Comparison of 1-μg and 250-μg Corticotropin Stimulation Tests for the Evaluation of Adrenal Function in Patients With Acquired Immunodeficiency Syndrome

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Many patients with acquired immunodeficiency syndrome (AIDS) have symptoms suggestive of adrenal insufficiency, but a normal 250-μg corticotropin (ACTH) stimulation test. We compared the results of 1-μg and standard 250-μg ACTH stimulation tests in patients with AIDS. Each patient was studied on 2 separate days. On day 1, 1 μg ACTH was given intravenously at 8 AM after an overnight fast and serum cortisol levels were measured at baseline, and 30 and 60 minutes after ACTH infusion. On day 2, the procedure was repeated with 250-μg ACTH. An absolute peak cortisol value of > 18 μg/dL and an increment of 7 μg/dL or more from baseline constituted a normal response. Among 31 patients, 16 (52%) had discrepant results: 14 (45%) had subnormal responses to 1 μg ACTH but normal responses to 250 μg ACTH (group 1); 2 (6%) had normal responses to 1 μg but subnormal responses to 250 μg (group 2) ACTH; 6 patients (19%) had concordant abnormal responses (group 3); and 9 (30%) had concordant normal responses (group 4). Eight patients of group 1 underwent a confirmatory insulin tolerance test (ITT); 4 of these patients had abnormal responses to ITT. Kappa statistic and McNemar's test were used to evaluate the data. A kappa statistic value of 0.095 and a *P* value less than.003 for the McNemar test indicate only random level of agreement and significant differences in the probability of positive result between the 2 ACTH tests. We conclude that discrepancies between the 1-μg and the 250-μg ACTH stimulation tests are common in patients with AIDS, with the likelihood of agreement with the "gold standard" ITT of only 50% for each test in our sample of patients. Larger studies are needed to further evaluate the use of these tests in patients with AIDS.

PATIENTS WITH acquired immunodeficiency syndrome (AIDS) exhibit a variety of abnormalities of hypothalamic-pituitary-adrenal (HPA) function. Baseline cortisol and corticotropin (ACTH) values in patients with human immunodeficiency virus (HIV) infection have been reported to be normal, elevated, or depressed.

The 250-μg ACTH stimulation test ("high-dose test" or HDT) is commonly used to assess adrenal function, although this test is not considered to be a "gold standard" for the evaluation of the HPA axis. The insulin tolerance test (ITT) and the metyrapone challenge test are the traditional reference tests.[1] The latter 2 tests, however, are rarely used because they are uncomfortable, can be dangerous, and require hospital admission and a physician's presence at bedside during the procedure. For these reasons, the HDT, which was first described by Wood et al in 1965[2] and originally designed to identify individuals with primary or secondary adrenal insufficiency,[3] has largely replaced the ITT or metyrapone test as a convenient alternative.

Several early studies comparing the HDT and the ITT in patients with hypothalamic-pituitary disease found an excellent correlation between the cortisol response 30 minutes after the administration of 250 μg ACTH and the response to hypoglycemia; however, a few discrepancies were noted.[4] Although the HDT test is highly specific, its sensitivity has been questioned. Several studies in patients with suspected secondary adrenal insufficiency have challenged the validity of the HDT, revealing discrepancies between the HDT and the ITT test in up to 35% of cases; these discrepancies could lead to the missed diagnosis of adrenal insufficiency.[5] Some reports described patients with clinical features suggestive of adrenal insufficiency that responded normally to HDT but had adrenal insufficiency diagnosed with ITT or metyrapone challenge.[1,2] In patients infected with HIV, plasma ACTH levels have been reported to be significantly increased (suggesting compromised adrenocortical function) during a 24-month follow-up even while cortisol response to HDT remained normal.[6-8]

In recent years, several investigators have described a low-dose ACTH stimulation test (LDT) using 1 μg ACTH.[1,5,6] The LDT may be more accurate in the diagnosis of adrenal insufficiency because subnormal levels of circulating ACTH may allow the adrenal cortex to remain responsive to a supraphysiological, but not physiological, doses of ACTH. The entire stored pool of endogenous ACTH in the anterior pituitary is approximately 600 μg. Thus, the conventional 250-μg ACTH (HDT) dose is a powerful adrenal stimulus. Far lower doses of ACTH, such as 1 μg, may provide a more physiologic level of stimulation to which only normal adrenals can respond.[8]

Thus, the purpose of this study was to compare the 1-μg ACTH stimulation test (LDT) with the standard HDT in a population of HIV-positive patients hospitalized for nonendocrine indications but suspected to have adrenal insufficiency.

MATERIALS AND METHODS

Subjects

This prospective study enrolled 31 consecutively hospitalized male patients. The study group was comprised of patients with documented HIV infection and symptoms suggestive of adrenal insufficiency, de-

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termined by the presence of at least 5of the following nine criteria symptoms: generalized weakness, anorexia, weight loss of more than 5% of body weight in the past 3 months, abdominal pain, diarrhea, orthostatic hypotension (drop in upright systolic blood pressure >10 mm Hg), mucocutaneous melanosis, hyponatremia (<130 mEq/L on 2 different occasions), and hypoglycemia (fasting blood glucose < 60 mg/dL).

Patients with a history of coronary artery disease, cerebrovascular disease, seizure, or mental disorder were excluded from the study. Also excluded were patients who were concurrently or in the prior 3 months taking medications known to interfere with the synthesis or metabolism of steroids, such as ketoconazole, rifampin, phenytoin, megestrol, or glucocorticoids.

Methods

The institutional review boards at St. Vincent's Medical Center and Weill Medical College of Cornell University approved the study. We identified the subjects during their hospitalization. After informed consent was obtained, subjects underwent testing for 2 days. On day 1 at 8 AM after an overnight fast and with the subject in a supine position an angiocatheter was inserted and a blood sample was drawn for basal serum cortisol level. Then 1 μg (1-24) ACTH (Cortrosyn, Organon, West Orange, NJ) was administered by intravenous bolus injection LDT. To prepare the 1-μg ACTH solution, a vial of 250-μg ACTH was diluted in 50 mL of normal saline to a concentration of 5 μg/mL and 0.2 mL of this solution was injected for the LDT. After the bolus was administered, blood samples were drawn by venipuncture for cortisol levels at 30 and 60 minutes. On day 2 at 8 AM, the procedure was repeated using 250 μg (1-24) ACTH (HDT). On day 3, those subjects who consented to an insulin tolerance test underwent this test after an 8-hour fast with a physician present at bedside during the entire procedure. While in a supine position, 10 U/kg of body weight of regular insulin was injected intravenously and blood samples were drawn for glucose and cortisol levels every 15 minutes for 90 minutes beginning at time 0, or more frequently if the patient developed symptoms suggestive of hypoglycemia. Blood sampling was continued for 30 minutes past the hypoglycemic nadir. The test was deemed adequate if serum glucose of 2.2 mmol/L (40 mg/dL) or below was documented. Intravenous glucose or orange juice by mouth was given to end the test when the hypoglycemic target was achieved or when patients could not tolerate the hypoglycemic symptoms.

All serum samples were centrifuged and stored either refrigerated at 2 to 8°C for up to 7 days or frozen at −20°C for up to 2 months. A glucose analyzer (Beckman Instruments, Fullerton, CA) was used to determine the serum glucose concentration for the ITT. The serum cortisol concentration was determined by solid-phase radioimmunoassay (Coat-A-Count Cortisol Kit, Diagnostic Products Corp, Los Angeles, CA).

A stimulation test response was considered normal if an absolute peak cortisol value of at least 18 μg/dL and an increment of 7 μg/dL or more from the baseline values at 30 or 60 minutes postinjection were achieved.

Statistical Analysis

The kappa statistic was used to determine the level of agreement between low- and high-dose ACTH tests. Values of kappa (κ) close to 1 indicate strong agreement, while values close to zero indicate only random agreement. McNemar's test was used to determine whether the likelihood of a positive result differed for the LDT and HDT.

RESULTS

Based on the results, the study population was divided into 4 groups. Fourteen patients (45%) had an abnormal response to the LDT but normal response to the HDT (group 1) (Fig 1). Eight of these patients underwent a confirmatory ITT; 4 of these had an abnormal ITT response, thus confirming the results of the LDT, while the other 4 had a normal response, thus confirming the results of HDT.

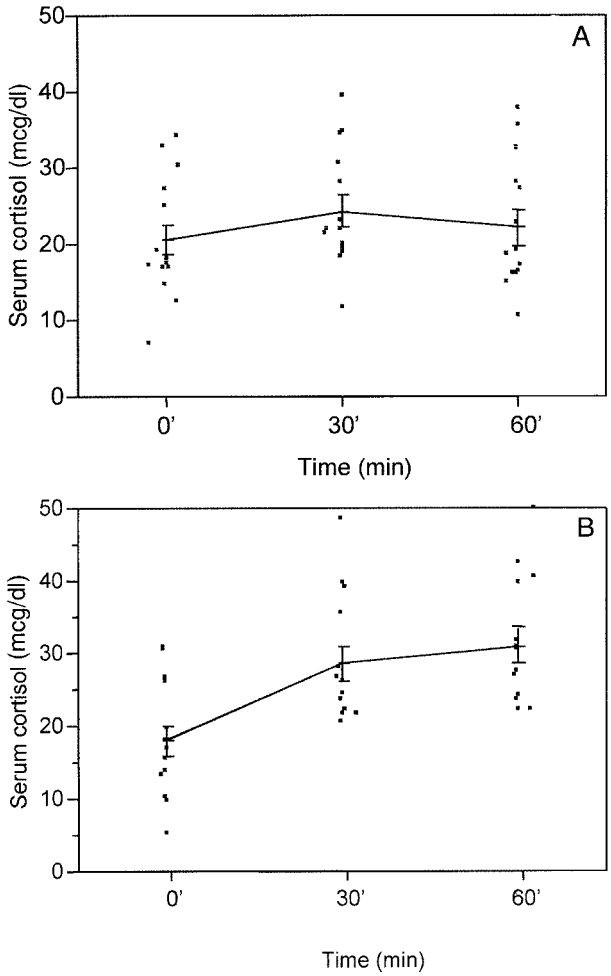

Fig 1. Serum cortisol levels (mean ± SEM, n = 14) during low-dose (A) and high-dose (B) ACTH stimulation tests in group 1 patients.

Two other subjects (6%) had a normal response to the LDT but an abnormal increment response to the HDT; neither had a confirmatory ITT performed because they did not consent to it (group 2) (Fig 2).

Six patients (19%) had concordant abnormal responses to both tests (group 3) (Fig 3); 2 of these underwent ITT and both were abnormal.

Nine patients (29%) had normal concordant responses for ACTH testing, and one of these subjects also had a normal confirmatory ITT (group 4) (Fig 4).

Thus, out of 31 subjects, 16 (52%, Groups 1 and 2) had discrepant results between the HDT and LDT test. The kappa statistic value for the low- versus high-dose ACTH stimulation tests was $\kappa = 0.095$ (suggesting only random agreement between the 2 tests) and the McNemar test yielded $P < .003$,

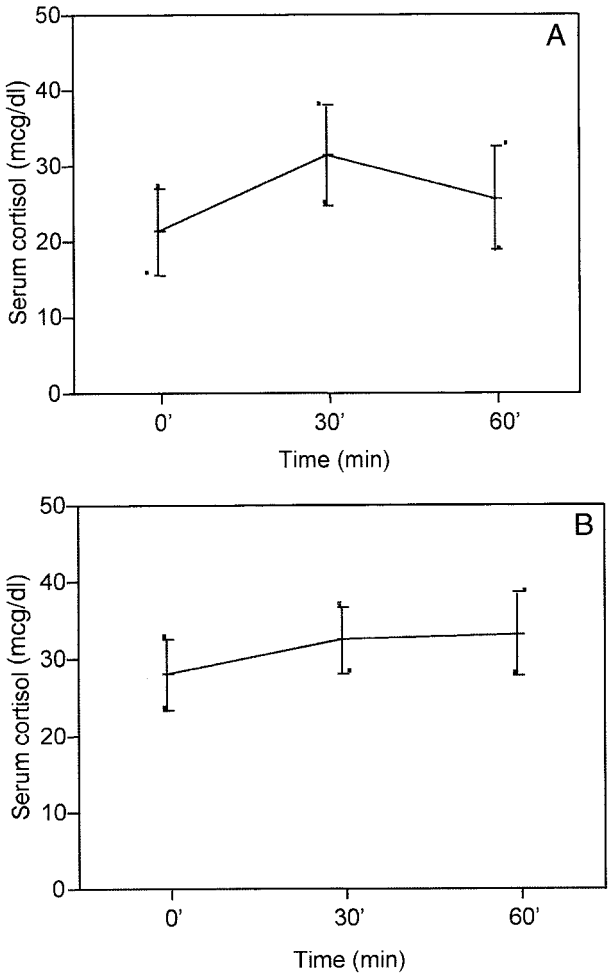

Fig 2. Serum cortisol levels (mean ± SEM, n= 2) during low-dose (A) and high-dose (B) ACTH stimulation tests in group 2 patients.

indicating that the probability of a positive result was significantly different for the 2 tests.

DISCUSSION

HIV targets most organs of the body including those of the endocrine system. The adrenal gland is a frequent site of pathologic involvement in HIV patients and adrenal insufficiency may be the most commonly occurring serious endocrine disease in this population.[3] The adrenal gland is the most common endocrine organ that is found to be abnormal at autopsy in AIDS patients, and is seen in 40% to 75% of cases.[4] Pituitary and hypothalamic abnormalities also have been seen in HIV disease at autopsy.[5]

The list of potential agents causing disease in the adrenal glands and hypothalamus/pituitary in patients with AIDS includes *Cytomegalovirus*, *Cryptococcus*, *Toxoplasma, Mycobacterium, Pneumocystis,* and *Histoplasma*. In addition, Kaposi's sarcoma metastases, lymphoma, and hemorrhage have been reported. Drugs that interfere with steroid metabolism are commonly used in patients with AIDS. These include rifampin, ketoconazole, and phenytoin.[9] Prolonged use of corticosteroids or megestrol acetate can further affect the adrenal glands in patients with AIDS.

In an attempt to correlate anatomic findings with function, many studies have examined biochemical alterations of the HPA axis in HIV-infected individuals. The results have varied, making a consensus difficult. Basal ACTH levels have been reported to be elevated, normal, or suppressed in conjunction with elevated, normal, or suppressed cortisol levels.[6-16]

The symptoms of adrenal insufficiency are generally subtle and nonspecific and could be easily overlooked in HIV-positive patients. Many patients with HIV or its associated opportunistic infections have a number of symptoms that may be consistent with adrenal insufficiency. These symptoms include fatigue, weakness, postural hypotension, diarrhea, and electrolyte disturbances[13] and may be attributed to the HIV itself or opportunistic infections. A study found that the classical symptoms and signs of adrenal insufficiency (fatigue, postural hypotension, and electrolyte disturbances) might be poor predictors of adrenal insufficiency in patients with AIDS.[17] For all of these

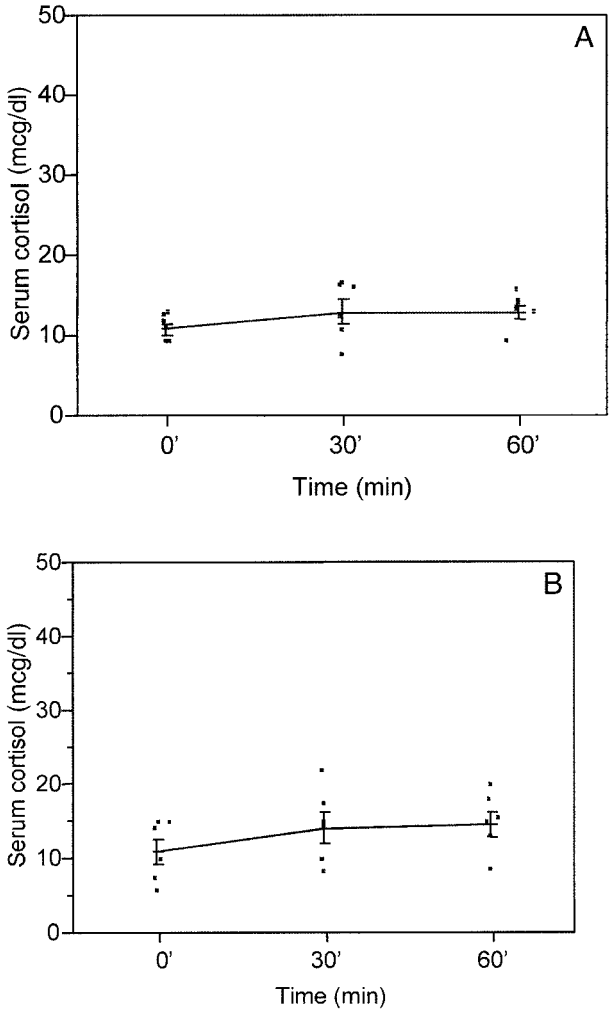

Fig 3. Serum cortisol levels (mean ± SEM, n = 6) during low-dose (A) and high-dose (B) ACTH stimulation tests in group 3 patients.

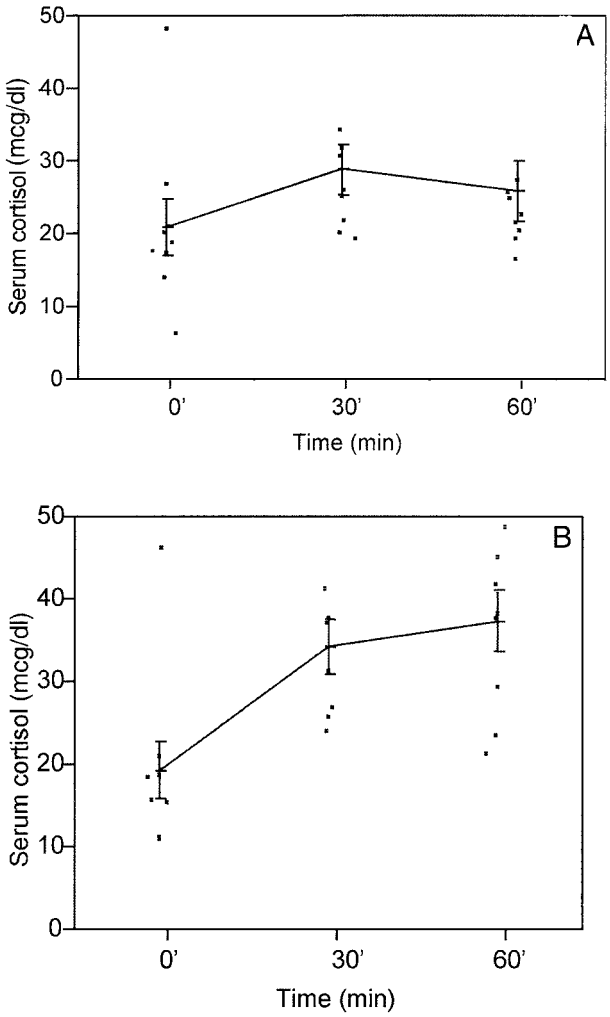

Fig 4. Serum cortisol levels (mean ± SEM, n = 9) during low-dose (A) and high-dose (B) ACTH stimulation tests in group 4 patients.

reasons, diagnostic ACTH stimulation tests are commonly employed in HIV-infected individuals.

Subnormal response to HDT stimulation has been described in up to 29% of patients with HIV infection during routine screening at various stages of disease. Thirty percent to 40% of patients with AIDS may have secondary adrenal insufficiency.[15] Thus, it is clear that the incidence of subnormal cortisol response is increased among patients with AIDS, especially end-stage disease.[8,11,12,16-24]

Our study demonstrated that discrepancies between the 1-μg and the 250-μg ACTH stimulation tests occur commonly in AIDS patients. Possible reasons for these discrepancies include failure to completely administer 1 μg ACTH, laboratory errors, and low sensitivity of the LDT or high false-negative rate of the HDT.

Our study has several limitations. Perhaps the main one is a relatively small number of patients who agreed to undergo ITT, which is considered a gold standard for assessment of HPA axis. However, the largest number of subjects who underwent ITT was in the most relevant groups: group 1 (normal responses to HDT, but abnormal response to LDT) and group 3 (concordant abnormal responses). In both groups, ITT was useful: when results of HDT and LDT were discrepant, each test had a 50% chance of concordance with ITT; when both tests were abnormal, ITT confirmed presence of adrenal insufficiency. Although a larger number of patients would have, in all likelihood, further strengthened our data, the kappa statistic values and the McNemar's test proved the data with the current number of patients to be valid.

Another possible weakness in our study is the standard dose of 0.1 U/kg of body weight of insulin used during ITT in all subjects. Although this standard dose may not account for differences in insulin sensitivity among the subjects, it does take into account the main variable which affects insulin sensitivity—the patient's weight. Besides, since hypoglycemia was achieved in all subjects who underwent ITT, our ITT results must be considered valid.

At this time, we recommend to begin the evaluation of the HPA axis function in AIDS patients with the HDT. If the response is abnormal, the individual is likely to have adrenal insufficiency. If response is normal but the clinical suspicion for adrenal insufficiency is high, we suggest performing a LDT. If the LDT is abnormal, consider performing an ITT as a confirmatory test. If the patient is unable to undergo or refuses ITT, consider using corticosteroid therapy empirically. The algorithm for this approach is presented in Fig 5.

In summary, discrepancies between HDT and LDT are com-

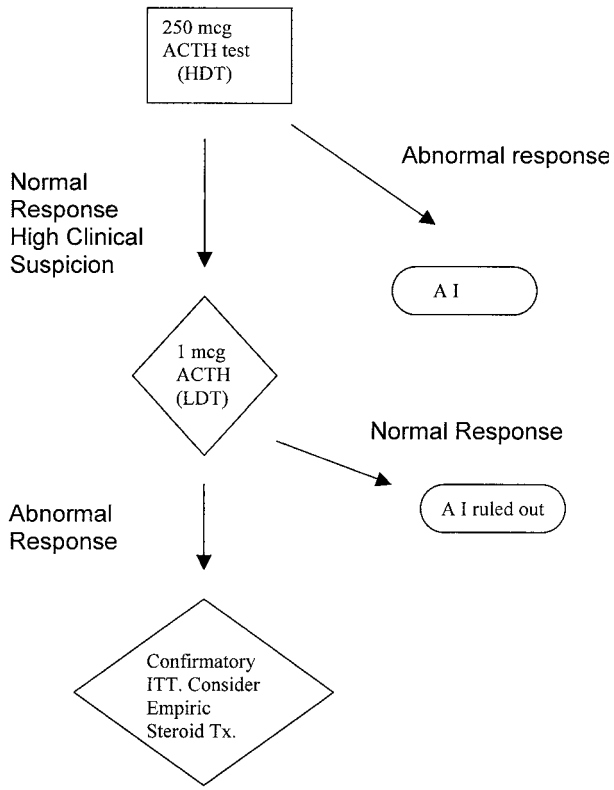

Fig 5. A proposed algorithm for evaluating a patient with AIDS for possible adrenal insufficiency. ITT, insulin tolerance test; AI, adrenal insufficiency.

mon in patients with HIV infection. Larger studies comparing the accuracy of 1-μg and 250-μg ACTH stimulation tests in patients with HIV infection and involving ITT as a gold standard in all subjects are needed to assess specificity and sensitivity of these 2 tests in patients with HIV infection.

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