SERUM FERRITIN CONCENTRATIONS IN TRANSFUSION DEPENDENT BETA-THALASSAEMIA

E George, H B Wong, R George, W A Ariffin

ABSTRACT

Patients on a moderate red cell transfusion programme have iron overload where the concentrations of the serum ferritin were inappropriate to increases in the transfusion load as a result of limitations of apoferritin synthesis and conversion of ferritin into haemosiderin. This study confirms the limitations for the use of estimations of the serum ferritin to evaluate the iron status in patients with expected high overload as would be seen in patients on many years of maintenance red cell transfusions in the absence of iron chelation therapy. Poor compliance, inadequate dosage of Desferal (deferoxamine), and the late initiation of iron chelation therapy were factors that were considered in the patients with failure of response to iron chelation.

Keywords: *transfusion dependent beta thalassaemia, serum ferritin.*

SINGAPORE MED J 1994; Vol 35: 62-64

INTRODUCTION

Ferritin, a high molecular weight iron containing protein, acts in the human body as an iron storage depot. Ferritin is the major iron storage protein of the liver, spleen, bone marrow, and other tissues of the body. Its two major functions are to remove excess iron from cells converting it into a harmless soluble form, and to provide a mobilisable reserve of iron which can be drawn when needed. Most of the ferritin is intracellular but the measurement of the circulating serum ferritin reflects the levels of the body's iron stores[1,2].

Homozygous beta-thalassaemia is an inherited red cell disorder characterised by severe anaemia beginning in the first year of life, bone marrow hyperplasia, a typical haemoglobin electrophoresis with markedly decreased or absent haemoglobin A, elevations of haemoglobins A_2 and F. Patients require maintenance red cell transfusions every 4 – 6 weeks. Iron overload has become the most important complication of thalassaemia with the advent of intensive red cell transfusion therapy which maintains the mean haemoglobin concentrations above 12 gm/dl. Patients on a low to moderate blood transfusion regimen have iron overload that may be aggravated by hypersplenism and compounded by increased intestinal absorption of iron.

Department of Pathology
Faculty of Medicine
Universiti Kebangsaan Malaysia
Jalan Raja Muda
50300 Kuala Lumpur
Malaysia

E George, FRCPA
Associate Professor

R George, FRCP
Associate Professor

W A Ariffin, FRCP
Associate Professor

Department of Paediatrics
National University Hospital
Lower Kent Ridge Road
Singapore 0511

H B Wong, MBBS, FAMS, FRCP (Edin), FRACP (Aust), FRCP (Glas), FRCP (Lond)
Emeritus Professor

Correspondence to: Dr E George

METHODS

Patients

Twenty-four patients with transfusion dependent thalassaemia from the Thalassaemia Clinic, Universiti Kebangsaan Malaysia, and from the Paediatric Department, General Hospital, Kuala Lumpur, were studied from 1989-1992. The study group consisted of 18 Chinese and 6 Malay patients, aged 2 to 11 years. A data profile chart was kept for each patient where information on liver profiles, serological assays and serum ferritin assays were recorded.

Serum ferritin

The serum samples were coded and stored at -20°C and analysed for serum ferritin in batches of 30 or more specimens on a routine basis. The quantitative determination of serum ferritin (SF) was by microparticle, enzyme immunoassay (MEIA, Imx system ferritin, Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064, USA) done in duplicate for each sample.

Statistical analysis

Student's t test was used to compare significance of laboratory data. To compare the features between the children at 1989 and 1992, non parametric analysis used the Wilcoxon rank-sum test. The Spearman's rank correlation was used to evaluate the relationship between the age of the patients to the levels of serum ferritin and serum alanine transminase (ALT). All data were expressed as means and SDs. Statistical significance was assessed at the $p<0.05$ level.

RESULTS

Haematological, biochemical and clinical data of the patients at January 1989 and January 1992 are shown on Tables I, II and III. A classical thalassaemia picture was seen in the peripheral blood films. The red cells showed marked anisopoikilocytosis, hypochromia, polychromasia, and microcytosis. There were target cells and misshapened cells. The range of Hb F seen was 35-85%. In a steady state, the mean pretransfusion and post-transfusion haemoglobin levels during the 4-year follow up were < 7.5 gm/dl and 11.5 gm/dl respectively.

All patients had serum ferritin levels > 350 ug/L. In 1989, only one (4%) of the patients had a serum ferritin level > 4000 ug/L. In contrast in 1992, seventeen (70%) had serum ferritin levels > 4000 ug/L. In the absence of chelation therapy to remove iron, in 1989 the patients showed a positive correlation of serum ferritin levels to age ($p=0.01$, $r_s=0.7$), but in 1992 there was a negative correlation ($p = 0.01$, $r_s = -0.9$). Only 2 (8.3%) of the patients in 1989 had serum alanine transaminases (ALT)

< 40 U/L and the serum ferritin levels had a positive correlation with the levels of ALT ($p = 0.01$, $r_s = -0.72$). There were only 2 patients who were on chelation therapy with Desferal (deferoxamine) which was given via pump subcutaneously at a dose of 35 mg/kg 3-5 times a week, which was commenced when the patients were 8 years old (Table I and II: B11HSF, and B16LWK). There was no significant difference in the serum ferritin levels of these patients who were on chelation therapy when the serum ferritin levels obtained in 1992 were compared with those of patients without chelation therapy ($p>0.1$). Five (20.8%) of the patients showed HBV markers, with a positive finding for HBsAg in three (12.5%).

Table I – Children with homozygous beta-thalassaemia, 1989 (n = 24)

Code	Age (yrs)	Sex	Race	Hb	SF	ALT	S	BT
B1 CYC	2	M	C	7.0	353.3	35.5	0	18
B2 CMC	2	F	C	6.8	560	45	0	18
B3 UA	2	F	M	7.1	730	40	0	18
B4 WSL	2	F	C	7.5	740	62	0	18
B5 N	3	F	M	7.7	530.7	67	0	30
B6 AL	4	F	C	6.5	804	82.5	0	42
B7 CCW	4	F	C	6.6	995	42	0	42
B8 CCY	5	M	C	6.5	1060	59	0	54
B9 LML	5	F	C	6.3	1140	50.5	0	54
B10 I	6	F	M	6.7	1178.4	56	0	69
B11 HSF	7	F	C	6.3	1240	114.5	0	84
B12 WYF	7	M	C	7.6	1500	48	0	84
B13 A	8	F	M	6.9	3826.7	54	0	99
B14 CSW	8	F	C	7.3	3550	162	/7	99
B15 HFP	9	M	C	7.9	2975	83	/7	114
B16 LWK*	9	M	C	7.5	1206.7	53	0	114
B17 LMP	9	M	C	7.5	1206.7	53	0	114
B18 SKW	9	M	C	6.6	4200	163	0	114
B19 LSK	10	F	C	6.3	3000	95.7	/7	132
B20 LJK	10	M	C	6.9	4000	121.7	/7	132
B21 SE	10	M	M	6.4	3600	162	/8	132
B22 A	11	F	M	7.4	2500	33.5	0	150
B23 CYC	11	M	C	8.2	3390	139	/7	150
B24 LLS	11	M	C	6.7	2215	43.5	/7	150

SF = serum ferritin µg/L, ALT = alanine transaminase U/L, BT = blood transfusion, IU = 350 ml, /Age at which splenectomy was done, 0 = Splenectomy not done. Hb = pretransfusion haemoglobin levels *chelation therapy from 8 years

Table II – Children with homozygous beta-thalassaemia, 1992 (n = 24)

Code	Age (yrs)	Sex	Race	Hb	SF	ALT	S	BT
B1 CYC	5	M	C	9.0	3406.3	100.5	0	54
B2 CMC	5	F	C	8.5	6042	45	0	54
B3 UA	5	F	M	6.7	6371.3	288	0	54
B4 WSL	5	F	C	6.4	3458.6	62	0	54
B5 N	6	F	M	7.9	6347.6	63	0	69
B6 AL	7	F	C	7.8	4468	84	0	84
B7 CCW	7	F	C	5.3	4490	65.5	0	84
B8 CCY	8	M	C	8.2	4540.2	108	0	99
B9 LML	8	F	C	6.3	5572.2	87	0	99
B10 I	9	F	M	8.9	2753.6	47	0	114
B11 HSF*	10	F	C	7.9	3031.4	69	/8	132
B12 WYF	10	M	C	8.0	5245.9	82	0	132
B13 A	11	F	M	5.8	2299.4	40	0	150
B14 CSW	11	F	C	8.8	5542.8	94	/7	150
B15 HFP	12	M	C	7.5	8351.4	76	/7	168
B16 LWK*	12	M	C	7.5	2719.4	28	0	168
B17 LMP	12	F	C	5.9	8552.4	46	0	168
B18 SKW	12	M	C	7.3	9045.9	180	/9	168
B19 LSK	13	F	C	7.1	6389.6	52	/7	189
B20 LJK	13	M	C	7.1	5493	147	/7	189
B21 SE	13	M	M	7.9	5522.3	140	/8	189
B22 A	14	F	M	8.9	2545.8	38	0	192
B23 CYC	14	M	C	9.7	6421.9	88	/7	320
B24 LLS	14	M	C	5.9	4219.6	62	/7	210

SF = serum ferritin µg/L, ALT = alanine transaminase U/L,
/Age at which splenectomy was done, 0 = Splenectomy not done.
BT = blood transfusion, IU = 350 ml, Hb = pretransfusion haemoglobin levels
*chelation therapy from 8 years

Table III – Serum ferritin, serum alanine transaminase, and haemoglobin levels in patients with transfusion dependent homozygous beta-thalassaemia (n = 24)

Mean ± SD	1989	1992
Age (years)	6.8 ± 3.2	9.7 ± 3.2
SF (ug/L)	2066.7 ± 1265.3	5557.1 ± 2041.4
ALT (u/L)	76 ± 41.5	90.3 ± 57
Hb (gm/dl)	6.9 ± 0.54	7.6 ± 1.2

SF: serum ferritin; ALT: serum alanine transaminase;
Hb: pretransfusion haemoglobin levels.

DISCUSSION

Homozygous beta-thalassaemia is described as a disease in which there is progressive iron overload from infancy to death in the early adulthood[3]. The iron is stored as ferritin and haemosiderin. The iron overload is the consequence of increased iron absorption and the large number of blood transfusions which these patients require. With the advent of intensive red cell transfusion therapy for this disease which maintain haemoglobin concentration higher than 12gm/dl, iron overload has become the most important complication of thalassaemia[4,5]. Intensive transfusion therapy also has been reported to suppress the increased intestinal absorption of iron which is seen in patients with increased red cell turnover as in thalassaemia[6]. In this study, patients received red cell transfusions that maintained the levels of the mean pre and post transfusion haemoglobins < 7.5 gm/dl and 11.5 gm/dl respectively. Hence, in this study an increased intestinal absorption of iron would be an inevitable cause contributing to raised serum ferritin levels[7]. In normal adults the concentration of serum ferritin is believed to be directly related to the available reticuloendothelial and parenchymal iron stores since the measurement of mobilisable iron stores by quantitative phlebotomy shows a good correlation with initial serum ferritin levels[8]. Serum ferritin in the two sexes were not analysed separately as sex difference is unimportant in children (patients in this study were less than 15 years old). Iron overload in homozygous beta-thalassaemia patients treated with maintenance red cell transfusion is an inescapable feature. Patients in our study showed raised levels of serum ferritin as early as when they were 2 years old following about 18-20 units of red cell transfusions (Table I). A simple relationship between serum ferritin and iron stores cannot be assumed particularly when the serum ferritin concentration is > 4000 ug/L or when more than 100 units of red cell transfusions have been administered[9]. Below this number of transfused units, the serum glycosylated ferritin correlated fairly well with the number of units given and iron load[10]. However, in the majority of homozygous beta-thalassaemia cases and as seen in our study, this amount had long been exceeded when the patients were 5-10 years of age (Tables I and II). The concentration of serum ferritin in patients with transfusion dependent beta-thalassaemia has been described to decline with increasing transfusion load[11]. In this study, there was a negative correlation of serum ferritin levels with age in 1992 ($p = < 0.01$, $r_s = -0.9$). Ferritin found in the serum has at least two different origins. Ferritin is secreted by cells that are stimulated to synthesise ferritin by excess iron – this is glycosylated ferritin which makes up to 80% of normal ferritin. The maximum rate for apoferritin synthesis in response to excess iron is reached after 100 units of red cell transfusions and as tissue iron accumulates, ferritin turns into haemosiderin which is not reflected in the serum ferritin level[12]. At this stage, accurate assessment of storage iron is possible through analysis of a biopsy sample of the liver[13].

Patients receiving multiple transfusions are at high risk for hepatitis B (HBV) and hepatitis C (HCV) infections. Positive markers for HBV infection was seen in five (20.8%) of the patients. In transfusion dependent homozygous beta-thalassaemia patients, liver function as reflected by the serum alanine amino transaminase (ALT) activity often deteriorates. In this study, in 1989 only two (8.3%) of the patients had ALT < 40 U/L. As tissue iron increases, parenchymal liver damage occurs, leading to the release of large quantities of nonglycosylated ferritin and grossly elevated total ferritin levels[14]. In addition, viral hepatitis aggravates damage to liver secondary to iron overload[15]. Splenectomy was needed for nine (37.5%) of the patients as a result of increasing needs for blood transfusions and for markedly enlarged spleens that caused distress to the patients. Removal of the spleen is associated with an increased risk of liver injury from iron overload and increased intestinal iron absorption in addition to the other adverse effects of splenectomy[16]. Only two patients were on iron chelation therapy with Desferal (deferoxamine) (Tables I and II: B11HSF, B16LWK). The chelation therapy was started when the patients were 8 years of age, at a dose of 35 mg/kg 3 – 5 times a week. Patients with adequate chelation therapy have been shown to have progressive reduction in their levels of serum ferritin[17,18]. In contrast to this finding, there were no significant differences in the serum ferritin levels while on iron chelation therapy when values obtained for 1992 were compared to the serum ferritin levels from patients without iron chelation therapy ($p > 0.1$). This suggested poor compliance, late initiation of chelation therapy (both commenced at age 8 years, with serum ferritin levels 1240 and 1206.7 ug/L respectively at onset), inadequate dosage of Desferal or inadequate duration of therapy for a negative balance to develop as patients have had only 2 and 4 years of chelation therapy respectively. The price of chelation therapy with Desferal (deferoxamine) is exorbitant: a vial (500 mg) costs M$13.50, and the average patient in Malaysia cannot afford life-long Desferal. Total management for transfusion dependent beta-thalassaemia in Malaysia currently is at its infancy. As programmes are set up, it is hoped that planners will include other tests in addition to assays of serum ferritin to evaluate the iron status of patients with beta-thalassaemia major who are on long term blood transfusion without iron chelation therapy.

In conclusion, this study has shown that though estimations of serum ferritin are convenient as a known method for the evaluation of iron in some clinical disorders, these tests are likely to be inappropriate for patients with beta-thalassaemia major who are on long term maintenance blood transfusion in the absence of adequate iron chelation therapy.

ACKNOWLEDGEMENTS

The study was supported by grants from Universiti Kebangsaan Malaysia (UKM 55/85) and from the Ministry of Science, Technology and Environment (IRPA: 03-07-03-024; 03-07-03-072). The serum ferritin study is a contribution from the M.D. programme, National University of Singapore of Dr E George.

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