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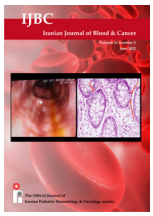

Original article

The protective role of deferoxamine in the prevention of doxorubicin-induced hepatic fibrosis in children: A randomized controlled clinical trial

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Abstract

Background: This study aimed to investigate the protective role of deferoxamine (DFO) in the prevention of doxorubicin (DOX)-induced hepatic fibrosis in children.

Methods: In this prospective randomized controlled trial, 61 treatment-naïve children (2-18 years) with different types of cancer who referred to a tertiary teaching hospital in the South of Iran were enrolled. They were randomly assigned to 3 groups; group 1 (control, n=21), group 2 (DFO 10 times DOX dose, n=20), group 3 (DFO 50mg/kg, n=20). DFO was administered as an 8-hour continuous intravenous infusion during and after DOX infusion in each chemotherapy cycle. Non-invasive serum markers of liver fibrosis, including AST-to-platelet ratio index (APRI), Fibrosis-4 (FIB-4) score and Fibro Test were measured in each individual. Besides, hepatic Fibro Scan was used after the last course of chemotherapy to estimate the fibrosis degree.

Results: Alanine aminotransferase was mildly increased after treatment compared to before treatment. The treatment with DFO 10 times DOX dose was associated with a significant decline in post-treatment APRI (adjusted odds ratio 0.17; 95% confidence interval 0.03- 0.84. P-value=0.015). The METAVIR fibro scores were in the F0-F1 zone in all participants, and the results were comparable in study groups. No adverse drug effects were reported in the treatment groups.

Conclusion: DOX may not lead to severe liver fibrosis if the maximum cumulative dose allowed is not exceeded. DFO at the dose of 10 times of DOX dose may have a potential protective role against liver fibrosis. More studies with longer follow-up are needed to further assess this issue.

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1. Introduction:

Anthracyclines are a group of chemotherapy agents which play a main role in the treatment of different types of childhood cancer[1, 2]. They include doxorubicin (DOX), daunorubicin, epirubicin, idarubicin, and mitoxantrone. It is speculated that the anti-neoplastic action of chemotherapy agents is

carried out through the induction of topoisomerase II, failure of DNA stands, and interference with DNA formation. Although the exact mechanism of acute DOX toxicity has yet been unknown, it is believed that it is mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, and lipid peroxidation of the cell membranes[3]. Induction of apoptosis and alteration of nitric oxide metabolism are other mechanisms that might be associated with DOX side effects [4, 5]. The toxicity of vital organs, including the heart and liver is a major limiting step in the widespread use of DOX, and a big concern for those who receive high cumulative doses of the drug[6].

On the other hand, Deferoxamine (DFO) is an iron chelator, and can bind to ferric ion by hydroxamic acid group, enhancing the drug excretion in the urine. Chelation of excess iron prevents its deposition and damage to several organs and tissues, including the liver[7]. It also prevents collagen accumulation and hepatic satellite cell activity, thus decreasing liver injury[8]. In addition, DFO is believed to have an anti-proliferative effect which may ultimately lead to cell cycle arrest and apoptosis [9]. This can also serve as an antioxidant, reducing liver fibrosis which is manifested as a declining trend in serum markers of liver fibrosis, including liver hydroxyproline and liver smooth muscle actin (α-SMA). Therefore, DFO decreases profibrogenic factors, and inhibits inflammatory cascade through different mechanisms[6, 7, 9, 10]. DFO is a historical iron-chelating agent that is generally used to treat transfusion-related iron overload in patients with thalassemia. The conventional dose of 50mg/kg(max dose:6gram/day) three to five times a week has an acceptable safety profile. Most of the side effects are limited to the site of subcutaneous injection, such as erythema, pain, and induration. More serious adverse effects, including ocular and ototoxicity are usually encountered in higher doses, especially when used for a long period [10].

There is a concern about whether iron-chelating agents may interfere with the anti-tumor activity of DOX. An in vitro study revealed that DFO may cause cytostasis through iron depletion. It also can inhibit breast tumor growth, and do not compromise the tumoricidal capability of DOX. DFO neither interferes, nor interactions with DOX [10,11].

There is no single antidote with proven efficacy in human beings to protect against DOX-induced hepatotoxicity. Although several studies in animal have shown some benefits using DFO, no clinical trial has been conducted in patients with cancer to assess its efficacy. Therefore, this study was designed to determine whether DFO has any beneficial role to prevent hepatotoxicity in children treated with DOX. The primary endpoint of this study was to compare hepatic fibrosis measurements in treated and non-treated groups. And the secondary endpoint was to assess whether different doses of DFO have different efficacy compared to the control subjects.

2. Materials and Methods

This single-center, prospective, parallel-group, and randomized-controlled trial was conducted in a referral oncology hospital in the south of Iran from July 2016 to April 2017. The population of the study included treatment-naïve children with the age range of 2–18 years and different types of cancer who were going to be treated with DOX as a part of their chemotherapy regimen. Patients with primary or metastatic liver tumors and those with underlying liver disease or active hepatitis B or C infections were excluded. Given that there was no previous experience with DFO regarding its hepatoprotective effect in cancer patients; it was assumed that patients treated with DFO would have a lower probability of DOX-associated liver fibrosis compared to the non-treated patients. Therefore, the authors used aspartate aminotransferase [AST]-to-Platelet Ratio Index (APRI) as an indirect marker of hepatic fibrosis, and compared APRI in the treatment groups with their control counterparts. A value of 0.5 was considered the cut-off point for discriminating mild fibrosis than no fibrosis [11]. A five percent decrease in the baseline APRI was regarded acceptable antifibrotic effect [12]. Accordingly, assuming the type I error of 0.05 and statistical power of 80%, the minimum sample size was estimated as 16 patients in each study group. It was decided to enroll at least 20 patients in each group to compensate for a possible 20% drop-out of the research due to the lack of enough follow-up. Since the beginning of the study, 123 new cases were admitted in this center though 85 patients were eligible to be enrolled among which 61 persons accepted to participate in the study. They were randomly allocated to three groups using a computer-generated block randomization sequence. It was done

by a statistician who was blind to the study protocol. Group 1 (n=21) consisted of patients who served as the control group. Patients allocated to group 2 (n=20) and group 3 (n=20) were pre-treated with DFO (Desferal®, Novartis, Switzerland), 10 times the DOX dose (equivalent to 10-20 mg/kg) and 50 mg/kg, respectively. Intravenous infusion of DFO was started 2 hours before chemotherapy which continued during DOX infusion (at least 4 hours), and for more 2 hours after the termination of the infusion, making up a total of 8 hours. This regimen was repeated in each chemotherapy course along with the infusion of DOX. Liver function tests (LFT), and complete blood count (CBC) tests were measured before and after the treatment. After the last course of chemotherapy, γ-glutamyltransferase (γGT), Haptoglobin, α 2 -macroglobulin (α2 MG) and apolipoprotein A1 (apoA1) were measured by Enzyme linked immunosorbent assay (ELISA) (Bioassay, China). Fibrosis-4 (FIB-4) score and AST-to-platelet ratio index (APRI) were calculated using the following formula:

$$\text{FIB-4 score} = \frac{\text{Age (Y)} \times \text{AST (U/L)}}{\text{Platelet count } (10^9/\text{L})} \times \frac{\text{Square root of}}{\text{(ALT) (U/L)}}$$

$$\text{APRI} = [\text{AST (U/L)} / 45] \times 100 / \text{platelet } (10^9/\text{L})$$

FIB-4 score ≤ 1.45 and APRI ≤ 0.5 were considered as no fibrosis, while the values more than 3.25 and 1.5 were regarded as significant and an indication of fibrosis, respectively [11, 13, 14]. FIB-4 Scores were dichotomized as zero for scores equal to or less than 1.45 and one for scores more than 1.45 [15]. FibroTest combines five standard biomarkers, including γGT, total bilirubin, α2 MG, apoA1, and Haptoglobin. These markers were interpreted depending on the age and sex. A cut-off value of 0.58 was assigned to delineate severe fibrosis [16]. Additionally, liver stiffness was measured in all patients by FibroScan or transient elastography (TE) (EchoseNS, Paris, France). Liver stiffness was expressed in kilopascals (kPa) and was computed for each subject as the median of 10 validated measurements according to the manufacturer's instructions. Measurements with an interquartile range of < 30% of the median value and a success rate of > 60% were considered reliable. Two-dimensional shear wave elastography (SWE) studies were performed using the Aixplorer ultrasound system (Supersonic Imagine SA, Aix-en-Provence, France) with a convex broadband probe (SC6-1, Super-Sonic Imagine). This technique has an advantage over others, since the probe can be installed on ultrasound machines. For quantitative measurements, a round region of interest was placed inside the SWE box, and minimum and maximum values of stiffness expressed in kPa were recorded. Four measurements were made, and the median value was recorded. The METAVIR fibrosis score, graded on a 5-point scale from 0 to 4, was used to delineate the degree of fibrosis (Table I). The scores were compared among the three groups. The normality of data was checked by Shapiro-Wilk test. Bivariate comparison of quantitative variables among three groups was done by ANOVA and Kruskal Wallis tests. Chi-square test was also used to compare qualitative variables among the three groups. A paired t-test, Wilcoxon signed-rank test, and McNemar's test were used to compare data before and after treatment in each group. The null hypothesis was assumed to be a decrease of less than 5% of APRI scores compared with their respective baselines. Then, a binary variable was generated considering the change value in the patient APRI score (i.e., APRI score after the intervention minus APRI score at baseline), in which patients with a decrease of more than five percent in their APRI scores were considered satisfactory outcome based on observations and others were defined as poor outcome. Binary Logistic regression was applied to estimate the independent (Adjusted for age, sex, and baseline APRI score) effect of the treatment in terms of adjusted odds ratio (OR) and its robust 95% confidence interval (95% CI). A two-sided p-value less than 0.05 or a one-sided p less than 0.025 k was considered significant. Data were analyzed using SPSS software (version 21.)

Figure 1 shows graphical abstract of the protective role of DFO in the prevention of DOX induced hepatic fibrosis.

2.2 Ethical consideration

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (code number: IR.SUMS.REC.1394.193.). The study protocol was registered in the Iranian Registry of Clinical Trials (registry number IRCT2016080315666N4). Informed written consent was obtained from either all participants or their parents.

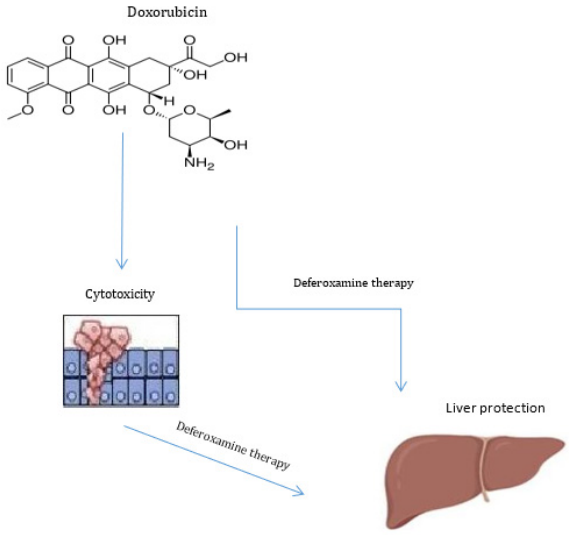

Figure 1: Graphical abstract of the protective role of deferoxamine in the prevention of doxorubicin-induced hepatic fibrosis.

3. Results

As the study was going on, one patient in group 2, and also 2 patients in group 3 died of cancer. 2 more patients in group 3 withdrew from the experiment and were excluded. Therefore, the study ended with 56 patients. No adverse drug reaction was reported while using DFO in treatment groups. The study flowchart is available in the supplement file (**Figure 2**). The patients in three groups were statistically compared in terms of age, sex, and duration of treatment (**Table 1**). Mean parameters of CBC and LFT, pre- and post-treatment with DFO among the 3 groups are shown in **Table 2**.

The measured background parameters are initially compared in **Table 3**. Similarly, no difference was observed following treatment between the study groups regarding these background characteristics (**Table 3**). When pre and post-treatment values of background variables were compared in each group, only total protein in group 3 (6 mg/dl vs 5.87 mg/dl, p = 0.047), ALT in group 2 (19 mg/dl vs 36 mg/dl), and group 3 (20.5 mg/dl vs 31.5 mg/dl) were significantly different (p = 0.03).

Regarding indirect serum biomarkers of liver fibrosis, percentage of at-risk patients according to the FIB-4 score was significantly decreased in group 3 following treatment with DFO (McNemar's test p =0.02).

There was no statistically significance association between group and FIB-4 score after the study period (p = 0.99). In terms of change in the APRI score, although treatment in group 3 had a significant antifibrotic effect compared with patients baseline APRI score (one-sided P- value= 0.015) (**Table 3**), no statistically independent effect was observed compared with the control group (adjusted OR, 0.327; 95% CI: 0.028–3.78). The comparison of odds of an acceptable ant fibrotic effect of treatment in group 2 compared with the control group was at 0.17 with a 95% CI of 0.03 to 0.84, adjusted for age, sex and patients' baseline APRI score.

The scores were not significantly associated with duration of treatment and DOX cumulative dose of the patients (data not shown). With regards to FibroTest, except for one patient in group 3 who exceeded the cut-off value of 0.58, other patients were within the safe zone of mild or no fibrosis. Figure III shows the comparison of Fibroscore among the three groups following treatment with DFO. All patients had METAVIR scores in the F0-F1 zones with no significant difference between the groups.

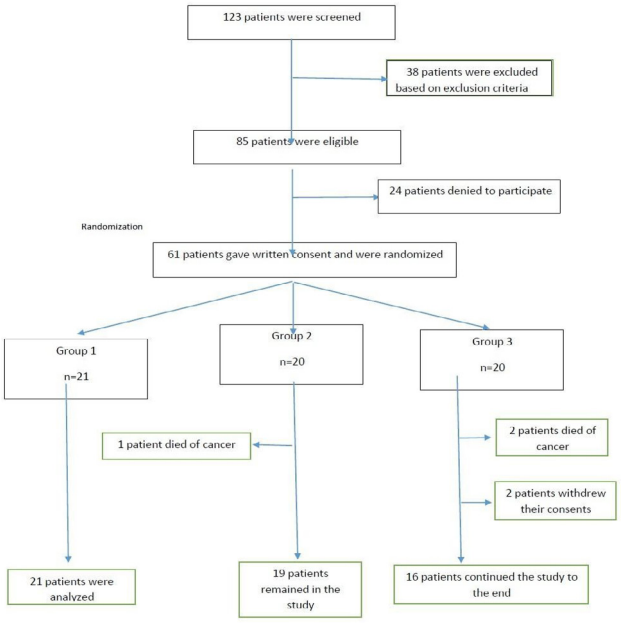

Figure 2: Population screening flowchart and randomization.

Table 1. Demographic data of the study population

Variable	Group 1	Group 2	Group 3	P-value
Number (Male/Female)	21(16/ 5)	19(15/ 4)	16(11/5)	0.62
Age (y) (Mean ± SD)	6.8 ± 4.8	7.9 ± 4.7	8.6 ± 4.8	0.7
Body surface area (/m2 (Mean ± SD)	0.93 ± 0.40	0.92 ± 0.35	1 ± 0.33	0.55
Malignancy (%) Leukemia Lymphoma the Other tumors	11(52.4%) 7(33.3%) 3(14.3%)	9(47.4%) 8(42.1%) 2(10.5%)	8(50%) 6(37.5%) 2(12.5%)	0.57
Duration of the treatment(Mo) (Mean ± SD)	14 ± 4	10 ± 3	12 ± 4	0.61
Doxorubicin cumulative dose (mg/m2) (Mean ± SD)	225 ± 132	224 ± 116	218 ± 130	0.68

Group 1: control group; group 2: treated with DFO (Deferoxamine) 10 times the DOX (Doxorubicin) dose; group 3: treated with DFO 50 mg/kg

Table 2. CBC and LFT, pre- and post-treatment with Deferoxamine

Pre-treatment values	Group 1 (n = 20)	Group 2 (n = 19)	Group 3 (n = 16)	P1 value
White blood cell (109/L)	11.15 ± 15.98	8.09 ± 7.65	18.47 ± 23.60	0.17
Hemoglobin (g/dl)	10.12 ± 2.71	9.55 ± 1.34	9.61 ± 2.61	0.69
Platelet (109/L)	181.19 ± 128.28	203.79 ± 120.39	160.750 ± 152.78	0.63
Total protein (g/dl)	6.03 (5.12–8.01)	5.90 (4.25–7.30)	6.10 (5.05–8.90)	0.57
Albumin (g/dl)	4.0 (3.10–5.40)	3.90 (2.70-5.00)	3.75 (3.00-4.50)	0.41
Total bilirubin (mg/dl)	0.50 (0.40–1.50)	0.53 (0.25–2.20)	0.52 (0.23–2.40)	0.53
Direct bilirubin (mg/dl)	0.10 (0.01–0.40)	0.10 (0.10–0.30)	0.10 (0.10–0.90)	0.42
Aspartate aminotransferase (U/dl)	33.0 (15.0–78.0)	31.0 (11.0-286.0)	32.50 (11.0–73.0)	0.35
Alanine aminotransferase (U/dl)	27.0 (8.0-136.0)	19.0 (11.0-317.0)	20.50 (5.0-115.0)	0.47
Alkaline phosphatase (U/dl)	367.0 (230.0-760.0)	370.0 (210.0-2850.0)	335.0 (246.0-952.0)	0.62

Group 1: control group; group 2: treated with DFO (Deferoxamine) 10 times the DOX(doxorubicine) dose; group 3: treated with DFO 50 mg/kg

Table 2. CBC and LFT, pre- and post-treatment with Deferoxamine

Post-treatment values	Group 1 (n=20)	Group 2 (n=19)	Group 3 (n=16)	P1 value
White blood cell (109/L)	4.48.±1.44	7.0±6.78	8.73±9.15	0.13
P2 value	0.07	0.68	0.10	
Hemoglobin (g/dl)	10.23±1.62	9.84±0.83	10.11±1.12	0.60
P2 value	0.82	0.40	0.44	
Platelet (109/L)	161.08±93.93	199.59±85.08	198.12±114.66	0.37
P2 value	0.28	0.88	0.31	
Total protein (g/dl)	6.0 (5.0-7.45)	5.80 (4.25–6.45)	5.87 (4.75-7.0)	0.52
P2 value	0.20	0.10	0.046	
Albumin (g/dl)	3.95 (3.05–4.50)	3.90 (2.90–4.35)	3.85 (2.75–4.25)	0.40
P2 value	0.56	0.80	0.85	
Total bilirubin (mg/dl)	0.5.0 (0.25–2.20)	0.55 (0.25–1.50)	0.57 (0.30–1.85)	0.35
P2 value	0.52	0.22	0.79	
Direct bilirubin (mg/dl)	0.10 (0.05–0.50)	0.11 (0.01–0.31)	0.15 (0.10–0.75)	0.65
P2 value	0.41	0.48	1.00	
Aspartate aminotransferase (U/dl)	32.50 (9.50–84.0)	29.50 (18.0-355.50)	26.25 (16.0-111.0)	0.47
P2 value	0.09	0.43	0.34	
Alanine aminotransferase (U/dl)	28.50 (14.0-106.50)	36.0 (23.0-698.50)	31.50 (18.0-225.0)	0.43
P2 value	0.42	0.03	0.03	
Alkaline phosphatase (U/dl)	324.0 (215.0-545.0)	380.0 (171.0-525.0)	331.25 (221.50–540.0)	0.57
P2 value	0.21	0.64	0.28	

Group 1: control group; group 2: treated with DFO (Deferoxamine) 10 times the DOX(doxorubicine) dose; group 3: treated with DFO 50 mg/kg
*Data related to parameters with normal distribution are summarized as mean and standard deviation, and those related to non-normal distribution are shown as median and interquartile range. P1 is related to the comparison of values between the 3 groups; P2 is related to the comparison of pre- and post-treatment values in each group.

Table 3. The comparison of indirect serum markers of liver fibrosis in the study groups*

	Group 1 (n=20)	Group 2 (n=19)	Group 3 (n=16)	P1 value
APRI				
Pre-treatment	0.62(0.31–1.53)	0.52(0.27–1.09)	0.57(0.24–3.59)	0.87
Post-treatment	0.66(0.33–0.89)	0.43(0.36–0.64)	0.38(0.25–0.66)	0.20
P2 value**	0.06	0.22	0.015	
FIB-4 score#				
Pre-treatment	0.27(0.09–0.71)	0.27(0.19–0.44)	0.38(0.21–1.68)	0.23
Post-treatment	0.17(0.11–0.52)	0.20(0.13–0.34)	0.21(0.11–0.40)	0.99
P2 value	0.725	0.191	0.020	
FibroTest	0.006 (0.003–0.02)	0.008 (0.004–0.02)	0.01 (0.005–0.02)	0.33

FIB-4: Fibrosis-4 score, APRI: AST-to-platelet ratio index
Group 1: control group; group 2: treated with DFO (Deferoxamine) 10 times the DOX(Doxorubicin) dose; group 3: treated with DFO 50 mg/kg; GT: gamma glutamyl transferase; α2MG: alpha2 macroglobulin; APOA1: apolipoprotein A1; APRI: AST-to-platelet ratio index.

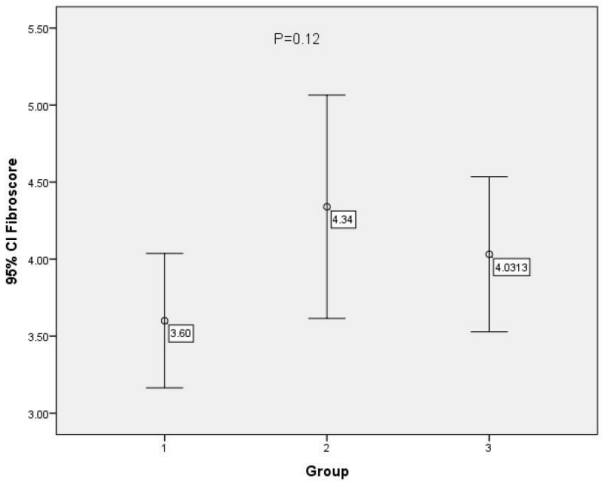

Figure 3: Assessment of liver stiffness by FibroScan in the three groups, regarding post-Deferoxamine treatment.

4. Discussion

This study was the first human clinical trial designed to investigate the protective role of DFO against DOX-induced liver injury in pediatric patients with cancer. This study could show that DOX didn't have significant toxicity to the liver tissue, at least in the short-term, though transient transaminases were seen in a subgroup of patients. There was no intergroup difference in the LFT parameters of patients treated with DFO and the group. Within each group, all parameters remained comparable except for a small rise in ALT level in groups 2 & 3 and a minor drop in total protein in group 3, but they were within the normal range (Table III). Due to the lack of similar clinical trials in the past, the comparison of our results with other findings was not possible.

The protective role of DFO against DOX-induced liver damage was previously studied in animal models. Saad et al., showed that pretreatment with DFO significantly reduce peroxidative damage in the myocardium, hepatic and renal tissues of rats who had an acute injection of DOX. The maximum effect was obtained with the dosage of 10-fold that of DOX. Higher dosing was not only more protective[12].

In this study, the researcher checked some indirect serum markers of fibrosis, and found that APRI and FIB-4 scores were significantly decreased in patients who were pre-treated with higher dose DFO (group 3, 50 mg/kg) (one-sided P values 0.015 and 0.02, respectively). However, statistical adjustment for age, sex, and baseline APRI revealed that only treatment in group 2 was independently accompanied with 83% decrease in the risk of DOX-related liver fibrosis. The application of these circulating factors to predict liver fibrosis is becoming more popular because they are available in many laboratories. Furthermore they are almost not expensive, and can be repeated several times. They may also indicate fibrosis in the whole liver, thus avoiding small sampling error which is a technical defect in percutaneous liver biopsy[13].

The predictive value of indirect serum markers of liver fibrosis was previously mentioned in conditions such as viral hepatitis and fatty liver disease[14, 1]. In this regard, Unalp-Ardia et al., similarly reported that APRI could be used to predict liver fibrosis with higher scores indicative of advanced liver disease [15]. Moreover, it has been claimed that the combination of APRI with FibroMeter may show an accurate lower-cost alternative to liver biopsy to evaluate fibrosis[16]. A meta-analysis showed that APRI > 1 had a sensitivity of 76% and a specificity of 72% to predict cirrhosis in patients infected with hepatitis C virus (HCV). For the significant fibrosis, an APRI threshold of 0.7 was 77% sensitive and 72% specific. They concluded that APRI might prevent the need for staging liver biopsy in a subset of patients with HCV infection[17].

Moreover, the FIB-4 index, as another non-invasive serum marker to delineate liver fibrosis, has been implicated in a variety of illnesses, including hepatitis B virus (HBV) infection. Mallet et al., [1]investigated the accuracy of the FIB-4 index in a group of chronic HBV-infected patients and concluded that a cut-off value ≤ 1.45 can differentiate moderate fibrosis from severe fibrosis with a negative predictive value of 86%, a sensitivity of 71.1% and a specificity of 73.1%. He asserted that it is even more precise than APRI to exclude significant fibrosis. Shah et al.,[14] reported that in patients with NAFLD, the FIB-4 index is superior to other non-invasive markers of fibrosis. In addition, a systematic review and meta-analysis showed that APRI and FIB-4 can identify hepatitis B-related fibrosis with a moderate sensitivity and accuracy. They suggested that an APRI threshold of 0.5 and 1.5 and an FIB4 threshold of 1.45 and 3.25 had acceptable sensitivity and specificity to delineate mild from

significant fibrosis [18]. It showed that treatment with higher dose DOX (50 mg/kg) may be associated with significant decrease in the FIB-4 score (Table III), though it could not reveal its independent association with the FIB-4 score.

In this study, the researcher checked FibroTest which is a commercially available algorithm combining different elements, and has been proven to have a high predictive value in advanced fibrosis. A cut-off value of 0.58 has been reported to associate with severe fibrosis ($F \geq 3$) [19]. Only one patient who was treated with high-dose DFO (group 3) exceeded this cut-off point, so the results were comparable in the 3 groups.

The same finding confirmed by TE showed that none of the patients in this study experienced significant fibrosis following DOX treatment. They all had a METAVIR score in the F0-F1 zones, compatible with no or mild fibrosis. Meanwhile, it was shown that DFO even further decreased serum markers of hepatic fibrosis when administered at a dose of 10 times DOX dose. It may be promising that DFO may play a role in reducing the chance of liver fibrosis in long term. Despite normal LFT, METAVIR score, APRI, FIB-4 index, and FibroTest, nobody can guarantee that these patients will be protected from liver fibrosis and cirrhosis when they reach adulthood. Therefore, there is always a concern that survivors of pediatric malignancy may suffer from multi-organ damage in the future, particularly if they are treated with toxic agents such as anthracyclines with proven cardiac and possibly hepatic and renal complications. It is also essential to consider that non-invasive predictors of liver fibrosis, such as serum markers and FibroScan are more reliable for the detection of advanced fibrosis, and are less sensitive in the early stages of liver fibrosis ($F \leq 2$)[13] . Although none of the patients in this study suffered from severe fibrosis as a result of the chemotherapy effect, it is highly recommended to have some follow -up procedures for such children, for at least a decade to investigate the long-term toxicity of chemotherapy agents on the liver and other organs.

The most effective dose of DFO in preventing DOX toxicity in various organs is not known. Our study compared the two different doses of DFO and showed that the dosing 10-times that of DOX (equivalent to 10-20 mg/kg), as iron chelator has a more beneficial role in reducing hepatotoxicity than the conventional dose (50 mg/kg) in thalassemia patients. Due to the lack of similar human studies, we could not compare our results with them. Further pharmacokinetic studies on a larger scale are required to find the optimum protective dose in the target organs.

This study had some strengths and limitations. Regarding the strengths, it was the first randomized clinical trial in patients with cancer, especially in the pediatric age group that assessed the role of DFO as a rescue therapy to prevent liver damage induced by DOX. Up to now, just few animal studies have been conducted in this regard, and this is the first human study. Secondly we tried to assess liver fibrosis with different modalities including FibroScan, LFT and non-invasive markers of liver fibrosis such as FibroTest, APRI and FIB-4 index. This probably helped us to increase the accuracy of this assessment.

On the other hand, the study faced some limitations. Firstly, the small sample size and the short follow- up period that could hinder the generalizability of the results. A larger multi-center study with longer follow up procedures is required to assess the reproducibility of the results. Secondly, the heterogeneity of the study population in terms of their primary diagnosis was another issue that may have confounded the results, because the chemotherapy protocols were not the same in all participants. Therefore, the interaction of other chemotherapy agents with DOX and their hepatic side effects cannot be overlooked. Lastly, tissue biopsy was not done to assess liver fibrosis in patients due to the ethical issues and the potential hazards of liver biopsy in cancer patients. Though FibroScan, and non-invasive serum markers of fibrosis are good alternatives to liver biopsy, they are more informative in advanced [20] fibrosis than in early stages of liver fibrosis. Moreover, it would have much more informative if we could have done the fibroscan before the study, and then compare the results with post-test data.

In the end, considering all the pros and cons of the study, these results may shed light on future research to solve the dark sides of this puzzle. It is highly advised to perform a large multi-center trial in a cohort of patients with similar malignancy such as leukemia to know whether DFO may be useful in the long-term protection of liver injuries related to anthracyclines.

5. Conclusion

DOX does not lead to severe fibrosis in the liver, provided that it would not exceed the maximum allowed cumulative dose. DFO at the dose of 10 times of DOX dose may have a potential protective role

against liver fibrosis. Larger multi-center studies are suggested to further assess this hypothesis.

Authors' contributions

Study conception and design were done by MB, GF, MF. SH, GF, MB, MS and analyzed and interpreted the data. Acquisition of data was done by MF, HMV, MB, GF and NS, MB and MD drafted the manuscript. The critical revision was done by all authors. All authors reviewed and approved the final manuscript.

Availability of data and materials

The data that support the findings of this study are available from Research Center of Shiraz University of Medical Science but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Research Center of Shiraz University of Medical Science.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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