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The Role of Morning Basal Serum Cortisol in Assessment of Hypothalamic Pituitary-Adrenal Axis

Abstract

Purpose: The use of morning basal serum cortisol levels as an alternative to dynamic testing for assessment of hypothalamic-pituitary-adrenal (HPA) axis has previously been reported. The purpose of this study was to determine the lower and upper cutoff values that would obviate subsequent HPA axis testing.

Methods: A single-centre, retrospective study from a tertiary care endocrinology clinic was conducted, analyzing data from 106 adult individuals referred for HPA axis testing who had undergone a 0800-0900 morning basal serum cortisol test followed by a standard dose (250 µg) adrenocorticotropin (ACTH) stimulation test. The ability of morning basal serum cortisol values to predict post-ACTH 30 or 60 minute peak cortisol value of >500 or >550 nmol/L was investigated.

Results: A morning basal cutoff of <128 nmol/L is sufficient for predicting a post-ACTH value < 550 nmol/L, and morning basal cutoff levels of >243 nmol/L and >266 nmol/L predict peak post-ACTH values of >500 and >550 nmol/L respectively, obviating the need for dynamic testing. Regression analysis further demonstrated the log-linear relationship between morning basal and peak levels, while also finding a significant decrease in peak post-ACTH levels for patients diagnosed with secondary hypothyroidism (76 nmol/L lower, $p=0.003$) or secondary hypogonadism (61 nmol/L lower, $p=0.02$). These data suggest that the risk of cortisol deficiency is significantly higher in individuals with additional pituitary insufficiencies. The odds ratios for cortisol deficiency in patients with history of isolated secondary hypothyroidism was 3.41 ($p=0.015$), with isolated secondary hypogonadism was 4.77 ($p=0.002$) and with both was 7.45 ($p=0.0002$).

Conclusion: Morning basal serum cortisol levels show promise as an effective screening test for HPA insufficiency for most patients. Clinicians should consider the high probability of HPA insufficiency in patients with one or more pituitary insufficiencies.

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A variety of tests have been reported in the literature to assess the hypothalamic-pituitary-adrenal axis (HPA) insufficiency. Although the insulin tolerance test (ITT) is mostly regarded as the gold standard, the adrenocorticotropin (ACTH) stimulation test has been proposed as a reliable alternative to the more cumbersome ITT [1-4]. Both the ITT and the ACTH test, generally regarded as 'dynamic tests', require medical supervision and the ITT is contraindicated in patients with seizure disorders, arrhythmias and ischemic heart disease, which impedes the utility of these tests as simple, effective screening tools. Studies have looked at the role of morning basal cortisol drawn between 0800-0900h as a screening test that typically reflects the highest physiologic levels attained throughout the day, usually peaking 30-45 minutes after awakening [5]. These studies have generally focused on specific patient populations of pre- [6,7] and post-pituitary surgeries [8,9] and consequently proposed variable lower and upper serum basal cortisol levels that would obviate the need for patients to undergo further dynamic testing. [8,10-13]. The primary purpose of this study was twofold: a) to assess the association between morning basal serum cortisol and serum cortisol levels after dynamic test using a standard dose (250 µg) ACTH stimulation test; and, b) to determine upper and lower cutoff values of morning basal cortisol that would obviate the need for dynamic testing in an outpatient setting.

Methods

The Queen Elizabeth II Health Sciences Centre is the sole tertiary care centre providing adult endocrinology services in the province of Nova Scotia, Canada. For this study, data was retrospectively collected from all patients referred to the adult (aged 18 years and older) endocrinology department between January 1, 2008 and December 31, 2012. All patients had undergone morning basal serum cortisol assessment between 0800h-0900h and, if they were on glucocorticoid therapy (hydrocortisone), this was done after holding the previous afternoon's (in patients taking thrice daily dose) and evening's doses, ensuring at least 24 hours since the last dose. Based on our previous protocol, a morning basal serum cortisol value of < 100 nmol/L was regarded as diagnostic of HPA insufficiency, a value of >300 nmol/L was regarded as normal, whereas those with morning basal serum cortisol values between 100-300 nmol/L underwent the 250µg ACTH stimulation test stimulation test and a peak (30- and/or 60- minute) serum cortisol value of >550nmol/L being regarded as a 'pass' based on previously published data [1,14]. All but 10 patients had no history of exogenous glucocorticoid therapy within the past 8 weeks, whereas the 10 patients were on glucocorticoid weaning ther-

apy with hydrocortisone (≤ 10 mg/m²/day). None of the patients had undergone an ACTH stimulation test within 12 weeks after pituitary surgery. In addition, the data on the function of hypothalamic-pituitary-thyroid (HPT) and the hypothalamic-pituitary-gonadal (HPG) axes at the time of initial presentation were also collected to assess the impact of these axes on the HPA assessment. Secondary hypothyroidism (SHT) was defined as a low serum free thyroxine (FT4) with either subnormal or inappropriately normal thyroid stimulating hormone (TSH), whereas secondary hypogonadism (SHG) was defined as a low morning serum total testosterone/estradiol with subnormal or inappropriately normal luteinizing (LH) and follicle stimulating hormones (FSH). Please refer to Appendix A for the reference ranges of our laboratory values. The Capital Health Research Ethics Board approved the study.

Serum cortisol, TSH, T4, LH, FSH, testosterone and estradiol were measured by radioimmunoassay, using Advia Centaur XP, Siemens Canada Limited, Mississauga, Ontario, Canada.

The data were analyzed using "R project" version 2.14.2. Morning basal cortisol levels were used to predict the peak post-ACTH 30 or 60 minute values as >500 nmol/L or >550 nmol/L using Receiver Operator Curves (ROC). One hundred percent sensitivity/specificity rates (the points at which a basal cortisol cutoff correctly classified all of the normal/abnormal peak post-ACTH values, respectively) for >500 and >550 nmol/L, as well as the optimal morning serum cortisol cutoff value were determined using the phi coefficient [15]. Simple and multiple linear regressions were performed to analyze the relationship between morning basal and peak serum cortisol levels, while controlling for the HPT and HPG values. For the regression, the peak cortisol values were log-transformed to account for non-normality.

Results

Patients

These data are summarized in Table 1. A total of 106 individuals (66 females and 40 males) were studied. The median age was 48 years and the range was 26-87 years. The primary diagnoses are listed in Table 1. Of all individuals, 18 patients had combined SHT and SHG, five had isolated SHT and six had isolated SHG. The diagnoses of SHT and SHG were made in all patients at the time of original presentation and all patients had been on stable, adequate replacement therapy with sex hormones and levothyroxine for at least 3 months at the time of ACTH testing. There were 28/106 patients who had previously undergone pituitary resection.

TABLE 1. Patient demographics and primary diagnoses

Total number of patients	106
Females/Males	66/40
Mean age (range) years	48 (26-87)
Diagnoses (n)	Pituitary tumour (55)
	Fatigue (16)
	glucocorticoid withdrawal (10)
	Pituitary tumour + radiation therapy (4)
	Non -pituitary sellar growth (6)
	Non-pituitary sellar growth + radiation therapy (6)
	Idiopathic pituitary insufficiency (5)
	Hypotension (2)
	Vomiting (1)
	Cranial radiation (1)

Morning basal serum cortisol vs. 250 µg ACTH test

The correlation between morning basal and peak serum cortisol was 0.52 and a log-transformation of the morning basal cortisol levels (Figure 1) indicated a log-linear relationship between morning basal and peak cortisol values. The ROC analysis (Figure 2) to assess the need for dynamic testing yielded slightly different morning basal cortisol values for peak post-ACTH values of >500 nmol/L or >550 nmol/L, as demonstrated in Table 2. The timing of peak cortisol, either 30 or 60 minutes post-ACTH, did not alter the ROC analysis significantly (data not shown). Using two different outcome levels of peak post-ACTH values (>500 nmol/L and >550 nmol/L), three potential cutoff levels for morning serum cortisol levels

were determined: the point that ensured all cases of normal post-ACTH were identified (100% sensitivity), the point that ensured all cases of abnormal post-ACTH were identified (100% specificity) and the optimal cutoff point, i.e., the cutoff point that ensured the most correct classifications.

In terms of morning basal cortisol *vs.* peak post-ACTH test, the peak value of > 550 nmol/L generated more robust cutoff values as compared with that of >500 nmol/L (see Table 2). While using the peak post-ACTH level >500 nmol/L, the lower cutoff for morning basal cortisol levels, which gave 100% specificity, was 52 nmol/L but only a few patients had basal levels below that point. The upper cutoff of 243 nmol/L was significantly more robust because all 17 patients with morning serum cortisol levels above that point had peak-ACTH levels above 500 nmol/L. For peak post-ACTH levels >550 nmol/L, the lower cutoff was more definitive because all 12 patients with morning serum cortisol levels below 128 nmol/L had peak levels below 550 nmol/L, and all 12 patients with morning serum cortisol levels above 266 nmol/L had peak levels above 550 nmol/L. Figure 1 presents these cutoff levels and the > 550 nmol/L line, demonstrating their predictive power.

The regression analysis confirmed our findings above, revealing the log-linear relationship demonstrated in Figure 1. The regression was significant ($p < 0.0001$), indicating that morning serum cortisol levels are a significant predictor of peak post-ACTH levels.

Risk of HPA insufficiency in patients with other pituitary hormone deficiencies

SHT and SHG had a significant effect on the post-ACTH levels, with SHT corresponding to a 77 nmol/L drop in peak

TABLE 2. ROC analysis

Post-ACTH Level (nmol/L)	AM Level (nmol/L)	Correct Normal	Correct Abnormal	Incorrect Normal	Incorrect Abnormal	Sens	Spec	NPV	PPV
>500	52	80	2	24	0	100.00	7.69	100.00	76.92
>500	243	17	26	0	63	21.25	100.00	29.21	100.00
>500	168	69	18	8	11	86.25	69.23	62.07	89.61
>550	128	63	12	31	0	100.00	27.91	100.00	67.02
>550	266	12	43	0	51	19.05	100.00	45.74	100.00
>550	153	61	20	23	2	96.83	46.51	90.91	72.62

AM Level=morning basal serum cortisol level

Sens - sensitivity

Spec - specificity

NPV – negative predictive value

PPV – positive predictive value

FIGURE 1. Log-linear relationship between morning basal and peak post-ACTH cortisol values

post-ACTH levels (95% CI: [26.9, 127.1], $p=0.003$) and SHG resulting in a 61 nmol/L drop (95% CI: [11.1, 111.4], $p=0.017$). There was no correlation between either condition and morning serum cortisol levels. The presence of additional pituitary insufficiencies increased the risk of HPA insufficiency. Odds ratios (OR) were calculated using the peak post-ACTH 30 or 60 minute cortisol value of >500 nmol/L. In patients with isolated SHT the OR of HPA insufficiency was 3.41 ($p = 0.015$), with isolated SHG was 4.77 ($p = 0.002$) and with combined SHT and SHG was 7.45 ($p = 0.0002$). Primary diagnosis including presence or absence of surgery did not have any effect on the association between morning basal cortisol and peak ACTH test.

Discussion

Previous studies have surmised that serum basal cortisol can be used effectively for predicting the integrity of the HPA axis in adults with reported lower cutoff values as 80 - 110 nmol/L and upper cutoff values as 250 – 494 nmol/L [8,10-13]. The lack of uniformity in these cutoff levels could in part be attributed to differences in study populations, variability of dynamic tests, different serum cortisol assays used, the cutoff of peak serum cortisol that was deemed indicative of a normal HPA axis response, and the clinical context in which the studies were done.

FIGURE 2. ROC curves using peak post-ACTH values of >500 nmol/L and >550 nmol/L.

A diverse group of patients was chosen for this study to provide a wider clinical context. A recent meta-analysis of 13 studies suggested that in ambulatory patients with no acute illness, but with suspected HP disorder, the cutoff value below 138 nmol/L best predicted HPA insufficiency in adults whereas an upper cutoff value of 365 nmol/L excluded HPA insufficiency [16]. As expected, our data showed that morning basal cortisol cutoff levels are dependent upon the peak post-ACTH value. This is an important issue as both values are reported in the literature and additional studies are required to further elucidate these differences. In our study, the lower and upper cutoff for morning basal cortisol against peak post-ACTH value of >500 nmol/L were 52 nmol/L and 243 nmol/L, respectively. Although this lower cutoff of 52 nmol/L is significantly lower than previous studies (80-138 nmol/L), it is less robust because only two of the 106 patients met this, whereas the upper cutoff of 243 nmol/L is more in keeping with the previously reported value of 250 nmol/L by Watts and Tindall [8], but significantly lower than the recent meta-analysis cutoff of 365 nmol/L [16]. On the other hand, if a peak cortisol level cutoff of >550 nmol/L was used, the lower and upper morning serum cortisol levels were 128 nmol/L and 266 nmol/L, respectively. The lower cutoff of 128 nmol/L is in line with the published data while the upper cutoff of 266 nmol/L is again markedly lower than the one reported in the meta-analysis [16].

The standard dose ACTH stimulation test was used exclusively in this study as previous studies have established it as an effective alternative to the more cumbersome ITT [3,4]. There are some limitations to the 250 µg ACTH stimulation test, particularly that it is less reliable in the first two months post-pituitary surgery [18]; however, in our population, none of the ACTH stimulation tests was done within twelve weeks of pituitary surgery. Of note, Watts and Tindall also used the standard dose ACTH stimulation test 5-7 days and 1 month after pituitary surgery [8], which may explain why their reported values are closer to ours. It has also been suggested that, the timing of the peak cortisol, i.e., 30 or 60 minutes after ACTH injection, may lead to diagnostic variability and consequently may have an impact on basal serum cortisol cutoff levels [14,17]; however, our data did not show a statistically significant effect of either 30 or 60 minutes post ACTH value on the ROC analysis for both the 500 or 550 nmol/L peak cortisol cutoff levels.

The standard 250 µg ACTH test, as opposed to the 'low dose' 1 µg ACTH, test was used exclusively in this study. Several studies have reviewed these tests reporting conflicting results with some suggesting the the 1 µg ACTH stimulation test is superior [16] while others showing them to be equivalent [19]. Future studies will need to be done to evaluate if the low dose ACTH stimulation test is a better predictor.

Although most studies have been conducted in a perioperative setting, in this study a diverse outpatient population was analysed, making it more clinically relevant to most outpatient settings.

Our data reveals a novel finding that suggests that the presence of additional pituitary hormone deficiencies increases the risk of HPA deficiency. Although in this retrospective study, data regarding growth hormone deficiency was not available, the risk of HPA deficiency was assessed in patients with SHT, SHG or both. In patients who had originally presented with SHT, the risk of HPA insufficiency was 44%, with SHG it was 50% and with both SHT and SHG it was 61%. It is also noteworthy that all patients were well established on stable replacement therapy at the time when these data were collected. Larger studies should be conducted to assess the adequate morning basal serum cortisol values that may provide the appropriate diagnostic assessment of HPA axis in patients with multiple pituitary insufficiencies. Until this is clarified, patients known to have at least one other known pituitary hormone deficiency should be regarded as having a high risk of HPA insufficiency.

Conclusion

In conclusion, our study further confirms the utility of morning basal cortisol as an effective screening test for HPA insufficiency. The appropriate morning basal cortisol values depend upon the cutoff values used for the ACTH stimulation test. Furthermore, the risk of HPA insufficiency is significantly higher in patients with additional pituitary insufficiencies, thus suggesting that a high index of vigilance should be maintained in such cases. Patients with known pituitary hormone deficiencies should undergo early dynamic HPA axis testing. Our data also suggest the need for larger prospective studies to confirm this association and assess the adequate morning basal cortisol values in individuals with one or more pituitary insufficiencies.

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Appendix A

Reference ranges:

Free thyroxine (FT₄) reference range: 10.0-19.0 pmol/L

Thyroid stimulating hormone (TSH) reference range: 0.35-5.50 mIU/L

Serum total testosterone reference range: 8.4-28.7 nmol/L

Serum estradiol in adult female:

Ovulating:

Follicular phase: <976 pmol/L

Midcycle phase: 433-1303 pmol/L

Luteal phase: 95-606 pmol/L

Postmenopausal:

Untreated <110 pmol/L

Treated: <341 pmol/L

Oral contraceptives: <374 pmol/L

Luteinizing hormone:

Female:

Normally menstruating

Follicular phase: 1.9-12.5 IU/L

Midcycle peak: 8.7-76.3 IU/L

Luteal phase: 0.5-16.9 IU/L

Pregnant: <0.1-1.5 IU/L

Postmenopausal: 15.9-54.0 IU/L

Contraceptives: 0.7-5.6 IU/L

Male:

20-70 years: 1.5-9.3

>70 years: 3.1-34.6 IU/L

FSH:

Female:

Normally menstruating

Follicular phase: 2.5-10.2 IU/L

Midcycle peak: 3.4-33.4 IU/L

Luteal phase: 1.5-9.1 IU/L

Pregnant: 0.0-0.2 IU/L

Postmenopausal: 23.0-116.3 IU/L

Male:

13-70 years: 1.4-18.1 IU/L