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MENOPAUSAL ANDROGEN EXCESS - ASSOCIATED CARDIO-METABOLIC RISK: CLUES FOR OVARIAN LEYDIG CELL TUMOUR (CASE REPORT AND MINI-REVIEW OF LITERATURE)

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Abstract

Background. Ovarian Leydig cell tumour is a very rare steroid hormones producing mass, causing clinical and biochemical hyperandrogenism. Even if the level of evidence is based on case studies, many authors (but not all) agree that raised androgens increase the cardio-metabolic risk thus early diagnosis and treatment are necessary On the other hand, the endocrine features pointing an ovarian tumour source of testosterone do not indicate the specific histological finding which needs a post-operative conformation.

Case presentation. We report a case of a 60-year-old woman with a 4-year history of progressive virilisation in association with hypertension, high number of red blood cells, impaired glucose tolerance and dyslipidemia. Total testosterone was 20 times above normal with suppressed gonadotropins, inadequate for menopause. Trans-vaginal ultrasound and pelvic and abdominal computerized axial tomography imaging revealed a right ovarian solid nodule, and no evidence of alteration in the adrenal glands. Total hysterectomy and bilateral salpingo-oophorectomy were performed. Histopathology and immunohistochemistry confirmed the diagnosis of Leydig cell tumour. After surgery, androgen levels returned to normal and the doses of anti-hypertensive drugs were reduced.

Conclusions. The hyperandrogenic state with elevated plasma testosterone and progressive signs of virilization raises suspicion of an ovarian androgen-secreting tumor. For a postmenopausal patient with hyperandrogenism the diagnosis of Leydig cell tumour should be considered. However, the exact diagnosis is provided by post-operative histological exam. Prolonged exposure to hyperandrogenism may generate cardiovascular abnormalities and metabolic syndrome which after tumor excision and removal of the source of androgen hormones are expected to significantly improve.

Key words: postmenopausal hyperandrogenism, virilization, ovarian Leydig cell tumor, cardio-metabolic abnormalities.

INTRODUCTION

Leydig cell tumors of the ovary account less than 0.1% of all ovarian tumors (1) and therefore their natural history, management and prognosis are difficult to determine. The clinical course of ovarian Leydig cell tumors is unknown, because there are not collected series in the literature and most of these tumors have been described in literature as case reports or series of cases as level of evidence. They secrete steroid hormones, especially testosterone, leading to hyperandrogenism. Persistent hyperandrogenism can induce systemic complications, which increase morbidity and mortality risk. No precise data are available concerning long-term effects of raised androgen levels. Until now few studies have examined the relationship between androgen levels and the cardio-metabolic risks in postmenopausal women.

MATERIAL AND METHOD

We aim to introduce a case of menopause tumour with ovarian androgen excess and associated cardio-metabolic risk in association with a brief literature review regarding this specific topic.

RESULTS

Case presentation

We present a case of a 60-year-old Caucasian non-smoking female who was admitted to a tertiary centre of endocrinology for a 4-year history of hyperandrogenism, progressive hirsutism, and cardio-metabolic abnormalities. Family clinical history was positive for high blood pressure (mother), but negative for endocrine conditions. With menarche at age of 14,

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she had regular menses and childbirth. Menstruation had ceased at age 47 without vasomotor symptoms.

Medical records

The patient became hypertensive at age of 44. High blood pressure was partially controlled under a combined hypotensive treatment (beta blocker, angiotensin converting enzyme inhibitor and diuretic) and complicated with a stroke at age of 59 (a brain scar of 6 x 28 mm with post-hemorrhagic appearance was discovered at magnetic resonance imagery), without evidence of clinical neurological complications. Since the age of 56, she was diagnosed with unexplained erythrocytosis (hemoglobin 18 g/dL; normal female range between 12 and 16 g/dL; hematocrit 53.8%; normal female range between 35 and 47%) and treated by regular phlebotomy. At the same time, high levels of free and total testosterone were found. In the last four years the patient reported a progressive and significant virilization process including androgenic alopecia, a male type beard, increase in body hair (abdomen and chest), deepening of the voice, plethoric facies, with unspecified etiology. She has been investigated in several medical centers, without receiving a specific treatment since no cause was identified.

On admission

On physical examination patient's body mass index (BMI) was of 26 kg/m^2 with an elevated blood pressure (185/85 mmHg). Severe virilization with male pattern of coarse hair distribution on the face, chest, abdomen, legs, and arms, male type beard, male-pattern alopecia (Ferriman - Gallwey score > 20), deepening of the voice, and loss of female fat distribution, muscular hypertrophy, plethoric face, and redness of upper chest were noted; there was no acne. Abdominal examination showed no abnormality except for mentioned hair and fat distribution. She had no features of Cushing syndrome.

The laboratory findings showed an abnormal hemogram with a high red cells mass (Table 1) and abnormalities of lipid profile as high cholesterol, high triglycerides and low HDL cholesterol, with impaired glucose tolerance (fasting glucose between 122 mg/dL and 137 mg/dL, 2-hours postprandial glucose of 188 mg/dL) (Table 2). Endocrine evaluation revealed a marked increase of total serum testosterone and an increase of delta 4-androstenedione and 17 hydroxy-progesterone concentrations. Serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were inadequately low for a postmenopausal woman. Serum levels of dehydroepiandrosterone-sulfate (DHEA-S), prolactin, Thyroid Stimulating Hormone (TSH) and cortisol were within the normal range. A negative dexamethasone suppression test excluded Cushing's syndrome. Bone profile assays were normal (including central Dual-Energy X-Ray Absorptiometry) except for low 25-hydroxivitamin D levels (13.31 ng/mL, normal levels above 30 ng/mL) (Table 3). Renal and liver functions were within normal limits. Sinus tachycardia and left ventricular hypertrophy were found on electrocardiogram. Gynecological examination revealed normal external genitalia. A painful and firm right adnexal mass was palpable. Trans-vaginal ultrasound showed a large uterus (93.8 x 52.6 mm) with a myomatosis pattern and an endometrial length of 8 mm. A heterogeneous mass of about 46 x 40 mm, with irregular shape, highly hyperechogenic (solid structure), and weak peripheral Doppler signal was seen in right adnexa. No ascites was present (Fig. 1). Computed tomography of the abdomen and pelvis demonstrated a round solid heterogeneous mass (43.7 mm × 28.8mm x 36.5 mm) arising from right ovary. No free fluid or significant lymphadenopathy was observed (Fig. 2). CA125 and HE4 biomarkers were normal

Table 1. Hemoglobin profile during follow-up

Parameter	Patient (56 years)	Reference ranges	Patient (57 years)	Reference ranges	Patient (60 years)	Reference ranges
Haemoglobin	**18**	12-16 g/dL	**18.3**	11.5-15 g/dL	**17.7**	12-15.5 g/dL
Hematocrit	**53.8**	35-47%	**54.5**	37-52%	**53.1**	36-48%

Table 2. Abnormalities of carbohydrate and lipid metabolism

Parameter	Patient's values	Reference ranges	Unit
Fasting glycaemia	**137**	70-99	mg/dL
Glycated haemoglobin HBA1c	5.9	4.8-5.9	%
Glycaemia 2-hour (OGTT)*	**188**	<140	mg/dL
Total cholesterol	**217**	0-199	mg/dL
HDL- cholesterol	**36**	40-60	mg/dL
Triglycerides	**184**	0-149	mg/dL

*OGTT: 75-gram oral glucose tolerance test.

Table 3. Results of hormonal evaluation during follow-up

Hormone	Patient (age 56)	Reference ranges	Patient (age 57)	Reference ranges	Patient (age 60)	Reference ranges (women)
Total testosterone	**13.75**	0.06-0.82 ng/mL	**1308**	<74ng/dL	**>16**	0.1-0.75 ng/mL
Free testosterone	**2.94**	0.1-1.7 pg/mL				
Δ4- Androstenedione					**4.54**	0.35-2.49 ng/mL
DHEA-S			93	35-430 µg/dL	174	8-188µg/dL
17-Hydroxyprogesterone					**3.61**	0.1-0.7 ng/mL
FSH					**0.1**	25.8-135 mUI/mL
LH			**0.128**	mUI/mL	**0.18**	7.7-58.5mUI/mL
ACTH	31.6	3-46 pg/mL			16	3-66pg/mL
Plasma morning cortisol	747.3	171-536 nmol/L			20.75	6.7-22.6 µg/dL
Plasma morning cortisol*					0.94	<1.8 µg/dL
TSH	1.03	0.27-4.2 µUI/mL			0.911	0.5-4.5 µUI/mL
Prolactin					3.9	2.74-19.64 µUI/mL
betaHCG					0	0.1-1 mUI/mL
Chromogranin A					56	20-125 ng/mL
NSE					11.6	0-12 ng/mL
25-hydroxy Vitamin D					13.31	30 ng/mL

DHEA-S: Dehydroepiandrosterone-sulphate; FSH: Follicle-stimulating hormone; LH: Luteinizing Hormone; ACTH: Adrenocorticotropic Hormone; TSH: Thyroid Stimulating Hormone; HCG: Human Chorionic Gonadotropin; NSE: Neuron-specific enolase; *after 1 mg Dexametasone suppression test.

Table 4. ROMA-score of patient

Parameter	Patient	Low risk level	Unit
CA 125	21.1	<35	UI/mL
HE 4	95.4	<140	pmol/L
ROMA (post-menopause)	24.65	<25.3	%

CA 125: Cancer associated carbohydrate antigen 125; HE 4: Human epididymis protein 4; ROMA: Risk of Ovarian Malignancy Algorithm.

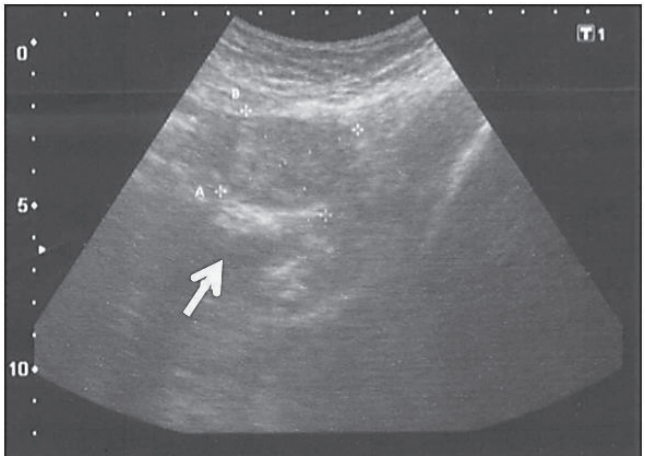

Figure 1. Preoperative transvaginal ultrasound. A heterogenous mass of 46 mm x 40 mm, with irregular shape, highly hyperechogenic and weak peripheral Doppler signal in right adnexa.

and ROMA-score was negative. Neuron-specific enolase (NSE) and chromogranin A were also negative (Tables 3 and 4). Signs of virilization, hormonal data and subsequently the finding of an ovarian mass on imaging suggested an androgen-producing tumor of the ovary as cause of hyperandrogenemia and correlated metabolic disturbances.

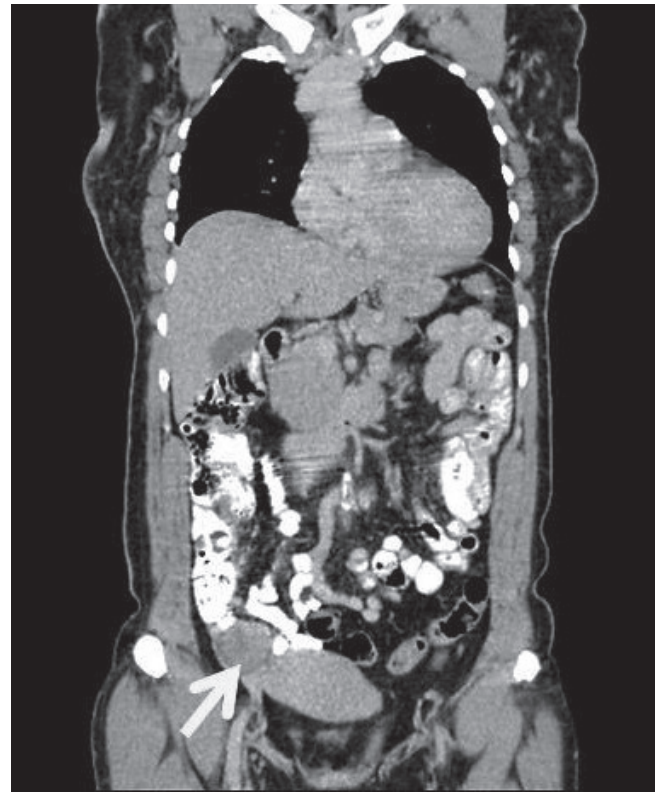

Figure 2. Computed tomography of the abdomen and pelvis with intravenous contrast showing a 43.7 mm × 28.8mm x 36.5 mm soft tissue lesion consistent with a right ovarian mass (arrow).

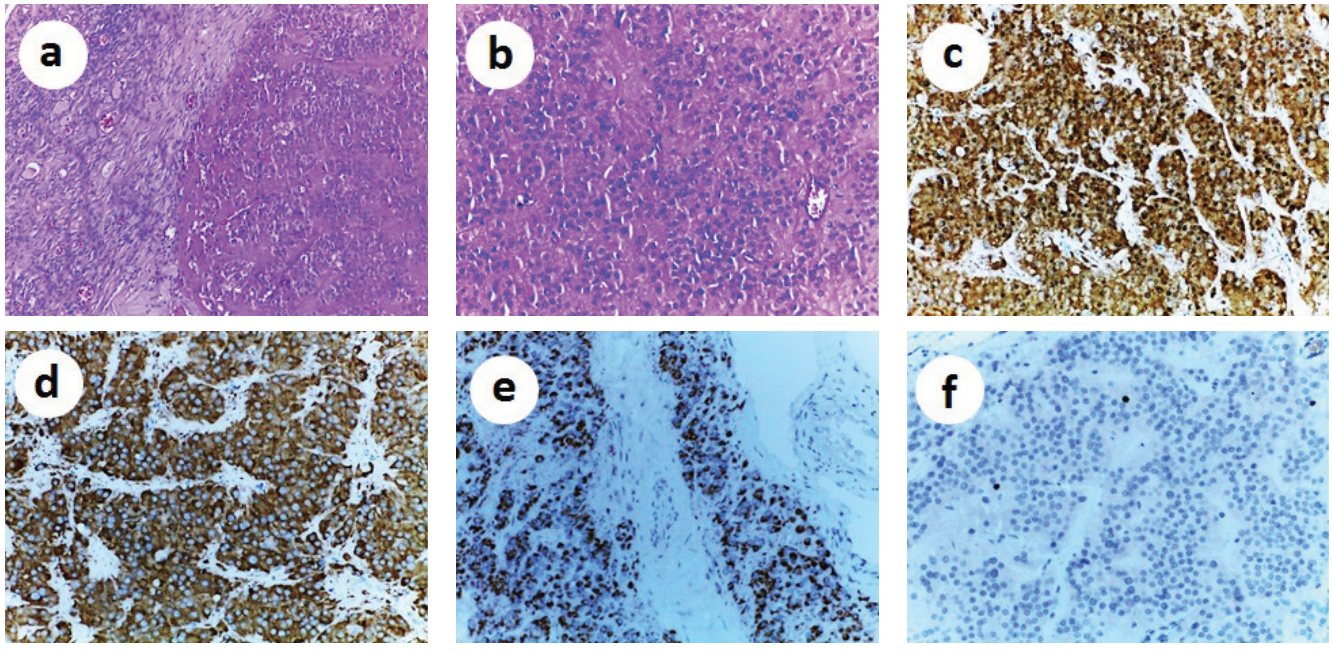

Figure 3. Ovary with a Leydig cell tumor. (A) Ovarian stroma with Leydig cells and spindle stromal cells in a rich vascular network. (HE stain, 10x). (B) Large polygonal or rounded tumor cells with abundant eosinophilic granular cytoplasm, round and central nuclei, with prominent nucleoli (HE stain, 20x). Diffuse positive staining for calretinin (C), inhibin (D) and melan A (E) (IHC, 20x). (F) Weak positive staining for Ki-67(IHC, 20x).

Surgical approach

With this diagnosis the patient was referred to surgery. Total hysterectomy with bilateral anexectomy was performed (through a classical approach), without complications. She was discharged after 7 days. She received anticoagulation for one more week. Pathological report showed a right ovarian solid tumor of 40 mm in association with diffuse uterine leiomyomatosis, without endometrial hyperplasia. Left ovary had serous cysts. Grossly, the right ovarian tumor was round - oval shaped and quite well circumscribed, with a smooth intact surface. Cross section showed a brown surface without necrosis, hemorrhage and cystic degeneration. On microscopic examination, the tumor cells were arranged predominantly in a diffuse pattern and anastomosing cords, in a spindle cell background and a rich vascular network. The tumor areas exhibiting polygonal or round cells with abundant eosinophilic granular cytoplasm, distinct cell borders, round and central nuclei and prominent nucleoli. Reinke crystals were not found in the specimen. Cellular atypia was not noted and mitotic figures were rare. Immunohistochemistry analyses were performed and the results showed diffuse positive staining for calretinin, inhibin, and melan A weak positive staining for MIC2/CD99 and Ki-67 (3%) and negative staining for Wilms tumor protein (WT1) and androgen receptor (AR) (Fig. 3). These findings are consistent with a gonadal steroid tumor, and specifically for a Leydig cell tumor.

Follow-up

Two weeks after tumor removal, the endocrine tests showed normalization of total plasma testosterone (0.22 ng/mL, normal levels between 0.029 and 0.409 ng/mL) while FSH started to increase consistent with ovarian status after androgen excess - related inhibition (7.41 mUI/mL). The doses of anti-hypertensive drugs were progressively reduced.

Narrative review of literature

We present a case with ovarian Leydig cell tumor as cause of hyperandrogenemia with potentially related cardio-metabolic abnormalities where the first sign discovered was increasing plasma levels of testosterone, and subsequently progressive hirsutism. Hyperandrogenism, the most common endocrine disorder in reproductive-aged women, is a rare condition after menopause that needs careful evaluation. A part from exogenous source of androgens, endogenous causes include adrenal and ovarian tumors. Measurement of total testosterone levels may help to identify the majority of ovarian androgen-secreting tumors, especially if the levels are very high.

It has been suggested that androgen excess increases the risk for cardiovascular diseases (myocardial infarction, stroke), obesity, metabolic syndrome and diabetes in postmenopausal women (2). For our postmenopausal patient, the development of signs of hyperandrogenism was accompanied by the

onset or exacerbation of cardio-metabolic abnormalities (erythrocytosis, high blood pressure complicated with a stroke, sinus tachycardia and left ventricular hypertrophy, dyslipidemia, impaired glucose tolerance). One of the most evident effects of increased testosterone levels is secondary erythrocytosis, occurred by stimulating endogenous serum erythropoietin levels and so hyperandrogenism in women can increase red blood cell mass, hemoglobin and hematocrit values, and thrombotic risk (3, 4). Mild hypertension or elevated preexistent hypertension can be due to raised haematocrit and post-operatory improvement is suggestive for hyperandrogenism component. In our case, the large number of red blood cells was discovered at the first admission in endocrinology service and the patient showed elevated blood pressure, which started before the detection of hyperandrogenism. Golden *et al.* found a strong association between free testosterone and the hyperinsulinemia and hyperglycemia, components of the metabolic syndrome in postmenopausal women, supporting a role of androgens in the regulation of glucose homeostasis (5). High androgens adversely alter the lipid profile with increase in LDL-cholesterol, decrease in HDL-cholesterol and increase in triglyceride levels (6). As mentioned previously, in our case impaired glucose tolerance and hyperlipidemia were present. This case reflects a possible relationship between androgen excess and cardio-metabolic risk. However, there is no special clue of histological type regarding the ovarian tumor before its removal and histological exam. This correlation has been described at much lower levels of androgens found in polycystic ovaries syndrome or in Cushing's syndrome - related hypercortisolemia (7). In addition, the presented case had vitamin D deficiency. Even though this may be incidental due to high prevalence in general population, especially in menopause, recent observations link hypovitaminosis D with metabolic syndrome or hyperandrogenic status as found in polycystic ovaries syndrome - related insulin resistance (8, 9).

In our case, androgen excess, signs of virilization, data imaging, and hormonal investigations correlated with pathological examination and immunocytochemistry have established the diagnosis of ovarian Leydig cell tumor. These tumors, usually small (less than 5 cm of diameter) and almost unilateral, are typically seen in postmenopausal women (the mean age is 58 years) as occurs in our case (10). Some tumors arise from ovarian hilus Leydig cells, others are located within the ovarian stroma and arise from non hilus Leydig cells, and in some cases it is impossible to determine the origin of tumoral Leydig cells (11). They produce steroids, especially testosterone, leading to hyperandrogenism . Evidence of virilization is seen in at least one-half of patients. In one-third of patients these tumors may exhibit a hyperestrogenic state, along with endometrial hyperplasia, vaginal bleeding, and ultimately endometrial adenocarcinoma (12-15). Origin of estrogenic changes may include estrogen secretion by the tumor, stromal hyperthecosis and peripheral conversion of androgen (16). In our case the ovarian tumor was associated with hyperandrogenism and virilization, without signs of hyperestrogenism.

Always the hyperandrogenic state with elevated plasma testosterone and progressive signs of virilization raises suspicion of a virilizing tumor. Extremely high levels of total testosterone on a female are very suggestive for a tumor production of either adrenal or ovarian site, which accounts for 5% of all hirsutism cases. Some authors suggested that at least three times above normal level testosterone is consistent with androgen-secreting tumours at the level of ovaries (17). In postmenopausal women, the ovarian causes of virilization are more common than adrenal ones. Adrenal tumours can be either adenomas or carcinomas and secrete other hormones in addition to testosterone, usually glucocorticoids and sometimes estrogens (18). Typically, androgens levels such as testosterone are not as high in adrenal Cushing's syndrome as in androgen producing ovarian tumours (19). In our case serum laboratory analysis revealed elevated free and total testosterone (more than 20 times) and slightly elevated delta 4-androstenedione and 17-hydroxyprogesterone, that indicated an ovarian origin of androgen release, and normal DHEA-S levels that ruled out an adrenal etiology for the hyperandrogenism.

Many of ovarian virilizing tumors are not diagnosed for years, although it seems that, in ovarian tumors, the time to onset of symptoms is usually faster than the other causes of hyperandrogenism (20). Symptoms and signs may present gradually with the onset of symptoms ranging from 5 to 7 years prior the diagnosis (21). Sometimes these tumors can behave in a clinically malignant fashion (22). Usually for the evaluation of androgen excess or virilization syndrome, the patient addresses to an endocrinologist, gynecologist or dermatologist. If androgen excess is extreme and prolonged, androgenic features are accompanied with virilization of the genitals and so, gynecological examination is essential to evaluate the presence of changes in the external genitalia like clitoromegaly or adnexal masses (22, 23). In our patient gynaecological

examination revealed normal external genitalia and a firm right adnexal mass. If clinically evaluated androgen excess seems related to an ovarian tumour, the specific underlined histological type as Leydig cell or Sertoli-Leydig cells containing tumour is confirmed only post-operative based on pathological findings (24, 25). Pre-operatory, no specific clue represents a clear cut evidence for histological profile (24, 26, 27). Dynamic tests such as GnRH (Gonadotropin-releasing hormone) analogue test are useful for confirming the tumour-related androgen excess as proof of cells able to contain the entire enzymatic profile to provide pathological steroidogenesis (28).

For elevated levels of testosterone, ultrasonography of the ovary and adrenal gland, computed tomography, and magnetic resonance imaging are important tools for the diagnosis (29). These tumors may be difficult to identify on imaging procedures , in part because they may be isoechoic to the uterus on ultrasound and isodense on computed tomography (30). For a postmenopausal patient with hyperandrogenism without ovarian or adrenal tumor in radiological images, it was proposed for diagnosis combined adrenal and ovarian venous catheterization (31). In our patient, both pelvic ultrasound and pelvic and abdominal computerized axial tomography confirmed the presence of a unilateral solid ovarian tumor without local invasion or metastasis and morpho-dimensional normality of the adrenal glands. Additionally, uterus larger than normal size in a postmenopausal woman was detected, with a myomatosis pattern and without endometrial hyperplasia. The presence of an ovarian tumor on a patient of menopausal age in association with particular aspects as uterine enlargement may suggest an ovarian cancer (32). However, the constellation of clinical risk factors potentially related to ovarian cancer such as early age at menarche, late menopause, smoking, chronic alcohol intake, obesity, family history of malignancy, contraceptive use, hormonal replacement therapy were negative for this patient. Also neuroendocrine tumors associated with ovarian steroid cell tumours were reported (33), therefore we tested for neuroendocrine markers as chromogranin A and Neuron-specific enolase. Neuroendocrine markers and ROMA-score were negative.

Leydig cell ovarian tumors are primarily managed surgically as other ovarian stromal tumors. Conservative surgery with unilateral oophorectomy is an option in young patients who desire future fertility. For those who have completed childbearing and for older women, total hysterectomy and bilateral salpingo-oophorectomy are the appropriate management options (34). In our case total hysterectomy and bilateral salpingo-oophorectomy were performed, with the consent of patient. It has been reported that the secretion of androgens by Leydig cell ovarian tumor can be inhibited by GnRH agonists that are able to induce suppression of secretions and apoptosis of tumoral cells (35). These effects seem to be related to the presence of GnRH receptors in the tumor cells (36). The use of GnRH agonist is an acceptable choice for treatment of postmenopausal hyperandrogenism in patients where ovarian origin of hyperandrogenism was not established, or in patients with many co-morbidities and high surgical and anaesthesia risk (37).

Leydig cell ovarian tumor is a histological and an immunohistochemical diagnosis. In our case the results of microscopic examination supported the diagnosis of this tumor. According to Deavers *et al.* inhibin and calretinin are the most sensitive markers for Leydig cell ovarian tumors (38). Also LHR, vimentin, cytokeratin, EMA, S100, and CD99 have been reported to be positive (39). Other markers such as HMB45, Chromogranin-A, AFP, carcinoembryonic antigen (CEA) and LeuM1 have been studied with inconclusive results (38, 39). In the present case immunohistochemistry revealed a positive reaction to calretinin, inhibin and melan A, a weak positive reaction to CD99, and a negative reaction to WT1 protein and androgen receptor. The Ki-67 labeling index was low (3%). These features were compatible with a Leydig cell ovarian tumor without histological signs of malignancy.

All cases of Leydig cell tumors reported until now have been benign, with an excellent prognosis and remission of symptoms after surgical treatment. In our case, two weeks after tumor removal, androgen levels had dropped to normal levels (0.22 ng/mL, normal levels between 0.029 and 0.409 ng/mL), while FSH started to increase consistent with ovarian status after androgen excess- related inhibition (7.41). The doses of anti-hypertensive drugs were reduced. We expect progressive reduction of hirsutism over time and improvement in signs of metabolic syndrome. Most of authors (but not all) agree regarding the high testosterone-related component of increased cardio-metabolic risk (40). The patient will be followed up closely and regularly with measurement of androgen hormone levels and pelvic ultrasound as markers of recurrence.

In conclusion, ovarian tumour-associated androgen excess may have long-term negative health

consequences and hence, early diagnosis and prompt treatment are required in postmenopausal women who present with hyperandrogenism. Despite the literature data which are controversial, this case report suggests that prolonged exposure to hyperandrogenism may generate cardiovascular abnormalities and metabolic syndrome with an expectance of improvement after normalization of androgen hormones levels.

Measurement of total testosterone levels represents a useful tool to identify the majority of ovarian androgen-secreting tumors in addition to clinical picture. However, the specific histological diagnosis as ovarian Leydig cell tumour, a rare variety of virilizing ovarian tumour, should be made based on microscopic pictures and immunohistochemistry testing of specific markers, such as inhibin and calretinin.

Conflict of interest

The authors declare that they have no conflict of interest.

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