The pituitary–adrenal axis in adult thalassaemic patients

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Abstract

Objective: We previously described in young thalassaemic patients an altered cortisol and ACTH responsiveness suggesting an impaired adrenocortical reserve. Owing to iron overload, a worsening of adrenal function should be expected in adult patients.

Design: In 124 adults with β-thalassaemia, urinary free cortisol (UFC) and plasma ACTH levels were determined and compared with those measured in 150 controls. In 45 patients, cortisol was measured in response to: i) tetracosactide 1 μg as an i.v. bolus (low-dose test, LDT) and ii) tetracosactide 250 μg infused i.v. over 8 h (high-dose test, HDT).

Results: UFC and serum cortisol were within the reference range in all patients. Conversely, basal plasma ACTH values were above the upper limit of the normal range in 19 patients. There were no statistically significant differences in the mean values of UFC, basal serum cortisol and plasma ACTH between patients and controls. A subnormal cortisol response to the LDT was registered in 18 out of 56 patients. Three of these patients also displayed a subnormal response to the HDT, together with elevated baseline plasma ACTH levels. In the LDT, a positive correlation was found between basal and peak cortisol values \( \rho \leq 0.0001 \). The latter were negatively correlated with basal ACTH values in both LDT \( \rho \leq 0.0001 \) and HDT \( \rho \leq 0.0001 \).

Conclusions: Adult thalassaemic patients often present a subtle impairment of adrenocortical function. This may become clinically relevant in case of major stressful events. Thus, we recommend an assessment of adrenocortical function in all adult thalassaemic patients.

Introduction

Lifelong blood transfusional regimen is the therapeutic mainstay of thalassaemia major (1). Despite the widespread use of effective iron-chelating drugs, secondary haemochromatosis remains one of the most important factors contributing to the number of endocrine complications in this disease. This contention is supported by the iron deposition in endocrine glands and the correlation between serum ferritin levels and diabetes or hypothyroidism in these patients. Among the endocrine dysfunctions, hypogonadism, growth retardation and bone demineralization are the most frequent in thalassaemia. As for the pituitary–adrenal axis, previous studies have reported a variable prevalence of impaired function, ranging from 0 to 45.8% depending on the degree of iron overload, but also on the tests used for diagnosis (2–7). Furthermore, the majority of studies addressing this issue have been performed in children or young adults. The adrenal hypofunction of thalassaemia seems to be caused by primary adrenal failure since most affected patients display high ACTH levels and impaired adrenal response to stimulation tests (8). In a previous study conducted in a small number of patients, we disclosed an enhanced ACTH response and a reduced cortisol rise after both insulin tolerance test (ITT) and corticotropin-releasing hormone (CRH) stimulations (9). These findings envisage a condition of suboptimal adrenal reserve even in the absence of clinical signs. Considering the greatly increased life expectancy of thalassaemic patients and the potential progression with age of the damages induced by iron overload, we elected to investigate the adrenal reserve in a large number of thalassaemic adults.

Methods

Patient population

One hundred and twenty-four adult thalassaemic patients (54 men and 70 women) aged 30.6 ± 0.59 years (range 18–50, median 30) and 150 control subjects (58 men and 92 women) aged 37.5 ± 1.40...
years (range 18–54, median 36) were included in the study. Ninety-eight of the patients were affected by β-thalassaemia major and 26 by β-thalassaemia intermedia on stable transfusional regimen. At the time of the study, their haemoglobin levels ranged from 7.5 to 12 g/dl.

At the moment of the study, 19 patients were receiving levodopa for primary hypothyroidism; 49 women and 40 men displayed secondary hypogonadism and were receiving oestradiol–progestogen or testosterone as appropriate; 18 patients, diagnosed with GH deficiency, had not yet been started on GH replacement. Thirty-five patients were affected by hepatitis C virus-related chronic liver disease: at the time of the study, none of them was on interferon treatment.

The large majority of control subjects presented ACTH and cortisol values fully comprised within the normal range and were therefore not submitted to further testing, whereas 22 subjects with either low-normal or high-normal hormonal measurements were tested with tetracosactide and displayed normal cortisol responses.

All patients and control subjects gave their informed consent to participate in the study, which has been approved by the ethics committee of our institution.

**Test procedures**

In all patients, an indwelling catheter was placed into a forearm vein between 0700 and 0800 h and continuously flushed with saline. Three blood samples were drawn at least 15 min apart for the measurement of basal ACTH. Forty-five of the patients, on separate occasions, underwent the following two tests: i) tetracosactide (Synacthen, Novartis) 1 µg as an i.v. bolus (low-dose test, LDT), and ii) tetracosactide 250 µg infused i.v. over 8 h (high-dose test, HDT). In another 11 patients only the LDT was performed, while an additional three subjects underwent only the HDT.

During the short test, blood samples for cortisol evaluation were collected 30 and 15 min prior to and 15, 30, 45, 60 and 90 min after the tetracosactide i.v. bolus, while during the prolonged test, blood samples for cortisol measurement were collected 30 and 15 min prior to and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min along the infusion. According to the literature (10–14), cortisol responses > 500 nmol/l following the two stimulation tests were considered normal. Furthermore, every patient performed two 24-h urine collections for free cortisol estimation.

Baseline blood samples for ACTH and cortisol measurement and 24-h urine collections for free cortisol estimation were also obtained in all control subjects.

Blood samples were centrifuged at 1000 g for 10 min at 4 °C, and plasma or serum was stored at −20 °C until assayed.

Ferritin levels were determined in all patients using basal blood samples.

**Biochemical assays**

Plasma ACTH was measured by IRMA (Allegro, Nichols Institute, San Juan Capistrano, CA, USA). Intra- and inter-assay coefficients of variation (CV) were 3.2 and 8.2% respectively. The sensitivity of the assay is 1 pmol/l.

Serum cortisol was measured by immunometric assay (Bryk-Sangtec Diagnostica, Dietzenbach, Germany). Intra- and inter-assay CV were 3.0 and 4.7%, respectively. The sensitivity of the assay is 13.5 nmol/l.

Urinary free cortisol (UFC) was measured by RIA (Diagnostic Products Corporation, Los Angeles, CA, USA) after urine extraction with dichloromethane. Intra- and inter-assay CV were 3.5 and 6.2% respectively. The sensitivity of the assay is 13.5 nmol/l.

Reference ranges in our laboratory are 2.2–11 pmol/l for ACTH, 138–690 nmol/l for serum cortisol and 27.6–220.7 nmol/l/24 h for UFC.

Serum ferritin was measured by electrochemiluminescence immunoassay (Roche Diagnostics). Intra- and inter-assay CV were 1.8 and 2.9% respectively. The sensitivity of the assay is 0.5 µg/l. Reference range is 30–400 µg/l in males and 13–150 µg/l in females.

**Statistical analysis**

In all comparisons and correlations, plasma ACTH was expressed as the mean of the values obtained from the three blood samples, serum cortisol was expressed as the mean of the two values obtained prior to tetracosactide injection, and UFC was expressed as the mean of the values obtained from the two 24-h urine collections. Results are presented as mean ± S.E.M. Student’s t-test for unpaired data was used for comparisons between groups. Linear regression analysis was used to evaluate associations between variables. Statistical analyses were performed with a commercially available software package (StatView, Abacus Concepts, Berkeley, CA, USA). A P value lower than 0.05 was considered statistically significant.

**Results**

Values of UFC and serum cortisol were within the reference range in all thalassaemic patients. Conversely, basal plasma ACTH values were above the upper limit of the normal range in 19 patients (15.3%). However, there were no statistically significant differences in the mean values of these parameters between thalassaemic patients and controls (115.09 ± 4.14 vs 107.36 ± 3.78 nmol/24 h, not significant (NS), for UFC; 353.7 ± 24.3 vs 351.0 ± 8.37 nmol/l, NS, for basal serum cortisol; 7.98 ± 0.61 vs 7.96 ± 0.77 pmol/l, NS, for plasma ACTH) (Fig. 1).

Mean serum ferritin of the patients was 1441.2 ± 140.89 µg/l.
The cortisol response to the LDT was subnormal in 18 out of 56 patients (32.1%). In this subgroup of patients, baseline ACTH ranged from 2.35 to 18.4 pmol/l, being normal in half of them. Their cortisol peak values after stimulation ranged from 238.2 to 479.21 nmol/l. Three of these 18 patients also displayed an insufficient cortisol response to the HDT; their baseline ACTH values were increased: 17.20, 26.64 and 61.75 pmol/l respectively. These three patients, all males affected by β-thalassaemia major, were started on glucocorticoid replacement therapy. Twenty-seven of the 38 patients showing normal cortisol responses to the LDT also underwent the HDT, displaying a normal cortisol rise. The hormonal response was also normal in the three patients in whom only the HDT was performed.

Among the other 16 patients with elevated ACTH, three displayed normal responses to both tests, another four showed subnormal responses to the LDT but normal responses to the HDT, while an additional two patients presented normal responses to the LDT and did not undergo the HDT. Dynamic evaluation could not be performed in the remaining seven patients, who were not available for further testing.

Thirteen thalassaemic women were on oestrogen replacement therapy at the moment of dynamic testing. Two of them, characterized by normal baseline ACTH levels, displayed a subnormal cortisol response to the LDT but fully normal responses to the HDT (cortisol peaks 1182.8 and 831.0 nmol/l respectively). Another one, displaying high basal ACTH (12.2 pmol/l), presented normal cortisol responses to LDT and HDT (peaks 626.0 and 925.2 nmol/l respectively). The remaining ten women showed normal basal values of serum cortisol and plasma ACTH, as well as fully normal cortisol responses to tetracosactide (peaks ranging from 753.4 to 1631.5 nmol/l).

In the whole group of patients, a strong positive correlation was found between baseline cortisol values and cortisol peak values during the LDT ($r = 0.776, P < 0.0001$), but not during the HDT ($r = 0.199, P = 0.185$; Fig. 2). Cortisol peaks after both tests were negatively correlated with baseline ACTH values ($r = -0.287, P < 0.0001$, and $r = -0.479, P < 0.0001$, after the LDT and the HDT respectively).

These correlations were still evident ($r = -0.300, P < 0.001$, and $r = -0.362, P < 0.0001$, after the LDT and the HDT respectively) when excluding as outlier the patient with markedly elevated (61.75 pmol/l) plasma ACTH levels (Fig. 3). Conversely, stimulated cortisol release was not correlated with either UFC or age of patients.

Lastly, no correlations were found between ferritin values and any of the following parameters: UFC, baseline serum cortisol and plasma ACTH; cortisol peaks after both stimulation tests.

**Discussion**

This study has demonstrated in a large series of adult thalassaemic patients, a not negligible proportion of impaired adrenal reserve. These subjects are characterized by urinary and serum cortisol still within the normal reference range, but elevated plasma ACTH and
insufficient cortisol responses to both low- and high-dose tetracosactide stimulations. To the best of our knowledge, this is the first study investigating adrenal glucocorticoid function in such a large population of thalassaemic adults.

Previous studies addressing this issue have been mainly performed in small groups of children or adolescents with β-thalassaemia. In these series, the observation of high baseline ACTH plasma levels was rather common, even when the response of the hypothalamic–pituitary–adrenal axis to dynamic testing was normal. In this context, normal ACTH and cortisol rises after splenectomy-induced stress and ITT were described in thalassaemic children displaying significantly elevated basal ACTH (15, 16). In our large series of adult patients, ACTH was abnormally elevated in 15.3% of the cases.

Along the same line, we previously demonstrated, in a group of young patients, a dissociated ACTH/cortisol responsiveness to both ITT and CRH, with enhanced ACTH and blunted cortisol rise respectively (9). These observations led us to hypothesize the existence of a subclinical impairment of adrenocortical function in patients with thalassaemia, of little or no clinical impact under basal conditions but of potential relevance during stressful events. This adrenal dysfunction appears to be mostly of primary origin, as indicated by our current observation of a negative correlation between basal ACTH values and cortisol peaks after both tetracosactide stimulation tests: indeed, in the whole series of patients, the subjects presenting the highest baseline ACTH values were those displaying the lowest cortisol responses to exogenous ACTH. However, a small number of patients in our series presented normal baseline ACTH values together with impaired cortisol responses to the LDT, a pattern which does not exclude pituitary ACTH deficiency; this condition could have been confirmed by a CRH test or an ITT, which unfortunately was not performed.

As concerns the diagnostic procedures employed in the present study, we elected to use the low-dose tetracosactide test and the HDT in order to explore the adrenal reserve after both acute and prolonged stimulation. Even though ITT has been considered the gold standard to disclose an impaired adrenal function, the ACTH stimulation test has been used for many years as an acceptable alternative with the advantage of lacking relevant adverse effects (10–14, 17–19). Different doses of synthetic ACTH, from 250 to 0.03 μg, have been used to optimize adrenal stimulation with the latter considered the minimal dose able to raise plasma cortisol values. Given the supraphysiological stimulation induced with the acute HDT, which allows ACTH levels at least double those measured during major physical stress (11, 20), this challenge gives rise to a high rate of false negative responses, thus appearing less suitable to detect mild adrenal dysfunction (21–23). Therefore, we performed in our patients a low-dose tetracosactide test, which is considered at the moment more reliable than the standard high-dose challenge in detecting adrenal insufficiency, by inducing an ACTH increase in the same magnitude as that obtained by ITT (24, 25) and major physical stress (26). In addition, to evaluate cortisol secretion in response to severe stressful conditions, we also carried out a CRH test, which confirmed our findings.

Figure 3 Correlations between baseline ACTH values and cortisol peaks following the two tetracosactide challenges (LDT, low-dose test; HDT, high-dose test). Open circles indicate patients with subnormal responses to tests. One patient is not indicated due to his exclusion as an outlier (ACTH 61.75 pmol/l).
out an 8-h infusion of 250 μg tetracosactide, which is considered a reliable tool to estimate the adrenal capacity to sustain a response after the exhaustion of cortisol stores (27). According to the literature, cortisol responses > 500 nmol/l were considered normal (10–14).

A subnormal cortisol rise after the 1 μg ACTH test was observed in one-third of our patients, with three of them (6.6%) displaying an impaired response to both tests. This pattern is similar to the so-called stage III subclinical hypoadrenalism described in non-thalassaemic patients with positive adrenal antibodies, characterized by normal serum cortisol, elevated plasma ACTH and impaired cortisol response to tetracosactide, considered an irreversible phase (28). Our three patients, diagnosed as having primary subclinical hypoadrenalism at high risk of acute insufficiency with stressful events, were cautiously started on glucocorticoid replacement therapy. Since we could not perform the stimulation tests in all patients with elevated baseline ACTH, the proportion of subjects with adrenocortical insufficiency might be even higher in our series.

The 1 μg tetracosactide test has been recently performed to assess adrenal function in a group of thalassaemic children and adolescents (6); combining subnormal cortisol responses with baseline cortisol levels below 400 nmol/l, the authors reported a prevalence of adrenal insufficiency of 45%. This figure appears to be overestimated, since the cut-off value of 400 nmol/l for basal morning cortisol is questionable. In all of our adult patients, including those subsequently considered amenable to glucocorticoid replacement, baseline serum and urinary cortisol were normal. This may reflect a reduced capacity of adrenal cortex to respond to acute increments of ACTH secretion with preserved baseline cortisol release (diminished adrenocortical reserve), although an impaired cortisol clearance secondary to liver dysfunction cannot be ruled out in our patients. Iron overload due to secondary haemosiderosis is thought to contribute to the pathogenesis of the endocrine complications of thalassaemia. In this context, a subnormal cortisol response to tetracosactide has recently been documented in a high percentage of thalassaemic adolescents submitted to excessive transfusion regimen with inadequate iron-chelating therapy (7). In our adult patients, the lack of correlation between serum ferritin and both baseline and stimulated parameters of adrenal function militates against a relevant role of iron overload in the pathogenesis of this endocrine complication. Moreover, imaging techniques seem to indicate that iron overload in thalassaemia chiefly involves the zona glomerulosa of the adrenal cortex (29). However, a single serum ferritin value may not reflect the real changes in the degree of iron deposition in the different phases of this chronic disease. Indeed, the amount of adrenal iron deposition is well correlated with the degree of liver iron accumulation but not with circulating ferritin (29).

Oestrogens are known to induce corticosteroid-binding globulin (CBG) (30), thus possibly causing falsely elevated serum cortisol levels. As a consequence, falsely normal cortisol responses to tetracosactide might have been observed in female patients with impaired adrenocortical function on oestrogen therapy. However, analysis of the data observed in our 13 thalassaemic women undergoing stimulation tests while on estrogen treatment does not support this contention in this subgroup of patients, with the exception of two women with normal baseline ACTH and subnormal cortisol response to the LDT, in whom secondary adrenal insufficiency cannot be ruled out. On the other hand, a liver insufficiency-dependent decrease in CBG production might have generated false positive results. However, the fact that our three patients with subnormal cortisol responses to both tests displayed only slightly elevated transaminase values with normal pseudocholinesterase levels and prothrombin time militates against this possibility.

No correlation was found in our study population, between cortisol responsiveness and the age of patients, which is an equivalent of disease duration. A comparison with the literature is not possible because of the young age of the patients investigated in others’ studies.

Based on the findings of this study, given the increased life expectancy of thalassaemic subjects and their possible exposure to stressful events, an evaluation of adrenal function appears to be recommended in these patients. From a clinical point of view, it is worth taking into account that manifestations of mild adrenal hypofunction might be masked by symptoms commonly reported by thalassaemic patients, such as asthenia, muscle weakness and arthralgias. Furthermore, due to the increased prevalence of surgery-related infectious complications reported in thalassaemia (31), preoperative administration of glucocorticoids should be considered before stressful events in patients with biochemical features suggestive for subclinical hypoadrenalism.

Finally, the present data should also be considered in the light of the high prevalence of GH deficiency previously reported in thalassaemic adults (32) and also documented in our present series. Indeed, by modulating the activity of 11β-hydroxysteroid dehydrogenase, GH may decrease serum and urinary cortisol concentrations and, when administered to non-thalassaemic hypopituitary patients, worsen a latent adrenocortical insufficiency (33). Thus, on the one hand, considering the number of GH-deficient thalassaemic patients comprised in this study, the prevalence of impaired adrenal function in thalassaemic adults might be even higher than that herein reported and, on the other hand, a careful evaluation of adrenal function appears mandatory in those thalassaemic patients who, diagnosed with severe GH deficiency, are to be started on GH treatment.
Declaration of interest
The authors of this paper declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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