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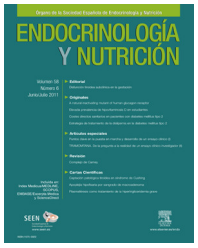

SCIENTIFIC LETTER

High serum testosterone concentrations in a diabetic woman with end-stage renal disease☆

Concentraciones séricas elevadas de testosterona en una mujer con diabetes e insuficiencia renal terminal

A simultaneous pancreas–kidney transplant (SPK) is indicated in patients with type 1 diabetes (T1D) and end-stage renal disease. In patients with type 2 diabetes (T2D) it is not generally indicated,[1] because steroid and immunosuppression treatment exacerbates insulin resistance and weight gain. However, in carefully selected patients with T2D who are not obese, SPK results may be similar to those observed in T1D.[2] Experiences with other types of diabetes are seldom reported although one study showed a favorable outcome in monogenic diabetes due to mutations in the HNF1A gene.[3]

The potential candidates for an SPK should be evaluated for any existing neoplasias which would contraindicate the transplant. In women with hyperandrogenism, tumors are rarely the underlying cause, but ruling them out is considered necessary, especially in cases in which clinical and analytical data, such as notably high levels of androgens, are observed.

We report the case of a patient with diabetes and end-stage renal disease who presented high serum testosterone levels during pre-transplant evaluation.

A 32-year-old woman with a 16-year history of diabetes on insulin treatment presented moderate diabetic retinopathy and end-stage diabetic nephropathy for the previous 3 years. Several members of her family had also been diagnosed with diabetes: her father was diagnosed at the age of 30, received insulin treatment and had renal impairment; her mother, her maternal grandmother and a paternal aunt were on hypoglycemic oral agents.

The patient used pre-mixed insulins (30/70) at breakfast and dinner and aspart insulin at lunch. This regime was modified to a basal-bolus scheme with glargine and aspart insulins. She also took candesartan, atorvastatin, calcitriol, omeprazole, amitriptyline and flunarizine.

She was referred for evaluation for an SPK. She reported increased hair growth over the previous two years, but no menstrual disturbances. She had never been pregnant. A physical examination revealed mild hirsutism (Ferriman–Gallwey 8), moderate frontal alopecia, hypotrophic breasts and normal external genitalia. Laboratory analysis revealed an HbA1c value of 6.5%, C-peptide (Cp) 3.79 ng/mL (normal values [*N*] 1.1–4.4), and negative anti-glutamic acid decarboxylase, anti-tyrosine phosphatase and anti-insulin antibodies. Genetic testing proved mutation p.R200W heterocygosis ''non-synonymous-coding'' on exon 3 of gene HFN1A.

Hormonal evaluation was as follows: total testosterone (TST) (follicular phase) 6.13 ng/mL (N in women <0.8 ng/mL), 17-OH progesterone 2.4 ng/mL (*N* 1.0–2.4), FSH 2.5 mU/mL (*N* 1.7–21.5), LH 7.1 mU/mL (*N* 1.0–12.6), androstendione 4.7 ng/mL (*N* 0.8–2.4), DHEA-S 4906 ng/mL (*N* 609–3400). Serum albumin and serum sex-hormone binding globulin (SHBG) levels were normal (4.4 g/dL and 28 nmol/L, respectively). Serum proteinogram was also normal, except for a mild increase in polyclonal gamma fraction: 1.5 g/dL (*N* 0.7–1.3).

Serial dilutions for TST were: 50% 2.0 ng/mL and 25% 1.37 ng/mL. These levels were confirmed by three different analyses in our laboratory using immunoassay Elecsys® Testo II (Siemens Healthcare Diagnostics Inc., USA): 6.99, 7.16 and 9.36 ng/mL. The result from another laboratory which used another immunoassay (Immulite 2000®: Siemens Healthcare Diagnostics Inc., USA) was 7.2 ng/mL. Free testosterone levels were evaluated in an external laboratory with radioimmuneanalysis (RIA Coat-A-Count Free Testosterone, Siemens Healthcare Diagnostics Inc., USA) in two different samples, showing normal results: 1.4 and 3.0 pg/mL (*N* 0.7–3.6).

Imaging techniques (abdominal ultrasonography, magnetic resonance [MRI] and computerized tomography angiography [CTA]) did not show evidence of alterations in the ovaries or adrenal glands. Transvaginal ultrasonography, however, showed a 13-mm mixed solid and cystic lesion in the central portion of the left ovary, with increased peripheral vascularization, although this lesion was not confirmed on a second evaluation. Tumor markers were normal.

Due to the presence of only mild androgenism and the absence of morphologic alterations, other options were considered. Serum testosterone levels were evaluated before (7.47 ng/mL) and after (7.1 ng/mL) hemodialysis. Ruling out the possibility of existing neoplasias was necessary before proceeding to the SPK, so catheterization of the ovarian venous sinuses was performed, which ruled out an

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ovarian origin. Finally, an 18-F-fluorodesoxiglucose positron-emission tomography (PET) was carried out, revealing no pathological uptake.

Given the previous results, the existence of an androgen-producing tumor could be confidently ruled out, and analytical interference was regarded as the most probable reason explaining the laboratory alterations. Since diabetes secondary to mutations in the HNF1A gene could be considered as an individual indication in itself, the SPK was performed. The patient is currently off insulin treatment, and presents an HbA1c level of 4.8%, Cp 3.56 ng/mL and serum creatinine 1.2 mg/dL (*N* 0.5–1.2).

Interestingly, when the laboratory evaluation was repeated, serum TST levels were normal (0.24 ng/mL), despite the same method being used (Elecsys® Testo II, Siemens Healthcare Diagnostics Inc., USA). Postransplant androgen levels were also notably lower. Samples taken prior to the transplant were evaluated once more using our routine method, confirming the elevated levels, but the values were normal when a method adapted to liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used.

The case that we present illustrates two peculiarities: a consideration of monogenic diabetes due to HNF1A mutation as a potential non-standardized indication for an SPK, and analytical alterations, which obliged us to rule out the possible existence of androgen-producing tumors.

In the past, the diagnosis of diabetes was based exclusively on phenotypic criteria, but, currently, Cp levels and pancreatic autoimmunity are also taken into consideration. Because Cp is excreted via the urinary pathway, a patient with diabetes and end-stage renal disease on hemodialysis with Cp levels <10 ng/mL was classically considered insulinopenic. This is the cut-off level currently accepted in SPK protocols in many centers. However, this may be an overestimation. In fact, insulinopenic patients may be relatively well differentiated, even in cases with severe renal impairment and Cp values as low as 0.6, 1.5 or 3 ng/mL.[2] A recent publication suggests that monogenic diabetes due to mutations in the HNF1A gene could be a potential indication for an SPK.[2]

In order to rule out the existence of a tumor as the underlying source of androgen excess in women, there are several imaging techniques available, such as abdominal CT or MRI, or transvaginal ultrasonography. If cases of negative imaging result, catheterization of the adrenal and ovarian venous sinus should be performed.[4] In the case that we present, because adrenal CT and MRI were normal, adrenal vein sampling was difficult, and transvaginal ultrasonography pointed to a possible ovarian lesion, we chose to proceed to ovarian selective catheterization. Ovarian production was then confidently ruled out.

Regarding changes in serum androgen levels associated with renal impairment or with hemodialysis, previous studies have reported lower levels.[5] Pre- and postransplant concentrations in our patient were similar.

Alterations in SHBG or other serum transporting proteins could be alternative possible reasons explaining elevated levels of TST, but normal free testosterone. For instance, in diabetes secondary to HNF4A mutations, transtiretine levels may be decreased.[6] This has not been described for mutations in HNF1A, although, on the other hand, C5–C8 complement levels may be altered[6]. Therefore, there is some evidence suggesting that genetic diabetes may affect hepatic protein production.

In summary, our patient presented mild hirsutism, regular menses, significant high levels of TST and normal free testosterone, all of which determined a convoluted differential diagnosis. Given the discrepancy between symptoms and laboratory results, the suspicion of analytical-assay interference was high, as this has been previously reported, for example, in cases involving heterophylic antibodies, rheumatoid factor, several drugs, conjugated steroids, immunoglobulins, etc.[7,8] All currently available direct immunoassays may provide falsely elevated levels of testosterone in certain samples. These interferences are mostly detected in women, since higher reference values in men may disguise their identification.[7] The reason for the interference remains unidentified in the majority of cases. A recent study described assay-interference in samples of women with renal impairment and hemodialysis using the same method as the one we used in our laboratory. However, serum androgen levels did not reach the markedly increased ones reported in our case.[9]

When assay interference is suspected, samples may be reanalyzed using other methods.[10] Nevertheless, the best option is to use a reference method. LC–MS/MS has proved to be a highly specific technique for the evaluation of serum testosterone and is useful in cases of low concentrations, such as those found in women and children.[7,9] In recent years, this method has become increasingly available in clinical laboratories working with steroid hormones, since immunoassays are not usually sensitive enough to detect variations <1 ng/mL.[9]

The evaluation of pretransplant samples from our patient using LC–MS/MS confirmed that testosterone serum concentrations had always been normal and that, therefore, the elevated levels previously found using an immunoassay method were in fact due to analytical interference. This interference disappeared after the SPK, so we believe that it was related to the presence of end-stage renal disease or to hemodialysis. If an appropriate laboratory method had been used from the beginning, invasive and costly diagnostic procedures could have been avoided, and the SPK could have been performed earlier.

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