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Therapeutic Drug Monitoring of Chelating Agent Deferoxamine for β-Thalassemia Major Patients

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ABSTRACT

Purpose: Therapeutic drug monitoring is used to prevent or decrease the risk associated with the toxic effects of medication. This study aims to evaluate the potential advantages of Therapeutic Drug Monitoring (TDM) of subcutaneous Deferoxamine injection and prevention of clinical problems in β-thalassaemia major patients. **Patients & Methods:** Fifty-four thalassemia patients were allocated into two groups; missing, and not missing deferoxamine dose. TDM of Deferoxamine injection and its clinical outcomes were critically studied under the following subheadings: assessment of the adequacy of Deferoxamine usage, serum peak and trough concentrations of Deferoxamine and ferroxamine with needed pharmacokinetics, cardiac parameters and biomarkers, biochemical and hematological indices, adverse effects/toxicity, urinary assessment of Fe, Zn, selenium, and copper levels, compliance to treatment, dose adjustment in correlation to therapeutic index and life style. **Results:** Demographic data showed no significant difference. Peak plasma concentrations were 144.83 ± 69 and 43.54 ± 39.16 μg/L, while trough concentrations were 33 ± 26.32 and 31.13 ± 21.58 μg/L of Deferoxamine and ferroxamine, respectively. The elimination rate constant was 0.0237 ± 0.00029 min^{-1}, half-life was 34 min, and distribution volume was 0.93 ± 0.078. Although cardiac parameters showed no significant differences, there were significant differences in CK-MB, and hsCRP levels; troponin I value could not be detected. Biochemical and hematological studies showed significant differences in Ferritin B, urea, SGPT, SGOT, alkaline phosphatase, serum albumin and serum calcium. Assessment of adverse effects/toxicity showed significant differences. The correlation of serum ferritin to therapeutic index, and the life style including Vitamin C and/or E administration were assessed for the compliance to treatment. **Conclusion:** Therapeutic monitoring of chelation therapy by Deferoxamine in β-thalassemia patients is necessary to ensure effective treatment, compliance, and to avoid adverse side effects and toxicity.

Keywords: Therapeutic Drug Monitoring; Deferoxamine; β-Thalassemia Major

1. Introduction

Therapeutic drug monitoring involves not only measuring drug concentrations, but also the clinical interpretation of the result. This requires knowledge of the pharmacokinetics, sampling time, drug history and the patient's clinical condition [1].

Homozygotes for beta-thalassemia may develop either thalassemia major or thalassemia intermedia. Thalassemia major patients come to medical attention within the first 2 years and require regular blood transfusion to survive. Differentiation of thalassemia major from thalassemia intermedia at presentation is a difficult and critical issue that should be strongly pursued [2].

Iron overload occurs when the intake of iron is increased over a prolonged period of time and is commonly seen in patients with beta-thalassaemia major, who receive frequent blood transfusions. The iron excess is initially stored in thereticuloendothelial system (which has a capacity of about 10 - 15 g), and then in all parenchymas [3], resulting in life-threatening complications, namely cardiopathy, liver and endocrine dysfunction and reduced patient's survival. The damage is characterized by excessive iron deposition. Without adequate iron chelation therapy, almost all patients with beta-thalassemia will accumulate potentially fatal iron levels [4].

Particular attention has been directed to the early di-

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agnosis and treatment of cardiac disease because of its critical role in determining the prognosis of individuals with β-thalassemia. Assessment of myocardial siderosis and monitoring of cardiac function combined with intensification of iron chelation can result in excellent long-term prognoses [5-7].

The most common secondary complications are those related to transfusional iron overload, which can be prevented by adequate iron chelation. After 10 to 12 transfusions, chelation therapy is initiated with Deferoxamine (DFO), which is derived from ferrioxamine B, a siderамine isolated in 1960 from *Streptomyces pilosus*. It has a high binding affinity for trivalent iron, which can be exploited to clinically remove excess iron from blood and tissue [8]. It is administered five to seven days a week from 12-hour continuous subcutaneous infusion via a portable pump. Recommended dosage depends on the individual's age and the serum ferritin concentration. The dose may be reduced if serum ferritin concentration is low. Deferoxamine therapy prevents the secondary effects of iron overload, resulting in a consistent decrease in morbidity and mortality [9]. The advantages of DFO are its high affinity for ferric iron, high efficiency in attaining negative iron balance and absence of iron redistribution. Its disadvantages are that it is not orally bio-available, fast rate of metabolism necessitating prolonged parenteral infusions, poor compliance, chelates zinc (sometimes causing clinical zinc deficiency) and high cost. Ascorbate repletion (daily dose not to exceed 100 - 150 mg) increases the amount of iron removed after DFO administration [10].

The primary signs of chelator toxicity are hearing loss, temporary loss of sight, cataracts, renal dysfunction, growth failure, and symptoms related to iron deficiency, (which may occur when total body iron is low but high doses of Deferoxamine are still being administered [11], and rarely, renal impairment and interstitial pneumonitis. DFO administration also increases susceptibility to Yersinia infections. The major drawback of DFO chelation therapy is low compliance resulting from complications of administration [12]. In addition to that, many trace metals are decreased, including selenium, zinc, and copper etc. [13].

Iron chelation is essential for patients with transfusion-dependent anemia, who inevitably develop iron overload and the risk of life-threatening end organ damage. The heart, along with liver and endocrine glands, is one of the main organs where iron deposition causes severe complications [10].

In clinical practice, the effectiveness of DFO chelation therapy is monitored by routine determination of serum ferritin concentration. However, serum ferritin concentration is not always reliable for evaluating iron burden because it is being influenced by other factors, the most important being the extent of liver damage [12]. Lack of response of an individual may result from inadequate dosing, high transfusion requirement, poor treatment adherence, or unfavorable clinical pharmacology of the chelation regime [14].

The indications for therapeutic drug monitoring have widened to include efficacy, compliance, toxicity avoidance, and therapy cessation monitoring [15]. A straightforward relationship between administered dose, serum concentration, clinical outcome, or adverse effects is often lacking. Nowadays, the focus lies in therapeutic drug monitoring and individualized therapy to find adequate treatment, to explain treatment failure or non-response, and to check patient compliance. However, extensive research in this field is still mandatory [11].

This study aimed to evaluate the potential advantages of TDM of chelating agent; subcutaneous Deferoxamine injection as part of medical treatments and prevention of clinical problems in β-thalassaemia major patients. The TDM assessment will be performed by determining the following: adequacy of Deferoxamine dose response, cardiac variables and biomarkers, biochemical and haematological tests, adverse effects/toxicity (systemic and topical) with urinary elements excretion, and patients' compliance to Deferoxamine treatment.

2. Patients & Methods

This study was a prospective, single-center investigation carried out in Sulaimani Thalassemia Center/Kurdistan Iraq, for the standard maintenance chelation monotherapy of subcutaneous Deferoxamine (20 - 50 mg/Kg/day), over 8 - 12 h administer at home. The study included 54 patients with β-thalassemia major, allocated to two groups; missing Deferoxamine dose versus non-missing dose, between March 2012 and February 2013. Inclusion criteria: Major β-Thalassemia patients that are regularly blood transfused and used subcutaneous Desferroxamin injection as an iron chelator. And those patients willing to participate in this study aging between 5 - 43 years old in both genders. While, exclusion criteria were: Patients who are shifting between Desferroxamin injection and Deferasirox tablet, pregnancy or expected pregnancy within six months, lactation, presence of active infection, history of tuberculosis, HIV, hepatitis B or C, severe pancreatitis (necrotizing pancreatitis or pancreatitis leading to multi organ failure), malignancy, ongoing treatment with other chelating agents, bone marrow suppression, and any inflammatory or infectious disease that induced high false serum ferritin levels. The Scientific and Review Board at the College of Pharmacy/Hawler Medical University approved the study protocol, and all parents or patients provided written informed consent, in accordance with the Declaration of Helsinki.

The TDM assessment was performed as discussed under the following subheadings.

2.1. Adequacy of Deferoxamine Dose Response Performed by Short Hospital Admissions

The drug was infused via a thin needle inserted subcutaneously and connected by an infusion line to a portable battery electrical device. Five ml of distilled water was added to a bottle containing 500 mg of Deferoxamine powder and shook well to make (10%) 5 ml clear solution. The infusion continued for 8 - 10 h and was given 5 - 7 times per week at a mean daily dose of 20 - 50 mg/kg body weights. The dose of Deferoxamine was adjusted according to body iron (serum ferritin level) and age. Dose adjustment was made with reference to the serum ferritin level using the therapeutic index (Porter JB. 1989); the aim was to keep the index 0.025 constant at all time. Obtaining prospective pharmacokinetic (PK) data; peak and trough concentrations of deferoxamine and ferroxamine using HPLC (Shimadzu, Koyota, Japan) analysis method, with needed pharmacokinetics; rate of elimination (Ke), half-life ($t_{1/2}$), and volume of distribution (Vd), in addition to the data concerning administrating both groups of patients, that is missing drug dose versus non-missing dose was the main objective of this research.

2.2. Cardiac Variables and Biomarkers

Cardiac variables determine are heart rate, systolic and diastolic B.P, ejection fraction, and mild dilated right ventricular) while biomarkers include Troponin I (VIDAS® TROPONIN l ULTRA "TNIU"), Ck-MB (VIDAS® CK-MB), and high sensitive CRP (*i*-CHROMA™ hs CRP)].

2.3. Biochemical and Hematological Tests

This was performed by measuring serum ferritin, serum-creatinine, blood urea, SGPT, SGOT, alkaline phosphate, total serum bilirubin, serum albumin, serum calcium, WBC, and platelets using SIEMENS ADVIA centaur®, FLEXOR equipment (which istype EL 200 automatic close system).

2.4. Adverse Effects/Toxicity

This was evaluated by closely monitoring patient for any systemic and topical adverse effects or toxicity from treatment, and by 24-h urine sample collection for measuring the urinary Fe, Zn, selenium, and copper elements excretion levels; using inductive coupled plasma-Optical emission spectrometer (ICP-OES) by Perkin Elmer (model Optima 2100 DV).

2.5. Patients' Compliance to Deferoxamine Treatment

In addition to the above tested parameters evaluated, relationship of dose treatment to serum ferritin and therapeutic index were also evaluated. Lifestyle, including administration of tea, and Vitamin C and/or E was as well determined.

3. Statistics

Data were analyzed using the statistical package for social sciences (SPSS version 17). Student t-test of two independent samples was used to compare between means, while Chi-square test of association was used to compare between proportions, $p \leq 0.05$ was considered as statistically significant.

4. Results

4.1. Demography

Fifty-four patients were included in this study, of which 19 males (63.3%) and 11 females (36.7%); 8 males (33.3%) and 16 females (66.7) were in G1 (missing DFO dose) and G2 (not missing DFO dose) respectively. Ages of patients in G1 and G2 were (14.7 ± 7.414) and (18.5 ± 10) respectively (**Table 1**).

Table 1. Demography of fifty-four thalassemia patients used for this analysis.

Variables		G1 (No. = 30)	G2 (No. = 24)	p value
Age (year)		14.7 ± 7.414	18.5 ± 10	0.115
Gender	Male No. (%)	19 (63.3%)	8 (33.3%)	0.028*
	Female No. (%)	11 (36.7 %)	16 (66.7%)	
Weight (Kg)		34.916 ± 14.614	37.808 ± 13.152	0.46
BMI		17.333 ± 2.996	18.234 ± 2.736	0.265
Height (cm)		138.633 ± 19.813	141.347 ± 16.848	0.6
Body temperature		35.772 ± 1.096	35.725 ± 1.048	0.87
Splenoctomy No. (%)		12 (41.4)	10 (41.7)	0.983

Each value represents the mean ± standard deviation or number and percentage, *$p < 0.05$.

4.2. TDM of DFO Chelation Therapy

4.2.1. Adequacy of DFO Dose

Variables measured under this section are; age of thalassemia diagnosis (year), duration of DFO administration (year), number of DFO vials administered by subcutaneous route per day and off days/week (Results are presented in **Table 2**) Patients who had one off day of the treatment/week were 16 (53.3%) and 13 (54.2%); followed by two off days/week, 9 (30%) and 8 (33.3%) and no off day of treatment, 3 (10%) and 3 (12.5%) for G1 and G2 respectively, while no DFO administration was 2 (6.7%) for G1 and 0 (0%) for G2, all with no statistical significant difference between them.

The pharmacokinetic parameters of DFO and FO, which include peak concentration mg/L, trough concentrations mg/L, rate of elimination, half life, and volume of distribution are as shown in **Table 3**.

4.2.2. Cardiac Variables and Biomarkers

There were no significant difference in the measured cardiac parameters between the two groups, except in CK-MB and high sensitive C-reactive protein which had values 3.58 ± 4.04 and 1.56 ± 1.2 μg/L for G1 and 3.93 ± 3.7 and 1.91 ± 1.9 mg/L for G2 respectively (**Table 4**).

Table 2. Data concerning the drug dosage.

Variables		G1 (No. = 30)	G2 (No. = 24)	p value
Age of diagnosis (year)		1.42 ± 1.66	2.03 ± 3.13	0.422
Duration of DFO used (year)		6.65 ± 4.21	6.76 ± 4.53	0.92
No. of DFO vials administered by S.C/day		2.25 ± 1.09	2.77 ± 1.05	0.086
Off days/week	No off days	3 (10%)	3 (12.5%)	0.78
	One off day	16 (53.3%)	13 (54.2%)	
	Two off days	9 (30%)	8 (33.3%)	
	No DFO administration	2 (6.7%)	0(0%)	

Each value represents the mean ± standard deviation or number and percentage.

Table 3. DFO pharmacokinetic parameters.

Variables	DFO	FO
Peak conc. (mg/L)	36.88 ± 6.8	15.83 ± 5.37
Trough conc. (mg/L)	$86.8 \times 10^{-7} \pm 7.23 \times 10^{-7}$	$83.95 \times 10^{-7} \pm 9.43 \times 10^{-7}$
Ke (min^{-1})	0.0237 ± 0.00029	0.022 ± 0.0006
t½ (min)	29.2 ± 0.36	31.45 ± 0.864
Vd (L/Kg)	0.93 ± 0.078	2.32 ± 0.61

Each value represents the mean ± standard deviation.

Table 4. Cardiac variables and biomarkers.

Variables	G1 (No. = 30)	G2 (No. = 24)	p value
Heart rate (beat/min)	90.433 ± 13.142	90.043 ± 12.178	0.912
Systolic B.P (mmHg)	98.846 ± 9.623	103.409 ± 12.089	0.152
Diastolic B.P (mmHg)	57.692 ± 8.629	61.818 ± 8.387	0.1
Ejection fraction (%)	65.3 ± 5.175	65 ± 6.518	0.74
Mild dilated right ventricular	3 (15%)	0 (0%)	0.535
Troponin (μg/L)	0.0013 ± 0.007	0.0142 ± 0.069	0.376
CK-MB (μg/L)	3.58 ± 4.04	1.56 ± 1.2	0.026*
High sensitive CRP (mg/L)	3.93 ± 3.7	1.91 ± 1.9	0.027*

Each value represents the mean ± standard deviation or number and percentage. *$p < 0.05$.

4.2.3. Biochemical and Hematological Assessment

The highest significant differences were seen in serum ferritin with value (6937.96 ± 2748.2) µg/L, and 3890.25 ± 2770.98) µg/L for G1 and G2 groups respectively. Blood urea (mg/dl) in patients ≤ 12 years old was 26.39 ± 5.69 and 20.74 ± 4.31, while for patients >12 years old it was (29.76 ± 7.21) and (23.16 ± 6.4), SGPT was (68.93 ± 31.61), and (46.35 ± 23.9) units/L, SGOT was (64.39 ± 30.7) and (47.78 ± 19.26) units/L, alkaline phosphatase (units/L) in patients ≤ 12 year olds was (526.84 ± 230.02) and (300.41 ± 148.17) while in patients >12 years old it was (365.05 ± 141.36) and (235.14 ± 97.85) in G1 and G2 respectively. Serum albumin also showed highest significant difference of 4.29 ± 0.3 and 4.7 ± 0.4) similar to serum calcium which gave 8.22 ± 0.9) mg/dl and 9.28 ± 0.88) mg/dl for G1 and G2 respectively, as showed in **Table 5**.

4.2.4. Monitoring of Adverse and Toxic Effect of DFO in Enrolled Major β-Thalassemia Patients

1) Urinary elements excretion

There were significant differences in urinary elements excreted. The value of Fe was (814.74 ± 654.49) and (3166.07 ± 2761.44) with (p = 0.018); Zn (384.66 ± 195.79) and (585.58 ± 361.84) with (p = 0.038) and Cu (62.63 ± 19.52) and (90.92 ± 57.34) with (p = 0.044), measured in µg/day in G1 and G2 respectively (**Figures 1-4**). Moreover the color of urine was reddish brown. Each value represents the mean ± standard deviation.

2) DFO adverse effects

Figure 5 showed that highest incidence of DFO adverse effects were arthralgia-myalgia, bone pain, growth retard, headache, dizziness, stomach pain, visual disturbance and hearing problem. There was significant different showed in diarrhea and fever between the two

Table 5. Biochemical and hematological assessment.

Variables		G1 (No. = 30)	G2 (No. = 24)	p value
Serum ferritin (ng/ml or µg/L)		6937.96 ± 2748.2	3890.25 ± 2770.98	0.001*
Serum creatinine (mg/dl)	≤12 year	0.36 ± 0.095	0.35 ± 0.107	0.79
	>12 year	0.48 ± 0.154	0.48 ± 0.144	0.939
Blood urea (mg/dl)	≤12 year	26.39 ± 5.69	20.74 ± 4.31	0.023*
	>12 year	29.76 ± 7.21	23.16 ± 6.4	0.03*
SGPT (units/L)		68.93 ± 31.61	46.35 ± 23.9	0.014*
SGOT (units/L)		64.39 ± 30.7	47.78 ± 19.26	0.045*
Alkaline phosphate (units/L)	≤12 year	526.84 ± 230.02	300.41 ± 148.17	0.043*
	>12 year	365.05 ± 141.36	235.14 ± 97.85	0.026*
Total serum bilirubin (mg/dl)		1.82 ± 1.14	2.11 ± 1.32	0.407
WBC (WBC/cmm)		12.45 ± 8.3	10.13 ± 8.28	0.344
Platelet in cells/µL	≤12 year	244459.72 ± 110964.93	315214.28 ± 113739.78	0.201
	>12 year	432030.3 ± 218042.79	456916.66 ± 274220.79	0.808
Serum albumin (g/dl)		4.29 ± 0.3	4.7 ± 0.4	0.001*
Serum calcium (mg/dl)		8.22 ± 0.9	9.28 ± 0.88	0.003*

Each value represents the mean ± standard deviation. *p < 0.05.

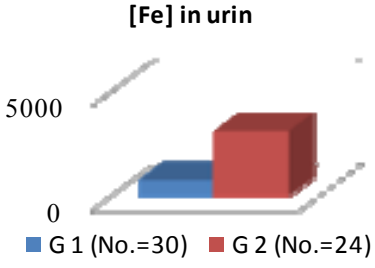


Figure 1. Urinary excretion of iron, *p < 0.05.

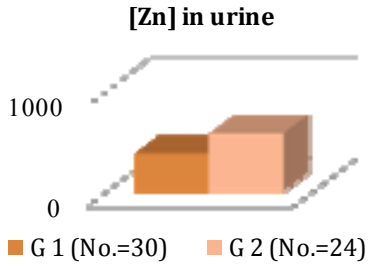


Figure 2. Urinary excretion of zinc, *p < 0.05.

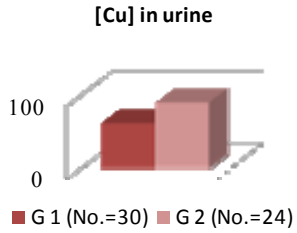


Figure 3 .Urinary excretion of copper, $^{*}p < 0.05$.

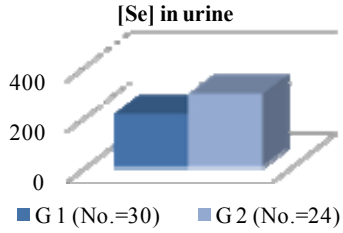
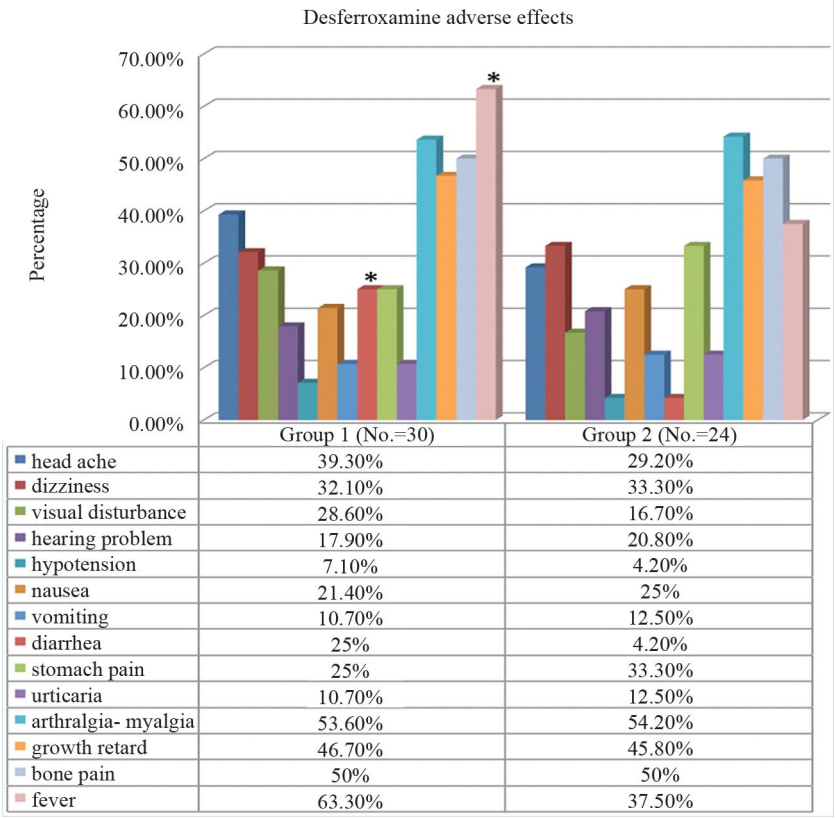


Figure 4. Urinary excretion of selenium, $^{*}p < 0.05$.



	Group 1 (No.=30)	Group 2 (No.=24)
head ache	39.30%	29.20%
dizziness	32.10%	33.30%
visual disturbance	28.60%	16.70%
hearing problem	17.90%	20.80%
hypotension	7.10%	4.20%
nausea	21.40%	25%
vomiting	10.70%	12.50%
diarrhea	25%	4.20%
stomach pain	25%	33.30%
urticaria	10.70%	12.50%
arthralgia- myalgia	53.60%	54.20%
growth retard	46.70%	45.80%
bone pain	50%	50%
fever	63.30%	37.50%

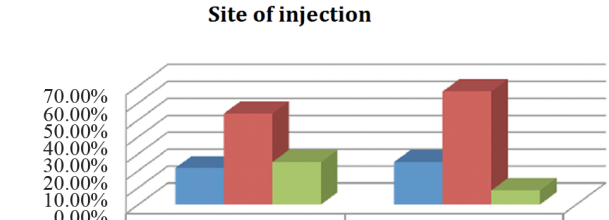
Figure 5. The percentage of DFO adverse effects, $^{*}p < 0.05$.

groups.

3) Distribution of the site of subcutaneous DFO injection and related adverse effects

Figure 6 showed the percentage distribution of the difference site of DFO subcutaneous injection. Patients that were administered via abdomen were 6 (21.4%) and 6 (25%); patients that used upper arm were 15 (53.6%) and 16 (66.7%) and patients that used both sites were 7 (25%) and 2 (8.3%) in G1 (missing DFO dose) and G2 (not missing DFO dose) respectively. There were no statistical significant differences between these two groups.

Adverse effects of S.C injection were swelling seen in 26 (92.9%) and 23 (95.8%) patients followed by infiltration in 26 (92.9%) and 20 (83.3%) patients; pain in 23 (82.1%) and 18 (75%) patients; pruritus in 24 (85.7%) and 19 (79.2%) patients and erythema in 20 (71.4%) and19 (79.2%) patients in both G1 and G2 respectively



	Group 1 (No.=30)	Group 2 (No.=24)
abdomen	21.40%	25%
upper arm	53.60%	66.7%
both	25%	8.30%

Figure 6. The distribution of the site of S.C DFO injection.

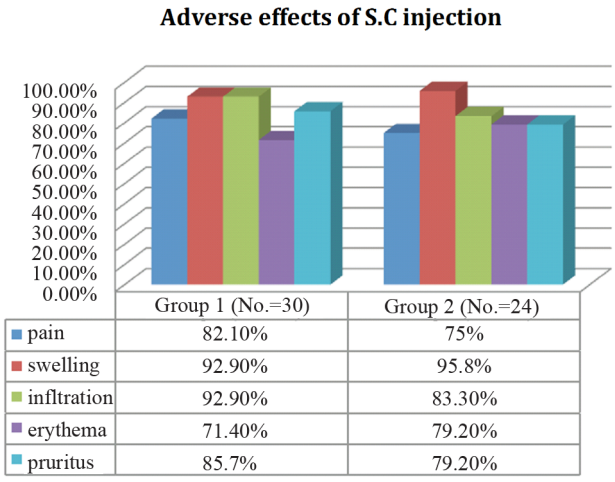
(**Figure 7**).

There was no statistical correlation between site of S.C DFO injection (abdomen, upper arm and both sites) and the injection site adverse effects (erythema, pruritus, pain,

swelling and infiltration) in both groups as showed in **Figure 8**.

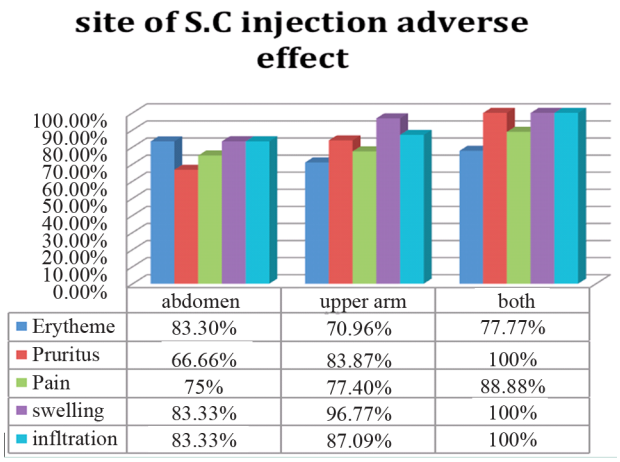
4) Compliance to treatment

Relations of DFO dose to serum ferritin and DFO therapeutic index, explain the compliance to treatment, which presented in **Table 6**.



	Group 1 (No.=30)	Group 2 (No.=24)
pain	82.10%	75%
swelling	92.90%	95.8%
infltration	92.90%	83.30%
erythema	71.40%	79.20%
pruritus	85.7%	79.20%

Figure 7. The percentage of S.C. injection adverse effects in both groups.



	abdomen	upper arm	both
Erytheme	83.30%	70.96%	77.77%
Pruritus	66.66%	83.87%	100%
Pain	75%	77.40%	88.88%
swelling	83.33%	96.77%	100%
infltration	83.33%	87.09%	100%

Figure 8. The site of S.C DFO injection (abdomen, upper arm and both sites) and side effects (erythema,pruritus, pain, swelling and infiltration) for enrolled patients.

Table 6. Relations of DFO dose to serum ferritin and DFO therapeutic index.

G1 (missing DFO Dose)				G2 (not missing DFO Dose)			
Dose of DFO mg/kg/day	Serum ferritin µg/L	Therapeutic index	Dose study	Dose of DFO mg/kg/day	Serum ferritin µg/L	Therapeutic index	Dose study
33.33	5257.8	0.0063	(1)	23.51	2991.77	0.0078	(1)
38.79	7328	0.0052	(1)	25.92	3970	0.0065	(1)
34	6748	0.005	(1)	30.3	4000.5	0.0075	(1)
35.21	1481	0.023	(2)	32.89	4115	0.0079	(1)
31.25	8965.25	0.0034	(1)	31.77	2857	0.011	(1)
35.71	6490	0.0055	(1)	66.66	2500	0.026	(2)
27.77	8518.75	0.0032	(1)	26.51	3817	0.0069	(1)
35.21	7578.25	0.0046	(1)	26.73	3189.25	0.0083	(1)
34.48	1954.75	0.017	(3)	34.6	1307.5	0.026	(2)
36.49	6315.4	0.0057	(1)	32.25	569.33	0.056	(2)
37.73	1244.25	0.03	(2)	28.84	3161	0.009	(1)
31.25	1488	0.021	(2)	29.41	3202.33	0.009	(1)
37.03	9265.25	0.0039	(1)	28.26	2667	0.01	(1)
20.92	3827.375	0.0054	(1)	43.19	1062.5	0.04	(2)
32.05	2545	0.012	(3)	48.78	1214.2	0.04	(2)
34.88	5818.33	0.0059	(1)	23.47	3599	0.0065	(1)
G1 (missing DFO Dose)				**G2 (not missing DFO Dose)**			
Dose of DFO mg/kg/day	**Serum ferritin µg/L**	**Therapeutic index**	**Dose study**	**Dose of DFO mg/kg/day**	**Serum ferritin µg/L**	**Therapeutic index**	**Dose study**
28.84	914	0.031	(2)	29.85	4010.9	0.0074	(1)

Continued

43.47	1170	0.037	(2)	33.89	1292.42	0.026	(2)
26.63	6093.33	0.0043	(1)	35.97	860.33	0.041	(2)
37.8	8011.83	0.0047	(1)	34.42	1409.2	0.024	(2)
33.33	6227	0.0053	(1)	43.47	4398.5	0.0098	(1)
23.98	1200	0.019	(3)	27.25	3572	0.0076	(1)
36.36	5000	0.0072	(1)	19.76	752.83	0.026	(2)
34.48	10102.83	0.0034	(1)	21.66	3484.5	0.0062	(1)
43.47	6145.6	0.007	(1)				
34.72	5804.2	0.0059	(1)				
40	14721.2	0.0027	(1)				

[(1)]Means low dose, calculated as (19) 70% and (15) 62.5% for patients in G1 and G2 respectively taking a lower dose than the standard recommended average daily dose. [(2)]Means high dose calculated as (5) 18.5% and (9) 37.5% for patients in G1 and G2 respectively taking a higher dose than the standard recommended average daily dose. [(3)]Means corrected dose calculated as (3) 11.5% for patients only in G1 taking a correct dose as the standard.

Life style of enrolled major β-Thalassemia patient

Table 7 illustrated these life style variables; exercise, eating prohibited food, drinking tea, missing Vitamin C or E in both G1 (missing DFO dose) and G2 (not missing DFO dose).

There was a highly significant difference between G1 and G2 in the percentage of patients that miss both Vitamin C and E (80 and 12.5%) and those that did not miss any (6.7 and 62.5%).

Table 7. Life style variables (exercise, eating prohibited food, drinking tea, missing Vitamin C or E) in both groups of patients.

Variables		G1 (No. = 30)%	G2 (No. = 24)%	p value
Exercise		15 (50)	9 (37.5)	0.358
Eating prohibited food		18 (60)	9 (37.5)	0.1
Drinking tea	Not drinking	2 (6.7)	1 (4.2)	0.816
	1 - 2 times	10 (33.3)	6 (25)	
	2 - 3 times	5 (16.7)	3 (12.5)	
	3 - 4 times	10 (33.3)	8 (33.3)	
	4 - 5 times	2 (6.7)	4 (16.7)	
	5 - 6 times	1 (3.3)	2 (8.3)	
Missing Vitamin C or E	Missing Vit.C	3 (10)	5 (20.8)	0.0*
	Missing Vit.E	1 (3.3)	1 (4.2)	
	Missing both	24 (80)	3 (12.5)	
	Not missing any	2 (6.7)	15 (62.5)	

Each value represents the number and percentage. *$p < 0.05$.

5. Discussion

Therapeutic drug monitoring (TDM) is the measurement of drugs and their active metabolites in patients receiving medications for the purpose of optimizing their therapeutic effect while minimizing adverse effects [16]. Patient demographic characteristics are critically important so that the contributions of age, disease state, ethnicity, and other variables to inter-individual variation in pharmacokinetics and pharmacodynamics can be considered.

An important part of therapeutic drug monitoring is the timing of the blood collection. When the drug is administered, the blood concentration increases until it reaches a peak and then the concentration begins to fall. The lowest concentration (trough) is usually just before the next dose. In this study the measured trough concentration of Deferoxamine and ferroxamine were 33 and 52.96 µg/L, respectively.

Deferoxamine plasma half-life was 20 - 30 min [4] while maximum peak plasma concentration was achieved after 4 half life (80 min). Deferoxamine and ferroxamine steady state concentrations were 144 and 42.44 µg/L, respectively, and the calculated rate of elimination (Ke) was (0.0237 ± 0.00029 min^{-1}), so the DFO $T_{1/2}$ was 34 min in enrolled patients at Sulaimani thalassemia center. The difference with other studies may be due to the blood sampling time for peak concentration, or may be the pharmacokinetics factors, such as protein binding.

One of the most important complications of regular blood transfusion is iron overload that eventually involves many organs like heart and cause myocardial injury [17]. Serum ferritin is a marker used to determine the development of cardiac complications [18] and the management of cardiac complications, which involves optimal transfusion therapy and strict compliance with chelation therapy [19]. CK-MB and troponin I are considered as one of these specific cardiac enzymes that are

used for evaluation of heart involvement [20,21]. The present study showed a highly statistically significant difference in serum ferritin level in the two groups studied (non compliant, and compliant), ($p < 0.01$).

According to Sakha *et al.* (2008) [17], studying LVEF for major thalassemia patients treated with Deferoxamine injection showed that some patients had normal left ventricular ejection fraction (LVEF = 50% - 70%), while others had reduced LVEF (20% - 45%). Cardiac markers (Troponin and CK-MB) are not helpful for the recognition of cardiac involvement in major thalassemia, and also do not predict cardiac damage of thalassemia patients, but may be helpful in serial measurement.

Echocardiography was performed in this study for enrolled patients in both groups, all had a normal left ventricular ejection fraction (LVEF = 50% - 70%), and there was no statistical difference between the two groups ($p = 0.74$). Those, who are adequately transfused but who are poorly chelated, may present with dominant right-sided heart involvement [22]. This study confirms that, only 3 patients (15%) from non-compliant group had mild dilated right ventricular, while there was no complain from the G2 (non missing dose) group.

The statistical analysis between the two studied groups showed no significant difference in troponin I level, which was within normal range; this result agrees with the results of Sakha *et al.* (2008) [17], but disagrees in regards to CK-MB. This study showed a statistical significant difference in CK-MB level, higher than normal level in non-compliant (missing dose) more than compliant group, which indicate myocardial injury.

Numerous prospective studies in healthy volunteers have confirmed that high-sensitivity CRP (hsCRP) predicts cardiovascular events (CVEs), while hsCRP is a strong independent predictor of risk of future MI, stroke, peripheral arterial disease, and vascular death [23].

High-sensitivity C-reactive protein may be clinically useful in identifying individuals who are at higher risk for CAD. A high sensitivity C-reactive protein level of less than 1.0 mg/L indicates low risk for cardiovascular disease, between 1.0 to 3.0 mg/L indicates moderate risk and more than 3.0 mg/L indicates high risk [24]. In this study high sensitive CRP showed a statistical significant difference between the two groups ($p = 0.027$), the results revealed that non-compliant patients (missing dose) are in high risk of developing atherosclerotic cardiovascular disease but compliant patients are in average risk, which is due to their levels of serum ferritin.

Iron overload is one of the major causes of morbidity in all patients with severe forms of thalassemia. Excess iron in vital organs, even in mild cases of iron overload, increases the risk of for example liver disease (cirrhosis, cancer). Various studies have found that Deferoxamine arrests progressive liver fibrosis even when taken in inadequate doses, as judged by reduction in liver iron concentration and improvement in liver function test [25]. There were significant increases in SGPT and SGOT levels in a group of patients that indicate liver damage because of iron overload in their body [26].

The level of liver enzymes was raised in non-compliant group than normal range; the raised mean level of all the studied liver enzymes (SGOT, SGPT, serum alkaline phosphatase) showed a statistically significant difference ($p > 0.05$), in non-compliant group compared to the compliant one.

There was a significant difference in blood urea between the studied groups, higher than normal range [27]. The results of this study agree with it, since there was a significant increase in serum urea in transfusion dependent β-thalassemia patients using Deferoxamine. Complications from continuous therapy may arise due to iron overload and toxicity of iron chelating therapy, which may affect many organs in the body including the renal system. Renal damage can be attributed to chronic anemia, iron over load and deferroxamine therapy [28]. Therefore monitoring of renal functions is recommended. Total serum bilirubin was measured in both groups and there was no statistical difference between them, but the value was higher than normal range, which may be explained by the study of Kassab-Chekira *et al.* (2003) [29], who mentioned that bilirubin increase observed in beta-thalassemia may be related to hemolysis process.

There was a significant decrease in serum creatinine in transfusion dependent β-thalassemia patients using Deferoxamine. This result agrees with the findings of Younus *et al.* (2012) [27] that determine low-level serum creatinine in groups of patients above 12 years old. This significant decrease in the mean serum creatinine level may be related to the lower body mass index due to growth retardation and lower muscle mass, usually encountered in β-thalassemia patients [30].

Growth retardation occurs in both groups at a rate of 46.7 and 45.8% respectively, but no statistical significant difference was shown between the two groups of patients. This means that growth retardation in these patients is multi factorial, that is, it may have occur due to iron overload, Deferoxamine use and/or serum zinc level which has adversely affected their growth velocity [31].

According to the study of Aziz *et al.* 2009 [32], albumin decreased significantly in patients with iron overload; this was similar to the results obtained by Livrea*et al.* (1996) [33] which shows that serum albumin was in the normal range in all thalassemia patients. This study agrees with the above-mentioned studies, in which measured serum albumin for all enrolled patients, were significantly lower in non-adherent patients more than those adherents to treatment, but both were within normal range.

Comparing the two studied groups, there was no sta-

tistical significant difference in WBCs and platelet count, but the WBCs in both groups were slightly higher. High platelet in both groups of patients may be due to the number of patients having splenoctomy. This result agrees with the study of Wirawan *et al*. (2004) [34], which observed that leukocytosis and thrombocytosis in post splenoctomy groups is due to no more pooling and phagocytosis of WBCs and platelets after splenoctomy [35].

Calcium level will be low in iron overload β-thalassemia patients due to hypoparathyroidism [36], because iron precipitation in tissues of parathyroid gland causes insufficient production of parathyroid and calcitonin hormones that regulate normal level of calcium in the blood [37]. This study showed that calcium level was significantly different in both groups.

According to the data published by Novartis pharmaceutical manufacturing factory in 2011 [38], Deferoxaminemesylate adequate average daily doses are 20- to 60-mg/kg-body weight. Patients with a serum ferritin level of less than 2000 μg/L require about 25 mg/kg/day; patients with a serum ferritin level between 2000 and 3000 μg/L require about 35 mg/kg/day and patients with higher serum ferritin levels may require up to 55 mg/kg/day. It is inadvisable to regularly exceed an average daily dose of 50 mg/kg/day except when very intensive chelation is needed in patients who are no longer growing. If ferritin values fall below 1000 μg/L, the risk of Desferal toxicity increases. Therefore, it is important to monitor these patients carefully and to consider lowering the total weekly dose.

Alternatively, the mean daily dose may be adjusted according to the ferritin value to keep the therapeutic index less than 0.025 (that is, mean daily dose of Desferal in mg/kg divided by the serum ferritin level in μg/L is below 0.025). The therapeutic index is a valuable tool in protecting the patient from excess chelation, but it is not a substitute for careful clinical monitoring.

According to the above dosage information, dose of Deferoxamine in these patients were studied and it showed that 66.66% of patients taking a lower dose than the standard recommended average daily dose required (unrelated to their serum ferritin levels) showed a high level of serum ferritin that eventually resulted in iron over load complications. Also, 27.45% of the patients who were administered a higher dose than the standard recommended average daily dose required, unrelated to their serum ferritin levels, showed Deferoxamine toxicity, while 5.88% of these patients were administered the exact right recommended dose of Deferoxamine

The study of urinary iron excretion was recommended for subcutaneous DFO infusion, the results from this study showed that the urinary iron excretion in both groups of patients was low compared to the study of Dubey *et al.* (1992) [39] ($p \leq 0.05$), because after infusion of Deferoxamine there was a significant increase of mean urinary and faecal iron excretion ($p < 0001$). On average, 45% of iron was eliminated through urine while 55% was through stool [40]. The data suggests that the amount of iron chelated *in vivo* is related to an increase in the size of an intermediate chelatable pool rather than the total amount of the iron load. The well-recognized delay in urinary iron excretion appears to be related to active tuular reabsorption of ferrioxamine [41].

Desferal has affinity for $Cu^{2\pm}$ and $Zn^{2\pm}$, and has some neurotoxic effects, which may be due to its ability to chelate copper or zinc. After the infusion of DFO, faecal and urinary zinc and copper excretion were significantly increased; the depletion of these elements may be responsible for the neurotoxic effect of the drug [40]. This study confirms that, there was a statistical significant difference between the two groups of patients; ($p \leq 0.05$) as regards urinary Zn and Cu excretion. Also the depletion of iron, zinc, and copper may be related to the retinal abnormalities [42,43]. More severe retinal or optic nerve toxicity, or both, that consisted of night blindness, field defects, visual loss, loss of color vision, and delayed visual evoked potential has been detected after intravenous or subcutaneous infusion of similar large doses of Deferoxamine for a prolonged period [40,44].

The most frequent adverse effects of DFO are local reactions at the site of infusion, such as pain, swelling, induration, erythema, burning, pruritus, wheals and rash, occasionally accompanied by fever, chills and malaise. Other complications mainly associated with high doses of DFO in young patients are low ferritin values [45]. This monitoring study found that there was no statistical significant difference between the two studied groups as regards the site of DFO S.C. injection; abdomen, upper arm or both abdomen and upper arm. Follow up of the enrolled patients, revealed the common side effects of DFO injection site to include pain, swelling, infiltration, erythema, and pruritus. There was no statistical correlation between sites used for injection and the side effects at the site of the injection; this indicates that Deferoxamine dose injection was not the cause of the side effects experience by patients.

It was also discovered from this study that the adverse effects of Deferoxamine include headache, dizziness, visual disturbance, hearing problem, nausea, vomiting, stomach pain, urticarial, arthralgia-myalgia, and bone pain ($\geq$10%), with no statistical significant difference between the studied groups. Hypotension was common ($\geq$1%), in both groups with no statistical significant difference between the two groups.

Diarrhea (G1, 25% and G2, 4.2%), and Pyrexia (G1, 63.3%; G2, 37.5%), which was very common in both groups of patients showed significant differences.

The patients with diarrhea and fever required appropriate stool samples, blood culture and serological testing for Yersinia infection [46], because of the increased risk of Yersinia infection in iron overload, which could increase further with DFO treatment [47]. Adverse effects like drowsiness, impotence, or nausea reduce adherence, which patients may not admit to [48].

6. Conclusions

This study, showed the importance of Therapeutic drug monitoring of DFO in β-thalassemia major patients. The adequacy of dose was not related to the poor drug response in the enrolled patients. CK-MB and high sensitive C-reactive protein was significantly higher, which indicated a high risk of developing a myocardial injury and atherosclerosis among non-compliant patients. Also there were significant increases in; serum ferritin level, Liver enzymes (SGOT, SGPT and Alkaline phosphatase), and bloodurea, while there was a significant decrease in serum calcium and albumin. In addition to that urinary Fe, Zn and Cu excretion was significantly higher, also diarrhea and fever was significantly higher in non-compliant patients.

The highest percentage of non-compliant patients, due to the drug's complicated and painful route of administration, so the role of clinical pharmacist is very important to be applied to these patients in any thalassemia center.

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