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COMPARATIVE STUDY OF THERAPEUTIC VALUES OF DIFFERENT IRON CHELATING AGENTS IN CHILDREN WITH BETA THALASSEMIA MAJOR

Adel A Hagag* and Nahla A Nosair

* Pediatrics and Clinical Pathology Departments, Faculty of Medicine, Tanta University, Egypt
Email: adelhagag20@yahoo.coom

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Abstract

Backgrounds: Beta thalassemia is hereditary blood disorder characterized by reduced or absent beta chains of hemoglobin resulting in imbalanced globin chain synthesis with early destruction of RBCs and anemia. Patients with thalassemia major become transfusion-dependent with subsequent iron overload. Effective and convenient iron chelation therapy remains one of the main targets of management of thalassemia major. **Objectives:** The aim of this work was to compare the efficacy of different iron chelating agents in treatment of iron overload in children with beta thalassemia major**. Patients and Methods:** The current study was conducted on 120 children with beta thalassemia major with serum ferritin level above 1000 ng/ml who were divided into 4 groups: Group A: 30 patients were treated with 8 hours intravenous infusion of desferrioxamine, 40 mg/kg/day, 6 days per week for 6 months. Group B: 30 patients were treated with subcutaneous infusion of desferrioxamine, 40 mg/kg/day, 6 days per week 8-12 hours per day at night using desferal pumb for 6 months. Group C: 30 patients were treated with oral deferiprone 75mg/kg/day in three divided doses daily for 6 months. Group D: 30 patients were treated with oral deferasirox 30 mg/kg/day in single dose on empty stomach daily 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. **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 , group B , group D and group C but without statistically significant differences between the four studied groups 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:** Iron chelators are cornerstone in treatment of iron overload in children with beta thalassemia. IV desferrioxamine therapy is more effective in treatment of iron overload compared with SC desferrioxamine, oral deferasirox and oral deferiprone with no statistically significant difference. **Recommendations:** IV desferrioxamine therapy should be considered for the treatment of iron overloaded in thalassemic patients. Further studies on large number of patients and for long duration are required to clarify the side effects of long term use of iron chelating agents.

Introduction

Beta thalassemia is hereditary blood disorder due to defect in beta globin gene with excess of free alpha globin chains which become abnormal components in maturing red blood cells leading to their destruction by the spleen with subsequent anemia[1]. Patients with beta-thalassemia major require regular blood transfusions to survive [2] and become transfusion-dependent. The primary long term complication of chronic RBCs transfusions is iron overload [3]. Excess iron is deposited in major organs resulting in organ damage [4]. Effective and convenient iron chelation therapy remains one of the main lines of treatment of thalassemia major [5].

Desferrioxamine (DFO) is hexadentate iron chelator that has been in clinical use since the 1970s and widely used as subcutaneous infusions since about 1980 [6]. The standard recommended dose is 20-40 mg/kg [7] by slow subcutaneous (SC) infusion over 8-12 hours using an infusion pump for 6 nights per week [8]. SC bolus of desferrioxamine twice daily may be indicated when an infusion pump is not available or when 10-hours infusion are not tolerated [9] and may be also considered if the patient is not at high risk of heart disease [10]. The optimal regimen of intravenous desferrioxamine for iron overload in high-risk β-thalassemia is still unknown but it is clear that 24-hours continuous intravenous desferrioxamine regimen and intermittent high dose of IV desferrioxamine for 8-10 hours per day are equally effective [11].

Deferiprone is a bidentate oral iron chelator that began clinical trials in UK in 1980. It was first licensed for use in thalassaemia in India, followed by European Union and other countries outside US and Canada, in 1990s [12]. According to official European licensing agency (EMEA), Deferiprone could be used as a second line drug for removing iron in patients who are unable to use Desferrioxamine or in whom DFO therapy has proven ineffective [10]. Deferiprone daily dose that had been evaluated most thoroughly is 75 mg/kg/day in three divided doses [13].

Deferasirox is an orally absorbed tridentate iron chelator [14] that has been licensed as first-line monotherapy for thalassemia major in over 70 countries worldwide including the US and the EU [15]. The drug is taken orally as a suspension in water, once daily, preferably before meal and is dissolved in water (or apple juice) using a non-metallic stirrer. The recommended daily dose of Deferasirox is 20-30 mg/kg [16].

Aim of the Work

The aim of this work was to compare the efficacy of different iron chelating drugs in treatment of iron overload in children with beta thalassemia major.

Patients and Methods

This study was done after approval from ethical committee of research center in Tanta 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, Tanta University Hospital in the period from January 2012 to August 2013. 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 were treated with 8 hours intravenous infusion of desferrioxamine, 40 mg/kg/day, 6 days per week for 6 months.

Group B:

30 patients were treated with subcutaneous infusion of desferrioxamine, 40 mg/kg/day, 6 days per week 8 hours per day at night using desferal pump for 6 months.

Group C:
30 patients were treated with oral deferiprone 75mg/kg/day in three divided doses daily for 6 months.
Group D:
30 patients were treated with oral deferasirox 30 mg/kg/day in single daily dose on empty stomach 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 [17,18], 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 [19], one ml was added to 2 ml hemolysate for Hb electrophoresis [20].

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.

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) **[21].**

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) **[22].**

Determination of serum ferritin:-
Serum ferritin was assessed by ELIZA [DRG® Ferritin ELISA (EIA-4292)] **[23].**

Statistical Analysis
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) **[24]**.

Results
♠ No significant differences between studied groups of patients as regard age, age of first transfusion, inter-transfusion 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 (IV desferrioxamine), group B (SC desferrioxamine), group D (oral deferasirox) and group C (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).
♠There were 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).

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

Items	Group A (No=30)	Group B (No=30)	Group C (No=30)	Group D (No=30)	ANOVA	
					F	P
Age	5.10 ± 1.61	5.68 ± 1.64	5.83 ± 0.92	4.91 ± 0.93	2.04	0.11
Age of 1st transfusion (months)	10.93 ±10.96	10.60 ± 6.67	12.07 ±7.19	8.60 ± 4.47	0.52	0.66
Inter-transfusion interval (days)	25.67 ± 6.51	31.67 ± 8.99	29.4 ± 10.6	30 ± 8.45	1.25	0.29
Clinical data	**Number (%)**	**Number (%)**	**Number (%)**	**Number (%)**	**Chi-square**	
					X^2	P
Pallor	30 (100%)	30(100%)	30(100%)	30(100%)	0.00	1.00
Jaundice	10 (33%)	20(66%)	18(60%)	10 (33%)	5.53	0.13
Hepatomegaly	30(100%)	30(100%)	30(100%)	30(100%)	0.00	1.00
Splenomegaly	18 (60%)	14 (46%)	17(57%)	16 (54%)	0.74	0.86
Splenectomy	3 (10%)	5 (16.6%)	4(13.3%)	5 (16.6%)	1.27	0.73

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

Ferritin (ng/ml)		Group A (No=30)	Group B (No=30)	Group C (No=30)	Group D (No=30)	ANOVA	
						F	P
Before (Mean ± SD)		3201.22 ± 2013.03	3280.88 ±1865.31	3030.37± 1538.36	3140.53 ± 1228.85	0.05	0.98
After (Mean ± SD)		1669.57 ± 790.4	2112.02 ±1440.29	2215.09 ± 1521.65	2226.80 ± 741.48	0.74	0.53
Differences		1531.65 ± 1441.2	1168.86 ± 816.13	815.28 ± 570.13	913.73 ± 742.02		
Paired t	**T**	4.116	5.547	5.538	4.769		
	P	0.001*	< 0.001*	< 0.001*	< 0.001*		
Serum iron (ug/dl)							
Before (Mean ± SD)		298.00 ±102.58	271.40 ± 142.69	278.07± 139.24	282.60 ± 89.30		
After (Mean ± SD)		174.85 ±61.52	177.73 ± 140.92	212.73 ± 103.21	209.00 ± 87.21		
Differences		123.15 ±80.97	93.67 ± 19.15	65.33 ±40.44	73.60 ±39.53		
Paired t	**T**	5.891	18.945	6.257	7.210		
	P	< 0.001*	< 0.001*	< 0.001*	< 0.001*		
Serum TIBC (ug/dl)							
Before (Mean ± SD)		213.47 ±80.36	217.67 ±68.48	217.20 ± 69.32	213.67 ± 45.04	0.01	0.99
After (Mean ± SD)		303.27	286.20 ±95.77	264.93 ±	267.20 ±	0.65	0.58

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		±97.97		75.08	74.06		
Differences		89.80 ± 60.57	68.53 ±59.99	47.73 ±17.27	53.53 ± 53.20		
Paired t	**T**	5.742	4.424	10.705	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		**Group A (No=30)**	**Group B (No=30)**	**Group C (No=30)**	**Group D (No=30)**	**ANOVA**	
						F	**P**
Before (Mean ± SD)		7.53 ±2.18	7.57 ±2.14	7.30 ±1.88	7.23 ±2.16	0.09	0.96
During (Mean ± SD)		7.77 ±2.02	7.83 ±1.72	7.30 ±1.88	8.43 ±2.13	0.86	0.46
Paired t	**T**	0.406	0.802	0.000	1.632		
	P	0.691	0.436	1.000	0.125		
ANC/mm^3							
Before (Mean ± SD)		3756.37 ± 327.42	3860.30 ± 311.66	3766.80 ± 327.56	3937.87 ± 323.51	1.05	0.37
During (Mean ± SD)		3777.6 ± 302.28	3881.17 ± 309.5	3758.40 ± 328.05	3973.47 ± 389.85	1.33	0.27
Paired t	**T**	1.802	0.324	0.802	0.850		
	P	0.093	0.751	0.436	0.409		
Platelets x 10^3/mm^3							
Before (Mean ± SD)		305.5 ± 35.39	294.40 ± 40.23	299.67 ± 38.95	301.20 ±25.71	0.25	0.86
During (Mean ± SD)		295.07 ± 39.04	275.67 ± 42.55	298.33 ± 46.83	283.87 ± 40.29	0.73	0.53
Paired t	**T**	2.030	1.190	0.087	1.574		
	P	0.075	0.254	0.932	0.138		

**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.

ALT (U/l)		**Group A (No=30)**	**Group B (No=30)**	**Group C (No=30)**	**Group D (No=30)**	**ANOVA**	
						F	**P**
Before (Mean ± SD)		74.80± 11.31	74.13± 12.49	72.96 ± 10.95	77.07 ± 11.37	0.33	0.79
During (Mean ± SD)		78.83 ± 13.29	75.33 ± 10.99	74.13 ± 9.11	78.60 ± 11.78	0.63	0.59
Paired t	**T**	0.915	0.277	0.417	0.437		
	P	0.376	0.786	0.683	0.668		
AST(U/l)							
Before (Mean ± SD)		81.13 ±7.52	82.80 ±6.78	80.61 ±4.07	81.93 ±6.36	0.34	0.79
During (Mean ± SD)		81.23 ±5.79	82.00 ±7.87	81.87 ±5.20	81.93 ±5.68	0.04	0.98
	T	0.040	0.396	0.736	0.474		

Paired t	**P**	0.969	0.698	0.474	1.000		
Serum creatinine (mg/dl)							
Before (Mean ± SD)		0.65± 0.07	0.68 ± 0.12	0.63 ± 0.10	0.65 ± 0.16	3.34	0.02
During (Mean ± SD)		0.63± 0.09	0.65 ± 0.12	0.61 ± 0.14	0.69 ± 0.13	2.56	0.064
Paired t	**T**	2.284	1.000	0.286	0.796		
	P	0.038	0.334	0.779	0.439		
Blood urea (mg/dl)							
		27.09 ± 4.85	31.93 ± 6.46	26.51 ± 5.45	26.27 ± 4.83	2.11	0.06
		27.47 ±5.61	25.67 ±4.22	30.53± 7.1	31.73 ± 5.15	2.38	0.057
Paired t	**T**	0.195	2.179	1.890	2.203		
	P	0.848	0.112	0.080	0.106		

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

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 [(14)]. Patients with thalassemia major become transfusion-dependent with excess iron deposited in major organs resulting in their damage [(4)].

This study was done to evaluate the efficacy of different iron chelators in treatment of iron overload in thalassemic children who were under follow up in Hematology Unit, Pediatric Department, Tanta University Hospital in the period from January 2012 to August 2013.

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

This is in agreement with **Fisher et al., 2013** [(25)] who found that both desferrioxamine (IV, SC) and oral iron chelators (deferiprone and deferasirox) produced significant reduction in iron overload however, they recommended desferrioxamine as first-line therapy and deferiprone or deferasirox when desferrioxamine is contraindicated or inadequate, **Poggiali et al., 2012** [(26)] who reported that desferrioxamine has higher efficacy over deferasirox and deferiprone, **Pepe et al., 2011** [(27)] who found that SC desferrioxamine was the best in reduction of serum ferritin when compared with oral deferiprone and oral deferasirox**,** and **Kattamis, 2007** [(28)] who found that IV desferrioxamine was more effective than SC desferrioxamine .

In contrary, this is not in agreement with **Bejaoui and Guirat, 2013** [(29)] who found more reduction in mean ferritin levels with oral deferasirox versus SC desferrioxamine with statistically significant difference, **Cappellini et al., 2006** [(30)] who found that oral deferasirox had comparable efficacy with SC desferrioxamine, **Maggio et al., 2002** [(31)] who found that oral deferiprone had comparable efficacy as SC desferrioxamine, and **Taher et al., 2001**[(32)] 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 **Cappellini et al., 2006** [(30)] who found no changes in mean values of blood count after oral deferasirox or SC desferrioxamine, **Gomber et al., 2004** [(33)] who found no changes in blood count after SC desferrioxamine or oral deferiprone, but this is not in agreement with **Hoffbrand et al., 2012** [(7)] who found neutropenia, agranulocytosis and thrombocytopenia after deferiprone and **Yang et al., 2007** [(34)] 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 **Pennell et al., 2006** [35] who found no differences with SC desferrioxamine and **Rombos et al., 2000** [36] who found no differences with oral deferiprone but not in agreement with **El Beshlawy et al., 2008** [37] who found elevation of ALT and AST after oral deferiprone and SC desferrioxamine therapy and **Cappellini et al., 2006** [30] who found elevated ALT and AST with oral deferasirox.

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 **Pennell et al., 2006** [35] who found no affection of kidney functions after oral deferiprone or SC desferrioxamine. However, **Cappellini et al., 2006** [30] found transient increase in serum creatinine ≥ 30% with doses of 20 mg/kg and 30 mg/kg of oral deferasirox and **Borgna-Pignatti et al., 2006** [38] found renal affection with oral deferiprone therapy.

It was demonstrated that IV desferrioxamine therapy was an effective and safe method for reduction of serum ferritin and treatment of iron overload in patients with thalassemia major [39, 40]. Many studies found that treatment with continuous intravenous desferrioxamine has been shown to improve myocardial iron even in the most overloaded hearts and symptomatic heart disease can be reversed with excellent long-term prognosis and consequently less drug toxicity using continuous intravenous treatment [15, 41]. So intensive desferrioxamine infusion is considered in patients with significant heart disease, persistently high serum ferritin, or LIC > 15 mg/gm dry weight [7].

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

From this study we concluded that: IV desferrioxamine is more effective in reduction of iron overload in beta thalassemia major when compared with SC desferrioxamine, oral deferiprone and deferasirox but with no statistically significant differences.

Recommendations

From the present study we can recommend further multicenter studies on large number of patients, for long duration with more advanced methods of assessment of iron status to measure iron burden with different iron chelators and to clarify their side effects.

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