Iron chelation therapy in Beta Thalassemia

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Background: Iron accumulation is an inevitable consequence of chronic blood transfusions and results in serious complications in the absence of chelation treatment to remove excess iron. Desferioxamine reduces morbidity and mortality although the administration schedule of slow , parentral infusions several days each week limits compliance and negatively affects long-term outcome so different strategies have been developed to overcome these problems such as deferiprone or deferasirox alone or dual chelator therapy.

Objectives: The aim of this work was to evaluate the effect of monotherapy and alternating therapy of iron chelators (deferioxamine, deferiprone, deferasirox) after six months of follow up of regular administration of these iron chelators.

Methods: This study was carried out on 120 children with beta thalassemia major with serum ferritin level above 1000 ng/ml who were divided into four groups. **Group I** received [Oral deferiprone (DFP) at 75 mg/kg/day for 4 days/week and subcutaneous desferioxamine (DFO) at 40 mg/kg/day for the other 3 days/week for 6 months]. **Group II** received [oral deferiprone only at 75 mg/kg/day in three divided doses for 6 months]. **Group III** received [subcutaneous desferoxamine only at 40 mg/kg/day daily 8-12 hours per day at night using desferal pumb for 6 months.

.Group IV received [oral desferasirox at a dose of 30 mg/kg/day, single dose daily, taken on an empty stomach at least 30 minutes before food for 6 months].

All thalassemic patients were subjected to complete history taking, thorough clinical examination and laboratory investigations including complete blood

count, serum ferritin, iron, TIBC, liver and kidney functions, 24 h urine iron excretion (UIE) throughout 6 months follow-up.

Results: There were significant reductions in serum ferritin and serum iron after treatment in all studied groups with the highest reduction in serum ferritin and serum iron in group A (alternating), group C (desferioxamine), group D (deferasirox) and group B (deferiprone) but without statistically significant differences between the four studied groups before and after chelation therapy. 24 h urinary iron shows a significant difference in group A and in significant difference in other groups of patients before and after chelation therapy.

There were no significant differences in the mean values of the parameters of CBC, liver enzymes and kidney function between the studied groups before and after chelation therapy.

Conclusion: Alternating DFO/DFP has some significant advantages over DFO monotherapy; it can keep a balanced iron load, targets different iron pools, is well accepted by the patients. This approach is more appropriate for well-chelated patients, who have difficulties in continuing DFO monotherapy.

Recommendations: The use of alternating therapy of desferoxamine and deferiprone when is poor compliance with infusion of DFO. Following up of patients with serum ferritin , 24 hr urinary iron to detect the long term effect on iron stores.

Key words: Thalassermia, Haemoglobinopathy, Iron overload, Iron chelation

Introduction

Thalassaemia is an autosomal genetic disease characterized by impaired synthesis of polypeptide chains of normal haemoglobin leading to anaemia. It remains one of the major health problems in Southeast Asia [(1)] In severe cases, in order to improve survival and quality of life, multiple blood transfusions are

required. Iron overload is the life limiting complication commonly found in thalassaemics, which may be due to ineffective erythropoiesis, increased gastrointestinal absorption, lack of physiologic mechanism for excreting excess iron, and above all multiple blood transfusions [2]

As the body has no effective means for removing iron, the only way to remove excess iron is to use iron binders (chelators), which allow iron excretion through the urine and/or stool. Iron chelating agents including deferoxamine, deferiprone, and deferasirox reduce iron overload in these patients in different degrees and therefore reduce morbidity and mortality, including cardiac complications [3]

Because of poor compliance with recommended subcutaneous regimens, the patients become massively iron overloaded and are at risk of early death, principally from cardiac complications, as the infusion can be troublesome, time consuming and painful [4]. The availability of oral iron chelators in recent years has enabled clinicians to tailor chelation therapy to the needs of the patient. Possible regimens are monotherapy with either deferiprone (DFO) or dual chelator therapy, whereby both drugs can be given on the same days (combination regimen) or on different days (alternating regimen)[5]

Aim of the work: was to evaluate the effect of monotherapy and alternating therapy of iron chelators (deferioxamine, deferiprone, deferasirox) after six months of follow up of regular administration of these iron chelators.

Patients and methods:

This study was done after approval from ethical committee of research center in Minia University Hospital and informed written parental consent from every case that participates in this research and was carried out on 120 children with beta thalassemia major under follow up in the Hematology Unit, Pediatric Department, Minia University Hospital in the period from November 2016 to November 2017. They were 68 males and 52 females with their age ranged from 4-7 years and mean age value of 5.43 ± 1.37

Inclusion criteria: Children with beta thalassemia major with serum ferritin level above 1000 ng/ml and not received iron chelation before this study and maintained on regular use of chelation during this study.

Exclusion criteria: Thalassemic children with serum ferritin level less than 1000 ng/ml. Thalassemic children with hepatitis A, B or C.

Patients were divided into 4 groups:

Group A: 30 patients received oral deferiprone (DFP) at 75 mg/kg/day for 4 days/week and subcutaneous desferioxamine (DFO) at 40 mg/kg/day for the other 3 days/ week for 6 months

Group B: 30 patients who received oral deferiprone only at 75 mg/kg/day in 3 divided doses for 6 months.

Group C: 30 patients who received subcutaneous desferoxamine only at 40 mg/kg/day daily for 6 months..

Group D: 30 patients who received oral desferasirox at a dose of 30 mg/kg/day, single dose daily, taken on an empty stomach at least 30 minutes before food for 6 months.

Patients in all groups were subjected to the following:

1. Complete history taking.

2. Thorough clinical examination with especial account on pallor, jaundice, mongloid facies, splenomegaly, hepatomegaly.

3. Laboratory investigations:

Specimen collection and handling:

Six ml of venous blood were collected using sterile needles through gentle venipuncture after sterilization of site of puncture by alcohol, and collected samples were divided into; 4 ml in a plain glass tube that were allowed to clot for 4 minutes and then centrifuged to separate serum which was used for estimation of serum iron, ferritin, TIBC, liver and kidney functions [6,7], one ml was delivered on 20 uL EDTA solution for complete blood count including reticulocytic count and differential count which was done on leishman stained peripheral blood smear with evaluation using ERMA PCE-210 N cell –counter [8], one ml was added to 2 ml hemolysate for Hb electrophoresis. Serum iron, ferritin, TIBC was done two times one before and one after 6 months of treatment while CBC, liver and kidney functions were done one before and once weekly during chelation therapy.

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Determination of serum iron:-

Iron that was dissociated from transferrin-iron complex by guanidine acetate solution and reduced by ascorbic acid reacts with ferrozine to give pink complex (according to procedure recommended by serum iron from Biomaghreb company) [6].

Determination of serum total iron binding capacity (TIBC):-

An excess of iron is added to the serum to saturate transferrin. The unbound iron is precipitated with basic magnesium carbonate (according to procedure recommended by the serum total iron binding capacity from Biomaghreb company) [7].

Determination of serum ferritin:-

Serum ferritin was assessed by ELIZA [DRG® Ferritin ELISA (EIA-4292)] [9]

Determination of 24 hours urinary iron excretion

24 hour urine sample was collected from every patient. Urinary iron excretion was measured by atomic absorption spectroscopy (agilent technologies 200 series AA 240 FSAA). 24-hour urine sample collected in iron-free plastic containers and 3-mL sample was added to 9 mL of 0.6 N HCl. After standing overnight, the contents were centrifuged at 3000 rpm by thermoscientific centrifuge. The iron content of the supernatant was determined directly by atomic absorption where the liquid sample is aspirated into a nebulizer (4-7 ml/min) and the formed aerosol is introduced into flame, where aerosol desolvation, evaporation and analyte atomization occur. A steady- state absorbance signal is measured.[10]

Statistical methods :

Collected data were organized, tabulated and statistically analyzed using the mean, standard deviation, unpaired T test, paired t test, Chi-square test and one way ANOVA by SPSS version 19 (Statistical Package for Social Studies)

Results

♠No significant differences between studied groups of patients as regard age, age of first transfusion, intertransfusion intervals and clinical manifestations (Table 1).

♠ There was highly significant reduction in serum ferritin and serum iron after chelation therapy in studied groups with the highest reduction in serum ferritin and serum iron in group A (alternating), group C (SC desferrioxamine), group D (oral deferasirox) and group B (oral deferiprone) with no statistically significant differences between the studied groups of patients before and after the 6 months duration of regular chelation therapy (Table 2).

♠Therewere no significant differences in white blood cells, absolute neutrophils and platelets counts, between the studied groups of patients before and during the 6months of chelation therapy (Table 3).

♠There were no significant differences in Alanine**aminotransferase (ALT), Aspartate aminotransferase (AST) and serum creatinine and blood urea between the studied groups of patients before and during 6months duration of regular chelation therapy (Table 4).**

♠There was no**significant difference as regard to urinary iron before chelation therapy in each studied group and also between the studied groups of patients after chelation therapy. But 24 h urinary iron shows a significant difference in group A and in significant difference in other groups of patients before and after chelation therapy (Table 5).**

Table (1): History and clinical manifestations of studied groups at the start of the study.

Items	Group A (N= 30)	Group B (N= 30)	Group C (N= 30)	Group D (N= 30)	ANOVA	
					F	P
Age of 1st transfusion (months)	5.10 ± 1.61	5.68 ± 1.64	5.83 ± 0.92	4.91 ± 0.93	2.04	0.11
Inter-transfusion interval (days)	10.93 ±10.96	10.60 ± 6.67	12.07 ±7.19	8.60 ± 4.47	0.52	0.66

Clinical data	A(n=30)		B(n=30)		C(n=30)		D(n=30)		Chi-square	
									X^2	P
	No	%	No	%	No	%	No	%		
Pallor	30	100	30	100	30	100	30	100	0.0	1.00
Jaundice	10	33.3	18	60	20	66.6	10	33.3	5.53	0.13
Hepatomegaly	30	100	30	100	30	100	30	100	0.0	1.00

Spleen Splenomegaly Splenectomy	18 3	60 10	17 4	57 13. 3	14 5	46 16.7	16 5	53.3 16.7	0.74 1.27	0.86 0.73

Table (2): Comparison of the mean values of serum iron status before and after chelation therapy in studied groups of patients.

Serum ferritin (ng/ ml)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		3201.22±2013.03	3030.37±1538.36	3280.88±1865.31	3140.53±1228.85	0.059	0.981
After		1669.57±790.40	2215.09±1521.65	2112.02±1440.298	2226.80±741.48	0.744	0.530
Difference		1531.65±1441.20	815.28±570.13	1168.86±816.13	913.73±742.02		
Paired t-test	t	4.116	4.769	5.547	54.769		
	p	0.001*	<0.001*	<0.001*	<0.001*		
Serum iron (ug/dl)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		298.00±102.58	278.07±139.24	271.40±142.69	282.60±89.30	0.132	0.941
After		174.85±61.52	212.73±103.21	177.73±140.92	209.00±87.21	0.576	0.633
Difference		123.15±80.97	65.33±40.44	93.67±19.15	73.60±39.53		
Paired t-test	t	5.891	6.257	18.945	7.210		
	p	<0.001*	<0.001*	<0.001*	<0.001*		
TIBC		Mean ±SD				ANOVA	

(ug/dl)		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		213.47± 80.36	217.20± 69.32	217.67± 68.48	213.67± 45.04	0.017	0.997
After		303.27± 97.97	264.93± 75.08	286.20± 95.77	267.20± 74.06	0.653	0.584
Difference		89.80± 60.57	47.73± 17.27	68.53± 59.99	53.53± 53.20		
Paired t-test	t	5.742	10.705	4.424	3.897		
	p	<0.001*	<0.001*	<0.001*	0.002*		

**Significant*

Table (3): Comparison of mean values of white blood cells, absolute neutrophils and platelets counts before and during 6 months of chelation therapy* in studied patients.

WBCs ($x10^3/mm^3$)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		7.57± 2.14	7.23± 2.16	7.53± 2.18	7.30± 1.88	0.097	0.961
After		7.83± 1.72	8.43± 2.13	7.77± 2.02	7.30± 1.88	0.861	0.467
Paired t-test	t	0.802	1.632	0.406	0.000		
	p	0.436	0.125	0.691	1.000		
ANC/ mm^3		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		3860.30±311.66	3937.87±323.51	3756.37±327.42	3766.80±327.56	1.055	0.375
After		3881.17±309.50	3973.47±389.85	3777.60±302.28	3758.40±328.05	1.335	0.272
Paired t-test	t	0.324	0.850	1.802	0.802		
	p	0.751	0..409	0.093	0.436		
platelets		Mean ±SD				ANOVA	

($x10^3/mm^3$)		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		294.40±40.23	301.20±25.71	305.5±35.39	299.67±38.95	0.251	0.860
After		275.67±42.55	283.87±40.29	295.07±39.04	298.33±46.83	0.738	0.534
Paired t-test	t	1.190	1.574	2.030	0.087		
	p	0.254	0.138	0.075	0.932		

**significant*, during 6 months of chelation therapy is the mean values of weekly done CBC parameters.

Table (4): Comparison of serum ALT, AST, creatinine and blood urea before and during 6 months of chelation therapy in studied patients

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ALT (lu/l)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		74.13±12.49	77.07±11.37	74.80±11.31	72.96±10.95	0.336	0.799
After		75.33±10.99	78.60±11.78	78.83±13.29	74.13±9.11	0.639	0.593
Paired t-test	t	0.277	0.437	0.915	0.417		
	p	0.786	0.668	0.376	0.683		
AST (lu/l)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		82.80±6.78	81.93±6.36	81.13±7.52	80.61±4.07	0.345	0.793
After		82.00±7.87	81.93±5.68	81.23±5.79	81.87±5.20	0.049	0.986
Paired t-test	t	0.396	0.474	0.040	0.736		
	p	0.698	1.000	0.969	0.474		
Blood urea (mg/dl)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		31.93±6.46	26.27±4.83	27.09±4.85	26.51±5.45	2.113	0.066
After		25.67±	31.73±	27.47±	30.53±	2.383	0.057

		4.22	5.15	5.61	7.18		
Paired t-test	t	2.179	2.203	0.195	1.890		
	p	0.112	0.106	0.848	0.080		

During 6 months of chelation therapy is the mean values of weekly done liver and renal function tests

Table (5): Comparsion of the mean values of urinary iron before and after chelation therapy in studied groups of patients.

Urinary iron in 24 h (mg/dl)		Mean ±SD				ANOVA	
		Group A(n=30)	Group B(n=30)	Group C(n=30)	Group D(n=30)	F	P
Before		33.0± 10.8	41.8± 12.5	37.1± 12.1	42.4± 8.2	2.33	0.088
After		39.3±9.9	44.5±7.9	43.4±6.9	45.6±7.9	0.39	0.760
Paired t-test	t	2.35	0.57	1.24	0.25		
	p	0.028*	0.224	0.268	0.154		

Discussion

Beta thalassemias are hereditary blood disorders caused by reduced or absent beta chains synthesis resulting in imbalanced globin chain with early destruction of RBCs and subsequent anemia [(11)] Patients with thalassemia major become transfusion-dependent with excess iron deposited in major organs resulting in their damage [(12)] This study was done to evaluate the effect of monotherapy and alternating therapy of iron chelators (deferioxamine, deferiprone, deferasirox) after six months of follow up of regular administration of these iron chelators in the Hematology unit of pediatric Department, Faculty of Medicine, Minia University Hospital during the period of November 2016 to November 2017.

In the present study, serum ferritin and iron levels were reduced after chelation therapy in all studied groups. The reduction of serum ferritin and serum iron was

highest in group A (alternating), group C (SC desferrioxamine), group D (oral deferasirox) and group B(oral deferiprone). There were no statistically significant differences between the studied groups before and after chelation therapy.

The effectiveness of the alternating DFO/ DFP was initially reported in **Mirbehbahani etal., 2015** [13] in a small controlled clinical study (n= 7, age 9.4±3.1 years), used DFO/DFP regimen similar to ours without DFO monotherapy for 6 months only. Baseline serum ferritin was 5536±5220 mg/dl vs.3778± mg / dl at the end of the study. At the six month of the therapy, a non-significant decline in serum ferritin was observed (P=0.08), and a significant reduction in LIC (Liver Iron Concentration) was also determined (P=0.03).

Also our study was in agreement with Waheed etal.,2014 [14] who studied 60 patients, the mean serum ferritin fell dramatically from 4500± 1250 ng/ ml at the start of the study to 1250± 750 ng/ ml (alternate therapy group; P<0.001) at the end of the study.

In contrary, this is not in agreement with Baksi and Pennell, 2014 **[15]** who found more reduction in mean ferritin levels with oral deferasirox versus SC desferrioxamine with statistically significant difference, Hoffbrand etal ., 2012 [16] who found that oral deferasirox had comparable efficacy with SC desferrioxamine, Totadri etal., 2015 [17] who found that oral deferiprone had comparable efficacy as SC desferrioxamine, and Sayani etal., 2016 [18]who found deferiprone more effective than SC desferrioxamine

In this study, there were no significant differences in the mean white blood cells, absolute neutrophils and platelets counts before and after chelation therapy in the studied groups. This is agreement with Song etal., 2014 [19]who found no changes in mean values of blood count after oral deferasirox or SC desferrioxamine, Arandi etal., 2015 [20]who found no changes in blood count after SC desferrioxamine or oral deferiprone, but this is not in agreement with Hoffbrand et al., 2012 [16] who found neutropenia, agranulocytosis and thrombocytopenia after deferiprone and Song etal.,

2014[19] who found neutropenia and thrombocytopenia after deferasirox

In this current study, there were no significant differences in ALT and AST before and after chelation therapy in the studied groups. This is agreement with Hosen etal., 2015 [21]who found no differences with SC desferrioxamine and Voskaridou etal., 2014 [22] who found no differences with oral deferiprone but not in agreement with Ratha and Altaei, 2013. [23] Who found elevation of ALT and AST after oral deferiprone and SC desferrioxamine therapy and Grady etal., 2012 [24]who found elevated ALT and AST with oral deferasirox

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In this work, there were no significant differences in serum creatinine and blood urea before and after chelation therapy. This result is in agreement with Yadav, 2016 [25] who found no affection of kidney functions after oral deferiprone or SC desferrioxamine. However, Pepe etal., 2011 [26]found transient increase in serum creatinine ≥ 30% with doses of 20 mg/kg and 30 mg/kg of oral deferasirox and Saliba etal., 2015 [27] found renal affection with oral deferiprone therapy.

24 h urinary iron shows a significant difference in group A and in significant difference in other groups of patients before and after chelation therapy.

This in agreement with Genc etal., 2016 [28] who reported that 24 hour UIE (Urinary Iron Excretion) increased significantly in alternating regimen (baseline 41±2.7 to

76±4.9 mg/24h at the end of the study, p<0.001). In the DFO monotherapy, 24 h UIE increased insignificantly, (p=0.15)

Variation between the results of this study and others could be explained by different number and mean age of studied patients, different presentation of thalassemia, different duration of the studies, variation of degree of iron overload, variation in dose and compliance with iron chelating agents and variation in the methods of evaluation of iron overload in different studies.

Conclusion

our findings demonstrate the therapeutic regimen (alternating DFO/DFP has some significant advantages over DFO monotherapy; it can keep a balanced iron load, targets different iron pools, is well accepted by the patients . This approach is more appropriate for well-chelated patients, who have difficulties in continuing DFO monotherapy. It is recommended Following up of patients with serum ferritin , 24 hr urinary iron to detect the long term effect on iron stores, Early and proper identification of poor compliance to the daily desferioxamine intake to select a more convenient merhod of chelation therapy and The use of alternating therapy of desferoxamine and deferiprone when is poor compliance with infusion of DFO.

Recommendations

The use of alternating therapy of desferoxamine and deferiprone when is poor compliance with infusion of DFO. Iron overload should be early and regularly assessed and managed as iron overload and its complications are the major causes of mortality among thalassemic patients.

Competing interests

No competing interests

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