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Ovarian vein sampling, and serum and urine testosterone monitoring in ovarian Leydig cell tumors: A report of two cases

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ABSTRACT

Background: Ovarian Leydig cell tumors are rare, testosterone-producing tumors that pose diagnostic challenges. *Cases:* A 36-year-old woman presented with 10 years of amenorrhea, facial hair growth and clitoromegaly. A 59-year-old woman presented after 2 years of voice deepening and terminal hair growth. Testosterone concentrations were elevated for both patients; however, imaging failed to identify ovarian or adrenal pathology. For the first patient, selective ovarian venous sampling was performed with results suggesting right ovarian testosterone production. Right ovarian Leydig cell tumors were found in both patients after salpingo-oophorectomy. Testosterone levels immediately declined following tumor removal.
Conclusion: Additional diagnostic modalities, such as ovarian venous sampling, should be considered when the etiology of hyperandrogenism cannot be identified through lab work or imaging. In addition, sequential post-operative testosterone levels in serum or urine can help confirm adequate removal of the ovarian tumor.

1. Introduction

Hyperandrogenism is one of the most common endocrine disorders and affects approximately 7% of women [1]. Manifestations of androgen excess include ovulatory dysfunction, hirsutism, virilization and alopecia. The differential diagnosis for hyperandrogenism is broad and includes ovarian and adrenal tumors, adverse effects from medication, polycystic ovary syndrome (PCOS), Cushing's syndrome, and adrenal hyperplasia [2]. Although PCOS accounts for 80% of hyperandrogenism in women, it is a diagnosis of exclusion, and ovarian and adrenal pathology must be ruled out [3]. Typically, total testosterone concentrations >200 ng/dl (normal range 9–55 ng/dl) or dehydroepiandrosterone-sulfate (DHEA-S) concentrations >900 μg/dl (normal range 63–380 μg/dl) raise concern for an ovarian or adrenal tumor in women [4]. Here we present two cases of rare Leydig cell tumors of the ovary causing hyperandrogenism, the diagnostic challenges associated with determining the source of the excess testosterone, and the use of serum testosterone measurements to confirm tumor removal.

2. Case 1

A 36-year-old nulligravida woman was referred for a second opinion regarding her hirsutism and amenorrhea. Her symptoms had begun ten years previously with an 18 kg weight gain, hair loss and thinning, deepening of her voice, facial hair growth necessitating hair removal and enlargement of her clitoris. She was initially diagnosed with PCOS. Further evaluation showed an elevated total testosterone level of 512 ng/dl. Transvaginal ultrasound showed no evidence of adnexal pathology. Additional laboratory testing showed an elevated hemoglobin A1C of 6.1% (pre-diabetic range 5.7–6.4%) and hyperlipidemia. The remainder of her results were within normal limits; DHEA-S 54 μg/dl, prolactin 13.4 ng/ml (normal range 3–27 ng/ml), 17-hydroxyprogesterone (17-OHP) 77.4 ng/dl (normal range 0–206 ng/dl) and thyroid-stimulating hormone (TSH) 1.48 mIU/L (normal range 0.35–3.60 mIU/ml). A positron emission tomography (PET) scan was performed in an attempt to localize a testosterone-producing tumor but was unremarkable. On examination, she exhibited a receding hair line, thin hair, acne, central obesity and clitoromegaly with a Ferriman-Gallwey score of 13. There was no indication of adrenal androgen production given a negative PET scan and normal DHEA-S level; therefore, ovarian testosterone production was suspected and selective ovarian vein sampling was performed. Results suggested right ovarian pathology as the etiology of excess testosterone production with a total testosterone of 1684 ng/dl and free testosterone of 528 ng/dl (normal range 0.08–0.74 ng/dl) from the right ovarian vein (Table 1). Due to aberrant venous anatomy,

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Table 1
Venous sampling and measurement of testosterone concentrations for Case 1.

Location	Total testosterone (ng/dl) (Normal range: 9–55 ng/dl)	Free testosterone (ng/dl) (Normal range: 0.08–0.74 ng/dl)
Right ovarian vein	1684	528
Left ovarian vein	n/a[a]	n/a*
Left renal vein	552	120
Superior vena cava	526	122
Peripheral	695	146

[a] Testosterone concentrations were not obtained from the left ovarian vein.

Table 2
Case 1 Testosterone concentrations pre-operatively, immediately post-operatively, prior to discharge and at post-operative visit.

Time points	Serum total testosterone (ng/dl) (Normal range: 9–55 ng/dl)	Serum free testosterone (ng/dl) (Normal range: 0.08–0.74 ng/dl)	Spot urinary testosterone (ng/dl)
0830	342	8.6	25
1028	241	6.6	14
1125	161	4.3	5
POD #3	<20	Undetectable	n/a[a]

[a] Urinary testosterone not obtained on POD 3.

entering the left ovarian vein was unsuccessful after multiple attempts and therefore the left renal vein was sampled. The patient subsequently underwent a laparoscopic right salpingo-oophorectomy. Intra-operatively, she was noted to have clitoromegaly with a clitoral length of 2 cm and width of 1 cm (clitoral index = 200 mm^2; mean clitoral index 18.5 mm^2). The right ovary appeared to be slightly larger than the left but was within normal limits (Fig. 1). Serial serum and urine testosterone measurements were obtained before, during and after surgery and testosterone concentrations were noted to decrease rapidly after removal of the ovary (Table 2). The patient was seen for follow-up on post-operative day (POD) 3, and reported a subjective decrease in the size of her clitoris. Her serum total testosterone was <20 ng/dl. Final pathology demonstrated a 1.5 cm × 1.1 cm moderately differentiated Leydig cell tumor with diffuse staining for inhibin in her right ovary (weight 11 g).

3. Case 2

A 59-year old gravida 2 para 2 woman was referred for elevated testosterone. The patient reported a deepening in her voice over the previous 2 years, occasional acne and course terminal hair on her upper thighs and hands. She had a Ferriman-Gallwey score of 11. She had undergone menopause at age 49 and denied post-menopausal bleeding.

Her total testosterone was elevated at 558 ng/dl. Transvaginal ultrasound demonstrated normal bilateral ovaries with an 8 mm left ovarian follicle. Laboratory results were within normal limits: prolactin 10.1 ng/ml, estradiol 31 pg/ml, TSH 1.49 mIU/L, free thyroxine (T4) 1.28 ng/dl (normal range 0.7–1.37 ng/dl), and DHEA-S 90 μg/dl. She was noted to have a mildly elevated 17-OHP level, at 277 ng/dl, and mildly elevated serum metanephrines, at 71 pg/ml (normal range 12–60 pg/ml). Accordingly, an adrenocorticotropic hormone (ACTH) stimulation test was performed and demonstrated an appropriate 17-OHP level. A computed tomography (CT) scan of the abdomen and pelvis demonstrated minimal thickening of the medial limb of the left adrenal gland, without any adrenal nodules and a 1 cm left ovarian cyst. A magnetic resonance imaging (MRI) scan was also performed and demonstrated a left adnexal 0.9 × 0.9 cm T2 bright non-enhancing cyst. The patient was offered selective ovarian vein sampling or a trial of gonadotropin-releasing hormone (GnRH) agonist therapy to suppress ovarian testosterone production; however, she declined. Due to her age, post-menopausal status, and the knowledge that an ovarian tumor may be small, bilateral salpingo-oophorectomy was recommended and the patient proceeded with surgery. She declined concurrent hysterectomy. Intra-operatively, both ovaries appeared normal. Total and free testosterone levels were noted to decrease rapidly after removal of the ovaries (Table 3). Final pathology demonstrated a 10 mm Leydig cell tumor within the *right* ovary (3 × 1.5 × 1 cm), weighing 4.5 g (Fig. 2). The patient was seen two weeks post-operatively, and her total testosterone level was <20 ng/dl.

4. Discussion

In both cases, pure Leydig cell tumors were diagnosed on pathology, which is noteworthy as steroid cell tumors account for only 0.1–0.2% of all ovarian tumors [5]. Pure Leydig cell tumors of the ovary are analogous to Leydig cells of the testes and are typically unilateral, small (<5–6 cm) and associated with virilization [6]. These tumors are typically benign and there have been only two documented cases of malignant Leydig cell tumors of the ovary [7,8]. Leydig cell tumors are diagnosed histologically, as they typically stain positive for inhibin and calretinin and contain pathognomonic intracytoplasmic crystals of Reinke [9].

For both patients, an initial workup failed to identify a source of excess testosterone production and, given concern about androgen-producing ovarian neoplasms, surgical management was indicated. For Case 1, in order to prevent premature menopause and loss of fertility from bilateral oophorectomy, ovarian vein sampling was utilized in an attempt to localize increased testosterone production. This method

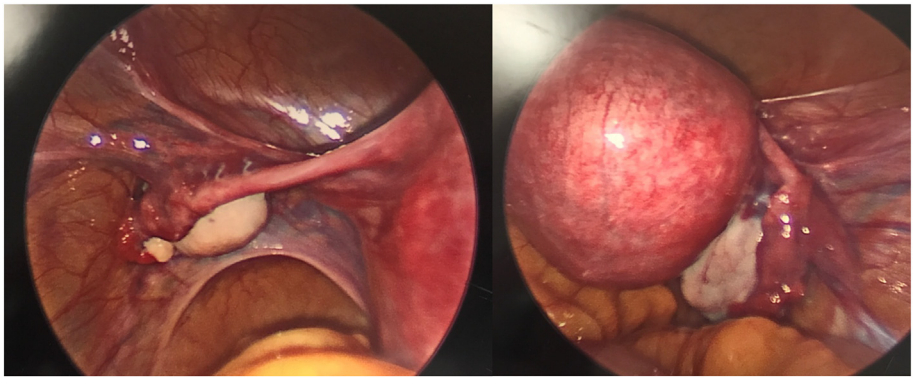

Fig. 1. Laparoscopic views. Left image: left adnexa with normal-appearing left ovary. Right image: uterus and right adnexa with slightly larger right ovary. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Case 2 serum total testosterone measurements pre-and post-operatively.

Time point	Total testosterone (ng/dl)
	Case 2 (Normal range:9–55 ng/dl)
Pre-operative (0834)	260
Immediately Post-operative	159
Prior to discharge (1254)	54
Post-operative appointment (13 days post-operatively)	≤20

has been shown to aid in the identification of small androgen-producing tumors that are not visible with standard imaging modalities. Testosterone in the right ovarian vein was found to be 2.4 times greater than peripheral testosterone concentrations. Although the left ovarian vein could not be entered, left ovarian vein testosterone concentrations were likely to have been significantly lower than those in the right ovarian vein since testosterone in the left renal vein, which drains the left ovarian vein, mirrored peripheral testosterone concentrations. It was previously considered necessary to perform bilateral catheterization of the ovarian veins and adrenal veins in order to adequately interpret results. However, successful catheterization of all four veins has been reported in only 27–45% of cases [10]. According to a study by Levens et al., when peripheral total testosterone exceeded >130 ng/dl, a right ovarian vein to left ovarian vein testosterone ratio > 1.44 detected 90% of right-sided ovarian testosterone-producing tumors [11]. Kuno et al. similarly identified a left ovarian Leydig cell tumor in a patient with signs of hyperandrogenism through selective ovarian sampling despite a normal workup and negative imaging [12].

For Case 2, bilateral salpingo-oophorectomy was performed since the patient was post-menopausal and ovarian pathology was suspected. However, if clinical management had been guided solely by imaging findings, in the setting of an otherwise normal hormonal workup, incorrect unilateral oophorectomy might have been performed. While oophorectomy is the standard of care for postmenopausal women with hyperandrogenism and findings suggestive of benign ovarian pathology, combined ovarian and adrenal sampling should be considered prior to surgery when imaging findings are inconclusive [13].

In Case 1, urinary steroid concentrations using mass spectrometry were obtained before, during and after surgery and urinary testosterone clearance was consistent with serum testosterone decline after tumor removal (Table 2). Urine mass spectrometry allows for quantification of multiple steroid metabolites and their precursors using a single sample, obtained in a non-invasive fashion, which can aid in the hormonal workup of hyperandrogenism.

When evaluating urinary steroid concentrations using direct immunoassay, cross-reactivity between steroids can occur since steroids are mainly excreted into urine as conjugates [14]. For this patient, testosterone was isolated and interference from similar steroid metabolites was decreased by the use of urine mass spectrometry. Mass spectrometry is not routinely used to measure steroid components in urine due to high

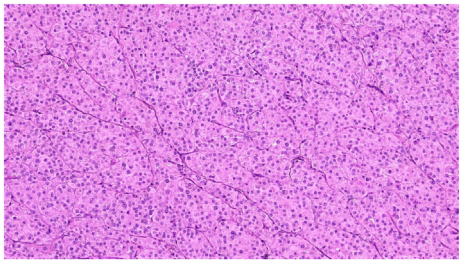

Fig. 2. Leydig cell tumor (Case 2) with uniform polygonal cells, eosinophilic cytoplasm and round central nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

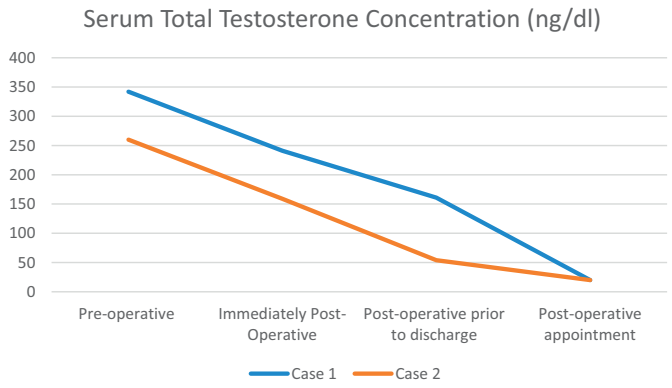

Fig. 3. Serum total testosterone concentrations pre-and post-operatively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cost, slow production rates and technical difficulty [15]. However, as technologic advancements allow for more ubiquitous use of this method, urinary steroid analysis by mass spectrometry may be considered as part of the workup of hyperandrogenism to establish a complete urinary steroid profile using a small sample, all while avoiding invasive blood draws.

Although Leydig cell tumors are rare, testosterone-producing tumors in women, this case report highlights the significant hyperandrogenism associated with these tumors and demonstrates the difficulty in diagnosis. This case report additionally emphasizes the rapid decline in testosterone, seen in both serum and urine measurements, after tumor removal. Within 2 h of tumor removal, serum testosterone had significantly decreased, and within 3 post-operative days had normalized (Fig. 3). Although data on the rate of expected testosterone decline after ovarian tumor removal is limited, incomplete tumor removal could be suspected if there is an insufficient decrease or plateau in serial testosterone levels, since the half-life of testosterone is 20 min [16]. To our knowledge, no case reports or studies have specifically evaluated testosterone clearance immediately after removal of testosterone-producing ovarian tumors. Therefore, physicians may consider implementing serial testosterone measurements before and after surgery when the source of hyperandrogenism is uncertain, in order to confirm adequate tumor removal.

Contributors

Grace Whiteley contributed to data analysis, and the writing of the manuscript.

Olivia Carpinello contributed to data collection and analysis, patient management, and the writing of the manuscript.

Micah J. Hill contributed to the writing of the manuscript.

Alan DeCherney contributed to patient management, and the writing of the manuscript.

All authors have approved the final article.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this case report.

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Patient Consent

Obtained.

Provenance and Peer Review

This case report was peer reviewed.

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