Plasma Adrenocorticotropic (ACTH) Values and Cortisol Response to 250 and 1 μg ACTH Stimulation in Patients with Hyperthyroidism before and after Carbimazole Therapy: Case-Control Comparative Study

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Context: Although the production and metabolic clearance rate of cortisol is increased during thyrotoxic state, the net effect on adrenocortical reserves is not clear.

Objective: We assessed circulating ACTH levels, cortisol binding globulin (CBG), and adrenocortical reserves in hyperthyroid patients (before and after carbimazole therapy) and healthy controls.

Design and Setting: This was a case-control investigative study in a tertiary care setting.

Patients and Methods: Plasma ACTH and free cortisol index (FCI; serum cortisol/CBG) were measured in 49 consecutive patients with hyperthyroidism and 50 controls. ACTH<sub>1-24</sub> stimulation tests (250 and 1 µg) were carried out in the first 20 patients and 15 controls. Peak FCI less than the mean –3 SD of healthy controls was considered subnormal. ACTH<sub>1-24</sub> stimulation tests were repeated in 24 patients in the euthyroid state.

Results: The mean basal plasma ACTH and FCI were higher and CBG was lower in thyrotoxic patients in comparison with controls. The peak cortisol was less than 18 µg/dl in 10 of 29 and 14 of 29 on 250 and 1 µg ACTH<sub>1-24</sub> stimulation. Peak FCI was subnormal only in three of 27 (11.1%) and two of 21 (7.4%) on 250 and 1 µg ACTH<sub>1-24</sub> stimulation, respectively. The mean plasma ACTH, basal FCI, and subnormal peak FCI (two of the three) normalized after euthyroidism. Plasma ACTH and FCI did not correlate with severity of thyrotoxicosis.

Conclusions: Up to 11% of thyrotoxics have subnormal peak FCI on ACTH<sub>1-24</sub> stimulation. Such changes occur despite high basal plasma ACTH and FCI. Use of FCI, rather than total cortisol, is required for the interpretation of cortisol values in thyrotoxicosis due to the variation in CBG. (J Clin Endocrinol Metab 92: 1693–1696, 2007)

Diarrhea and weight loss are prominent clinical features of thyrotoxicosis (1, 2). Similar symptoms are observed in patients with adrenocortical insufficiency (3). Although the metabolic clearance and production rate of the cortisol is enhanced during thyrotoxicosis (4), its net effect on adrenocortical reserves is not clear. In thyrotoxicosis, serum cortisol binding globulin (CBG) levels are reduced and return to normal after induction of the euthyroid state (9, 10). Reduction in cortisol reserves in thyrotoxic patients and its normalization after euthyroidism (5–8) could be due to variation related to CBG. In stress, like thyrotoxicosis, circulating ACTH values are expected to increase and more so in presence of adrenal insufficiency. We assessed plasma ACTH and adrenocortical response to 250 and 1 µg of ACTH<sub>1-24</sub> stimulation in patients with thyrotoxicosis before and after attainment of euthyroidism, taking into consideration changes in CBG levels, in the interpretation of cortisol response.

Subjects and Methods

Study included 49 consecutive patients with hyperthyroidism [39 females; mean ± sp age, 38.0 ± 12.3 yr; body mass index (BMI), 20.1 ± 4.0 kg/m<sup>2</sup>; median duration of symptoms, 12 months] attending endocrine clinic of the All India Institute of Medical Sciences and 50 healthy controls. Patients with thyrotoxic crisis and history of recent glucocorticoid intake were excluded. Hyperthyroidism was diagnosed based on the Volpe’s criteria with Wayne’s score greater than 19, serum total T<sub>4</sub> and TSH in the hyperthyroid range, and high <sup>131</sup>I uptake (11). Forty-two patients had diffuse thyromegaly, five had multinodular goiter, and two had solitary toxic nodule on 99mTc pertechnetate thyroid scan. The healthy controls were volunteers (mean age, 35.4 ± 7.6 yr; BMI, 22.8 ± 3.2 kg/m<sup>2</sup>) who had normal serum total T<sub>4</sub> and TSH. Ten milliliters of venous blood were drawn from all study subjects at 0800 h for measurement of baseline plasma ACTH, serum total cortisol, T<sub>4</sub>, TSH, thyroid peroxidase antibodies (TPOAb), and CBG. Intravenous ACTH<sub>1-24</sub> (250 and 1 µg; Ciba-Geigy, Basel, Switzerland) stimulation tests were performed in the initial 29 consecutive patients and 15 healthy controls.

One microgram ACTH<sub>1-24</sub> per milliliter saline was prepared afresh before each test by mixing 250 µg ACTH<sub>1-24</sub> in 250 ml of 0.9% saline in a plastic container (12). One and 250 µg ACTH<sub>1-24</sub> stimulation tests were performed between 0800 and 0900 h on d 1 and 2, respectively. Blood was drawn for cortisol estimation at −15, 0, +30, +60, and +90 min after ACTH bolus. The free cortisol index (FCI) was calculated by serum cortisol/CBG (nanomoles per milligram).

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Abbreviations: AUC, Area under curve; BMI, body mass index; CBG, cortisol binding globulin; FCI, free cortisol index; TPOAb, thyroid peroxidase antibodies. JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
Definitions of subnormal cortisol response

The mean peak FCI during 250 and 1 μg ACTH<sub>1-24</sub> stimulation tests in healthy controls was 12.4 ± 1.4 and 9.9 ± 1.04 nmol/mg, respectively. Peak FCI in 250 and 1 μg ACTH<sub>1-24</sub> stimulation tests were considered subnormal in thyrotoxicosis if the value was less than the mean -3 SD of healthy controls (~8.2 and 6.8 nmol/mg, respectively).

Treatment

Patients were treated with carbimazole (10–15 mg, three times a day). Serum total T<sub>4</sub> was measured at an interval of every 2 months. The mean duration to achieve euthyroidism was 4 ± 1 months. Twenty-four patients were reassessed after clinical (BMI, 22.5 ± 3.8 kg/m<sup>2</sup>) and biochemical euthyroidism (mean interval 7 ± 1 months), and repeat tests were performed including fasting serum CBG, plasma ACTH, and ACTH<sub>1-24</sub> stimulation. The Institutional Ethics Committee of All India Institute of Medical Sciences approved the study protocol, and written informed consent was obtained from all subjects.

Hormone assays

Serum and plasma samples were stored at −20 C and assays were performed together in multiple batches by electrochemiluminescence (Elecsys 2010; Roche, Mannheim, Germany). CBG and plasma ACTH were measured by RIA (Biosource Technologies, Inc., Nivelles, Belgium) and immunoradiometric kit (DiaSorin, Stillwater, MN). The normal range for plasma ACTH, 0800 h serum cortisol, total T<sub>4</sub>, TSH, TPOAb, and other hormones were measured by RIA (Elecsys 2010; Roche, Mannheim, Germany). CBG and plasma ACTH were performed together in multiple batches by electrochemiluminescence

Statistical analysis

SPSS software (version 11.5; SPSS, Inc., Chicago, IL) was used for statistical analyses. The data are given as mean ± SD. ANOVA followed by Bonferroni post hoc test was used to compare mean of various variables in different groups. Paired t test was used to compare differences in the mean of variables before and after carbimazole therapy. Fisher’s exact or χ<sup>2</sup> tests with Yates’s correction were used to assess the differences in the frequency of nonparametric variables. Pearson’s correlation coefficients were used for correlation analysis. The area under curve (AUC) for FCI was calculated by the trapezoidal rule. P < 0.05 was considered significant.

Results

Table 1 shows the clinical characteristics, mean plasma ACTH, serum CBG, total cortisol, and FCI at baseline and after ACTH stimulation in thyrotoxic (before and after treatment) and the controls group.

Serum CBG, plasma ACTH, and FCI in thyrotoxic state

The mean serum CBG, basal plasma ACTH value (median, 32.0 pg/ml; range 11.0–400.0 pg/ml), and frequency of supranormal plasma ACTH values were higher in thyrotoxic patients than healthy controls (Table 1). Although the mean basal cortisol was comparable between thyrotoxic patients and the controls, the mean basal FCI was significantly higher in thyrotoxic patients. All nine thyrotoxic patients and two healthy controls with supranormal plasma ACTH had nor-

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<th>TABLE 1. Comparison of baseline characteristics, CBG, and FCI in patients with thyrotoxicosis before and after carbimazole therapy and healthy controls (ANOVA)</th>
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<td>Parameters</td>
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<td>Mean TSH (μIU/ml)&lt;sup&gt;†&lt;/sup&gt;</td>
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<tr>
<td>Peak cortisol &lt; 18.0 μg/dl (%)&lt;sup&gt;**&lt;/sup&gt;</td>
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Data in the same row with different superscript letters are significantly different from each other. Basal cortisol and basal FCI are the mean of 0800 h serum cortisol values on the days of 1 and 250 μg ACTH stimulation. SDS, SD score. FCI = cortisol/CBG. SI unit conversion factors: cortisol, 1 μg/dl = 27.59 nmol/liter; ACTH, 1 pg/ml = 0.2202 pmol/liter.

<sup>a</sup> P values were calculated after log transformation.

<sup>* and **, n is different from the whole group.
Adrenocortical Reserves in Hyperthyroidism

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Changes in serum CBG, plasma ACTH, and FCI after carbimazole therapy

The mean plasma ACTH and basal FCI decreased and mean serum CBG significantly increased after attainment of euthyroidism. The mean CBG, plasma ACTH, and basal FCI in thyrotoxic patients after euthyroidism were not significantly different from that in healthy controls (Table 1). The mean FCI response including its peak and AUC after 250 and 1 µg ACTH1–24 stimulation were comparable before and after treatment. Two of the three thyrotoxic subjects with subnormal peak FCI on 250 µg ACTH1–24 test showed normal plasma ACTH and peak FCI response after euthyroidism. The third patient with subnormal peak FCI and elevated plasma ACTH during the euthyroid state at 6 months showed normal plasma ACTH at 10 months of follow-up.

Severity of hyperthyroidism and adrenocortical reserves

All 49 patients were stratified in two groups at a median serum total T4 level of 22.2 µg/dl. The median plasma ACTH [28.0 pg/ml, range (11–110 pg/ml) vs. 34.0 pg/ml (range 11–400 pg/ml), P = 0.18], serum CBG (47.3 ± 17.1 vs. 44.1 ± 12.7 mg/liter, P = 0.59), and basal FCI (7.4 ± 3.2 vs. 8.7 ± 4.0 nmol/mg, P = 0.35) were comparable in the two groups. Similarly mean FCI at +30, +60, and +90 min, peak and AUC, and the frequency of subnormal peak FCI after 250 and 1 µg ACTH1–24 stimulation were comparable in the two groups. Pearson’s test showed no relationship among total T4, plasma ACTH, and basal and peak FCI in the thyrotoxic state.

Discussion

Holst in 1935 first reported increase in the adrenal size on autopsies of thyrotoxic patients (13). Adrenal glands were moderately enlarged on computerized tomography in Graves’ disease (7). Although increased circulating ACTH in thyrotoxicosis is linked to skin hyperpigmentation, there are limited data on plasma ACTH levels in them. Hilton et al. (14) reported increased ACTH-like activity in thyrotoxicosis. Subsequently Yamakita et al. (8) and Gao et al. (15) reported mild elevation of plasma ACTH in Graves’ disease that normalized after antithyroid drugs. In the current study, the mean plasma ACTH and FCI values were higher in patients with thyrotoxicosis than that in healthy controls and normalized after attainment of euthyroidism. Presence of adrenal enlargement and supranormal plasma ACTH coupled with significantly higher basal FCI suggest that pituitary-adrenal axis is stimulated in thyrotoxic state.

On the other hand, there are reports of subnormal peak cortisol on ACTH1–24 stimulation tests in thyrotoxic subjects (5–8). Peak cortisol less than 18 µg/dl during ACTH1–24 stimulation is considered subnormal (16). In the current study, the peak cortisol was also less than 18.0 µg/dl in 34.5 and 48.3% of thyrotoxic patients during 250 and 1 µg ACTH1–24 stimulation tests, respectively.

Several investigators suggested adjustment for variation in serum CBG in interpretation of cortisol values (17–19). Measurement of free cortisol would exclude effects related to alteration in serum CBG. Ratio of total serum cortisol and...
CBG, i.e. FCI, has also been shown to correlate with free cortisol levels (18). Recently le Roux et al. (19) compared peak FCI and peak total cortisol values in 31 subjects during the perioperative period of major surgeries. Peak FCI was normal in all seven subjects in whom peak cortisol was less than 18.0 μg/dl (19). There is no study in which adrenocortical reserves have been assessed using FCI in ACTH1–24 stimulation studies in thyrotoxic patients. The observation of the current study showing significant reduction in CBG in thyrotoxic patients and its normalization after euthyroidism is in accordance with the previous studies (9, 10). Interestingly, majority of the patients who had peak cortisol values less than 18 μg/dl on 250 and 1 μg ACTH1–24 stimulation showed normal peak FCI response. Using peak FCI less than 3 sd score of the healthy controls as the definition of subnormal peak FCI during ACTH1–24 stimulation test, only three of 27 thyrotoxic patients (11%) had subnormal response on 250 μg ACTH1–24 stimulation.

Overall, the observations of high circulating ACTH and basal FCI in thyrotoxicosis but impaired peak FCI response in 11% support adrenal hyperactivity with the possibility of adrenal exhaustion in a subset of them. Abnormalities observed in the pituitary-adrenal axis were reversible after attainment of euthyroidism. Coexistent autoimmune adrenalitis is reported in up to 5% of patients with Graves’ disease (20). Reversible nature of abnormalities in peak FCI suggests autoimmune adrenalitis as the unlikely cause of subnormal reserves.

Thus, up to 11% of thyrotoxic patients have subnormal peak FCI on ACTH1–24 stimulation. Such changes occur despite high basal plasma ACTH and FCI. Use of FCI, rather than total cortisol, is required for the interpretation of cortisol values in thyrotoxicosis due to the variation in CBG. Further studies involving direct measurement of free cortisol would be helpful in assessing adrenocortical reserves in patients with thyrotoxicosis.

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