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# The relationship between iron bone marrow stores and response to treatment in pediatric acute lymphoblastic leukemia

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**Abstract**

Iron is an intracellular element whose accumulation in the body is associated with tissue damage. This study examines the effect of iron on pediatric acute lymphoblastic leukemia (ALL) and its "response to treatment." At the end of the first year of treatment, bone marrow iron store (BMIS) was evaluated in children with ALL and the relationship between iron store and minimal residual disease was investigated. Moreover, the 3-year disease-free survival (3-DFS) of patients was determined. Patients' BMIS were compared with that of subjects with normal bone marrow. The study examined 93 children, including 78 Pre-B and 15 T-cell ALL patients. BMIS did not differ between the children with ALL and those with no evidence of cancer. BMIS was increased in 26.6% of patients at the end of the first year of treatment. Drug resistance and BM relapses were more prevalent in cases with high BMIS in both Pre-B and T-cell groups. Bone marrow iron store is not considered a risk factor for childhood ALL. However, high levels of BMIS are associated with poor response to treatment and the risk of relapse. Bone marrow iron store control during treatment can therefore help achieve better outcomes and improve the chances of recovery.

**Abbreviations:** 3-DFS = 3-year disease-free survival, ALL = acute lymphoblastic leukemia, BM = bone marrow, BMIS = bone marrow iron store, CI = confidence interval, IgH = immunoglobulin heavy chain, ITP = idiopathic thrombocytopenic purpura, LDH = lactate dehydrogenase, MRD = minimal residual disease, OR = odds ratio, PCR = polymerase chain reaction, rHuEPO = recombinant human erythropoietin, WBC = white blood cell.

**Keywords:** acute lymphoblastic leukaemia, bone marrow iron store, risk of relapse

## 1. Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease in which the immature lymphoid cells proliferate uncontrollably and replace the normal bone marrow cells.[1] About 80% of ALL patients responded properly to chemotherapy, but 20% to 30% experienced disease relapse.[2,3] Drug resistance is the main factor contributing to disease relapse, although many other factors, including genetic and environmental ones, may also be involved. Identifying these factors can help in disease prevention and the selection of more suitable treatments.[4]

Iron is an essential mineral for humans and is necessary for cell proliferation, survival, and metabolism.[5] Iron has different absorption mechanisms; however, there are no physiological mechanisms for its removal, and its excess accumulation can have destructive consequences.[6] Iron overload is not common in healthy children, as they have lower iron stores than adults, and iron stores slowly but progressively increases with age.[7,8] Iron carriers bind to iron and prevent its damaging effects on the body tissues; nevertheless, these carriers have a limited capacity. After the saturation of iron-binding proteins and carriers, free iron radicals necessarily bind to other proteins and low-weight molecules and exert undesirable effects.[9] It is suggested that iron radicals may have a role in both carcinogenesis and the development of drug resistance.[10–14] A growing body of evidence shows that iron excess may lead to some malignant diseases, including lung, ovary, colorectal, and hepatic cancers.[15–17] Moreover, a down regulatory effect of iron on p53 is recently observed, which not only may contribute to tumorigenesis, but also have impact on drug response to chemotherapy.[18,19] Furthermore, published data have demonstrated the inhibitory effect of iron chelators on tumor growth.[18,20,21] In contrast, some other evidences suggest that iron has a protective role for the cells (fighting oxidative tissue damage by nitrogen oxidase).[22–24] In addition, it is reported that the in-vitro in flux of iron into the cell can help prevent and control cancer.[25,26] This lack of consensus has made it impossible to decide on a specific strategy for determining the ideal iron store in different types of cancer.

Given the lack of consensus about the effects of iron on the development or progression of cancer types and the lack of clinical studies on the effects of iron on the response to treatment in children with ALL, the present study was conducted to

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examine the effect of iron stores during treatment on the response to treatment.

## 2. Materials and methods

### 2.1. Study groups

This research was approved by the Ethic Committee of Isfahan University of Medical Sciences (protocol number 394,879). All the patients with a diagnosis of Philadelphia-negative ALL admitted to Sayed Al-Shohada Hospital from October 2009 to January 2013 were included in this study. All the patients with Burkitt-type ALL, infantile ALL, or Philadelphia-positive translocation were excluded because of the different treatment protocols. Data on age, gender, disease phenotype, preliminary tests (lactate dehydrogenase [LDH] level and white blood cell [WBC] count), organomegaly, extramedullary involvement at diagnosis, frequency of severe bleeding, and units of platelet and packed cell transfused in the first year of treatment (if applicable) were extracted from the patients' medical records. The patients were treated based on the Australian and New Zealand Children's Cancer Study Group ALL study 8 protocol (http://www.anzctr.org.au/trial_view.aspx?ID=1568). At the end of the first year, minimal residual disease (MRD) was evaluated based on the disappearance or persistence of monoclonality for IgH or T-cell Gamma receptor gene rearrangement by PCR methods. Persistent monoclonality along with any evidence of disease relapse in the first year was taken to indicate resistance to treatment. The patients' 3-year disease-free survival (3-DFS) was also determined.

Transfusion rate

According to our transfusion protocol, packed cell transfusion is recommended for noncomplicated leukemia patients with a hemoglobin level of less than 8 g/dL, and those having any pulmonary or heart disease with a hemoglobin level of over 10 g/dL. The relation of packed cell transfusion rate and increasing body iron store was also evaluated.

### 2.2. Bone marrow iron assessment

Bone Marrow Iron Store (BMIS) was chosen as the measure of the body's iron stores.[27] Written consents regarding iron assessment were obtained from the parents of patients. Bone marrow slides were prepared in the initial diagnosis and after the first year of treatment (often prepared to evaluate the treatment progress). Provided bone marrow (BM) particles were present, slides were reviewed for assessing their BMIS. Perl Prussian blue stain was used for this assessment and scoring was carried out with respect to hemosiderin-laden macrophages.[28] After the staining, 1 or 2 slides (based on the number of slides available) were assessed and graded by the conventional Gale method, from very low storage (grade 0) to very heavy iron storage (grade 6), according to protocol. BMIS were evaluated for each patient in a separate double-blind form by 2 researchers, and the mean score was recorded as the final summary of the findings (Fig. 1). If there was a score difference of more than 1 grade, a third score was applied by a different and uninvolved researcher. Finally, the patients were divided into 3 groups based on their BMIS:

Group A: low to normal BMIS (grades 0–2)
Group B: moderate-heavy to heavy BMIS (grades 3–4)
Group C: heavy and very heavy BMIS (grades 5–6)

### 2.3. The control group

The bone marrow slides of the patients with thrombocytopenia but completely normal BM elements (a definite diagnosis of idiopathic thrombocytopenia with no exceptions) were used in the control group as they were convenient and suitable samples for BMIS evaluation in noncancer age match patients. These slides were only stained and scored, with respect to their iron stores, if the BM particles were present and after the ruling out malignancy.

Ultimately, BMIS of the group of patients at the beginning of the disease was compared with that of the control group, the relationship between BMIS and response after 1 year of treatment (the MRD status or relapse in the first year) was examined, and the 3-DFS was also determined.

### 2.4. Statistical analysis

The $\chi^2$ test was used to evaluate the relationship between iron stores and the response to treatment. To determine the effectiveness of the intervention, the paired *t* test and odds ratio (OR) were used at a confidence interval (CI) of 95%. The data obtained were analyzed in STATA-13 at a significance level of 5%.

## 3. Results

### 3.1. Patient details

A total of 106 children, aged 1 to 15 years old at diagnosis, were admitted to the selected hospital with a diagnosis of ALL during the period examined in this study. Eleven patients were excluded from the study including 2 patients who died due to infection, 4 patients whose BM slides could not be assessed due to the absence of BM particles or the poor quality of staining, and 5 patients with Burkitt-type leukemia who were treated with different protocols. From the total of 95 non-Burkitt leukemia patients, the MRD status of 92 patients with monoclonal pattern of IgH or γ receptor T-cell gene rearrangement was assessed. In 1 patient with t(4,11), remission occurred after the third induction and the patient was therefore added to the resistant group (making for a total of 93 patients). Table 1 represents the details of the patients. From this population, the correlation between BMIS and leukemogenesis was evaluated in 30 patients (out of the total of 40 admitted after 2011). The BM slides of these patients which were collected from the beginning of the disease were successfully assessed (with regard to the presence or absence of particles on the slides). The bone marrow slides of 30 patients with idiopathic thrombocytopenic purpura (ITP) were considered controls and assessed in terms of their BMIS.

### 3.2. Bone marrow iron store

Bone marrow iron store of 30 leukemia patients at diagnosis (46.6% male) was compared with 30 control group (53.3% male). Mean age in both groups was similar (5.2 ± 2.7 year vs 5.6 ± 2.6 respectably; $P = .380$). At the time of diagnosis, the iron store of the patient group did not differ significantly from that of the control group (80% vs 83% in group A, $P = .322$), but in patient group, the mean BMIS increased significantly after the first year of treatment ($P = .0107$) (Table 2).

### 3.3. The relationship between iron stores (after the first year of treatment) and drug resistance

In the Pre-B group, evaluating the response to treatment after the first year of treatment was possible in 78 patients and resistance to

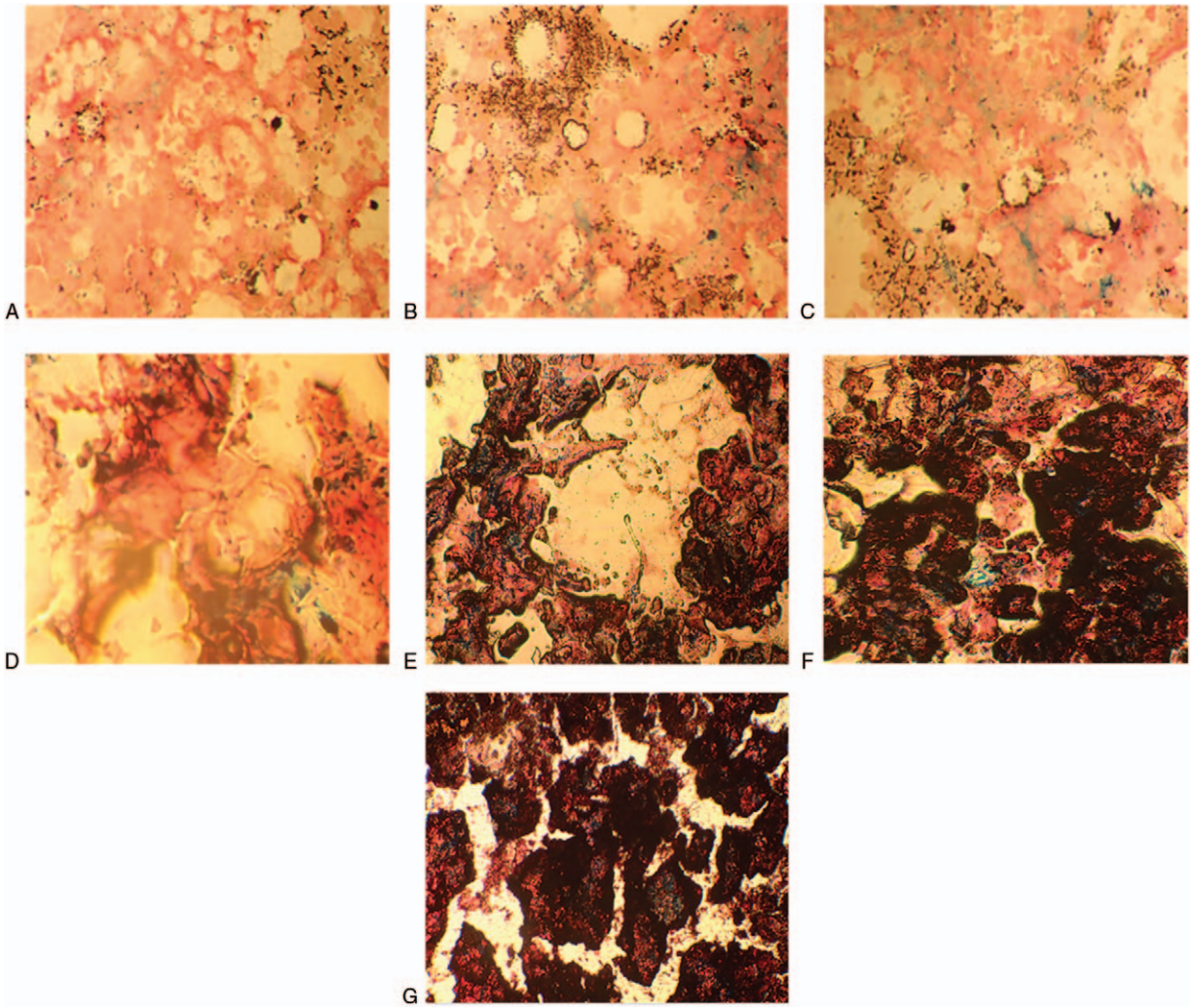


**Figure 1.** Perl Prussian blue staining for the evaluation of bone marrow iron storage (×400). A, Low iron storage. B–D, Intermediate iron storage. E–G, High iron storage.

treatment was observed in 39.5%. Drug resistance by the end of the first year (MRD+ or relapse) was less prevalent in group A than in non-group A (Table 3). To remove the underlying factors, 21 cases who had received more than 30 cc/kg/y of blood transfusion were excluded from the study. After the exclusion of these 21 cases, the development of resistance to treatment was still more prevalent in non-group A ($P = .010$). Logistic regression indicated that patients at low level of iron had 3.6 times more chance of positive response to treatment than patients at moderate or high level of iron (OR = 4.56, 95% CI: 1.39–14.97, $P = .012$). Age-adjusted odds ratio also indicated that patients at low level of iron had 4.7 times more chance of positive response to treatment than patients at moderate or high level of iron (OR = 4.69, 95% CI: 1.41–15.64, $P = .012$) (Fig. 2). Bone marrow relapses were observed in 8% of the Pre-B ALL patients during the first 3 years of treatment; however, this problem was more common in non-group A (15% in non-group A vs 4% in group A).

From the 78 Pre-B cell patients, 31 patients were MRD positive (measured at the end of the first year of treatment) and 47 children were MRD negative. Patients were followed up for 3 years and none of those with MRD negative showed relapse. Bone marrow iron store was significantly lower in patients with MRD negative compared with those with BM relapse at the end of the third year of follow-up ($P = .027$). Logistic regression indicated that patients at low level of iron had 5.5 times excess chance of 3-year disease-free survival than patients at moderate or high level of iron (OR = 6.5, 95% CI: 1.1–40.7, $P = .044$). Age-adjusted odds ratio also indicated that patients at low level of iron had 6.5 times excess chance of 3-year disease-free survival than patients at moderate or high level of iron (OR = 7.5, 95% CI: 1.1–51.6, $P = .040$) (Fig. 3).

Regarding the total number of 15 T-cell patients, 60% showed resistance to therapy after 1 year of treatment based on the identified persistent IgH or T-cell gamma receptor monoclonality (Table 1). Out of the aforementioned 15 patients, 13 were followed up for 3 years. Among them, 3 patients underwent bone marrow transplantation, 4 were categorized into group-A BMIS and 6 into non-group A BMIS. One patient (25%) from the 4 group-A BMIS and 4 (75%) from the 6 non-group A BMIS relapsed within the 3 years of follow-up. By removing 6 cases with a high blood transfusion, the risk of resistance to treatment in non-group A BMIS patients, increased from 33% to 66%, although the difference was not significant (OR = 4.17; 95% CI: 0.33–65.64; $P = .189$).

**Table 1**

**Characteristics of 93 children with diagnosis of acute lymphoblastic leukemia (T or Pre-B lineage) admitted at Sayed Al-shohada Hospital from October 2009 until January 2013.**

| | | | Patients characteristics |
|---|---|---|---|
| Sex | | Male | 55 |
| | | Female | 38 |
| Age (mean ±SD) | | Group A (n = 58) | 5.8 ± 3.5 |
| | | (n = 35) Non-group A | 5.3 ± 3.1 |
| Immunophenotype | | T-cell linage | 15 |
| | | Pre-B and early Pre-B linage | 78 |
| Cytogenetic analysis (pre-B cell group) | | t(1;19)(q23;p13) | 1 |
| | | t (4;11)(q21;q23) | 2 |
| | | t (12;21)(p13;q22) | 8 |
| | | others | 67 |
| Response to treatment (1 y) | Good response | Pre-B | 47 |
| | | T-cell | 6 |
| | Low response | Pre-B | 31 |
| | | T-cell | 9 |
| 3-y disease-free survival | Yes | Pre-B | 47 (100%)* |
| | | T-cell | 8 (89%)* |
| | No | Pre-B | 7 (9.2% of total) |
| | | T-cell | 5 (33% of total) |
| Relapse | Bone marrow | Pre-B | 6 |
| | | T-cell | 2 |
| | CNS | Pre-B | 1 |
| | | T-cell | 3 |
| | Other sites | Pre-B | 0 |
| | | T-cell | 0 |
| Bone marrow iron store (BMIS) | Group A | Pre-B | 51 |
| | | T-cell | 7 |
| | | Total | 58 (62%) |
| | Group B | Pre-B | 20 |
| | | T-cell | 5 |
| | | Total | 25 (27%) |
| | Group C | Pre-B | 7 |
| | | T-cell | 3 |
| | | Total | 10 (11%) |

* Data were calculated for patients with minimal residual disease (MRD) negative results after 1-y treatment. MRD+ patients were excluded from data analysis since intensified treatment was needed to be applied.

Overall, increased iron overload (grade≥3) raised the risk of resistance to treatment and BM relapse in all the patients (Table 1 and Fig. 2). Results showed no significant relationship between the patient's 3-year disease-free survival and any of the prognostic factors including chromosomal translocations t(1;19)(q23;p13), t(4;11)(q21;q23), and t(12;21)(p13;q22) initial white blood cell count, age, sex, or LDH levels measured at diagnosis.

## 4. Discussion

This study found no evidence on the role of iron in inducing childhood ALL, and BMIS was similar in the patient and control groups. There is no consensus on the carcinogenicity of iron in the

**Table 2**

**Categorizing patients according to the grade of bone marrow iron store (BMIS).**

| | | Control group (n) | Patients at diagnosis (n) | Patients after 1 y of treatment (n) |
|---|---|---|---|---|
| Grade of BMIS | 0 | 1 | 1 | 1 |
| | 1 | 16 | 13 | 6 |
| | 2 | 8 | 10 | 14 |
| | 3 | 2 | 2 | 4 |
| | 4 | 2 | 3 | 2 |
| | 5 | 1 | 1 | 3 |
| | 6 | 0 | 0 | 0 |
| | Sum | 30 | 30 | 30 |

There was no difference between the control and patient group at the onset of the disease according to their BMIS ($P > .05$). Paired $t$ test showed a significant increase in BMIS after 1 y of treatment ($P = .01$).

BMIS = bone marrow iron store, n = number.

**Table 3**

**Minimal residual disease positivity after "1 y treatment" was more frequent in patients with intermediate and high levels of iron storage in pre-B cell acute lymphoblastic leukemia ($P = .003$) and total patients group ($P = .001$).**

| BMIS response to treatment | | Low iron storage | Intermediate iron storage | High iron storage | Total |
|---|---|---|---|---|---|
| Good response | Pre-B | 37 | 9 | 1 | 47 |
| | Total (T cell and pre-B) | 41 | 10 | 2 | 53 |
| Low response | Pre-B | 14 | 11 | 6 | 31 |
| | Total (T cell and pre-B) | 17 | 15 | 8 | 40 |
| Total | Pre-B | 51 | 20 | 7 | 78 |
| | Total (T cell and pre B) | 58 | 25 | 10 | 93 |

BMIS = bone marrow iron store.

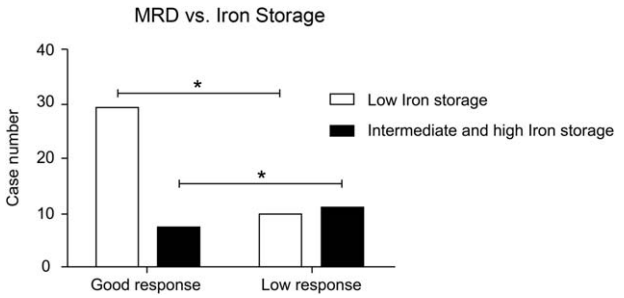


**Figure 2.** The relationship between level of "bone marrow iron store" and "response to treatment" in pediatric Pre-B ALL after 1-year treatment (excluding data of patients with high transfusion therapy) is relatively strong and statistically significant ($P = .010$).

literature. Most studies on the carcinogenicity of iron are either related to adult cancers in which the length of tissue contact time with this oxidizing agent is higher or, if dealing with pediatrics, are related to the co-occurrence of lymphoid leukemia with a hemochromatosis gene, which causes iron overload from an early age.[10,14,29,30] It is necessary to make distinctions between the carcinogenicity of a substance and the role of a carcinogenic substance in the development of a particular cancer at a specific age. In the present study, most of the children, including the patients (at diagnosis) and the controls, had low iron stores (83% of the total of 60 children in both groups), which is consistent with the findings of other studies.[7,8] Although the data in this study reject the role of iron in the development of pediatric ALL, the evidence is not sufficient for making conclusions about the carcinogenicity of iron because of the small sample size.

This study shows that a BMIS decrease is associated with an increased chance of response to treatment. Given the potential relationship between the amount of blood transfusion and increased iron store, transfusions higher than 30 cc/kg/y were excluded, but the risk of drug resistance further increased. Several studies have examined the relationship between high body iron store and the development of drug resistance. Some of these studies provide clear evidence about the ability of reduced iron store to accelerate apoptosis, reduce resistance to treatment, and ultimately improve the response to treatment.[13,31–33] Although the present findings are in line with the cited studies, none of them have investigated the role of iron in pediatric ALL. In the present study, regardless of the disease's phenotype, the chances of 3-DFS and response to treatment (by the end of the first year) show a definite reduction with the increase in BMIS. Regardless of whether or not iron-lowering medications can be used to treat this cancer type and achieve better responses to treatment, measures can be taken to prevent increase in the body's iron store.

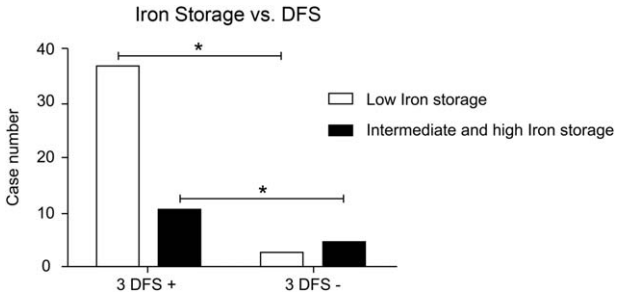


**Figure 3.** The relationship between level of "bone marrow iron store" and 3-years disease-free survival in pediatric Pre-B ALL is statistically significant ($P = .027$).

Many children who receive chemotherapy may at times need packed cell transfusion due to the complications of chemotherapy; this need is not the same in all children. In this study, during the first year of treatment, the need for this transfusion varied from 10 to 150 cc/kg; nevertheless, the majority (70%) of these children required less than 30 cc/kg of the transfusion. Iron storage gradually increased during the first year in more than 20% of the children with ALL in this study. There are similar results in the literature about iron overload in ALL children under treatment. Some studies link iron overload in these children to packed cell transfusion and argue that intensive treatment increases the need for blood transfusion and thereby the iron store.[34,35] In contrast, some studies have observed an increase in iron load only in patients who needed BM transplants.[36,37] In the present study, the increase in iron storage was not limited to blood transfusion and was also observed in patients with low blood transfusions. Nevertheless, contrary to the aforementioned studies, the present study used BMIS assessment instead of ferritin, as it is the best way to assess iron stores in the body.[38] In addition to blood transfusion, accessory factors such as excessive parental obsession with the children's nutrition can play a role. Nevertheless, perspective and clinical trials and molecular studies performed to elucidate the pathogenesis of the disease can pave the way for care plans devised for children with ALL. The evidence on the relationship between the development of resistance to treatment and increased iron stores may justify the need to control body's iron store by reducing blood transfusion, adjusting one's diet, and potentially considering iron chelation therapy in certain cases.[35]

Serum ferritin is an index of intracellular ferritin and is not necessarily related to body iron store; it especially increases in cases of inflammation with no link to the body's iron store.[39] Given that in most treatment plans BM aspiration is performed repeatedly in some stages of the treatment for assessment purposes, BM aspiration slides can be examined to evaluate BMISs. Anemia is a common finding in patients with cancer and there are several mechanisms for its incidence; however, regardless of the mechanism by which it is developed, modifying hemoglobin levels are emphasized for the condition.[40,41] Some evidence suggests that, in patients with anemia, the effect of hypoxia reduces the response to treatment.[42] Not all cases of anemia are due to iron deficiency and, although iron deficiency may affect the development of resistance to treatment through the noted mechanism, iron overload has a similar effect as well.[35] The administration of recombinant human erythropoietin (rHuEPO) to increase hemoglobin is perhaps a good strategy under these conditions and, due to the resultant reduced frequency of blood transfusion, it may not be associated with any increase in iron stores.[43]

## 5. Conclusion

Data in this study shows that BMIS is not associated with the incidence of pediatric ALL; however, during the treatment, iron stores gradually increase in some cases. The increase in iron stores may be due to the frequent blood transfusions or the parents' excessive care for the children. Increased BMIS is associated with the incidence of resistance to treatment at the end of the first year of the treatment as well as the risk of relapse. Controlling the iron store status can be associated with better treatment outcomes.

### 5.1. Study limitations

Quantitative evaluation of MRD was not feasible in this study. On the other hand, analysis of the hemochromatosis genes according to their mRNA profiles was not available in the patients' samples. Moreover, investigating larger populations of ALL patients in prospective cohort studies may help intensify the validity of results provided in this study.

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