Adrenal antibodies detect asymptomatic auto-immune adrenal insufficiency in young women with spontaneous premature ovarian failure

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BACKGROUND: Auto-immune adrenal insufficiency is a potentially fatal disorder. Young women with spontaneous premature ovarian failure (POF) are at increased risk of developing this condition. METHODS: We further characterized auto-immune adrenal insufficiency in this population by conducting an in-depth cross-sectional evaluation of adrenal function in a series of 123 women. RESULTS: We uncovered a new diagnosis of adrenal insufficiency in four women [3.2%, 95% confidence interval (CI) 0.2–6.4%]. All four tested positive for adrenal antibodies as detected by a clinically available indirect immunofluorescence assay. A positive adrenal antibody test was highly associated with adrenal insufficiency while a negative test was associated with normal adrenal function in all cases \( (P < 0.001) \). Adrenal antibodies increased the pretest probability of adrenal insufficiency from 3.2 to 67%. As a screening method the cortisol response during a standard adrenocorticotrophic hormone (ACTH) stimulation test gave two false positive results (1.7%, upper 95% confidence limit 5.0%). CONCLUSIONS: Our findings suggest that measuring adrenal antibodies would be an effective screening method by which to detect auto-immune adrenal insufficiency in young women with spontaneous POF. The standard ACTH stimulation test should be reserved to confirm adrenal insufficiency in women with adrenal antibodies, or those with signs and symptoms of adrenal insufficiency.

Key words: Addison disease/adrenal antibodies/adrenal insufficiency/21-hydroxylase antibodies/POF

Introduction

Primary auto-immune adrenal insufficiency is a potentially fatal condition that has an insidious onset and presents with non-specific symptoms. The diagnosis can be easily missed (Brosnan and Gowing, 1996; Oelkers, 1996; al Sabri et al., 1997). In some cases adrenal insufficiency first becomes clinically evident with an abrupt onset of adrenal crisis during an elective surgical procedure (Aono et al., 1999), as was the case in two women with premature ovarian failure (POF) that we have seen previously (Kim et al., 1997).

Women with spontaneous POF are at increased risk of developing adrenal insufficiency. Primary auto-immune adrenal insufficiency is known to occur in association with spontaneous POF with an estimated prevalence of between 2 and 10%. (Irvine et al., 1968; Irvine and Barnes, 1974; LaBarbera et al., 1988; Betterle et al., 1993). In contrast, in the general population of developed countries the prevalence of adrenal insufficiency is approximately one in 10 000 (Kong and Jeffcoate, 1994; Oelkers, 1996; Willis and Vince, 1997).

There is controversy regarding the best screening approach to detect auto-immune adrenal insufficiency in young women with spontaneous POF (Jaffe, 1991; Kim et al., 1997; Speroff et al., 1999). Here we further characterize the clinical entity of auto-immune adrenal insufficiency developed in association with spontaneous POF. We report our clinical and laboratory findings in a series of 123 women who underwent an in-depth evaluation of adrenal function.

Materials and methods

Subjects

Between December 1996 and March 2000 we performed screening for primary adrenal insufficiency and adrenal auto-immunity on 123 consecutive women. The women were referred to our clinical research centre to participate in a study of spontaneous POF. Infertility and amenorrhoea were the major concerns of all these patients and they generally considered themselves to be otherwise in good health. None of the participants carried a previous diagnosis of adrenal insufficiency. Our institutional review board approved the study and all participants signed an informed consent.

Referring physicians made the diagnosis of POF based on the following criteria: development of at least 4 months of amenorrhoea before age 40, associated with two serum FSH levels >40 IU/l
(drawn at least 1 month apart). Women with POF as a result of surgery, radiation, chemotherapy, or known karyotype abnormalities were not included in the study.

The demographics of the participants were as follows: 85% were Caucasian, 9% African American, 4% Asian and 2% Hispanic; their median age was 33 years (range 19–42); median time since diagnosis of POF was 3 years (range 4 months–20 years). The following co-morbidities were evident: 10 women had hypothyroidism (8%), 16 women had euthyroid Hashimoto thyroiditis (13%), three women had treated Graves disease (2.4%), two had osteoporosis, two had experienced depression in the past, two had asthma in remission, one had discoid lupus, and one had eczema.

All participants discontinued estrogen/progestin replacement therapy at least 2 weeks prior to our evaluation. All patients had a complete history and physical examination. We reviewed available medical records with special emphasis on finding documented symptoms, signs or test results consistent with adrenal insufficiency. On admission all patients had a routine blood count, serum electrolytes, including K and Na, creatinine, blood urea nitrogram (BUN), blood sugar, urine pregnancy test and urinalysis.

Tests to evaluate adrenocortical function
A standard adrenocorticotropic hormone (ACTH) stimulation test was performed between 0800 and 1000 h in a fasting state. The patients were given 250 μg cosyntropin (Cortrosyn®; Organon Inc., West Orange, NJ, USA) i.v. and blood for cortisol was drawn at time 0, 30 and 60 min. The normal value for the cortisol response was defined as reaching ≥550 nmol/l (20 μg/dl) at any time during the test (Tsigos et al., 1996). We made a diagnosis of cortisol insufficiency if the standard ACTH stimulation test was confirmed to be abnormal on repeat testing.

The mineralo-corticoid axis was evaluated by measuring plasma renin activity (PRA) and serum aldosterone after at least 2 h in the supine position. All patients were on a diet containing 4–8 g of sodium per day. When PRA was above the upper limit of normal we calculated the aldosterone/plasma renin activity ratio (A/PRA). Values ≤109, if aldosterone and PRA are expressed in SI units, indicated primary aldosteronism.

Patients were diagnosed with adrenal insufficiency if they demonstrated cortisol insufficiency, aldosterone insufficiency, or both conditions as defined above.

Serum cortisol was determined by TDx/TDxFLx assay (Abbott Laboratories, Abbott Park, IL, USA). The normal morning values were 210–620 nmol/l (7.5–22.5 μg/dl). Plasma aldosterone was measured by immunoradiometric assay (IRMA) (Endocrine Sciences Inc., Calabasas Hills, CA, USA). Normal morning levels were 2.2–13.3 pmol/l (10–60 pg/ml). Serum aldosterone was measured by radioimmunoassay (RIA) (Nichols Institute, San Juan Capistrano, CA, USA). Reference morning values on a normal salt diet were 55.5–250 pmol/l (2.5–9 ng/dl) for supine position. PRA was determined by measurement of generated angiotensin I. (RIA; NEN Life Science Products, Boston, MA, USA). Reference values for PRA on a normal salt diet were 0.22–0.67 ng/l/h (0.8–2.4 ng/ml/h) in supine position.

Tests of adrenocortical autoimmunity
We employed a clinically available indirect immunofluorescence assay to determine adrenal antibody titres (Quest Diagnostics, San Capistrano, CA, USA). Briefly, dilutions of patient serum were applied to monkey adrenal gland sections (Mardx Diagnostics, Carlbad, CA, USA; product 10-6304). Sections were then exposed to fluorescein-tagged anti-human gamma globulin and analysed with fluorescent microscopy. If a positive pattern was noted the titre was taken to 1:40 and a higher titre was simply reported as >1:40. A titre of <1:10 was reported as normal.

In a subset of women we measured antibodies against recombinant human adrenal 21-hydroxylase (Endocrine Sciences, Calabasas Hills, CA, USA). Patient serum was incubated with radiolabelled recombinant human 21-hydroxylase, bound antibodies were precipitated by protein A, and the radioactivity of the precipitate was measured (normal <1.2 IU/ml).

Statistical analysis
Association was tested by Fisher’s exact test (Statistica for Windows, StatSoft Inc., Tulsa, OK, USA) and confidence intervals (CI) were calculated with α set at 0.05.

Results
In four of the 123 women tested we uncovered previously unsuspected adrenal insufficiency (3.2%; 95% CI: 0.2, 6.4, Table I). All four had positive adrenal and 21-hydroxylase antibodies (Table I). One of these patients had profoundly abnormal adrenal function tests despite the fact that she was asymptomatic on initial systems review. Her blood pressure was 94/61 and her pulse was 75. Her serum electrolytes were within normal limits (Table I, patient 1). Two other patients had a clearly abnormal standard ACTH stimulation test and an abnormal mineralocorticoid axis (Table I, patients 2 and 3). One patient had a clearly abnormal mineralocorticoid axis and an elevated baseline plasma ACTH yet her standard ACTH stimulation test remained normal on repeated testing (Table I, patient 4). This patient had an elevated PRA and a low aldosterone/PRA ratio indicating primary mineralocorticoid insufficiency.

When used as a primary screening test in this population the standard ACTH stimulation test yielded two false positive results indicating adrenal insufficiency, when in fact the women had a completely normal adrenal function. In both cases the women had a negative adrenal antibody test, a normal standard ACTH stimulation test on repeat examination, as well as a normal basal ACTH level and a normal aldosterone/PRA ratio (Table II). This yields a false positive rate of 1.7% when a single standard ACTH stimulation test is employed as a screening method (2/119; upper 95% confidence limit of 5%).

When used as a primary screening test in this population the morning serum cortisol level was misleadingly in the normal range (>206 nmol/l or 7.5 μg/dl) in three of four women subsequently shown to have adrenal insufficiency. Only one of 11 women with a morning cortisol below normal was found to have adrenal insufficiency (Table III). If a morning cortisol level >550 nmol/l (20 μg/dl) is accepted as evidence of adequate adrenal function, the test was able to rule out adrenal insufficiency in only 12 of 119 women with normal adrenal function (10%). In most cases the measurement of morning cortisol added no useful information, and in 10 cases performing this test as a screening method would have led to an unnecessary ACTH stimulation test. The finding of a low morning serum cortisol level was not significantly associated with adrenal insufficiency and thus this test would not be expected to perform well as a primary screening method (Table III).
Adrenal insufficiency is a potentially fatal condition that is readily treatable. Signs and symptoms are a late manifestation of the disease. Patient number one in Table I tests at the time of our testing (Table I, patients 5 and 6). We found a high concordance between adrenal antibodies detected by indirect immunofluorescence and immunoprecipitation of radiolabelled 21-hydroxylase (100%; 107/107; lower 95% confidence limit 97%). Of the six women who tested positive for adrenal antibodies, three had no other evidence of auto-immunity and three had associated thyroid auto-immune disease (two had Hashimoto thyroiditis with hypothyroidism and one had Graves disease).

Adrenal antibodies were highly associated with impaired adrenal function as were the results of the single standard ACTH stimulation test used as a screening method (Table III). A positive adrenal antibody test increased the pretest probability of detecting adrenal insufficiency from 3–67%.

**Discussion**

With an in-depth evaluation of adrenal function we found a 3.2% prevalence of previously undetected adrenal insufficiency among 123 consecutive young women with spontaneous POF (95% CI 0.2–6.0%). This is a 300-fold increase compared with the general population.

Auto-immune adrenal insufficiency is a potentially fatal condition that is readily treatable. Signs and symptoms are a late manifestation of the disease. Patient number one in Table I
clearly illustrates this point. She had markedly elevated serum ACTH levels and low cortisol levels despite the absence of recognizable symptoms, hyperpigmentation, or electrolyte abnormalities. Of special note is the absence of hyperpigmentation in the presence of such markedly elevated ACTH levels. This might be explained by a short duration of the adrenal insufficiency, by the patient's fair complexion or by her limited exposure to sunlight.

Our findings support a recommendation that before any elective surgical procedure all women with spontaneous POF should be screened for the presence of asymptomatic adrenal insufficiency. Furthermore, because not all surgery is elective, our findings justify a recommendation to screen routinely all women with spontaneous POF for this condition.

Our study suggests that serum adrenal antibodies would be a useful early marker to detect primary auto-immune adrenal insufficiency in young women with spontaneous POF. Circulating adrenal antibodies are known to be elevated early in the course of auto-immune adrenal disease (Turkington and Lebovitz, 1967; Irvine et al., 1968; Betterle et al., 1987; Belvisi et al., 1993; Hoek et al., 1997; Betterle and Volpato, 1998). We found that in women with spontaneous POF adrenal antibodies are highly associated with the presence of primary adrenal insufficiency ($P < 0.001$). The commercially available adrenal antibody tests performed well in our series demonstrating a sensitivity of 1.0, a specificity of 0.98, a positive predictive value of 0.67 and a negative predictive value of 1.0. These test characteristics should be confirmed in a larger series.

In addition to detecting the current presence of autoimmune adrenal insufficiency in a patient, based on other published series, the adrenal antibody test has the added benefit of predicting women at risk for developing adrenal insufficiency. Patients with auto-immune polyglandular syndrome type 1 (APS1) and positive adrenal antibodies carry the highest risk of developing adrenal insufficiency (close to 100%) (Betterle et al., 1987, 1997). Adult patients with a positive adrenal antibody test and other organ-specific endocrine diseases (without APS1) have a 20–50% estimated risk of developing adrenal insufficiency within 5–10 years (Betterle et al., 1987, 1997; Laureti et al., 1998).

Our experience that commercially available assays employing indirect immunofluorescence or immunoprecipitation of radiolabelled 21-hydroxylase are equally effective is in agreement with the findings of other investigators (Betterle et al., 1999).

The results reported here suggest that measuring morning serum cortisol should not be relied upon as an effective means by which to screen for the presence of adrenal insufficiency in young women with spontaneous POF. Reliance upon this test could lead to false reassurance and a delay in diagnosis; the test failed to detect three women with asymptomatic adrenal insufficiency (all three had positive adrenal antibodies). Furthermore, the morning serum cortisol test is associated with an unacceptably high false positive rate, as it was below the normal range in 10 women who had normal adrenal function. We found in our record review that almost 25% of our patients had been screened for adrenal insufficiency by measurement of morning serum cortisol, as recommended by some textbooks (Speroff et al., 1994; Mishell et al., 1997). Our experience suggests that morning serum cortisol should not be used as a screening test to detect adrenal insufficiency.

The standard ACTH stimulation test was developed as a diagnostic tool to detect clinically significant adrenal insufficiency. The findings reported here suggest that the standard ACTH stimulation test should not be used as a routine screening test to detect adrenal insufficiency in asymptomatic young women with spontaneous POF. The test was falsely positive in two women with absolutely normal adrenal function. False positive results provided by a definitive diagnostic test, when used as a screening test, create the risk of unnecessary treatment. Based on our experience we recommend that the standard ACTH stimulation test should be reserved as a diagnostic test to confirm adrenal insufficiency in women with a high pretest probability for the disease, i.e., those women with signs and symptoms of adrenal insufficiency or a positive adrenal antibody test.

In conclusion, women with spontaneous POF are at increased risk for auto-immune adrenal insufficiency. In our view clinicians should routinely screen for this condition when the diagnosis of spontaneous POF is confirmed. A careful history and physical examination and the measurement of serum adrenal antibodies appears to be the best approach. The standard ACTH stimulation test should be reserved as a diagnostic test to confirm the presence of adrenal insufficiency.

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References
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