Early iron overload in beta-thalassaemia major: when to start chelation therapy?

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SUMMARY Twenty-eight children with β -thalassaemia major aged between 11 and 48 months were given intensive transfusions. Serum iron, transferrin saturation, serum ferritin, non-transferrin iron, and subcutaneous desferrioxamine-induced urinary iron excretion were measured. The results showed that even children with a limited number of transfusions had severe iron overload as indicated, in particular, by the raised serum ferritin levels and the high excretion rates after subcutaneous infusion of desferrioxamine. The desferrioxamine test was useful, even in very young children, in assessing response to chelation therapy thus enabling such treatment to be started early to prevent harm from iron overload.

Severe iron overload develops progressively in patients with transfusion-dependent homozygous β -thalassaemia, so that there is a need for continuous chelation therapy. The recent use of subcutaneous desferrioxamine infusion now enables chelation therapy to be started early even though its effectiveness in preventing toxic iron accumulation in β -thalassaemia major children still has to be proved.¹⁻⁷

The purpose of this work was to study serum iron, transferrin saturation, serum ferritin, the non-specific iron fraction,⁸ and urinary iron excretion in polytransfused patients with β -thalassaemia major, aged between 11 and 48 months, before they were started on chelation therapy, in order to assess at what age iron overload and early signs of iron toxicity develop and how soon chelation therapy should be started.

Patients

Twenty-eight children with β -thalassaemia major, aged between 11 and 48 months, not yet given chelation therapy, were studied. Each was given regular intensive transfusion, starting at age 6-12 months. The annual mean Hb level was higher than 12.9 g/dl (Table 1). None had been splenectomised. Beta-thalassaemia major was diagnosed by routine tests, by the degree of anaemia before blood transfusion, and by the parents' history. On the hypothesis that the patients had vitamin C deficiency (we did

not have the facilities to measure levels of vitamin C), each child was given 100 mg ascorbic acid by mouth daily, for a week, before the desferrioxamine (Desferal) test. In the test, desferrioxamine was given by slow (8 hours) subcutaneous infusion: first 20 mg/kg for 3 days and then 40 mg/kg for 3 days. Samples of urine were collected for 3 days and the daily average urinary iron excretion rate was calculated.

Methods

All serum samples were obtained before vitamin C administration for the desferrioxamine test was started. To avoid iron contamination disposable iron-free equipment was used throughout the study. All glassware was pretreated for 6 hours in 2N hydrochloric acid solution and rinsed several times in iron-free distilled water.

Levels of serum iron and total iron binding capacity were determined by colorimetric analysis, as recommended by ICSH.⁹ Latent iron binding capacity was measured with a saturated solution

Table 1 Annual mean Hb levels (mean $\pm$ SD) maintained by transfusion in thalassaemia major children

Ages (months)	Number of children	Haemoglobin (g/dl)
11-24	7	13.3 $\pm$ 0.4
25-36	8	13.6 $\pm$ 0.6
37-48	13	12.9 $\pm$ 1.0

containing a volume of $^{59}\text{FeCl}_2$ solution sufficient to give about 10 000 counts/ml per minute in the final solution, as described by ICSH.¹⁰ Serum ferritin was measured by a two-site immunoradiometric assay (Ramco). The sera containing the highest serum ferritin concentrations had to be diluted 1 : 20.

Unbound iron was measured in duplicate serum samples using the method of Herskho *et al.*⁸ All sera were obtained from patients before being given iron chelation therapy for 3 days. Briefly, 1 ml of serum was mixed with 0.5 ml of pH 7.45 buffer containing 5 mmol/l tris and 0.16 mol/l sodium chloride and 0.5 ml 0.01 mol/l EDTA, pH 7.5. The mixture was incubated at room temperature for 10 minutes.

The solution was then placed in a CF 25 Amicon filter cone (Amicon Corporation, Lexington, Massachusetts) and centrifuged at $400 \times g$ for 20 min. The CF 25 Amicon filter cones retain 95% of molecules with molecular weight greater than 25 000. About 1 ml of filtrate was obtained from each sample. Filtrate iron concentration was measured by atomic absorption spectroscopy.

Before use, the filters were soaked in EDTA for 2 hours in order to remove any iron contamination. Urinary iron excretion was evaluated by atomic absorption spectroscopy too. Red cells indices were determined in a Coulter electronic cell counter.

Osmotic fragility was measured by adding hypotonic saline buffered to pH 7.4 and haemolysis was read photoelectrically.

Hb A₂ was quantitated by DEAE-cellulose chromatography¹¹ and Hb F by alkali denaturation.¹²

Statistical methods. The coefficient correlation (*r*) between two independent variables was calculated.

Results

The details of all the patients, their ages, their ages at the first blood transfusion, the total amount of blood transfused, and the levels of iron at the time of the study are shown in Table 2.

Mean values for serum iron, transferrin saturation, serum ferritin, and urinary iron excretion are shown in Table 3. All levels were high, even in the youngest group of children. Desferrioxamine-induced urinary iron excretion after a dose of 20 mg/kg resulted in high levels only in children between 37 and 48 months of age, but after a dose of 40 mg/kg the levels were very high in all children. There was no correlation between serum ferritin and the age of the patients. There was a weak correlation between desferrioxamine-induced urinary iron excretion (20 mg/kg) and the age of patients ($P < 0.05$), but not after a dose of 40 mg/kg.

Table 2 Data for the 28 children

Case	Age (months)	Age at which first transfusion given (months)	Total blood transfused (ml)	Post-transfusion values				
				Serum iron ($\mu\text{g}/100 \text{ ml}$)	Transferrin saturation (%)	Ferritin (ng/ml)	Urinary iron excretion (mg/24 h)	
							Desferrioxamine (20 mg/kg)	Desferrioxamine (40 mg/kg)
1	11	11	544	244	100	730		
2	17	2	2440	244	100	3300	2.5	10.5
3	17	2	2632	211	85	1480		
4	18	8	3080	197		1440		
5	19	6	2800	57	36	660	2.4	4.6
6	22	2	2928	174	51	4500		
7	24	5	2688	255	100	4000	2	4.1
8	26	8	3400	215	100	2100	4.2	7.1
9	26	3	4192	155	49	1700	0.9	1.6
10	28	4	4216	216	100	2900	1	8.7
11	28	4	4424	255	100	2600		
12	29	6	2584	208	100	1300	3.1	4.2
13	32	6	3722	107	58	1500	1	3
14	33	3	6480	234	100	2500		
15	36	11	5760	300	100	1320		
16	37	12	4250	205	89	1320	3.7	7
17	38	6	8184	175	50	4000	5.9	8.5
18	39	7	5920	155	47	1800		
19	41	11	6376	188	88	2900	2.8	12
20	44	6	10176	222	72	3300	3.2	10.7
21	45	6	5520	155	71	1040	1.2	2.7
22	47	24	5184	174	87	2250	6.1	6.1
23	47	5	10320			4200	5.7	8.9
24	48	14	10064	208	100	4300	6.5	16.3
25	48	16	8824	175	100	2700	2.1	10.5
26	44	3	12000	231	100	4300		
27	48	4	10400	155	74	2600	6.2	9.3
28	48	11	9120	247	100	2100	6	7.4

Table 3 Mean serum iron, transferrin saturation, serum ferritin, and urinary excretion in patients with β -thalassaemia major (mean $\pm$ SD)

Age (months)	Number of children	Serum iron (μ g/100 ml)	Transferrin saturation (%)	Ferritin (ng/ml)	Urinary iron excretion (mg/24 h)	
					Desferrioxamine (20 mg/kg)	Desferrioxamine (40 mg/kg)
11-24	7	197 $\pm$ 75	79 $\pm$ 28	2301 $\pm$ 1596	2.3 $\pm$ 0.3*	6.4 $\pm$ 3.6
25-36	8	211 $\pm$ 59	88 $\pm$ 22	1990 $\pm$ 623	2.2 $\pm$ 1.8†	4.7 $\pm$ 3.0
37-48	13	191 $\pm$ 31	81 $\pm$ 19	2831 $\pm$ 1128	4.5 $\pm$ 1.9‡	9.0 $\pm$ 3.5

*Performed in 3; †performed in 5; ‡performed in 11.

There was a significant correlation between the amount of blood transfused and the serum ferritin level ($P < 0.01$) (Fig. 1) and between the amount of blood transfused and desferrioxamine-induced urinary iron excretion for both doses ($P < 0.01$) (Fig. 2). A highly significant correlation between

serum ferritin and 40 mg/kg desferrioxamine-induced urinary iron excretion rate ($P < 0.01$) was observed, but not after 20 mg/kg (Fig. 3).

Non-transferrin iron was detected in 11 of the 27 patients, being present in those in whom the transferrins were completely iron saturated (Table 4). The non-specific iron concentrations were in the range of 9 and 28 μ g/dl.

There was no correlation between non-specific iron and either serum ferritin or desferrioxamine-induced iron excretion.

Discussion

Our findings show that there can be severe iron overload in children younger than 4 years old. The intensive transfusion regimens to which the children were subjected led us to assume that the major endogenous factor influencing iron absorption, erythropoiesis, is minimal in such young children, so that roughly the entire iron load could be attributed to the blood received.¹³ This is confirmed by the significant correlation between serum ferritin, which reflects iron stored, and the amount of blood

Fig. 1 Correlation between serum ferritin and amount of blood transfused.

Fig. 2 Correlation between blood transfused and desferrioxamine-induced urinary iron excretion.

Fig. 3 Correlation between serum ferritin and desferrioxamine-induced urinary iron excretion.

Table 4 Non-transferrin iron in patients with β -thalassaemia major

Age (months)	Number of children	Patients with non-transferrin iron
11-24	7*	1
25-36	8	4
37-48	13	6

*Not performed in one patient.

transfused. The high correlation between desferrioxamine-induced urinary iron excretion (after either 20 or 40 mg/kg), which reflects the chelatable iron pool, and the amount of blood transfused also supports this view. Moreover, the positive relationship between serum ferritin and urinary iron excretion after desferrioxamine confirms that both these parameters are directly related to iron load.

It should be noted that transferrin saturation, serum ferritin, and urinary iron excretion after subcutaneous infusion of desferrioxamine (particularly after 40 mg/kg) were greatly raised in very young children. Some of them even showed iron overload after only a few blood units and we feel that an early start of chelation therapy in such children may delay the iron load-induced tissue damage (as was shown by Modell¹⁵). The finding of non-specific iron in children younger than 4 years extends Hershko's observations. He found non-transferrin iron, considered to be an index of iron toxicity, in patients with β -thalassaemia major and intermedia aged between 6 and 30 years who had secondary haemochromatosis.⁸ Similarly, Batey *et al.*¹⁴ recently found non-transferrin iron in the

sera of patients with severe iron overload caused by idiopathic haemochromatosis.

The presence of non-transferrin iron in the sera of these patients confirms that iron overload and iron toxicity develop very early in children with β -thalassaemia major. The lack of correlation between non-transferrin iron and either serum ferritin or urinary iron excretion may be due to the fact that there were few patients studied or that the iron overload was at an early stage.

Our findings show that β -thalassaemia major children, even those younger than 2 years, can have a severe iron overload and that regular subcutaneous infusion of desferrioxamine could greatly improve survival and should be started as soon as possible—that is from about the 3rd transfusion—according to the response of the patient to the desferrioxamine test performed with both doses (20 and 40 mg/kg a day).

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