Plasma cortisol response to 1-24 adrenocorticotropin in patients with treated/untreated sellar & suprasellar mass lesions

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Background & objectives: One microgram short synacthene test is widely recommended as a screening test for evaluation of hypothalamo-pituitary-adrenocortical axis in patients with secondary adrenal insufficiency. Information on adequacy of cortisol response to this dose at different periods of the day in patients with hypothalamic-pituitary disorders is not available. Hence, this study was designed to assess the adequacy of cortisol response to 1 µg 1-24 adrenocorticotropicin (ACTH) at 0800 h and 1600 h in patients with sellar and suprasellar mass lesions.

Methods: Thirty five consecutive patients with sellar and suprasellar mass lesions with mean age of 43.0±14.4 yr and 36 healthy controls with mean age of 32.3±9.0 yr were studied after obtaining informed consent. Maintenance doses of glucocorticoids in these patients were discontinued appropriately. On day 1, prestimulated and stimulated plasma cortisol samples at 0800 h and at 30 and 60 min following IV bolus of 1 µg 1-24 ACTH were collected. While on day 3, plasma cortisol samples were similarly collected at 1600 h. Cortisol estimation was done by a sensitive and specific radioimmunoassay. Stimulated plasma cortisol of 500 nmol/l or higher was defined as a normal response.

Results: In healthy controls, the prestimulated and peak cortisol levels at 0800 h (377.5±93.3 and 729.1±183.2 nmol/l) were higher (P<0.001 and P<0.01) than those at 1600 h (230.1±75.7 and 665.8±138.6 nmol/l). All subjects had a cortisol response of 500 nmol/l or higher in response to 1 µg 1-24 ACTH both at 0800 and 1600 h. In the patients' group, the prestimulated plasma cortisol at 0800 h (250.3±169.7 nmol/l) was higher (P<0.001) than that at 1600 h (166.3±128.9 nmol/l), while the peak cortisol response was comparable (P>0.05) in the morning as well as in the evening (490.9±309.4 vs 464.8±318.4). In 27 patients (77%) the morning and evening stimulated cortisol response to 1 µg 1-24 ACTH was consistent (normal in 13 and subnormal in 14) but was discrepant in the remaining 8 (23%). In 7 of these 8 patients, cortisol response was normal at 0800 h but not at 1600 h, while in only one, normal response was seen at 1600 h but not at 0800 h.

Interpretation & conclusion: The demonstration of normal peak cortisol response to 1 µg 1-24 ACTH at 0800 h but not at 1600 h in substantial number of patients with sellar and suprasellar mass lesions suggests preference to morning for performing this test.

Key words Adrenocorticotropic - cortisol - sellar and suprasellar mass lesions

Laboratory evaluation of hypothalmo-pituitary-adrenocortical (HPA) axis is obligatory in patients with sellar and suprasellar disorders before and following surgery and/or radio-ablation to assess the adrenocortical reserve. Dynamic tests to evaluate HPA axis include insulin induced hypoglycaemia (IIH), 250µg/1µg 1-24 adrenocorticotropicin (ACTH) and metyrapone. A peak cortisol response of 480-600 nmol/l to insulin hypoglycaemia or 1-24 ACTH or 11-deoxycortisol response of 200 nmol/l to metyrapone have been reported to indicate normal adrenocortical reserve and suggest that the integrity of HPA axis is preserved. Among all these dynamic tests IIH has been recognized as the reference test for evaluation of HPA axis.
Both IIH and 250 µg ACTH stimulation tests can be performed at any time of the day\(^4,5\). The peak cortisol response to either of them is similar in the morning as well as in the evening\(^4,5\). The dose of 250 µg 1-24 ACTH is considered supraphysiological while 1 µg 1-24 ACTH, near physiological\(^6-8\). In an earlier study, we observed plasma cortisol responses to 1 µg 1-24 ACTH and IIH is comparable, with sensitivity of 83.3 per cent and specificity of 95 per cent\(^6\).

One microgram 1-24 ACTH stimulation in healthy individuals evokes normal cortisol response in the morning as well as in the evening\(^9\). However, cortisol response to 1 µg ACTH has not been evaluated in patients with hypothalamic-pituitary disorders at different times of the day. This study was conducted to find out whether this test can be performed at any time of the day in patients with secondary ACTH deficiency due to hypothalamic-pituitary disorders.

**Material & Methods**

The study was conducted in the endocrine outpatient clinic of Nehru Hospital, attached to Postgraduate Institute of Medical Education and Research, Chandigarh between June 2000 to January 2002. Thirty five consecutive patients (21 men and 14 women) with sellar and suprasellar mass lesions were included in the study. Their age ranged from 14 to 72 yr with a mean (±SD) of 43.0±14.4 yr. Sixteen patients had somatotropinoma, 11 had non-functioning pituitary tumour, 3 each had prolactinoma and craniopharyngioma, and one each had clivus chordoma and lymphocytic hypophysitis. All the pituitary adenomas were macroadenomas. The tests were done in 3 patients pre-operatively, and in 32 following trans-sphenoidal/trans-frontal surgery with (6) or without (26) postoperative radio-ablative therapy. The time interval between surgery and assessment of adrenal reserve ranged between 3 months to 4 yr and, after irradiation 2 to 5 yr. Three patients in pre-operative group were not on glucocorticoid replacement, while patients in the post-operative group were on glucocorticoid maintenance, that was omitted (hydrocortisone for 2 days and prednisolone for 5 days) prior to the tests.

Thirty six healthy resident doctors and staff members served as controls. They included 23 men and 13 women, age 22-54 yr with mean age of 32.2±9.0 (±SD) yr. The sample size (including number of patients and controls) was calculated on the basis of our previous study\(^9\) keeping type 1 error (\(\alpha\)) of 0.05, a sample size of 35 in each group was found to provide power (1-\(\beta\)) of at least 0.8. The study protocol was approved by the Institute's Ethics Committee and informed consent was obtained from all.

A dilute solution of 1-24 ACTH (Synacthene, Novartis, Basel, Switzerland) was prepared by adding 25 µg of stock solution (250 µg/ml) to 25 ml of normal saline in a sterile glass vial and was stored at 4°C. One milliliter of this reconstituted synacthene, (1 µg/ml) was used as intravenous (iv) bolus in the test.

On day 1, the test was performed at 0800 h and on day 3 at 1600 h. After inserting the intravenous canula into one of the anterior cubital veins and keeping in situ for 20 min, the '0' h sample (pre-injection) was collected. Further samples were obtained at 30 and 60 min following iv bolus injection of 1 µg ACTH. Plasma cortisol was estimated by a specific non-chromatographic radioimmunoassay using antiserum to cortisol-3-BSA and \(^3\)H cortisol\(^10\). The sensitivity of the assay was 5 nmol/l and the intra and inter-assay coefficient of variation was <8 per cent.

**Statistical analysis:** Statistical analysis was performed by using statistical package for social sciences (SPSS) for windows, release 10.0.1 (SPSS Inc., Chicago IL). Results were expressed as mean ±SD unless specified otherwise. Chi-square test (Pearson or Fisher exact test) was used for comparing catagorical variables. Where data were not normally distributed appropriate non-parametric test like Mann Whitney U test for independent samples and Wilcoxon signed rank test for related samples was used. All t values were calculated as two tailed and \(P\) value of <0.05 was taken as statistically significant.

**Results**

In healthy subjects, the prestimulated plasma cortisol at 0800 h (377.5±93.3 nmol/l, range 180 to 645) was higher (\(P<0.001\)) than that at 1600 h (230.1±75.7 nmol/l, range 124 to 430) indicating normal diurnal variation. The peak cortisol response was 500 nmol/l or higher in response to 1 µg 1-24 ACTH both at 0800 and 1600 h in all healthy subjects. The peak plasma cortisol response
at 0800 h (729.1±183.2 nmol/l, range 520 to 1200) was higher (P<0.01) than that at 1600 h (665.8±138.6 nmol/l, range 500 to 1000).

In the patients' group, the prestimulated plasma cortisol at 0800 h (250.3±169.7 nmol/l, range 10 to 640) was higher (P<0.001) than that at 1600 h (166.3±128.9 nmol/l, range 10 to 680). The peak plasma cortisol response at 0800 h (490.9±309.4 nmol/l range 19 to 1200) was comparable to that at 1600 h (464.8±318.4 nmol/l range 26 to 1300).

In 27 patients (77%), the peak cortisol response was consistent both at 0800 and 1600 h (normal in 13, deficient in 14) but was discrepant in the remaining 8 patients (23%) (Table). In 7 of them the response was normal at 0800 h but not at 1600 h while in one, normal response was seen at 1600 h but not at 0800 h. Seven of them had higher basal cortisol at 0800 h than that at 1600 h. No significant correlation between prestimulated and peak cortisol response at 0800 h (r=0.43, P=0.29) as well as at 1600 h (r=0.38, P=0.36) was noted.

No adverse event was observed during and/or after the test in any of the patients or healthy subjects.

**Discussion**

One microgram short synacthene test (SST) is physiological, free of any adverse event and cost-effective, therefore is being increasingly projected as a screening test for evaluation of HPA axis in patients with ACTH deficiency. Whether the test can be performed at any time of the day as in the case of 250 µg SST, has not been examined as yet. Dickstein et al observed the optimal peak cortisol response to 1µg 1-24 ACTH at 0800 h in healthy subjects who received overnight dexamethasone. It was, therefore, assumed that basal cortisol does not influence the stimulated response and that the 1 µg 1-24 ACTH stimulation test can be performed at any time of the day.

In our study, peak cortisol response to 1 µg 1-24 ACTH was consistent both at 0800 and 1600 h in 27 patients, but was discrepant in 8 patients. Seven of them had normal cortisol response (³500 nmol/l) at 0800 h but not at 1600 h and basal cortisol level in them was higher at 0800 h than at 1600 h. The higher peak cortisol response in the morning, compared to that in the evening could be due to physiologic dose of 1-24 ACTH used and due to the influence of basal cortisol on the stimulated response. Cortisol response to 1 µg 1-24 ACTH was higher in the morning than in the evening whereas this variability in response was lost with 250 µg 1-24 ACTH. Several investigators have documented the influence of basal cortisol on stimulated cortisol response at different periods of the day. Daidoh et al reported maximal cortisol response at 1200h to a dose as low as 0.5µg 1-24 ACTH as iv bolus with basal cortisol of 200 nmol/l. Dickstein et al reported submaximal cortisol response to 0.6 µg 1-24 ACTH both at 0800 and 1600 h in healthy subjects pretreated with...
dexamethasone, thereby, eliminating influence of basal cortisol. Thus, basal cortisol influences peak cortisol response to 1-24 ACTH, while pretreatment with dexamethasone perhaps affects it by inhibiting endogenous ACTH drive and steroidogenesis in adrenal cortex.\footnote{15}

The stimulated plasma cortisol response to define optimal adrenal reserve in different studies ranged from 480 to 600 nmol/l\footnote{3,8,16-18}. The variable cut-offs are related to analytical methods used and observed values in controls. In our study, peak cortisol level of 3500 nmol/l as a criterion of optimal response was based on observed response in healthy subjects where a specific radioimmunoassay for cortisol was employed.\footnote{9}

In the present study the mean age of patients was significantly higher ($P<0.001$) than healthy controls. Therefore it is prudent to look at the effect of age on cortisol levels. However, many studies have shown that there were no alterations in basal as well as stimulated cortisol levels. However, many studies have shown that

There is no diurnal variation of cortisol response to sub maximal the time interval for assessment of HPA axis following pituitary surgery using 1µg 1-24 ACTH stimulation test has been a matter of debate\footnote{21-23}. An interval of 12 wk or more is considered sufficient for atrophy of adrenal glands in absence of endogenous ACTH drive\footnote{22,23}. False positive results are likely to be obtained in early period of evolution of adrenal atrophy.\footnote{22,23}. Our patients had minimum lag time of 12 wk after surgery and 2 yr after irradiation prior to evaluation.

Documentation of normal peak cortisol response to 1 µg 1-24 ACTH at 0800 h but not at 1600 h in substantial number of patients with sellar and suprasellar disorders suggests that 1 µg SST can be performed in the morning hours than at other times of the day.

References

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