Case Report

LIMITATIONS OF BASAL CORTISOL IN THE DIAGNOSIS OF CUSHING SYNDROME

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ABSTRACT

Objective: Cushing syndrome (CS) is one of the most challenging diseases to diagnose due to the difficulties that may arise during laboratory test interpretations. A random serum cortisol level is often obtained by a general practitioner as a first step in the work-up of suspected CS patients. In this respect, it is rarely useful and has limitations.

Methods: We report an extremely unusual case of a female patient who presented with adrenocorticotropic hormone–independent CS and corticosteroid-binding globulin (CBG) deficiency.

Results: The patient was initially misdiagnosed with and treated for adrenal insufficiency because of persistently low basal cortisol levels, in detriment of her exacerbated Cushing features and symptoms.

Conclusion: We describe the limitations of using basal cortisol in the diagnosis of CS and review the differential diagnosis of patients with CS who have low basal cortisol. CBG variants may explain the findings of high urinary and salivary cortisol, in the absence of increased serum cortisol. **(AACE Clinical Case Rep. 2019;5:e91-e94)**

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Abbreviations:
ACTH = adrenocorticotropic hormone; **CBG** = corticosteroid-binding globulin; **CS** = Cushing syndrome; **RV** = reference value

INTRODUCTION

Cushing syndrome (CS) is caused by chronic and inappropriate exposure to excess glucocorticoids, which leads to increased rates of mortality and morbidity (1,2). The different international guidelines for the diagnosis of CS are similar on major aspects of the diagnosis of CS, but there are few challenging patients. We report an unusual case of a female patient who presented with adrenocorticotropic hormone (ACTH)-independent CS, firstly misdiagnosed as adrenal insufficiency. The aim of this case report is to describe the limitations of using basal cortisol in the diagnosis of CS and review the differential diagnosis of patients with CS who have low basal cortisol.

CASE REPORT

A 41-year-old female patient presented with significant muscle weakness that limited her ability to rise, facial plethora, and pathologic fractures (both humerus and two ribs) over the past 4 years. Biochemical assessment revealed repeated low basal 8 AM serum cortisol of 1.1 and 1.3 μg/dL (30.3 and 35.8 nmol/L, respectively) (reference value [RV], 6.7 to 22.6 μg/dL [184 to 623 nmol/L]) measured using electrochemiluminescence immunoassay (Cobas, Roche 2010, Mannheim, Germany). The inter- and intra-assay coefficients of variation were 1.6% and 1.3%, respectively. Originally diagnosed with and treated for adrenal insufficiency, her symptoms worsened. This diagnosis was made by the original clinician and based only

on basal serum cortisol levels. No confirmatory stimulation test was performed. She developed easy bruising, weight gain, and depression. Due to persistent symptoms, she was referred to our department.

On physical examination, a cushingoid appearance with a full moon face, upper chest plethora, buffalo hump, central body fat distribution with muscle atrophy of lower limbs, and evident muscle weakness were observed. These findings strongly suggested the presence of hypercortisolism. Laboratory evaluation revealed a 24-hour urinary free cortisol level of 200 and 1,324 μg (552 and 3,654 nmol, respectively) (RV, 10 to 100 μg [27.6 to 276 nmol]; measured by electrochemiluminescence immunoassay), 11 PM salivary cortisol of 1.36 and 0.42 μg/dL (37.5 and 11.5 nmol/L, respectively) (RV, <0.35 μg/dL [<9.6 nmol/L]; measured by electrochemiluminescence assay), confirming diagnosis of CS. A dexamethasone suppression test was not performed because of the low basal serum cortisol levels. ACTH levels measured in plasma at 8 AM on two distinct occasions were 5 pg/mL (1.1 pmol/L) (RV, 5 to 46 pg/mL [1.1 to 10.1 pmol/L]), indicating an adrenal origin for CS. A computed tomography scan showed a low-density lesion of 3 cm in the left adrenal gland. Oral ketoconazole was prescribed and unilateral adrenalectomy performed. Histologic examination was consistent with the diagnosis of a 3.4 × 2.0 × 1.5 cm benign adrenocortical adenoma. Glucocorticoid coverage was required after adrenalectomy. One month after adrenalectomy, basal serum cortisol level was 0.5 μg/dL (13.7 nmol/L) while taking 5 mg of oral prednisone daily. Additional investigations revealed normal serum albumin levels and a serum corticosteroid-binding globulin (CBG) concentration of 35 μg/mL (601.3 nmol/L) (RV, 40 to 154 μg/mL [687.2 to 2,645.7 nmol/L]). Hepatic and renal functions were normal, and proteinuria was absent. Prior to CS, the patient's medical history was unremarkable. She denied using any medication, including any form of glucocorticoids, during the last 2 years.

DISCUSSION

The patient was inappropriately diagnosed with adrenal insufficiency, based on early morning basal cortisol concentrations, despite her clinical features of hypercortisolemia. Although different international guidelines strongly recommend confirmatory testing with stimulation tests, our patient did not undergo an ACTH stimulation test (with basal plasma ACTH level) and/or imaging of the adrenal gland before glucocorticosteroid therapy was initiated by the original physician. This resulted in misconduct and malpractice, with the patient's correct diagnosis and treatment being delayed.

CS is difficult to diagnose, with many pitfalls in laboratory interpretation. A random serum cortisol level is often obtained by a general practitioner as a first step in the work-up of suspected CS patients. In this respect, it is rarely useful and has limitations. Because CS is a rare disorder, if no criteria are applied before investigation, the risk of false-negative test results is high. Thus, it is imperative to know which patients to investigate. Biochemical hypercortisolism must be confirmed before any attempt at differential diagnosis (3).

The evaluation of patients with suspected CS requires an understanding of the proper use and limitations of the tests commonly included in the diagnostic work-up. Only patients with specific features of hypercortisolemia must be investigated. False-positive results are reduced if case detection is limited to individuals with an increased pretest probability of having the disorder. Signs that should raise concern for CS include easy bruising, facial plethora, and violaceous striae wider than 1 cm or proximal myopathy, particularly if associated with other clinical finding such as moon face, buffalo hump, supraclavicular fullness, poor skin healing, as well as unusual features for age (osteoporosis and hypertension) (2,4).

A normal laboratory finding amidst a high predictive pretest value should not urge physicians to challenge their clinical intuition and establish a diagnosis only based on test results, without taking into account their clinical suspicion. The first step to diagnose CS, after clinical suspicion, aims to exclude exogenous use of glucocorticoids and then confirm hypercortisolism with functional tests that evaluate different aspects of the hypothalamus-pituitary-adrenal axis (2).

Cortisol is secreted in a pulsatile manner with a circadian rhythmicity; therefore, due to an overlap in similar values between normal subjects and CS patients, basal serum cortisol is not used for the diagnosis of CS (5,6). Even increased levels of morning basal serum cortisol have a low sensitivity for the diagnosis of CS (7). Although some studies have tried to find a cut-off for basal serum cortisol in CS (7,8), different guidelines do not recommend its use in the diagnosis of CS (2,3). Additionally, basal serum cortisol cannot be used for patients who have low CBG levels. Because 80% of cortisol is bound to CBG, and because assays measure total protein-bound cortisol, a decrease in binding proteins will accordingly alter serum cortisol concentrations (9,10). In contrast, both urinary and salivary cortisol tests reflect circulating free and biologically active cortisol and, therefore, they are not influenced by CBG concentrations.

Levels of CBG are decreased in states of glucocorticoid excess, such as in CS, due to glucocorticoid receptor–dependent inhibition of CBG production and secretion (11). CBG gene mutations have been described in humans (12). The Leuven and Lyon mutations reduce the CBG-cortisol binding affinity, and the null mutation results in complete or partial reduction in CBG levels. In Lyon heterozygotes, there is an unexplained 20% reduction in immunoreactive-CBG levels that can be due to reduced CBG synthesis or increased CBG degradation and a 40% reduction in total

cortisol levels due to low cortisol binding affinity (12). These variants suggest that glucocorticoid deficiency has been associated with affinity-altering or CBG level–reducing mutations (13). Therefore, a mutation in the CBG gene explains the persistently low basal serum cortisol found in our patient. Extremely low serum cortisol levels can be seen with a mildly low CBG, as seen in our case report and described in a heterozygous Lyon CBG mutation (12) and in patients with heterozygous null CBG mutation (13).

Additionally, polymorphisms in *CBG* can also exist and may contribute to low levels of total cortisol. Single-nucleotide polymorphisms in *CBG* can explain why CBG levels in some individuals are approximately 50% lower than normal (14).

Unusual combinations of CS and CBG deficiency have been described in two case reports published to date (15,16). Although evident hypercortisolism signs and symptoms were present, almost collapsed basal serum cortisol levels were observed continually. Also, 24-hour urinary free and late-night salivary cortisol levels, which are not influenced by conditions that alter CBG, were high. The patient did not use any medication or have any conditions that could modify CBG levels. The level of deficiency in CBG probably indicates a heterozygous state, similar to those found in family members who harbor a heterozygous CBG gene mutation (12,13). No other family member was investigated.

In a small group of patients with a type of CS known as cyclic CS, cortisol secretion is only periodically increased. The syndrome may be suspected in patients with signs and symptoms of CS with normal or subnormal cortisol levels intercycle or with fluctuating cortisol values. Our patient did not present with clinical features suggestive of cyclic CS (17). Interestingly, wide variations in cortisol levels can be seen in CS and do not necessarily relate to cyclic CS (18).

Another differential diagnosis could be iatrogenic CS with low serum cortisol values caused by suppression of the endogenous hypothalamic-pituitary-adrenal axis. This scenario consists of glucocorticoid administration, including inhaled glucocorticoids and the use of phytocosmetic products (19,20). In these circumstances, if the exogenous glucocorticoid is abruptly ceased, true hypocortisolism with low basal serum cortisol can arise in a patient who may still have stigmata of CS. Finally, other causes of low cortisol may be the presence of heterophile antibodies and food-dependent CS during fasting (21,22).

CONCLUSION

In conclusion, serum basal cortisol levels should not be used as a screening test for the presence of CS. Normal test results should not urge physicians to challenge their clinical suspicion and establish a diagnosis only based on test results. Taken together, our results indicate that CBG variants may explain the findings of high urinary and salivary cortisol, in the absence of increased serum cortisol. The correct management of hypothalamic-pituitary-adrenal axis disorders requires consultation of an expert endocrinologist with sufficient knowledge of the laboratory hormonal analysis, as correct diagnosis relies on correct interpretation of these techniques.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

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