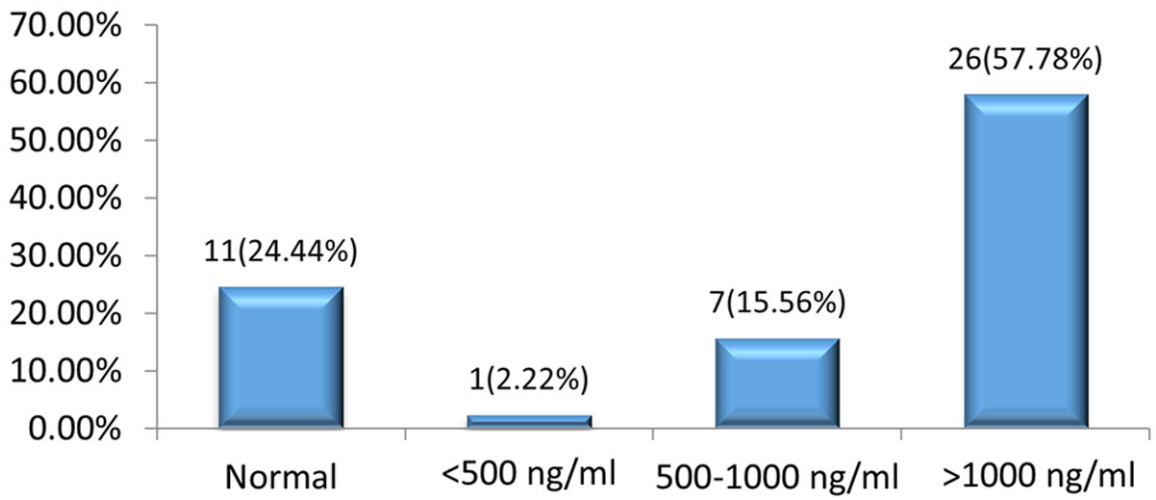

Figure 3. Ferritin level of study subjects at 6-month follow up period.

statistically significant (**Figure 3**). There was a strong correlation between initial subclinical hypothyroidism and final ferritin level with a *P*-value of 0.001.

It was evident that 75% of patients with high baseline serum TSH had high endpoint ferritin levels, whereas 62.5% of them had a level above 500 ng/ml. As expected, the final ferritin level depended on the number of packed cell transfusions received during treatment (*P*-value 0.002). 74.42% of subjects receiving more than 100 ml/kg packed cell transfusions had ferritin levels above 1000 ng/ml. It was found that the final ferritin level was a strong predictor of the status of liver dysfunction at six month follow-up period (*P*-value 0.034). A statistically significant 85.71% of patients having deranged liver function test with SGPT and SGOT level above three times normal limit had serum ferritin level above 1000 ng/ml.

Myocardial dysfunction in the form of diastolic dysfunction at six-month follow-up period was evident to have a statistically significant association with baseline Liver function (*P*-value 0.019). Patients having diastolic dysfunction had a 66.67% chance of having baseline liver function derangement, whereas 16.7% had Transaminitis above three times normal.

Another strong association between 2 D echo findings of iron overload was endpoint serum TSH level (*P*-value 0.05). Base Serum TSH and baseline 2 D Echocardiography of 49 subjects had a strong positive correlation with a Pearson coefficient of 0.587. The final echo-cardiographic finding at the end of 6 months follow-up period with baseline serum TSH showed a Pearson coefficient of correlation of 0.651.

Baseline Liver function also had a strong correlation with baseline serum TSH with a Pearson coefficient of 0.461.

Outcome predictors for mortality showed a strong statistical correlation with deranged baseline serum TSH and had a positive Pearson coefficient of 0.964. Study subject receiving a greater number of transfusions during the study period had statistically significant final serum TSH level (Pearson coefficient 0.853) and echocardiographic changes (Pearson coefficient 0.841).

Discussion

In our study, we found a high level of ferritin level in 65% of subjects at treatment start and 76% at an endpoint which is slightly lower than the results seen in the study by Srinivasan, Rusia, Anand and Sood [25] but much higher than the prevalence of 24% as in the study of Nair [26]. Mean ferritin level was 725 ng/ml at baseline and six-month follow-up period 1268 ng/ml, with about 57% had ferritin level above 1000 ng/ml have a strong correlation to basal ferritin level (*P*-value 0.005). The final ferritin level correlated strongly with the final number of packed cell transfusion (*P*-value 0.0002),

and those who received above 100 ml/kg of packed cell transfusion had a higher probability of high ferritin level above 1000 ng/ml, which was lower than the level observed in the cross-sectional study by Nair of 100 ml/kg of the packed cell. The mean volume of blood transfused was 25 ml/kg before starting treatment and 45 ml/kg at sixsix-month follow-ups.

In the study of Goyet over three years follow up period, there was 14% cardiac and 66% of hepatic iron overload, which was evident radiologically [27]. In contrast, in our study, we found no radiological evidence of iron overload in the liver in the 6 six months follow-up period. Still, the liver's functional derangement was evident biochemically in 13.7% before starting treatment and 31.8% at six-month follow-up period. Biochemical derangement of liver function was up to 22% in the study by Parker [28]. Cardiovascular system affection by iron overload had a higher burden in our study than reported by Goyet. Echocardiography detected diastolic dysfunction in 2% of patients at baseline before starting treatment and increased to 22% in 6 months follow up period. No patient had systolic dysfunction or developed any significant reduction in the ejection fraction over the study period, which might take an extended period to develop than the diastolic dysfunction [15]. In our study, no structural damage of the pancreas was seen radio-logically. The Fasting blood sugar level of all subjects remained in the normal range over the study period. Hypothyroidism was in the form of subclinical hypothyroidism. The percentage of subclinical hypothyroidism increased from 22.8% to 48.8% during treatment. Clinical hypothyroidism was not seen, and no patient developed low T4 and T3 levels during the follow-up period. Mortality was 15% of total study subjects during the 12-month study period. The most common cause of mortality was septic shock with disseminated intravascular coagulation and the commonest terminal event was respiratory failure.

In terms of the outcome of mortality, the female gender was found to have better survival for age above five years. Initial serum ferritin level above 500 ng/ml, initial packed cell transfusion above 50 ml/kg before starting treatment, and significantly deranged liver function were a poor prognostic factor.

Baseline Serum ferritin level was dependent on the packed cell transfusion only. In contrast, the final serum ferritin level showed a significant correlation with baseline ferritin, packed cell transfusion received, baseline serum TSH level. Diastolic dysfunction of the heart as detected by 2-D Echocardiography showed a strong correlation with baseline and final serum TSH level, liver dysfunction, number of packed cell transfusion. Our study highlighted iron overload as a severe complication related to therapy in patients of hematological malignancy and bone marrow failure syndromes even before starting treatment. A serum ferritin level of 500 ng/ml before treatment starts is associated with significant morbidity and mortality, although 1000 ng/ml is taken as significant iron overload in all the studies.

As the organ damage is multifactorial in children receiving chemotherapy, the major limitation of our study is the lack of standardization of results across patients and removal of confounding factors. Larger sample size with age matched case-control study is required to predict odds ratio between iron overload and organ damage in immune-compromised cohort. Iron overload is also an important and neglected cause of morbidity and mortality in these patients, which needs attention.

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We as all authors, certify that the work has not been published and is not considered for publication elsewhere. Due ethical consideration was taken for this manuscript preparation. The institutional ethical clearance was obtained vide reference number. VVMC/SJH/Thesis/October/2017/-166.

Disclosure of conflict of interest

None.

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