Original Article

Cortisol response to low dose versus standard dose (back-to-back) adrenocorticotrophic stimulation tests in children and young adults with thalassemia major

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ABSTRACT

Background: Thalassemia major patients with repeated blood transfusion have high prevalence of endocrinopathies due to iron overload. **Materials and Methods:** We examined the adrenocortical function in 23 thalassemic patients (10 children and 13 young adults) aged 8-26 years. Serum cortisol and dehydroepiandrosterone sulfate (DHEA-S) concentrations were determined in each subject before blood transfusion both in basal condition and after low dose (LD) (1 μg), followed by standard dose (SD) (250 μg, respectively) with synthetic corticotrophin beta 1-24 ACTH (Synacthen, Ciba). Normal controls were a group of 13 age- and sex-matched normal subjects. **Results:** Using a peak total cortisol cutoff level of 550 nmol/L and increments of 200 μg above basal cortisol, adrenal insufficiency (AI) was demonstrated in 8 patients (34.7%) after the LD ACTH and in 2 patients (8.7%) after SD cosyntropin (ACTH) test, but none of the controls. Using a peak total cortisol cutoff level of 420 nmol/L and increments of 200 μg above basal cortisol, AI was demonstrated in 5 patients (21.7%) after the LD ACTH and in 2 patients after SD ACTH test (8.7%), but none of controls. All patients with biochemical AI were asymptomatic with normal serum sodium and potassium concentrations and had no history suggestive of adrenal pathology. The peak cortisol concentrations in thalassemic patients with impaired adrenal function both after 1 μg and 250 μg cosyntropin (294 ± 51 nmol/L and 307 ± 58.6) were significantly lower than those with patients with normal (454 ± 79.7 nmol/L and 546.1 ± 92.2 nmol/L, respectively) and controls (460.2 ± 133.4 nmol/L and 554.3 ± 165.8 nmol/L, respectively). Adolescents and young adults, but not children with thalassaemia, had significantly lower peak cortisol concentration after SD ACTH versus controls. Peak cortisol response to LD ACTH was correlated significantly with peak cortisol response to SD in all patients ($r = 0.83$, $P < 0.0001$). In adolescents and young adults with thalassemia, DHEA-S levels before and after LD ACTH stimulation were significantly lower and the cortisol/DHEA-S ratios were significantly higher than the controls. **Conclusion:** The use of LD ACTH test diagnoses more adrenal abnormalities versus SD ACTH in thalassemic patients. The relatively high prevalence of AI in thalassemic adolescents and young adults necessitates that these patients have to be investigated for AI before major surgery and those with impaired cortisol secretion should receive stress doses of corticosteroids during the stressful event.

Key words: Cortisol, dehydroepiandrosterone sulfate, low dose adrenocorticotropic hormone test, standard dose adrenocorticotropic hormone test, thalassemia

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Introduction

Thalassemia major (TM) patients with repeated blood transfusion, especially those with inadequate iron chelation, have high prevalence of endocrinopathies, attributed to iron overload of the different endocrine glands.[1-4] Thalassemia patients have universal deposition of iron

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both in the adrenal gland as well as in the pituitary gland, with potential risk of developing central and/or peripheral adrenal insufficiency (AI).

Several studies reported a significant prevalence of subclinical "biochemical" AI, ranging from 18-45% in patients with thalassemia. This may have been attributed to the severity of thalassemia, iron overload status, the test used for assessing adrenal function, and the cutoff value used to define subnormal cortisol response.[5-8] In addition, other adrenal conditions such as adrenal incidentaloma, adrenal extramedullary hematopoiesis, and adrenal tumors have been reported in thalassemia.[9-12]

Although the diagnosis of overt adrenal failure is generally straightforward, identification of those asymptomatic patients with subclinical disturbance of the hypothalamic pituitary adrenal (HPA) axis is still a diagnostic challenge. The HPA axis response to insulin hypoglycemia (ITT) is still considered the gold standard in the evaluation of suspected AI.[13-16] However, ITT is contraindicated in patients with cardiovascular disease or patients with multiple pituitary hormone deficiencies like thalassemic patients, who have high prevalence of cardiomyopathy and endocrinopathies. Therefore, alternative tests to evaluate the HPA have been proposed, such short standard ACTH tests. Since the adrenal gland can respond to exogenous ACTH administration when there is sufficient endogenous ACTH reserve, the short synacthen test (SST) has been introduced to test the HPA in patients with secondary AI or after corticosteroid withdrawal.[11] More recently, a low–dose (1 μg) ACTH (LD) test was developed primarily to unmask the central causes of AI, and it has been shown to be more close and sensitive than the standard ACTH test in detecting more subtle insufficiency of the HPA axis. Therefore, the 1 μg ACTH test may be more appropriate for screening for secondary cortisol insufficiency in thalassemic patients, but ITT is still required by others as a definite test for diagnosing AI.[5,17-20] In many studies, good correlation was found between the responses to ACTH and ITT.[20-25]

Recently, it has been shown that patients with impaired HPA function have a more severe loss in dehydroepiandrosterone sulfate (DHEA-S) secretion than that of glucocorticoids. Measurements of serum DHEA-S levels during LD simulation provide additional valuable information that improves the diagnostic accuracy of LD ACTH test in patients suspected to have central AI.[26] Patients with thalassemia may have a dissociated secretion of cortisol and adrenal androgens.[6]

The aim of this study was to compare cortisol and DHEA-S responses to LD and SD ACTH tests in children and adolescents with TM in relation to their age serum ferritin versus age-matched normal controls.

Materials and Methods

This cross-sectional study was conducted at the Departments of Pediatrics and Hematology, Hamad Medical Center (HMC). The study was approved by the ethical committee of HMC and informed consents were obtained from the parents and, when appropriate, from the patients. Ten thalassemic children and 13 adolescents were randomly recruited. They had been receiving blood transfusion at a regular basis every 4 week. Iron chelation therapy was suboptimal in most of them. Patients were excluded if they had impaired glucose tolerance or diabetes mellitus, had been taking glucocorticoids, sex steroids, or drugs known to affect adrenal function, and/or had been diagnosed as having AI, liver disease, or thyroid disorder before enrollment. Thirteen normal healthy children and adolescents with normal variant short stature and normal thyroid function and normal growth hormone response to provocation by clonidine served as controls.

Data collection

Demographic data included age, gender, height (Ht) and HtSDS, and weight and body mass index (BMI) were calculated by using the WHO Standard Growth Data.[27] In the morning before blood transfusion, blood samples for Hb, hematocrit, hepatic transaminases, ferritin, and albumin were obtained before the testing. Ferritin levels were calculated from the average data for the past 3 years. Ferritin level <1000 ng/ml was considered good iron chelation and >2000 ng/ml was considered bad control.

Patients and controls underwent intravascular (i.v.) 1 μg cosyntropin (LD) and 250 μg (SD) cosyntropin (ACTH) tests. Blood samples for total cortisol were collected before and at 30 after i.v. injection of 1 μg cosyntropin (Cortrosyn; Organon, Oss, Holland). One microgram cosyntropin was prepared using one vial of 0.25 mg cosyntropin diluted in sterile normal saline solution to a concentration of 5 μg/ml. The dilution was kept in use under 4°C without light exposure for not longer than 2 months, under which, its stability was previously demonstrated. One microgram cosyntropin was injected directly through the short (approximately 1.5-inch long) i.v. catheter, followed by 10 ml of normal saline solution flushing to minimize adherence of the medication to plastic tubing.[28,29] Then, a standard dose (250 μg) i.v. ACTH was performed and plasma cortisol concentration determined after another 30 min. Individuals who had peak total cortisol of <420 nmol/L (16 μg/dl) and increments

<200 nmol/L after ACTH were considered adrenal insufficient according to a recent meta-analysis.[30]

Serum total cortisol was analyzed using an Immulite 1000 cortisol chemiluminescence immunoassay (Diagnostic Products Corporation, Los Angeles, CA). The interassay and intraassay coefficient of variation was 5.4% and 5.7%, respectively. DHEA-S concentration in the serum was directly measured using the CoatA Count DHEA-S04 Kit (Diagnostic Products Corporation, Los Angels, USA); the interassay and intraassay coefficient variation (CVs) of RIAs for DHEA-S were 7.8% and 8.4%, respectively.

In our laboratory, normal morning cortisol concentrations were 138-432 nmol/l, while the cutoff for a normal cortisol response was >550 nmol/l to ITT, SD ACTH test, and >420 nmol/l to LD ACTH. These cutoff points were established in 40 normal adult subjects after ITT, 46 after SD ACTH, and 37 after LD ACTH tests. Data are given as the mean ± SD.

Data are presented as mean (SD) and median (range) wherever appropriate. Comparison of different variables in various groups was done using student *t*-test and Wilcoxon rank sum test for normal and non-parametric variables, respectively. Spearman's correlation test was used for correlating nonparametric variables. For all tests, a probability (*P*) less than 0.05 was considered significant.

Results

There were 36 children and adolescents enrolled in the study, 23 of them had TM (non-splenectomised), and 13 healthy age-matched individuals served as controls. TM patients were significantly shorter and had higher serum ferritin and alanine transferase (ALT) concentrations versus controls [Table 1].

Basal cortisol levels did not differ significantly between patients with TM and controls [Table 2]. Using a peak total cortisol cutoff level of 550 nmol/L and increment > 200 nmol/L diagnosed AI in 6 adolescents and young adult patients and 2 children with TM after the LD ACTH (38.7%) and only 2 thalassemic young adults with AI after the SD ACTH (8.7%). Using a peak total cortisol cutoff level of 420 nmol/L and increment >200 nmol/L diagnosed AI in 5 adolescents and young adult patients with TM (21.7%), but none of the children after 1 μg cosyntropin and only 2 thalassemic young adults after the SD ACTH (8.7%) [Table 3]. Peak cortisol response to SD ACTH test was significantly lower in thalassemic adolescents compared to the control group ($P = 0.0455$).

All patients with biochemical AI were asymptomatic and had no history of adrenal crisis. There was no significant difference in any clinical characteristic between the patients with impaired adrenal function and those with normal adrenal function (symptoms e.g., weakness, asthenia, and pigmentation, growth, or electrolyte disturbance).

Thalassemic adolescents had significantly lower basal and ACTH-stimulated DHEA-S levels and higher baseline cortisol to DHEA molar ratio that increased further with LD ACTH stimulation compared to controls [Table 4].

Cortisol responses to LD ACTH were correlated significantly with responses to SD ACTH tests ($r = 0.83$, $P < 0.001$) [Figure 1]. The average serum ferritin

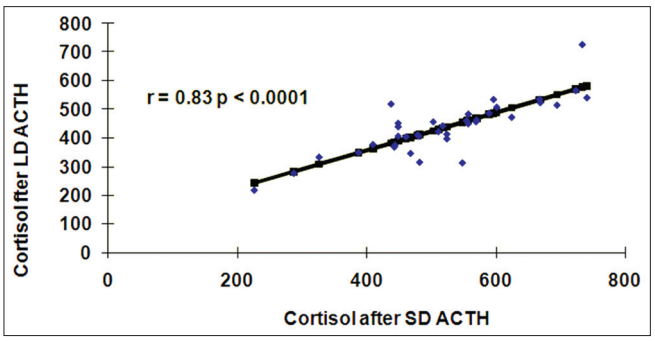

Figure 1: Correlations between cortisol response to LD and SD ACTH tests

Table 1: Anthropometric and lab data of patients and controls

	Thalassemia	Controls
Age at enrollment (y)	16.5+/-6.6	16.2+/-1.6
Age at diagnosis	2.5+/-0.6	
BMI SDS	(-0.5)+/-0.4	0.3+/-0.5
HtSDS	(-1.8)+/-0.43*	(-1.3)+/-0.5
Ferritin (ng/ml)	2200+/-689*	56+/-15
Albumin (g/L)	44.5+/-3.8	46.5+/-4.2
ALT (U/L)	41.4+/-19.5*	18+/-5

*P<0.05 thalassemia versus controls, ALT: Alanine aminotransferase, HtSDS: Height standard deviation scores, BMI: Body mass index, SDS: Standard deviation scores

Table 2: Basal and ACTH stimulated cortisol levels in patients with thalassemia vs controls

	Basal	Cortisol after LD ACTH	Cortisol after SD ACTH
Children with TM (N=10)			
Mean	127.5	441.6	517.7
SD	46.4	39.6	57.1
Adolescents with TM (N=13)			
Mean	241.2	408.1	441*
SD	155.4	70.7	113.1
Controls (N=13)			
Mean	191.3	468.5	562.7
SD	142.2	120.5	154.3

*P<0.05 patients vs controls, LD: Low dose (1 ug ACTH), SD: Standard dose (250 ug ACTH), ACTH: Adrenocorticotropic hormone, TM: Thalassemia major

concentrations were significantly correlated with the cortisol response to LD [Figure 2] and SD ACTH (P = 0.048 and 0.044, respectively). However, neither basal nor stimulated cortisol levels were correlated with the age of the patient [Table 5].

Discussion

A basal serum cortisol concentration >300 nmol/L makes it unlikely that the patient has clinically important HPA insufficiency. In contrast, a value <80 nmol/L makes AI very likely.[31,32]

A major problem with relying on unstimulated serum cortisol values as the basis for the diagnosis of AI is that cortisol secretion is episodic. Furthermore, the normal range is broad and a patient can have pituitary or AI, but maintain basal cortisol secretion within the range of normal. For these reasons, dynamic function tests should be performed when there is doubt about the status of HPA function. An early morning serum cortisol value can be very helpful in excluding AI.

In this study, two thalassemic patients had basal AM cortisol level <80 nmol/L, both had cortisol response <550 nmol/L after both LD and SD ACTH, and one of them had cortisol response < 420 nmol/L after both LD and SD ACTH tests.

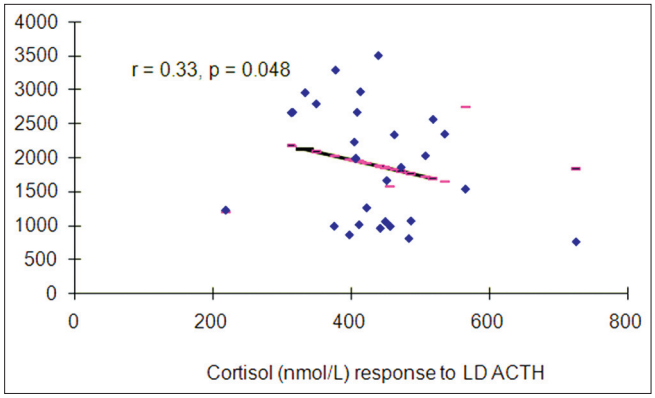

Figure 2: Correlations between ferritin and cortisol response to LD ACTH test

The LD ACTH test (1 μg/1.73 m^2) stimulates maximal adrenocortical secretion up to 30 min post-injection, and, in normal subjects, it results in a peak plasma ACTH concentration about twice that of insulin-induced ones.[33] A subnormal response to the LD or SD ACTH stimulation test is diagnostic of primary or secondary AI, whereas a normal response excludes both disorders.[34] The low-dose ACTH stimulation test is more sensitive in detecting AI, primary, secondary, and tertiary than the high-dose test.[35,36]

It may detect more subtle deficiency in cortisol secretion than the ITT test because plasma ACTH does not increase as much[35] and appears to be superior to standard-dose test for diagnosing chronic HPA insufficiency[30]; however, the clinical importance of this finding is not documented.

Using the apparently more "physiologic" LD ACTH and a normal peak total cortisol cutoff level of 550 nmol/L and increment >200 nmol/L, we diagnosed a prevalence of AI of 8/23 (34.7%) of thalassemic patients (6 adolescents and 2 children), while using the SD test using the same cutoff levels diagnosed 8.7% (2/23) thalassemic adolescents. Therefore, about 75% of patients who failed the LD ACTH had normal peak total cortisol levels after the SD ACTH.

Lowering the normal peak cutoff levels to 420 nmol/L and increments >200 nmol/L decreased the prevalence of AI to 21.7% (5 adolescents) after the LD test and 8.7% (2 adolescents) after the SD dose test. Therefore, about 40% of patients who failed the LD ACTH had normal peak total cortisol levels after the SD ACTH.

The significant diminished adrenal secretion of DHEA-S in adolescents with thalassemia both before and after

Table 3: Prevalence of adrenal sufficiency in thalassemic patients using two different cutoff peak levels

	Number	C-LD <550 nmol/L	C-LD <550 and peak <200 nmol/L	C-SD <550 nmol/L	C-SD <550 and peak <200 nmol/L
Thalassemia >12 years	13	11	6	8	2
Thalassemia 8-12 years	10	9	2	4	0
Thalassemia total	23	20	8	12	2
Controls	13	6	0	4	0
	Number	**C-LD <420 nmol/L**	**C-LD <420 and peak <200 nmol/L**	**C-SD <420 nmol/L**	**C-SD <420 and peak <200 nmol/L**
Thalassemia >12 years	13	6	5	4	2
Thalassemia 8-12 years	10	1	0	1	0
Thalassemia total	23	7	5	5	2
Controls	13	2	0	2	0

P<0.05 patients versus controls, LD: Low dose (1 μg) ACTH, SD: Standard dose (250 μg) ACTH

Table 4: Cortisol-DHEAS ratio before versus after LD and SD ACTH stimulation

	Basal C/DHEAS ratio	C/DHEAS ratio after LD	C/DHEAS ratio after SD
Controls			
Mean	30.4	65.1	76
SD	10.1	26.2	28.8
Thalassemics			
Mean	70.3*	114.6*	122.6*
SD	50.3	72.2	77.3

C: Cortisol, DHEAS: Dehydroepiandrostendione sulfate, LD: After low dose ACTH, SD: After standard dose ACTH *P<0.05 thalassemics versus controls, ACTH: Adrenocorticotropic hormone

Table 5: Correlations between variables in thalassemic patients

	Age	Basal	C-LD	C-SD	Ferritin
Age	1				
Basal-C	0.2195	1			
C-LD	−0.31*	0.51*	1		
C-SD	−0.239	0.52*	0.87*	1	
Ferritin	0.78*	0.0471	−0.32*	−0.33*	1

*P<0.05, C: Cortisol, LD: Low dose ACTH, SD: Standard dose ACTH

ACTH stimulation versus controls gives additional direct evidence of significant pathology in the adrenal gland. Adolescents and young adults with thalassemia had dissociated secretion of cortisol and adrenal androgens. Recently, it has been shown that patients with impaired HPA function have a more severe loss in DHEA secretion than that of glucocorticoids. Measurements of serum DHEA-S levels during LD ACTH simulation provided additional valuable information that improved the diagnostic accuracy of LDC in patients suspected to have central AI.[26] In this study, thalassemic adolescents had a higher baseline cortisol to DHEA molar ratio, which increased further with LD ACTH stimulation compared to controls, adding more evidence to impaired HPA function in these patients.

Despite this high prevalence of AI, none of our patients with AI had symptoms or signs suggestive of adrenal pathology or electrolyte abnormalities. In support, several studies reported a significant prevalence of "biochemical" AI, ranging from 18-45% in patients with thalassemia comparable to our results.[5-8] In addition, there was no significant difference in any clinical characteristic between the patients with impaired adrenal function and those with normal adrenal function in our study and others.[6,7,37,38] Impaired HPA axis function secondary to hemochromatosis of pituitary gland and/or adrenal glands is the presumed etiology of AI in thalassemic patients. However, other mechanisms have been proposed to underlie adrenal dysfunction in patients with thalassemia.[39-42]

In this study, adolescents with TM had higher prevalence of AI compared to children with TM, and the peak cortisol response to SD ACTH stimulation was significantly lower compared to controls. Longer duration of iron overload and its deleterious effect on the pituitary-adrenal axis explains this finding. Although serum ferritin does not always accurately reflect tissue iron overload, the presence of significant negative correlation between the average serum ferritin level (3 years) and the cortisol response to LD and SD cosyntropin tests (P = 0.048 and 0.044, respectively) supported this view. However, neither basal nor stimulated cortisol levels were correlated with the age of the patient. This supports the key importance of proper chelation for preventing adrenal dysfunction. In all our thalassemic patients, albumin level was normal and they had only normal or mild elevation of ALT. In addition there was no significant correlation between ALT and cortisol responses to LD or SD ACTH tests. In addition, a normal CBG level has been reported in patients with thalassemia.[5]

The important question that necessitates a clear answer is "Can thalassemic patients with biochemical AI tolerate physical and/or surgical stress?" Clinical AI or adrenal crisis appear to be rare during the course of thalassemia.[5] Nevertheless, data on patients with thalassemia before and after surgery showed that although they had higher basal ACTH before operation versus non-thalassemic individuals, thalassemic patients had no significant elevation of ACTH upon surgical stress versus normal controls.[43] Another study showed defective rhythmicity of ACTH secretion in beta-thalassemia.[42]

In conclusion, the prevalence of partial AI is considerably high in thalassemic adolescents, and the use of LD ACTH test diagnoses more adrenal abnormalities versus SD ACTH. Therefore, thalassemic patients have to be carefully investigated for AI before major surgery and those with impaired cortisol secretion should receive stress doses of corticosteroids during the stressful event.

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