



## ARAŞTIRMA / RESEARCH

# Transfusion associated iron overload in childhood cancer survivors

Çocukluk çağı kanserlerinde transfüzyon ile ilişkili aşırı demir yükü

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### Abstract

**Purpose:** We aimed to evaluate iron burden and possible target organ damage in childhood cancer survivors

**Materials and Methods:** Files of patients treated for cancer and completed treatment at least 6 months ago were retrospectively evaluated for treatment duration, duration after cessation of treatment, relapse status, erythrocyte transfusion number given during treatment, liver function tests, ferritin levels, T\*2 Magnetic resonance imaging (MRI) results.

**Results:** 118 patients with solid tumors and hematological malignancies were included in the study. There were 44 (37.2%) female, 74 (62.8%) male patients with a mean age of 10.71 ± 4.64 years. There was a weak negative correlation between time after cessation of treatment and ferritin levels. A statistically significant weak positive correlation between ferritin levels and number of erythrocyte transfusions were. Ferritin levels were significantly higher in patients who had relapse (9/118) compared to patients with no relapse (109/118).

**Conclusion:** Transfusion associated iron overload measured by ferritin level was higher patients who were more frequently transfused during treatment. However, abnormal iron accumulation in liver was not detected using qualitative analysis with T\*2 MRI method.

**Key words:** Iron overload, childhood cancer, ferritin, T\*2MRI

### Öz

**Amaç:** Bu çalışmada kanser tedavisini tamamlamış çocuklarda demir yükünü ve olası hedef organ hasarının değerlendirilmesi amaçlanmıştır.

**Gereç ve Yöntem:** Kanser tedavisinin tamamlanması üzerinden en az 6 ay geçmiş olan hastaların dosyaları tedavi süresi, tedavi kesildikten sonra geçen süre, nüks durumu, tedavi sırasında verilen eritrosit süspansiyonu sayısı, karaciğer fonksiyon testleri, ferritin düzeyi, T2\*Manyetik Rezonans Görüntüleme (MRG) bulguları yönünden retrospektif olarak değerlendirildi.

**Bulgular:** Solid tümör ve hematolojik malignite tanı 118 hasta çalışmaya dahil edildi. Hastaların 44'ü (%37,2) kız, 74'ü (%62,8) erkek idi. Ortalama yaş 10,71±4,64 yılı, Ferritin düzeyleri ile tedavi kesildikten sonra geçen süre arasında negatif yönde zayıf korelasyon saptandı. Tedavi süresince verilen eritrosit süspansiyonu sayısı ile ferritin düzeyleri arasında pozitif yönde zayıf korelasyon saptandı. Ferritin düzeyi nüks olan hastalarda (9/118) olmayanlara (109/118) göre istatistiksel olarak anlamlı şekilde yüksek bulundu.

**Sonuç:** Demir yükünün değerlendirilmesi amacıyla bakılan ferritin düzeyleri tedavi sırasında daha sık transfüzyon yapılan hastalarda daha yüksek bulunmuştur. Bununla birlikte, T2\*MRG ile karaciğerde anormal demir birikimi tespit edilmemiştir.

**Anahtar kelimeler:** Demir yükü, çocukluk çağı kanserleri, ferritin, T2\*MRG

## INTRODUCTION

As there is no physiological pathway to increase iron excretion in humans, iron metabolism is tightly

controlled<sup>1,2</sup>. Excess iron can accumulate and cause tissue damage as a result of formation of reactive oxygen radicals<sup>2</sup>. Iron overload resulting from therapeutic transfusions has been extensively studied in patients with transfusion dependent chronic

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anemia. The presence of chronic iron overload in these patients frequently results in cardiac, liver and endocrine dysfunction. In addition to that, iron overload has been shown in large epidemiologic studies to increase the risk of cancer<sup>3,4</sup>.

Cancer treatment's most important goal in all patients is to minimize the long-term morbidity associated with treatment while keeping the survival rate at the highest level<sup>5</sup>. It is necessary to make sure that the benefit-loss analysis is done correctly<sup>6</sup>. Indeed, patients may be lost due to the side effects of cancer treatment. Hargrave et al. determined that treatment-related deaths occurred most often with preventable causes, such as bacterial and fungal infections, and hemorrhages<sup>7</sup>.

An equilibrium between the risk of transfusion-related damage and the patient's benefit must be established when an erythrocyte transfusion is performed<sup>8</sup>. One of these risks are transfusion induced iron overload<sup>9</sup>. Erythrocyte transfusion increases blood hemoglobin content and oxygen transport capacity and reduces tissue hypoxia<sup>10</sup>. Adequate transfusion support should be provided to relieve the anemic complications observed during these treatments. However, considering the increased cure rates, the long-term effects of iatrogenic iron accumulation in various organs and the potential effects on quality of life are also imponderable<sup>11</sup>. An increase in the number of transfusions leads to iron accumulation in the body as a result of complications such as ineffective erythropoiesis and increased gastrointestinal system iron absorption<sup>12,13</sup>. Studies have shown that excess iron is first deposited in the liver and pancreas<sup>14</sup>.

Increased treatment intensity in pediatric cancer protocols has been associated with an increased need for red blood cell transfusions, which is subsequently resulted in iron overload that is measured with serum ferritin levels<sup>15,16</sup>. It is important to identify the survivors of childhood cancer who are at risk for iron overload in order to prevent irreversible liver damage. Schempp et al found that there was a statistically significant positive correlation between ferritin level of >1000 ng/mL and iron accumulation index determined by T2 \* MRI in pediatric patients<sup>9</sup>.

Herein we retrospectively evaluated survivors of childhood cancer for iron overload at least 6 months after the time of treatment cessation by using serum ferritin measurement and T2\*MRI in patients with a

ferritin level of >250 ng/mL.

## MATERIALS AND METHODS

The study was approved by Cukurova University Ethics Committee (04/11/2016, Decision number. 26). The files of the patients admitted to pediatric oncology departments of Sağlık Bilimleri Univeristy, Adana Numune Education and Research Hospital and Çukurova University Faculty of Medicine Balcalı Hospital between June 2015-May 2016 were retrospectively evaluated. Patients who completed their treatment at least 6 months ago were eligible.

Serum ferritin levels, liver function tests (Aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total and direct bilirubin, alkaline phosphatase, albumin), the treatment duration, erythrocyte transfusion numbers used during treatment, the time after cessation of treatment were obtained from patients' files.

Liver iron content (LIC) was measured in patients with a ferritin level of >250ng/mL by hepatic magnetic resonance imaging (MRI) (FerriScan) with qualitative methods. Excessive amounts of iron in the tissue can be revealed by MRI characterized by shortening of T2 relaxation time resulting from superparamagnetic effect of iron<sup>17</sup>. The activity of MRI at the time of fermentation is based on the acceleration of T2 relaxation resulting from interaction between water molecules and high molecular weight iron complexes such as ferritin and hemosiderin<sup>18,19</sup>.

## Statistical analysis

Subject and treatment characteristics were compared using the  $\chi^2$ , Mann Whitney U, Fisher exact, or Kruskal-Wallis test, as appropriate. The relationship between variables was assessed by Pearson or Spearman correlation analysis according to whether the variables were normally distributed or not. All tests were 2-tailed, and a *p* value of <0.05 considered to be statistically significant. Data analysis was completed using IBM SPSS Statistics program (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

## RESULTS

A total of 118 patients were included in the study. The median follow-up time was 4.41 years (Range:

1.25-13.66 years). There were 74 male and 44 female patients (M:F ratio = 1.68). The mean age at diagnosis was  $10.85 \pm 0.57$  years and mean age at time of study was  $10.71 \pm 4.64$ . Hodgkin lymphoma was detected in 23 (19.5%), Non-Hodgkin's lymphoma in 21 (17.8%), Wilms tumor in 16 (13.6%) and acute lymphoblastic leukemia in 15, 9 (7.6%) acute myeloid leukemia, 9 (7.6%) non-rhabdomyosarcoma soft tissue tumors, 7 (5.9%)

neuroblastoma, 1 (0.8%) retinoblastoma, Langerhans cell histiocytosis in 4 (3.4%), central nervous system tumors in 4 (3.4%), rhabdomyosarcoma in 4 (3.4%), Ewing sarcoma in 2, Hepatoblastoma in 1 (0.8%), osteosarcoma in 1 (0.8%) and germ cell tumor in 1 (0.8%) patients.

Demographic data and clinical features of the patients were given in Table 1.

**Table 1. Demographic and clinical features of the patients**

	n=118	%
Sex		
Male	74	62.8
Female	44	37.2
Mean age at diagnosis	10.85±0.57 (Range:1-20 years)	
Mean age at time of study	10.71±4.64 (Range:2-20 years)	
Median ferritin level	43.3ng/mL (Range:4.8-1300 ng/mL)	
Median treatment duration	1.7 years (1-4 years)	
Median time aftercessation of treatment	2 years (1-13 years)	
Median follow-up time	4.41 years (1.25-13.66 years)	
Mean number of erythrocyte transfusion	2.19±0.23	
Relapse status		
Relapse+	9/118	
Relapse -	109/118	
Diagnosis	n	%
Acute Myeloid Leukemia	9	19.5
Acute Lymphoblastic Leukemia	15	17.8
Hodgkin Lymphoma	23	13.6
Non-Hodgkin's Lymphoma	21	12.7
Central Nervous System Tumors	4	7.6
Neuroblastoma	7	7.6
Retinoblastoma	1	7.6
Wilms tumor	16	5.9
Hepatoblastoma	1	3.4
Osteosarcoma	1	3.4
Ewing sarcoma	2	1.7
Rhabdomyosarcoma	4	0.8
Non-Rhabdomyosarcoma Soft Tissue Tumors	9	0.8
Germ Cell Tumor	1	0.8
Langerhans Cell Histiocytosis	4	0.8

Median ferritin level was 43.3 ng/ml (Range: 4.8-1300 ng/mL, normal reference values:12-150 ng/mL ) in the whole study population. Patients were divided in to two groups according to their diagnosis as hematological malignancy (Hodgkin and Non-Hodgkin lymphoma, acute lymphoblastic or myeloid leukemia) and solid tumors. There was no statistically significant difference according to ferritin levels between these two groups ( $p=0.7$ ). There was no statistically significant relation between treatment duration and ferritin levels. However, it was found that as the time passed after

cessation of treatment increased, the level of ferritin decreased. There was a weak negative correlation between these two variables ( $r=-0,24$ ) ( $p<0,001$ ). The mean number of erythrocyte suspensions patients received during the treatment period was  $2.19 \pm 0.23$ . It was observed that ferritin levels increased as the number of transfusions increased and there was statistically significant weak positive correlation between these variables ( $r = + 0.354$ ) ( $p < 0.01$ ). Nine patients were found to be treated for relapsed disease. Median ferritin level was 151 ng/mL in these patients whereas it was 34 ng/mL in

patients who had no relapse. Ferritin levels were significantly higher in patients who had relapse compared to patients with no relapse ( $p=0.01$ ).

No association was found between serum ferritin levels and the presence of liver dysfunction. As a result of qualitative evaluation of T2 \* MRI performed to detect iron accumulation in the liver in 9 patients with ferritin level > 250 ng/mL, abnormal iron accumulation in the liver was not detected in none of the patients.

## DISCUSSION

Transfusion therapy is one of the most important part of supportive care in children with cancer. The incidence of anemia in children receiving cancer treatment is reported as 80% in Europe, with the highest prevalence being found in leukemia and lymphomas<sup>20</sup>. Erythrocyte transfusion is the treatment for anemia which occurs during childhood cancer treatment. Although there are no indications definitively determined for transfusion, the decision can be based on the general condition of the patient, the ability to compensate for anemia, cardiopulmonary risk factors, and the expected duration of anemia<sup>20</sup>.

Transfusion-associated iron overload is an important complication of supportive treatment in children with cancer. Patients with solid tumors requiring intensive chemotherapy and those with bone marrow involvement and who are treated with hematopoietic stem cell transplantation need more frequent transfusion<sup>20</sup>. Ferritin level is the cheapest and easiest method to measure iron burden. Studies have shown that ferritin correlates well with iron accumulation in the liver but doesn't correlate with cardiac iron accumulation. Recent years, non-invasive iron measurement methods such as SQUID and T2 \* MRI have also been used<sup>20</sup>. Schempp et al. analyzed 63 children with a history of receiving at least one erythrocyte suspension during treatment. In this study, evaluation was made at least 24 months after the completion of treatment and median ferritin level was found 179 ng/mL in allogeneic stem cell transplant patients, 96 ng/mL in autologous stem cell transplant patients, and 60 ng/mL in patients without stem cell transplantation. Ferritin levels were >1000 ng/mL in 7 of 27 patients who underwent allogeneic transplantation and ferritin levels were >1000 ng/mL only in one of the patients without stem cell transplantation. It has

also been shown that the transfusion volume correlated with the ferritin level<sup>9</sup>. Landier et al. found ferritin levels >500 ng/mL in a quarter of 370 children who completed cancer treatment<sup>21</sup>.

Most studies investigating iron burden in children with cancer were done with patients who have leukemia<sup>15,21-24</sup>. In a study by Eng and Fish, 107 ALL patients were included and the iron load in these patients was calculated by the amount of erythrocyte suspension per kilogram during the treatment. This suggests that the patients with high-risk ALL get more intensive treatment so that more frequent anemia occurs in these patients resulting in increased need for transfusion and iron overload<sup>22</sup>.

Nottage et al. analyzed the number of transfusion volume, cumulative transfusion volume, and weight-adjusted transfusion volume of 881 patients diagnosed with hematological malignancy and patients with hematopoietic stem cell transplantation and AML have been shown to have taken more transfusion and consequently had greater iron burden<sup>23</sup>. In our study patients with hematologic malignancy (AML, ALL, HL, NHL) accounted for more than half of the whole group and median ferritin level was found 43.3 ng/ml which was close to the median ferritin level in patients without stem cell transplantation reported by Schempp et al<sup>9</sup>. However, there were only two patients with ferritin levels of >1000 ng/ml in our study. Patients with allogeneic stem cell transplantation were shown to have the highest ferritin levels in the study by Schempp et al<sup>9</sup>. There were no patients with stem cell transplantation in our group and this might be the possible reason for having few patients with high levels of ferritin.

Another important finding in our study was statistically significant negative correlation between time after the cessation of treatment and level of ferritin which means time passed after cessation of treatment increased, the level of ferritin decreased. This finding may indicate that children with cancer who completed their treatment, might have used their iron store for their growth. Halonen et al. measured serum ferritin, iron, transferrin iron saturation levels at the end of treatment and 3 years after discontinuation of treatment; they showed that the initially measured high levels were reported to be normal at follow-up measurements. The reason for this was shown as the use of iron deposits in children who have recovered due to ongoing growth<sup>24</sup>.

As the patients duration of treatment increases, the bone marrow suppression effect of chemotherapy becomes more prominent and higher levels of ferritin is expected in these patients because of increased transfusion need. However, there was no statistically significant correlation between treatment duration and ferritin in our study. Eng and Fish and Ruccione et al. have shown in their study that transfusion numbers in terms of iron deposition were determinative and that the prolongation of treatment duration alone did not have an effect on iron burden<sup>15,22</sup>. Similarly we observed a positive correlation between the number of transfusions and ferritin levels in our study. Ruccione et al reported that the number of transfusions, transfusion volume, and projected iron burden in patients receiving intensive therapy have been shown to exceed normal limits<sup>15</sup>.

Despite low percentage of patients with relapsed disease in our study, we found significantly higher ferritin levels than the relapse-free group. It was thought that the high ferritin levels in these patients might have been due to intensive chemotherapy regimens and prolonged treatment. In literature there was no study analyzing the effect of relapse on iron burden in childhood cancers.

In the only study using MRI method in the assessment of liver iron accumulation due to transfusions in childhood cancers, MRI was performed in 8 patients with an average ferritin level of 1337 ng/ml and it was reported that there was a statistically significant positive correlation between ferritin level and iron accumulation index determined by T2 \* MRI method<sup>9</sup>.

In our study, no abnormal iron accumulation was detected in patients with a ferritin level > 250 ng/ml with qualitative evaluation performed by the T2\*MRI method. Our study's one of the major limitations was the availability of qualitative methods instead of quantitative methods. Some abnormal findings with T2\*MRI might have been found with quantitative measurement. The other limitations were retrospective design of the study and lack of a control group.

The results obtained from these studies will provide adequate information to determine the amount of transfusion that poses a risk for iron deposition and to determine the critical ferritin level for establishment of this abnormal iron deposition. Treatment methods such as phlebotomy or

chelation may be used to prevent target organ damage in a more precisely identified group at risk. Large-scale multicenter studies are needed to address most appropriate imaging and laboratory methods for assessing iron overload in survivors of childhood cancer.

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