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Original Articles

Adrenal Insufficiency in Paediatric Transfusion Dependent Thalassaemia Major in Hong Kong: A Pilot Study

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Abstract Endocrinopathies including hypogonadism, diabetes mellitus and hypothyroidism have been well described in transfusion dependent thalassaemic patients. Adrenal insufficiency, on the other hand, was thought to be rare. Recent studies conducted in India, Thailand and Italy have confirmed that primary and secondary adrenal insufficiency occur too, as a result of iron overloading. In this prospective study, local transfusion dependent thalassaemic patients were recruited for adrenal function testing. Out of 28 patients, 10 (36%) were found to have adrenal insufficiency. Four of them have primary adrenal insufficiency and 6 have secondary (pituitary/hypothalamic) adrenal insufficiency. The prevalence of adrenal insufficiency is statistically related to age and years of blood transfusion, but does not correlate with the serum ferritin levels. These patients are advised to have hydrocortisone stress cover during illnesses and surgery to prevent crises.

Key words Adrenal insufficiency; Iron; Thalassaemia major

Introduction

Thalassaemia belongs to a group of recessively inherited haemoglobin disorders, and is characterised by defects in production of globin chains, leading to synthesis of abnormally configured and unstable haemoglobin, premature destruction of red cells, with accumulation of abnormal globin chain complexes in erythroid precursors including the bone marrow, liver and spleen, hindering effective erythropoiesis. Prior to implementation of effective treatment, most patients died before their adolescence.[1]

Life-long packed red cell transfusions have drastically reduced the mortality of such patients, allowing growth and decreasing ineffective erythropoiesis.[2] However, the consequences of iron overloading with deposition of non-transferrin-bound iron into the various endocrine organs continue to bring about considerable morbidity. Hypogonadism, growth retardation and impaired glucose tolerance have been well described.[3-5]

There is growing interest on exploring the adrenal adequacy of such iron-overloaded patients. Traditionally, it was believed that adrenal insufficiency will be amongst the least common and last to occur endocrinopathy due to iron overload in thalassaemia patients. However, although frank adrenal crises are rare, recent overseas studies in Asian and Mediterranean countries[6-8] have shown that adrenal insufficiency (AI) is not uncommon in thalassaemia major patients and even in non-transfusion dependent HbE/

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thalassaemia patients.[9] The purpose of this pilot study is to investigate on the prevalence of adrenal insufficiency in local transfusion dependent thalassaemia patients and to identify factors of significant correlation with the complication, with a view to generate useful data for the planning of further studies as appropriate for the better management of the condition in our local patients.

Method

The population studied included paediatric transfusion dependent thalassaemia patients aged 18 years or below requiring regular blood transfusion in the Department of Paediatrics, Prince of Wales Hospital and the Department of Paediatrics and Adolescent Health, Princess Margaret Hospital.

Written informed consent was obtained from patients and parents of patients who were less than 18 years old before enrolment. The study was conducted in accordance with the protocol and in compliance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and Good Clinical Practice (GCP).

Data Collection

The current age, age at time of diagnosis of thalassaemia, duration of transfusion, height and weight with centiles were recorded. Latest thyroid function and fasting glucose data, calcium, phosphate and alkaline phosphatase levels were collected. The result of the latest renal function test performed within one year was obtained. The mean haemoglobin level from the last two years and mean ferritin level from the last ten years was calculated. Magnetic resonance imaging of the liver and heart, which was performed in older patients, was studied.

Hormonal Tests and Procedures

ACTH (Adrenocorticotropin) stimulation tests were employed to diagnose adrenal insufficiency. All patients underwent the low dose short ACTH test. If the cortisol response was subnormal, the standard dose short ACTH test was then performed. All patients were admitted into hospital at 8AM and an indwelling catheter was inserted between 0800 and 0900h.

Basal Cortisol and ACTH Levels

Blood samples for basal cortisol and paired ACTH were obtained after a 30 minutes rest after insertion of venous catheter.

Low Dose Short ACTH Test

All patients then underwent the low dose short ACTH test. One microgram of ACTH was prepared by diluting one vial of 250 microgram ACTH into 250 mL sterile normal saline. 1 mL (=1 microgram ACTH) of the solution is then injected as intravenous bolus. Basal samples for cortisol were collected as 0 minute sample and then at 30 and 60 minute intervals. Children whose cortisol reached above 500 nmol/L were considered to have normal functioning hypothalamic-pituitary-adrenal axis.

Standard Dose Short ACTH Test

Children whose cortisol failed to reach 500 nmol/L in the low dose short ACTH test underwent the 250 microgram standard dose ACTH test to distinguish between primary and secondary adrenal insufficiency. Those who failed the low dose ACTH test but achieved cortisol above 500 nmol/L in the standard dose ACTH test were considered to have secondary adrenal insufficiency. Those whose exhibit an elevated baseline ACTH level together with failure to reach a peak cortisol response of 500 nmol/L in both tests were considered to have primary adrenal insufficiency.

Those who fail to elicit a satisfactory response with both low dose and standard dose ACTH test, and has no elevation in baseline ACTH are diagnosed as long standing secondary adrenal insufficiency. Due to chronic lack of stimulation of the pituitary, the adrenals undergo partial atrophy and thus fail to respond to both tests.

Biochemical Assays for Research Outcomes

Cortisol levels are measured by chemilumininescent assay (Beckman Coulter, Brea, California, USA).

ACTH levels are measured by double antibody RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA).

Ferritin levels are measured by immunoassay (Beckman Coulter, Brea, California, USA).

Research Outcomes

The primary outcome is the prevalence of adrenal

insufficiency in transfusion dependent thalassaemia major patients. The secondary outcomes include the correlation between the incidence of adrenal insufficiency and the age of patient, years of transfusion, the ferritin levels and the type of chelation therapy for iron removal.

Statistical Analysis

The data shall be expressed in mean +/- SD or median where appropriate. Regression analysis was used to establish statistical relationship between variables. Statistical significance is taken at p value <0.05. Confidence intervals are taken as 95%.

Results

Population Characteristics (Table 1)

Twenty-eight paediatric thalassaemia patients (14 boys, 14 girls) from Department of Paediatrics, Prince of Wales Hospital and the Department of Paediatrics and Adolescent Health, Princess Margaret Hospital were recruited in the study. They were between 3 to 18 years of age. All have been transfusion dependent for at least 2 years, and at the time of study, required blood transfusion every 3 to 8 weeks. Eight were transfused once every 3 weeks, 19 were transfused once every 4 weeks, and 1 was transfused once every 8 weeks. Twenty were affected by beta thalassaemia major, 3 had haemoglobin Barts disease, 1 had HbH disease, 1 had HbH Constant Spring disease, 1 had beta^0/ beta$^+$ disease, and 2 had HbE/beta thalassaemia. Four patients received chelation therapy of subcutaneous desferrioxamine therapy, 8 patients were on deferiprone, and 7 were on deferasirox. Eight patients received combination therapy of deferiprone and desferrioxamine. One patient had not yet been started on iron chelation therapy (Table 2).

The low dose ACTH stimulation test identified ten out of the 28 patients (36%) to suffer from adrenal insufficiency. The lowest cortisol level achieved from the low dose ACTH test was 261 nmol/L.

Of those who failed the low dose ACTH stimulation test and who underwent the standard dose ACTH test, 3 (30%) suffer from primary adrenal insufficiency, and seven (70%) from secondary adrenal insufficiency. Among these seven patients, one of them failed both the low dose and high dose ACTH stimulation test whilst the ACTH was not increased, She is considered to be suffering from long standing secondary adrenal insufficiency. She has haemoglobin Barts disease and has been transfused since birth.

The youngest patient noted to have adrenal insufficiency in our study is 9 years old. The mean pre-transfusion haemoglobin level in this study group ranged from 8.3 g/dL to 10.8 g/dL. There was no statistically significant correlation between the mean pre-transfusion haemoglobin levels and the cortisol response in the low dose ACTH test. Those children with adrenal insufficiency did have significantly lower 9AM baseline cortisol level compared

Table 1 Patient characteristics (N=28)

Parameter	Value
Thalassaemia types, n (%)	
Beta thalassaemia major	20 (75)
Hb Barts disease	3 (10)
HbE/beta thalassaemia	2 (7)
HbH disease	1 (3.5)
Beta^0/Beta$^+$ disease	1 (3.5)
HbH constant spring	1 (3.5)
Age in years, mean±SD (range)	11.552±5.5070 (3-18)
Male: female, n (%)	14 (50): 14(50)
Splenectomised, n (%)	3 (10)
Age at start of transfusion in years median (IQR, min, max)	0.83 (0.375-1.5, 0, 72)
Pre transfusion haemoglobin levels in g/dL, mean±SD (range)	9.8±0.99 (7.2-11.6)
Transfusion frequency median (once every__ week), (range)	4 (3-8)
Serum ferritin level in ng/ml median (IQR, min, max)	1800 (1310-2252, 193, 7390)
<1500	9
1500-2500	13
2500-3500	4
>3500	2
Current chelator, n (%)	
Desferrioxamine	4
Deferiprone	8
Desferrioxamine + deferiprone	8
Deferasirox	7
Nil chelation	1
Current chelator dose in mg/kg per day mean±SD (range)	
Desferrioxamine	23.5±8.0 (13.8-30.0)
Deferiprone	76.1±17.3 (43-100)
Desferrioxamine	23.8±9.9 (13-39)
+ deferiprone	82.9±11.0 (65-93)
Deferasirox	21.4±6.3 (9-30)

with those with normal function (p=0.045, 95% CI 0.016-1.24) (Figure 1). Twenty-two patients have serum ferritin <2500 ng/ml. There was no statistical significant correlation between ferritin levels with baseline cortisol or cortisol responses post stimulation. None of the patients have chronic hepatitis B or C infection.

The cortisol response mounted had been found to correlate inversely with patient's age (p=0.018, 95% CI -28.9-3.03) (Figure 2) and years of regular transfusion. (p=0.016, 95% CI -28.5 - -3.24) (Figure 3).

Magnetic resonance imaging (MRI) of the liver to assess hepatic iron overloading was performed in seventeen patients, at the age of 9 years to 18 years. Mild iron overloading (hepatic T2* 2.7-6.3 ms) was found in fourteen of these patients. Half (7 out of 14) of these patients are adrenal insufficient. MRI of the heart was performed in 19 patients, and 7 patients had MRI T2* relaxation time below 20 ms, signifying pathological accumulation of iron in the

Table 2 Demographic data and results of hormonal tests

Thal type	Age (Yr)	Tranfusion (Yr)	Transfusion frequency (Q___weeks)	Basal cortisol (nmol/L)	Peak cortisol LDSST (n>500 nmol/L)	ACTH (N<10.2 pmol/L)	Peak cortisol SDSST (n>500 nmol/L)	Adrenal insufficiency
β thal major	13	12	4	341	493	11.8	342	Primary
β thal major	16	15	3	229	377	21.5	379	Primary
Hb barts	16	16	4	124	328	19.2	491	Primary
β thal major	13	13	3	137	465	3.6	438	Chronic secondary
β thal major	16	15	4	302	355	6.2	538	Secondary
Beta HbE	17	15	8	384	384	7.4	876	Secondary
β thal major	9	8	4	226	261	Undetectable	531	Secondary
β thal major	18	17	4	222	393	5.72	657	Secondary
β thal major	16	12	4	113	396	7.355	620	Secondary
β thal major	15	15	4	128	492	5.176	689	Secondary
β thal major	16	15	4	595	884	5.1		
β thal major	16	15	3	312	725	2.7		
β thal major	7	6	4	182	895	Undetectable		
β thal major	16	15	4	309	587	3		
$Beta^0/beta^+$	7	3	4	306	880	2		
HbE/beta	5	4	4	522	843	2		
β thal major	3	2	3	343	923	4.2		
HbH CS	3	1	3	291	793	2.9		
β thal major	18	15	4	179	740	Undetectable		
β thal major	3	2	4	249	658	8.99		
β thal major	10	9	3	232	600	3.81		
β thal major	7	6	3	107	742	4.63		
β thal major	18	18	4	261	709	4.09		
β thal major	18	18	4	228	681	Undetectable		
β thal major	10	9	3	194	596	5.99		
Hb barts	4	4	4	293	610	5.49		
Hb barts	7	7	4	113	576	3.541		
β thal major	15	14	4	156	632	3.28		

myocardium. Two of these patients have adrenal insufficiency.

Of the 28 candidates, one patient has type 2 diabetes mellitus, currently on metformin. She also suffers from delayed puberty and amenorrhoea from secondary hypogonadism. She is the only patient with hypogonadism. Her adrenal function is intact. Three other patients have impaired fasting glucose, and one of them is adrenal insufficient. None of the 28 patients have deranged thyroid function. Three patients have short stature (defined as height <-2 SD) below normal for age and sex, and they all have concomitant adrenal insufficiency.

All subjects have normal blood calcium, phosphate and alkaline phosphatase, which indicate normal parathyroid function. The blood electrolyte levels are normal in all patients, signifying an intact renin-angiotensin-aldosterone system.

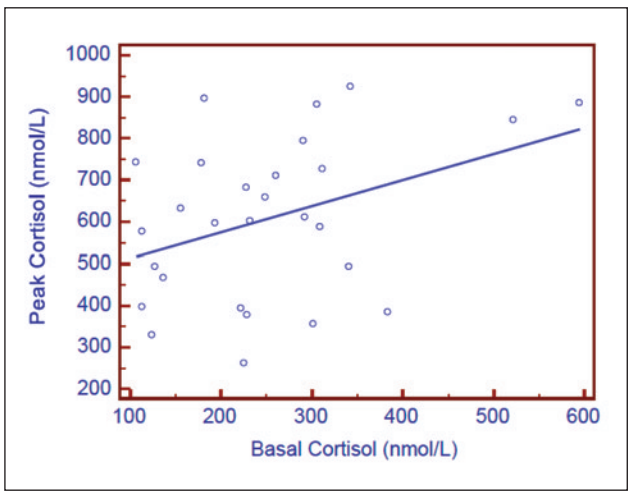

Figure 1 Linear correlation between basal cortisol levels and peak cortisol responses achieved in 1 microgram ACTH test.

Discussion

This study showed that adrenal insufficiency was present in 36% of the transfusion dependent thalassaemia patients, and these results are similar to that found in reports from other countries. We have also found that there is increased prevalence of adrenal insufficiency in patients of older age groups and longer duration of transfusion, but there is no statistical correlation between ferritin levels and maximal cortisol responses.

The study on the pituitary adrenal reserve in adult thalassaemic patients in Thailand showed subnormal adrenal responses to low dose ACTH test in 32.1% of patients.[10] The ferritin level has, similarly, failed to predict the likelihood of adrenal insufficiency in these patients.

The gold standard to test the integrity of the hypothalamic-pituitary-adrenal axis is the insulin tolerance test, as it tests the entire axis. Hypoglycaemia potently stimulates the production of CRH from the hypothalamus

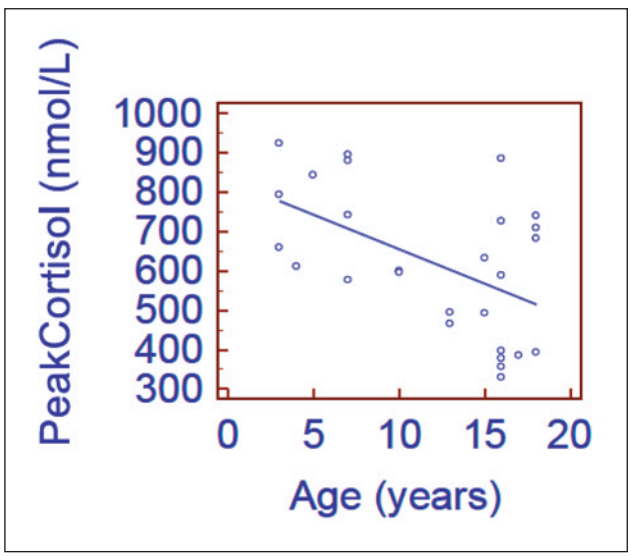

Figure 2 Inverse correlation between age of patient and peak cortisol level achieved in 1 microgram ACTH test.

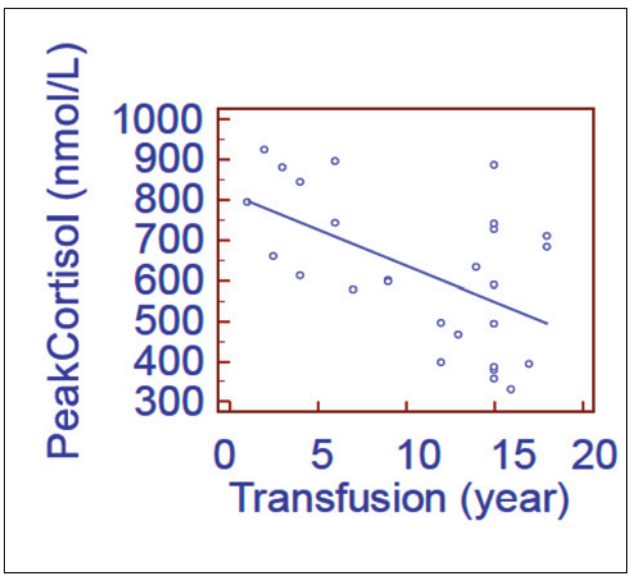

Figure 3 Inverse correlation between years of transfusion and peak cortisol level achieved in 1 microgram ACTH test.

and ACTH from the pituitary gland, producing cortisol to be measured. Its shortcoming, however, is that this test is potentially hazardous, can precipitate hypoglycaemic seizures and is preferentially avoided in children. The 250 microgram standard dose test, using a supraphysiological dose of synthetic ACTH to stimulate the pituitary gland, was found to have consistent correlation with insulin tolerance test.[11] Thus it has come to popularity as an alternative screening test for adrenal insufficiency. The rationale for using 250 microgram ACTH test is that the adrenal glands requires regular stimulation by ACTH to be functional. When the secretion of endogenous ACTH fails, the zona fasciculata of the adrenal glands undergoes atrophy and is unable to respond to exogenous ACTH.[12] Subsequent reports have demonstrated lack of sensitivity of the 250 mcg ACTH test in detecting mild secondary adrenal failure, [12,13] and it is logical to believe that the supra-physiological doses of ACTH, being at least double the ACTH value which can be induced physiologically in stressful conditions,[14] can lead to stimulation of the partially atrophied adrenal glands and give falsely reassuring results. The 1 microgram low dose ACTH is now adopted as this level of ACTH is comparable to that produced in insulin tolerance test[13] and in acute stress.[15] Several large studies have demonstrated the use of 1 microgram ACTH to have good correlation against insulin tolerance test for patients with pituitary disease[16,17] and is a sensitive test to diagnose mild secondary hypoadrenalism.

In this study we employed the use of 1 microgram ACTH test to screen for adrenal insufficiency. Those who fail the 1 microgram ACTH test are deemed adrenal insufficient, and undergo the 250 microgram ACTH test. Should they fail the 250 microgram ACTH test and have raised ACTH level, they are diagnosed to have primary adrenal insufficiency or longstanding secondary adrenal insufficiency if ACTH is not elevated.

Adrenal insufficiency, as in the other endocrine abnormalities in thalassaemia, is most commonly a consequence of iron overloading from blood transfusion. In these patients, even before transfusion is started, production of abnormal globin chains results in anaemia and ineffective erythropoiesis, and erythyroid hyperplasia sets in. This leads to downregulation in the hepcidin levels and an increase in ferroportin. Ferroportin is a transmembrane protein responsible for inducing macrophages to release iron into the blood stream and increases iron absorption from the gastrointestinal tract. Thus iron overload in thalassaemic patients often occur even before regular blood transfusion.[18]

There are no mechanisms to regulate iron loss. In men, only minimal amount of iron is lost through sweat, skin cells and occasionally via the gastrointestinal tract at around 1 mg/day. In women the loss of iron via menstrual blood is a mere additional 1 mg/day. Iron transfused in blood is mostly stored and results in iron overloading.

As iron overload progresses, the transferrin capacity is saturated. The non-transferrin bound iron is toxic as it promotes the formation of free hydroxyl radicals which leads to lipid peroxidation, cell death and fibrosis.

Different methods have been used to evaluate the severity of iron overload. Since the liver has the largest capacity to store iron, the iron concentration in the liver gives a good reference of iron balance of the body.[19]

Traditionally, the gold standard for liver iron concentration is by direct liver biopsy but this is invasive, labour intensive and prone to sampling error.[20] Serum ferritin has been found to correlate well with liver iron concentrations[21] and thus serum ferritin is often adopted as a surrogate marker for iron stores in the body. Unfortunately, ferritin is also raised in patients with inflammatory states, infections, liver dysfunction and malignancies, and in these cases the ferritin can be raised disproportionately higher than the degree of iron overload.

In our group of patients, there is no significant correlation between serum ferritin levels and the quantitative cortisol levels achieved in the low dose ACTH test.

Compared with liver biopsy, MRI of the liver using either R2 (1/T2) or R2* (1/T2*) pulse sequences has the advantage of being non-invasive, and the sampling error due to uneven deposition of iron in the liver is also reduced. MRI liver is now the preferable method to measure liver iron concentrations. This method has been validated against liver iron concentration from liver biopsy and found to have strong linear correlations for regularly transfused thalassaemic major and thalassaemia intermedia patients.[22] Seventeen out of our 28 (60.7%) have undergone MRI liver imaging and 14 of those tested (82%) have been found to have some degree of hepatic iron overloading. Half (7 out of 14) of these patients are adrenal insufficient. However correlation between degree of MRI liver siderosis and peak cortisol results is difficult since not all patients have undergone the MRI liver imaging.

Concerning iron deposition in endocrine organs, there has been increasing interest in using MRI to measure pituitary size and MRI R2 pituitary iron deposition to predict the severity of hypogonadism. So far, results have shown that iron deposition occur in the first decade of life in 25% of patients, before clinical symptoms of

hypogonadism can be apparent, as evident by decreased T2 relaxation times of the pituitary gland.[23] Furthermore, MRI pituitary R2 correlates significantly with serum ferritin, MRI liver, heart and pancreatic iron concentration.[22]

So far no studies have been performed to correlate pituitary iron deposition with secondary adrenal insufficiency and more studies need to be performed to see if secondary adrenal insufficiency is associated with significant MRI abnormalities in the pituitary. As for iron deposition in the adrenals, a study performed on 35 Greek beta thalassaemia major identified that 24 of them demonstrated adrenal hypointensity in MRI adrenal T2*. These hypointensities correlated significantly with hepatic siderosis.[24] These results are in line with a large autopsy series in iron -overloaded subjects, which showed a positive correlation between liver and adrenal haemosiderosis.[25]

Iron deposition in the adrenal glands, in fact, is observed mostly in the zona glomerulosa, the site of minerocorticoid production, and less frequently involves the zona fasicularis, the site of cortisol production.[25] In our patients, none of them have deranged sodium or potassium levels, thus the renin-angiotensin-aldosterone system is likely intact.

Although adrenal insufficiency is not uncommon in thalassaemia patients, frank adrenal crises are rare. Banani et al performed a study on cortisol and ACTH responses on 27 paediatric thalassaemic patients undergoing splenectomy and compared them with non-thalassaemic controls undergoing laparotomy, and found normal cortisol responses in these patients, though the ACTH appears more significantly elevated for the thalassaemia group. Thus they postulated that the adrenal reserve may be partially insufficient, with undue pressure on the pituitary.[26]

Conclusions

Both secondary and primary adrenal insufficiencies have been found in our local transfusion-dependent thalassaemic population They required glucocorticoid replacement during febrile illnesses and major surgery, and should be re-tested again 1-2 years later for progress monitoring. Cortisol levels can be monitored during genuine stress periods to better gauge the adequacy of adrenal reserve.

One major limitation of this study is the small sample size. Local data for other endocrinopathies in thalassaemia major patients has already been very well described.[27] This study was done in the hope to increase awareness of possible adrenal insufficiency in these groups of patients. Larger studies are needed to set the recommended age to start adrenal function testing, since adrenal insufficiency occurs only in the older age groups in our study. Imaging studies of the pituitary can be performed to look for correlations between pituitary T2* relaxation times and secondary adrenal insufficiency.

Declaration of Interest

The authors have report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89:1187-93.
2. Rund D, Rachmilewitz E. Beta Thalssaemia. NEJM 2005;353: 1135-46.
3. Adil A, Sobani ZA, Jabbar A, Adil SN, Awan S. Endocrine complications in patients of beta thalassaemia major in a tertiary care hospital in Pakistan. J Pak Med Assoc 2012;62:307-10.
4. Costin G, Kogut MD, Hyman CB, Ortega JA. Endocrine abnormalities in thalassaemia major. Am J Dis Child 1979;133: 497-502.
5. Gamberini MR, De Sanctis V, Gilli G. Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major followed from 1980 to 2007 in the Ferrara Centre. Pediatr Endocrinol Rev 2008;6 Suppl 1:158-69.
6. Srivatsa A, Marwaha RK, Muraldharan R, Trehan A. Assessment of adrenal endocrine function in Asian thalassaemics. Indian Pediatr 2005;42:31-5.
7. Jaruratanasirikul S, Tanchotikul S, Wongcharnchailert M, Laosombat V, Sangsupavanich P, Leetanaporn K. A low dose adrenocorticotropin test for the evaluation of adrenal function in children with B thalassaemia receiving hypertransfusion with suboptimal iron chelating therapy. J Pediatr Endocrinol Metab 2007;20:1183-8.
8. Masala A, Meloni T, Gallisai D, et al. Endocrine functioning in multitransfused prepubertal patients with homozygous beta thalassaemia. J Clin Endocrinol Metab 1984;58:667-70.
9. Nakavachara P, Viprakasitt V. Adrenal insufficiency is prevalent in HbE/beta thalssaemia paediatric patients irrespective of their clinical severity and transfusion requirement. Clin Endocrinol (Oxf) 2013;79:776-83.
10. Scacchi M, Danesi L, Cattaneo A, et al. The pituitary-adrenal axis in adult thalassaemic patients. Eur J Endocrinol 2010; 162:43-8.
11. Hurel SJ, Thompson CJ, Watson MJ, Harris MM, Baylis PH, Kendall-Taylor P. The short Synacthen and insulin stress tests in the assessment of the hypothalamic-pituitary-adrenal axis. Clin Endocrinol (Oxf) 1996;44:141-6.

12. Abdu TA, Elhadd TA, Neary R, Clayton RN. Comparison of the low dose short synacthen test (1 microg), the conventional dose short synacthen test (250 microg), and the insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in patients with pituitary disease. J Clin Endocrinol Metab 1999; 84:838-43.
13. Nye EJ, Grice JE, Hockings GI, et al. Adrenocorticotropin stimulation tests in patients with hypothalamic-pituitary disease: low dose, standard high dose and 8-h infusion tests. Clin Endocrinol (Oxf) 2001;55:625-33.
14. Oelkers W. Dose response aspects in the clinical assessment of the hypothalamo-pituitary-adrenal axis, and the low dose adrenocorticotropin test. Eur J Endocrinol 1996;135:27-33.
15. Dickstein G, Arad E, Schechner C. Low dose ACTH stimulation test. Endocrinologist 1997;7:285-93.
16. Soule SG, Fahie-Wilson M, Tomlinson S. Failure of short ACTH test to unequivocally diagnose longstanding symptomatic hypoadrenalism. Clin Endocrinol (Oxf) 1996;44:137-40.
17. Cunningham SK, Moore A, Mckenna TJ. Normal cortisol response to corticotrophin in patients with secondary adrenal failure. Arch Intern Med 1983;143:2276-9.
18. Origa R, Galanello R, Ganz T, et al. Liver iron concentrations and urinary hepcidin in beta thalssaemia. Haematologica 2007;92: 583-8.
19. Ramacha A, Sanz C, Contreras E, et al. Guidelines on haemoviligance of post-transfusional iron overload. Blood Transfus 2013;11:128-39.
20. Ambu, R, Crisponi G, Sciot R, et al, Uneven hepatic iron and phosphorus distribution in beta thalassaemia. J Hepatol 1995;23: 544-9.
21. Jensen PD. Evaluation of iron overload. Br J Haematol 2004; 124:697-711.
22. Christoforidis A, Haritandi A, Perifanis V, et al. MRI for the determination of pituitary iron overload in children and young adults with beta-thalassaemia major. Eur J Radiol 2007;62: 138-42.
23. Noetzli LJ, Panigrahy A, Mittelman SD, et al. Pituitary iron and volume predict hypogonadism in transfusional iron overload. Am J Hematol 2012;87:167-71.
24. Drakonaki E, Papakonstantinou O, Maris T, Vasiliadou A, Papadakis A, Gourtsoyiannis N. Adrenal glands in beta-thalassemia major: magnetic resonance (MR) imaging features and correlation with iron stores. Eur Radiol 2005;15:2462-8.
25. McDonald R, Mallory GK. Haemachromatosis and haemosiderosis - a study of 211 autopsied cases. Arch Int Med 105:686-700.
26. Scacchi M, Danesi L, Cattaneo A, et al. The pituitary-adrenal axis in adult thalassaemic patients. Eur J Endocrinol 2010;162:43-8.
27. Au WY, Lee V, Lau CW, et al. A synopsis of current care of thalassaemia major patients in Hong Kong. Hong Kong Med J 2011;17:261-6.