Relationship of endocrinopathy to iron chelation status in young patients with thalassaemia major

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Abstract
Disturbances of growth and development in patients with thalassaemia receiving hypertransfusion programmes are well recognised and are most likely to be due to iron overload. The extent of endocrine dysfunction was investigated in a group of 18 patients thought to have been treated by acceptable modern standards, 11 of whom could be considered as well chelated. Assessment of growth and puberty showed a wide variation in height SD scores with five patients having significantly short stature. Most patients are progressing through puberty normally with the exception of two boys with marked pubertal delay. The most prominent finding was that growth hormone responses to glucagon stimulation were significantly impaired in all of the patients with iron overload. Basal endocrine assessment showed primary hypothyroidism in two patients aged 16·8 and 12·9 years with plasma thyroxine concentrations of 86 and 59 nmol/l (normal range 65–165 nmol/l) and plasma thyroid stimulating hormone 10·2 and 30·3 mU/l (normal range 0·5–5 mU/l). One patient had diabetes mellitus. These results show that even when ideal management is sought a significant amount of endocrine damage occurs; surveillance of these patients is thus essential.

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Endocrine dysfunction is well recognised in patients with transfusion dependent thalassaemia and is thought to reflect the consequence of iron overload.[1–4] The use of iron chelating drugs has been shown to delay the development of iron induced damage of cardiac and liver tissue, resulting in improved survival.[5 6] The ability of desferrioxamine to prevent endocrine damage is less clear, yet with patients surviving into their fourth decade the consequences of endocrine failure become increasingly important.

It is generally considered that the prevention of excessive iron overload will improve the prognosis for the late sequelae of iron toxicity. Intensive iron chelation treatment has now been widely used for the last 10–15 years. The first cohort of patients receiving hypertransfusion treatment and subcutaneous desferrioxamine since early childhood is now approaching and entering puberty. We therefore took the opportunity to investigate the effects of long term subcutaneous iron chelation treatment on growth, development, and endocrine function in our patients.

Patients and methods

Eighteen patients (11 boys, seven girls) with transfusion dependent thalassaemia were studied. Twelve were of Asian and six Mediterranean origin. All were healthy at the time of assessment. Chronological age ranged from 6·7 to 17·7 years (median 12·8). All patients were receiving a hypertransfusion regimen with pretransfusion haemoglobin maintained at concentrations greater than 105 g/l. The median age at the start of transfusion was 6 months (range 3–120 months) and the mean (SD) transfusion requirement was 0·75 (0·18) units/kg/year.

CHELATION TREATMENT

Subcutaneous desferrioxamine was started in early childhood in most patients (median age 36 months, range 17–96 months); the mean (SD) ferritin concentration at the start of chelation treatment was 1164 (655) μg/l. At the time of this study, all patients were using desferrioxamine at least four nights a week as an eight hour subcutaneous infusion. The mean (SD) desferrioxamine dose was 17 (4·7) g/kg/year. In recent years home chelation treatment has been supervised by two clinical nurse specialists. Iron overload was assessed by monitoring serum ferritin concentrations at least three times a year from the start of transfusion treatment. Splenectomy was performed in five patients at an average age of 10 years due to hypersplenism. All patients were receiving vitamin C. Liver function tests including aspartate transaminase, alkaline phosphatase, and bilirubin were within normal limits for all but two patients, one of whom has active hepatitis C.

AUXOLOGY

Standing height was measured using a Harpenden stadiometer by a single observer (RGG). Pubertal staging and bone age determination, which was performed by the same observer, were assessed according to the criteria of Tanner and coworkers.[7–9]

ENDOCRINE ASSESSMENT

Standard commercial reagents used in the endocrine laboratories of the Hospital for Sick Children and St Bartholomew's Hospital were used for the determination of hormone

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Characteristics of the two groups of patients with
thalassaemia

Group	Mean age (years)	Sex ratio (M:F)	Mean (SD) ferritin concentration (μg/l)
A (n=11)	12	6:5	1595 (392)
B (n=7)	13·2	5:2	3285 (853)

concentrations. Plasma concentrations of thyroxine, prolactin, oestradiol, testosterone, dihydrotestosterone, dehydroepiandrosterone, androstenedione, and luteinising and follicle stimulating hormone were determined by radioimmunoassay. Thyroid stimulating hormone (TSH) and growth hormone were determined by immunoradiometric assay.

Dynamic endocrine tests were performed on the day care unit. Growth hormone concentrations were measured at −15, 0, 30, 60, 90, 120, 150, and 180 minutes after an intramuscular injection of glucagon (15 μg/kg). Patients whose bone age was greater than 10 years at the time of assessment received stilboestrol 1 mg twice a day for two days before testing. Thyrotrophin releasing hormone and gonadotrophin releasing hormone tests were performed together, TSH, luteinising hormone, and follicle stimulating hormone concentrations being measured at 0, 20, and 60 minutes after bolus intravenous doses of thyrotropin releasing hormone (200 μg) and gonadotrophin releasing hormone (100 μg).

Endocrine and exocrine pancreatic functions were not tested.

Assessment of parathyroid function was limited to serum calcium and phosphorus; parathyroid hormone concentrations were not routinely measured. Student's t test was used for statistical analysis of the data; SD scores were calculated in the usual way.

Results

Patients were divided into well and poorly chelated groups according to five year mean ferritin concentrations. An average ferritin concentration less than 2500 μg/l was considered to reflect adequate chelation. Ferritin was measured every four months over a minimum period of five years; the lowest concentrations each subject achieved in each time

period were used in the calculation of the mean ferritin concentrations (see table). Spuriously increased concentrations were ignored. A desferrioxamine excretion test performed in the patient with hepatitis C confirmed heavy iron overload. Eleven of 18 patients in our cohort were considered well chelated with a mean (SD) ferritin concentration of 1595 (392) μg/l (group A). In the poorly chelated group (group B) the mean (SD) ferritin concentration was 3285 (853) μg/l. The mean (SD) age of the well chelated group was 12 (2·8) years; that of the poorly chelated group was 13·2 (3·3) years. There was no difference between the two groups in terms of age, sex, or race.

HEIGHTS

Figure 1 compares the SD score for height for boys and girls with the mean SD score and range of mid-parental heights. A wide range of heights is seen; however, five patients show significant short stature (>2 SD from mean). The mean (SD) heights of boys (150 (14) cm) and girls (134 (16) cm) in our cohort are higher than the average corrected heights of their parents. The mean SD score of the well chelated children (group A) is −1·23 (1·04), that of their parents is −1·6 (0·82), whereas the mean SD score of the poorly chelated children (group B) is −1·0 (0·8) and their parents −1·15 (1·6). There is no statistical difference in these two groups. Bone age was delayed in seven patients compared with chronological age (mean (SD) ratio (chronological age/bone age) 1·1 (0·1)) and was advanced in three patients compared with their chronological age (mean (SD) ratio 0·9 (0·05)).

PUBERTAL STATUS

All but two of the boys are progressing through puberty normally or have reached full maturity. Gonadotrophin concentrations were within normal limits and reflected the pubertal stage of the patient. The two boys with delay are in Tanner stage 2 at 16 and 16·5 years of age and have prepubertal gonadotrophin concentrations on dynamic testing. All of the girls are progressing through puberty normally.

ENDOCRINE ASSESSMENT

Baseline endocrine tests showed two patients with primary hypothyroidism who were clinically euthyroid (thyroxine 86 and 59 nmol/l, normal range 65–165; TSH 10·2 and 30·3 mU/l, normal range 0·5–5·0 respectively). One of these patients was in the well chelated group; the other was considered poorly chelated. The latter patient also developed insulin dependent diabetes mellitus and has hepatitis C. Cortisol, prolactin, testosterone, oestradiol, and adrenal androgen results were within normal limits. Serum calcium and phosphorus were normal in all patients.
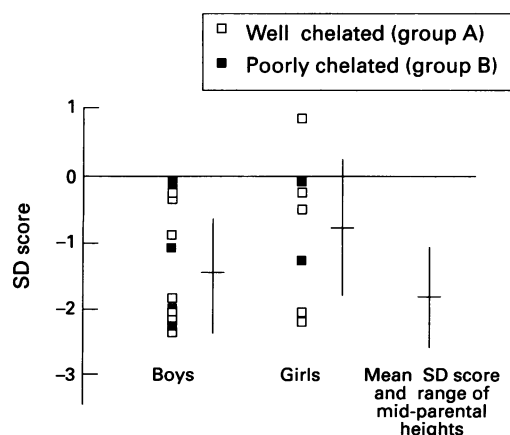

Figure 1   Height SD score values in patients with
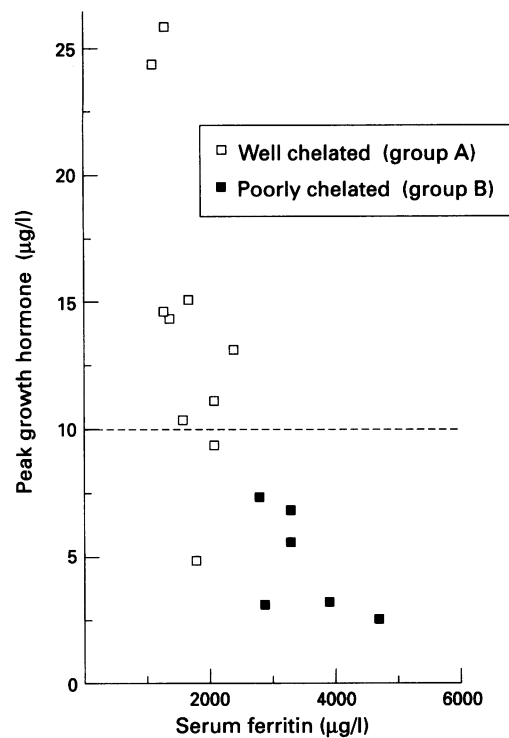thalassaemia using standards of Tanner and Whitehouse.[9]

Figure 2   Relationship between peak growth hormone concentration during provocation test and mean serum ferritin concentrations (1 μg/l growth hormone=2 mU/l).

Growth hormone secretion was tested dynamically in 16 of 18 patients. The well chelated group had a mean (SD) growth hormone peak of 14·2 (6·8) μg/l (28·4 (13·6) mU/l) whereas the poorly chelated group had a mean (SD) level of 5·0 (1·8) μg/l (10·1 (3·6) mU/l). The difference between the two groups is significant using Student's t test (p<0·01). The growth hormone peak for each patient is plotted against their mean serum ferritin concentration; an inverse relation between increasing ferritin concentration and decreasing growth hormone peak is seen. A peak growth hormone concentration of less than 10 μg/l (20 mU/l) is considered to represent growth hormone deficiency, thus those children who are poorly chelated have a significant amount of growth hormone deficiency compared with the well chelated group (p<0·01). All but two of the well chelated group had normal growth hormone secretion, explaining the relatively large SD. After these investigations three patients are now receiving growth hormone treatment.

Discussion

The modern treatment of transfusion dependent thalassaemia has evolved from the supposition that strict control of iron concentrations by chelation treatment will prevent endocrine and other organ damage caused by iron overload.[2] [10] Preliminary results for patients who have received long term intensive iron chelation treatment are encouraging.[11] Most patients need to use desferrioxamine as a subcutaneous eight to 12 hour infusion at least five nights a week to achieve optimum iron removal and iron

balance. Local reactions at the site of injection are common and uncomfortable. Good compliance with this time consuming, expensive, and unpleasant treatment is difficult to achieve, particularly as children become older.[12] An alternative treatment approach for some patients is bone marrow transplantation, which obviates the need for long term transfusion and iron chelation treatment. Although acceptable short term results have been published,[13] there are few data on the late effects or quality of life in those transplanted.

Despite a strong commitment to achieving iron balance using clinical nurse specialists visiting the home and regular outpatient visits, we find that only 60% of our group are well chelated, with an aggregate average serum ferritin concentration less than 2000 μg/l.

The SD scores for height show that all but one of the group are below average height compared with Tanner standards. The average height SD score for boys and girls with thalassaemia was higher than the SD score for the corrected mid-parental heights, however, which suggests that although the group are overall smaller than average, this is in part due to genetic factors. The ethnic and socioeconomic make up of our group, with many living in the inner city, may also be a contributory factor to short stature. Height standards for ethnic groups are unfortunately not available. Three boys and two girls had heights of more than two standard deviations less than the mean. There was no difference in the SD scores at assessment between the well and poorly chelated groups. Growth failure has been documented in patients starting subcutaneous desferrioxamine at 8 months of age before the onset of iron accumulation, rather than when the serum ferritin has reached 1000 μg/l.[14] The median age for initiation of chelation treatment in our group was 36 months (range 17–96 months) and none had an initial ferritin concentration less than 1000 μg/l; it is thus unlikely that this effect of desferrioxamine contributed to our findings of growth failure in most of our patients.

Pubertal delay is common in patients with thalassaemia, especially if iron chelation treatment is started late.[15] Twelve of 18 patients in our cohort are in a Tanner stage 2 of puberty or greater, most of these (75%) showing normal pubertal progression. The exceptions are two boys who are still in stage 2 of puberty at 16 and 16·5 years of age, both having prepubertal serum gonadotrophin concentrations on dynamic testing. These two patients are poorly chelated. The older patient has started replacement testosterone treatment.

Primary hypothyroidism was detected in two patients (11%) on baseline endocrine testing, both of whom were clinically euthyroid. This complication occurs in over 17% of iron overloaded patients.[15] This was an isolated finding in one of our patients in the well chelated group, however, emphasising the importance of monitoring thyroid function regularly in all transfusion dependent patients with thalassaemia.[16]

During this study one patient was admitted in diabetic ketoacidosis and is now insulin dependent. This patient is poorly compliant with an average serum ferritin concentration of 5013 μg/l over a 10 year period, but also has recently diagnosed hepatitis C. This is a recognised complication of transfusion and has been suggested to be a predisposing factor to the development of diabetes in iron overloaded patients.[17] This patient has significant problems: he is short (SD $-2\cdot3$), has not entered puberty at $16\cdot5$ years, is hypothyroid, has insulin dependent diabetes mellitus, and is growth hormone deficient.

The most striking abnormality detected in this survey was the extent of growth hormone deficiency. Eight of 16 patients evaluated by provocative testing had peak growth hormone concentrations less than 10 μg/l (20 mU/l), six of whom were in the poorly chelated group, suggesting that growth hormone deficiency in these patients was related to the effects of iron overload. In our cohort there appears to be an inverse relation between serum ferritin and peak growth hormone concentrations (fig 2). The higher the mean serum ferritin concentration, the lower the peak growth hormone value, reflecting the effect of iron overload. The growth hormone deficient patients, who have growth potential, have been started on growth hormone replacement treatment, which has been shown to be effective in such patients.[18] One of the patients in the well chelated group who has growth hormone deficiency has significant short stature with an SD score of $-2\cdot15$; her mid-parental SD score is $-0\cdot71$ and her sister is growing normally. The aetiology of her isolated growth hormone deficiency is unclear. Although the duration of iron overload is predominantly related to age, there is no clear relation between age and growth hormone deficiency in our group. Although growth retardation is common in thalassaemia, provocative testing of growth hormone response has shown normal[4 19 20] and abnormal responses.[18 21-23] Normal responses to a growth hormone provocation test contrasting with abnormal 24 hour profiles of growth hormone secretion has been reported, consistent with socalled growth hormone neurosecretory dysfunction.[19]

Abnormalities of growth are unlikely to be due to growth hormone deficiency alone. Several groups have reported markedly reduced concentrations of plasma insulin-like growth factor (IGF)-I, which does not appear to be due to abnormalities in the liver receptor for growth hormone but may be due to impaired hepatic IGF-I synthesis.[24 25] Abnormal IGF-I generation has been noted, suggesting that if iron deposition is responsible it must be an early event as many patients could be considered well chelated.[26 27] It remains to be seen to what extent growth hormone replacement will improve the growth and development of our patients.

In summary, despite the availability of effective iron chelating drugs and our best efforts, seven of the 18 patients can be considered to be poorly chelated and nine of these

18 patients had clinical or laboratory evidence of endocrine dysfunction. The degree of growth hormone deficiency was an unexpected finding and in our cohort is significantly correlated with iron overload. We conclude that all patients with transfusion dependent thalassaemia require close monitoring of growth and development and regular endocrine assessment. This is likely to be best achieved in a joint endocrine/haemoglobin-opathy clinic.

Bone marrow transplantation is now a well established alternative treatment for thalassaemia major. Few studies have been published to date on the endocrine outcome of such patients. If endocrinopathy, which appears inevitable in many conventionally treated patients, can be prevented by successful bone marrow transplantation, a strong argument exists for the further development of this treatment. Further follow up studies of endocrine outcome in patients receiving conventional treatment and those undergoing bone marrow transplantation are needed.

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Causes of mononucleosis

Doctors in Madrid studied 124 children with clinical and haematological evidence of infectious mononucleosis (Ana Lajo and colleagues, *Pediatric Infectious Disease Journal* 1994; **13:** 56–60). They found that 104 had serologically proved Epstein-Barr virus (EBV) infection and 20 cytomegalovirus (CMV) infection. The two infections were not distinguishable clinically, although those due to CMV were more often in children under 4 years old. Ninety three per cent of EBV cases and 75% of CMV cases had cervical lymphadenopathy. Heterophil antibody tests were positive in half of the EBV infections but in none of those due to CMV. Only 13·5% of children under 4 years of age with EBV infection had heterophil antibodies.

Complications occurred in six (6%) of the children with EBV infection. Three developed Gianotti-Crosti syndrome*, two Bell's palsy, and one severe airway obstruction. In those with CMV infection four (20%) had complications: two with thrombocytopenia, and one each with severe neutropenia and interstitial pneumonia.

Infectious mononucleosis may be due to either of these viruses but usually it will not be important to distinguish between them.

ARCHIVIST

*Fernando Gianotti (born 1920) and Agostino Crosti (born 1896) were Italian dermatologists. They described papular acrodermatitis of childhood. This usually affects children between 1 and 4 years of age and is a benign and self limiting condition characterised by crops of small red papules distributed peripherally on the limbs sometimes with vesicles and with a variable degree of itching. It is often associated with hepatitis B infection but also occurs with other viruses including hepatitis A, enteroviruses, adenoviruses, EBV, CMV, parainfluenza viruses, and rotavirus.