Discriminating between virilizing ovary tumors and ovary hyperthecosis in postmenopausal women: clinical data, hormonal profiles and image studies

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Abstract

Background: The presence of virilizing signs associated with high serum androgen levels in postmenopausal women is rare. Virilizing ovarian tumors (VOTs) and ovarian stromal hyperthecosis (OH) are the most common etiologies in virilized postmenopausal women. The differential diagnosis between these two conditions is often difficult.

Objective: To evaluate the contribution of clinical features, hormonal profiles and radiological studies to the differential diagnosis of VOT and OH.

Design: A retrospective study.

Setting: A tertiary center.

Main outcome measures: Clinical data, hormonal status (T, E2, LH and FSH), pelvic images (transvaginal sonography and MRI) and anatomopathology were reviewed.

Patients: Thirty-four postmenopausal women with a diagnosis of VOT (13 women) and OH (21 women) were evaluated retrospectively.

Results: Clinical signs of hyperandrogenism were more prevalent in the VOT group than the OH group. Although the VOT group showed higher T and E2 levels and lower gonadotropin levels than the OH group, a great overlap occurred among the hormone levels. A pelvic MRI provided an accurate differentiation of these two conditions.

Conclusion: In this group of patients, the main features contributing to the differential diagnosis of VOT and OH were serum levels of testosterone and gonadotropins and the presence of an ovarian nodule identified on the MRI. Although the association of clinical, hormonal and radiological features contributes to the differential diagnosis of these two conditions, histopathological analysis remains the gold standard for the diagnosis of ovarian hyperandrogenism in postmenopausal women.

European Journal of
Endocrinology
(2017) **177**, 93–102

Introduction

Several disorders may cause clinical manifestations of hyperandrogenism in postmenopausal women. Despite the presence of clinical features, the altered hormonal profile and accessibility to imaging studies, the differential diagnosis of these disorders remains difficult.

The most common causes of virilization in postmenopausal women are ovarian disorders, virilizing ovarian tumors (VOTs) and ovarian hyperthecosis (OH). Different types of VOTs have been described, especially tumors derived from ovarian stroma, which show a low malignancy potential (1). OH is characterized by ovarian stromal hyperplasia associated with cellular luteinization (2). The pathophysiology of OH has not yet been fully elucidated, but it is believed that the stimulation of ovarian stromal cells by elevated gonadotropins in postmenopause, especially luteinizing hormone (LH), may be involved in this process (3, 4, 5, 6). Several authors have observed a significant reduction in serum levels of testosterone after gonadotropin-releasing hormone analog (GnRHa) administration in postmenopausal patients with virilization and an atomopathological study has confirmed the presence of OH (7, 8). These findings indicate a role for continuous ovarian stimulation by elevated gonadotrophins in the pathophysiological process of OH in menopausal women.

The presence of hyperinsulinemia and insulin resistance is also suggested as factors involved in the pathophysiology of OH. Nagamani *et al.* observed that women with OH have a significant degree of insulin resistance, not related to obesity, and suggested that the presence of hyperinsulinemia would act as a stimulatory factor for the production of ovarian androgens, possibly through the induction of stromal luteinization (9). These authors found a significant correlation between peripheral insulin levels and serum levels of testosterone, androstenedione and dihydrotestosterone collected in the ovarian veins. Similar to the findings by Nagamani, other authors have also associated OH with the presence of insulin resistance and alterations in fasting glucose (10, 11).

Some evidence indicates that VOTs are also gonadotropin-dependent conditions (12).

Few studies of hyperandrogenic postmenopausal women have been described. Sarfati *et al.* evaluated the hormonal profile of 22 postmenopausal women who had various conditions, such as neoplastic diseases, including adrenal and ovarian tumors and non-tumor disorders. Comparison of the hormonal levels in these conditions showed higher testosterone levels and lower FSH levels in women with tumor disorders (13).

Studies of patients with VOTs or OH are mainly reported as case reports, or the studies have incorporated a small series of patients. This context has contributed to a poor characterization of the virilizing ovarian disorders in postmenopausal women (5, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23).

We report here the results of a retrospective study of a selected series of postmenopausal women with a histopathological diagnosis of VOT or OH reviewed by an expert pathologist. Clinical data, hormonal profile and pelvic radiological images of 34 women were reviewed.

Patients and methods

This study was approved by the Ethical Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP).

Thirty-four postmenopausal women with histologically confirmed VOT and OH were identified after a retrospective medical record review. All patients were diagnosed with clinical hyperandrogenism and referred to the Endocrinology Unit of HCFMUSP between 1999 and 2013. Although the study was retrospective, one of the examiners (J A M M) evaluated and followed up with all the patients.

The diagnoses of VOT and OH were reviewed and confirmed by an expert in gynecological pathology. The diagnosis of the ovarian tumor type was confirmed by specific criteria and classified according to the World Health Organization 2014 (24). The diagnosis of OH was confirmed by the presence of lutein cells grouped into nests or scattered into the stroma with a typical background of stromal hyperplasia (2).

All women had clinical features, a hormonal profile and an ovarian morphological assessment by pelvic radiological images (transvaginal ultrasound (US) and/or pelvic magnetic resonance image (MRI)) evaluation. A positron emission tomography/computed tomography (PET-CT) scan was performed in five patients.

The clinical data evaluated were menstrual patterns before menopause, number of pregnancies, age at menopause, age at diagnosis, the rate of disease progression, the elapsed time from the onset of symptoms until diagnosis, clinical signs of hyperandrogenism (hirsutism, androgenic alopecia, clitoromegaly, deepening of the voice and muscle hypertrophy). Hirsutism was defined according to the modified Ferriman-Gallwey score ≥ 8 (25). Androgenic alopecia was evaluated by the

Ludwig scale, and the presence or absence of alopecia was used as a criterion of virilization (26). Clitoromegaly was considered as a clitoral body length equal or greater than 2 cm (27). Deepening of the voice and muscle hypertrophy are subjective criteria. These features were considered present if the patient reported the change, and the conditions were also recognized by two different examiners.

Patients with hyperandrogenism and a diagnosis different from VOT or OH were not included in the study.

Hormone measurements

The hormonal evaluation included the measurement of total serum testosterone (T), estradiol (E2), LH and FSH that were measured by immunofluorometric assay (AutoDELFIA, Wallac Oy, Turku, Finland). The intra- and inter-assay coefficients of variation (CV) were <5.0% and <6.0% respectively. Hormonal levels were measured on at least two occasions.

The GnRH analog suppression test (3.75 mg of leuprolide acetate, IM, every 30 days, for 3 months) was performed, and serum levels of LH, FSH, E2 and T were evaluated before and 30 days after the last GnRH α injection.

Imaging studies

Ovarian morphology was evaluated by transvaginal ultrasound (US) in all except two women. A pelvic MRI was performed on 16 women (nine women with OH and seven women with VOT), and the results of 11 images (five women with OH and six women with VOT) were available for blinded review by a single radiologist (B R H). A PET-CT study was performed on five women (four women with OH and one woman with VOT).

Ovarian volume $\geq 4.0 \text{ cm}^3$ was considered an increased ovary size in postmenopausal women (28, 29). The presence of ovarian asymmetry was defined as the largest ovary size greater than or equal to twice the smallest ovary size, and this finding in the imaging studies was suggestive of the presence of an ovarian tumor (30).

Statistical analysis

Descriptive statistics were performed for each variable, and quantitative results are presented as the mean values $\pm$ s.d. The significance of clinical characteristics was evaluated

using Fisher's exact test. A comparison of the quantitative variables between VOT and OH was performed using Student's *t*-test or Mann-Whitney non-parametric test.

The receiver operating characteristic (ROC) curve was performed to determine the optimal cut-off hormonal values and the sensitivity (Se) and specificity (Sp) of the values for a VOT diagnosis.

Results

A total of 34 women with a VOT or OH diagnosis were studied (Table 1). Thirteen women aged 52–77 years were diagnosed with VOT, and 21 women aged 52–80 years were diagnosed with OH. All tumors were unilateral, and OH was bilateral in 20 women and unilateral in one patient who had previously submitted to unilateral oophorectomy.

Three women of the VOT group and two women of the OH group presented with surgical menopause after hysterectomy for myoma. All women studied had a history of monthly menstrual cycles and only two women, one patient in the VOT group and the other patient in the OH group, reported infertility. Premature ovarian failure (at the age of 33 years) was reported by one patient in the VOT group. The presence of clinical signs of hyperandrogenism prior to menopause was reported by two patients with OH.

The clinical data of the natural history evaluated did not demonstrate clinical significance between the two groups of patients, except for a higher number of pregnancies in the VOT group than that in the OH group ($P=0.04$). The elapsed time from the onset of signals and symptoms of the disease until the diagnosis was 4 to 120 months in the VOT group and 12 to 126 months in the OH group ($P=0.630$).

All women had hirsutism, and no difference in the hirsutism score between the two groups ($P=0.749$) was identified. In addition to hirsutism, alopecia was the most frequent hyperandrogenic sign and was present in all women with VOT and in 16 of the 21 women with OH ($P=0.132$). The presence of clitoromegaly was not significantly different between the two groups of patients ($P=0.141$).

The clinical signs such as deepening of the voice ($P<0.001$) and muscle hypertrophy ($P=0.01$) were more prevalent in the VOT group than those in the OH group.

The comparative data of clinical and hormonal parameters are presented in Tables 2 and 3.

There was no difference concerning BMI between the patients with VOT and OH (30.8 ± 5.7 vs 31.6 ± 4.4

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Table 1 Clinical data of 34 postmenopausal women with ovarian hyperandrogenism.

						Signs				
Patients	Age (y)	Meno (y)	ETD (y)	MC	Preg (n)	H	A	C	DV	MH
Tumor										
1	58	50	8	M	2	15	+	+	+	−
2	58	45	10	M	2	23	+	+	+	+
3	62	51	8	M	7	18	+	+	+	+
4	53	40 ¹	8	M	3	23	+	+	+	+
5	65	45	4	M	6	14	+	+	+	+
6	56	33 ²	2	M	3	20	+	+	+	+
7	57	38 ¹	3	M	3	22	+	+	+	−
8	65	43 ¹	0.3	M	6	11	+	+	+	−
9	77	55	10	M	12	31	+	+	+	+
10	52	45	1	M	2	20	+	−	−	−
11	56	48	9	M	5	14	+	−	−	+
12	73	46	4	M	5	10	+	+	+	+
13	53	45	3	M	3	25	+	+	+	N
Hyperthecosis										
14	55	52	3	M	7	22	+	−	−	−
15	55	52	2	M	1	29	+	−	−	−
16	70	50	11	M	5	10	−	+	−	−
17	52	49	2	M	2	21	+	−	−	−
18	62	50	4	M	2	20	−	−	−	−
19	55	40	15	M	3	27	+	−	−	−
20	70	50	15	M	6	21	+	+	−	−
21	54	31 ¹	4	M	0	28	−	+	−	+
22	52	41	2	M	1	12	+	+	−	+
23	61	45 ¹	3	M	N	14	+	+	−	−
24	56	49	6	M	5	19	+	+	−	−
25	56	46	5	M	2	8	+	−	−	−
26	67	51	1	M	3	21	+	+	−	−
27	74	54	2	M	2	2	+	+	−	−
28	56	51	32	M	0	15	+	+	+	+
29	68	53	4	M	7	11	−	+	−	−
30	80	49	5	M	0	26	+	+	−	−
31	59	48	4	M	3	18	−	−	−	−
32	69	53	10	M	4	14	+	N	−	−
33	62	48	10	M	1	28	+	N	+	+
34 ³	52	50	18	M	0	15	+	−	−	−

Meno, menopause; ETD, elapsed time until diagnoses; MC, menstrual cycle; M, monthly; Preg (n), number of pregnancies; H, hirsutism; A, androgenic alopecia; DV, deepening of the voice; C, clitoromegaly; MH, muscle hypertrophy; N, not described; ¹History of hysterectomy due to myomas; ²History of early menopause at 33 years of age; ³Right oophorectomy at 38 years of age because of hirsutism and unknown pathological diagnosis.

respectively, $P=0.653$) (Table 2). Obesity and overweight were diagnosed in 84% of women with VOT and in 95% of women with OH.

Six of 13 women with VOT and 15 of 21 women with OH were diagnosed with type 2 diabetes (DM2) at the first visit to investigate the clinical picture of hyperandrogenism. No statistical significance was observed between VOT and OH patients concerning fasting glucose and fasting insulin.

Hormonal profile

The hormonal profile revealed significant differences in the T and E₂ mean levels; higher levels were observed in

the VOT group than that in the OH group (560 ng/dL vs 182 ng/dL, $P<0.01$ and 65 pg/mL vs 26 pg/mL, $P<0.01$ respectively). Additionally, the LH and FSH mean levels were higher in the OH group than that in the VOT group (25 IU/L vs 9 IU/L, $P<0.001$ and 45 IU/L vs 14 IU/L, $P<0.001$ respectively).

The best cut-off value for hormonal levels to differentiate VOT from OH in our patient groups was $T>312.5$ ng/dL (Se=76.9%; Sp=90.5%), $LH<10.8$ IU/L (Se=76.9%; Sp=90.5%) and $FSH<22.3$ IU/dL (Se=77%; Sp=85.7%).

Two women in the VOT group and 13 women in the OH group were submitted to a GnRHa suppression test, and the suppression of the gonadotropic axis was

Table 2 Comparison of the clinical profile of patients diagnosed with a virilizing ovarian tumor and ovarian hyperthecosis.

	VOT	OH	P value
Age at diagnosis (y)	60.4±7.8	61.2±8.1	0.943*
Onset of symptoms (y)	55.1±8.1	53.7±11.6	0.943*
Elapsed time until diagnosis (y)	5.4±3.5	7.4±7.5	0.630*
Number of pregnancies	4.5±2.8	2.7±2.3	0.048*
Hirsutism	13/13	20/21	>0.999 [§]
Androgenic alopecia	13/13	16/21	0.132 [§]
Clitoromegaly	11/13	11/19	0.141 [§]
Deepening of the voice	11/13	2/21	<0.001 [§]
Muscle hypertrophy	8/12	4/21	0.010 [§]
Body mass index (kg/m ²)	30.8±5.7	31.6±4.4	0.653**

VOT, virilizing ovarian tumor; OH, ovarian hyperthecosis;

*P values from the Mann-Whitney test; **P values from Student's t-test;

[§]P values from Fisher's exact test.

observed in all 15 subjects (the median of LH and FSH after the GnRHa test in VOT vs OH groups were 1.05 IU/L and 9.7 IU/L vs 0.87 IU/L and 8.0 IU/L respectively). The median T levels were reduced to a minimum of 50% after the GnRHa test (from 538 ng/dL to 12.5 ng/dL for the VOT group and from 182.4 ng/dL to 12.7 ng/dL for the OH group).

Radiological characteristics

In the group of patients with VOT, transvaginal US correctly identified the side of the ovarian tumor in five of eleven women (45.5%). The most common transvaginal US finding was a complex cyst present in three of the five patients; in each of the other two patients, a solid nodule was identified. In this group of hyperandrogenic

postmenopausal women, the transvaginal US accuracy of the VOT diagnosis was 48% (Table 4).

In the group of OH patients, transvaginal US identified the ovaries bilaterally in 14 women, unilaterally in six women (one patient previously underwent an oophorectomy), and the ovaries were not identified in one patient. Bilateral increases in the size of the ovaries were observed in eight women diagnosed with OH. The presence of a unilateral solid nodule was observed in three patients (one patient previously underwent an oophorectomy), a bilateral solid nodule was observed in four patients, and asymmetric ovaries without solid or complex cystic lesions were observed in one patient. All seven ovarian nodules detected by transvaginal US in the OH patients were not histologically confirmed (Table 5).

Pelvic MRIs of six patients with VOT were reviewed, and in all patients, nodules were identified on the reviewed images, except in one patient who had a steroid cell tumor (patient 9). The presence of five unilateral nodules (four solid lesions and one solid-cystic lesion) was confirmed; four nodules presented in a hypervascular pattern. The patient without ovarian nodules on MRI presented with asymmetrical ovaries, a suggestive criterion of tumor presence (Table 4).

A hypointense signal of T1-weighted sequences with enhancement after contrast was observed in all ovarian lesions of VOT patients. The signal intensity on T2-weighted sequences was variable (a hyperintense signal in two nodules, a hypointense signal in one nodule and different intensity signals in the other two nodules).

In the OH group, an MRI was performed in nine women, but only five images were available for review. In this group of patients, a pelvic MRI revealed the presence of a unilateral nodule in one patient, an asymmetric ovary

Table 3 Comparison of the hormonal profile and the fasting glucose and insulin levels of patients diagnosed with a virilizing ovarian tumor and ovarian hyperthecosis.

Hormones	Virilizing ovarian tumor		Hyperthecosis		P value
	Mean (±sd)	Median	Mean (±sd)	Median	
Testosterone (ng/dL)	560±434	493	182±89	173	<0.001
Estradiol (pg/mL)	65±64	45	26±9	25	<0.001
LH (IU/L)	9±9	8	25±12	23	<0.001
FSH (IU/L)	14±10	10	45±25	40	<0.001
Androstenedione (ng/mL)	3±2	2	2.5±1.7	2	0.788
DHEAS (ng/mL)	974±531	889	749±447	1	0.222
17OHP (ng/mL)	3±4	2	1.4±0.9	1	0.146
Insulin (μU/mL)	17±10	13	20±10	18	0.520
Glucose (mg/dL)	118±61	96	122±34	112	0.820

*P values from the Mann-Whitney test; Conversion factors to SI units: T, ng/dL to nmol/L, multiply by 0.0347; E₂, pg/mL to pmol/L, multiply by 3.671; Androstenedione, ng/mL to nmol/L, multiply by 3.18; DHEAS, ng/mL to μmol/L, multiply by 0.0371; Insulin, μU/mL to pmol/L, multiply by 7.175; Glucose, mg/dL to mmol/L, multiply by 0.0555.

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Table 4 Pelvic transvaginal ultrasound and magnetic resonance comparison and correlation with anatomopathology of patients with an ovarian androgen-producing tumor.

Pt	Pelvic ultrasound		Magnetic resonance of pelvis		Anatomopathology		
	RO (cm ³)	LO (cm ³)	RO (cm ³)	LO (cm ³)	RO (cm ³)	LO (cm ³)	Size/kind
1	5.2	8.3	6.4 (solid nodule, 2.0 cm)	4.5	Leydig Tu ± OH	OH	1.8 cm/solid
2	9.0	7.0	8.4 (solid nodule, 2.4 cm)	0.8	Leydig Tu ± OH	OH	NM/solid
3	10.0	6.7	–	–	OH	Leydig Tu + OH	2.0 cm/solid
4*	–	–	–	–	OH	Leydig Tu + OH	2.5 cm/solid
5	374.0 (Cyst, 9.6 cm with hypervasc solid vegetation)	1.4	302.3 (solid-cystic, 3.2 cm)	3.2	Hilar Leydig Tu	Not surgically located	4.0 cm/cystic
6	Not visualized	↑	–	–	OH	Steroidic cell Tu NOE + OH	NM/solid
7	Previous surgical removal	31.0 (complex cyst, 4.3 cm, hypovasc)	–	–	Previous surgical removal	Steroidic cell Tu NOE	5.0 cm/solid
8	–	–	0.8	10.1 (solid nodule, 2.4 cm, hypervasc)	Not surgically located	Steroidic cell Tu NOE	3.5 cm/solid
9	12.0	8.0	9.7	19.6	OH	Steroidic cell Tu NOE	NM/solid
10	16.0 (solid nodule, 2.6 cm, hypervasc)	3.0	24.9 (solid nodule, 2.6 cm)	10.5	Thecoma ± OH	OH	2.5 cm/solid
11	Not visualized	Not visualized	–	–	Sertoli-Leydig Tu (MD) + OH	OH	1.8 cm/solid
12	10.3 (solid nodule, 1.6 cm)	1.8	–	–	Sertoli-Leydig Tu (MD) + OH	OH	4.5 cm/solid
13	22.5 (cyst with parietal vegetation of 2.2 cm)	Not visualized	30.0	Not visualized	Sertoli-Leydig Tu (MD) + OH	Normal	4.0 cm/ solid

Pt, patients; RO, right ovary; LO, left ovary; Size/Type, size and histological type of tumor; Tu, tumor; OH, ovary hyperthecosis; NOS, not otherwise specified; NM, not measured; MD, moderately differentiated; Hypovasc, hypovascularized; Hypervasc, hypervascularized; normovasc, normovascularized; *, no image available.

in one patient and ovaries with increased size bilaterally in three women (Table 5). A hypointense signal on T2- and T1-weighted sequences without enhancement after contrast was observed in the ovarian parenchyma of OH patients, except in the patient (patient 14) that presented with a solid nodule with enhancement after contrast, a feature observed in the tumor lesions.

In this group of hyperandrogenic postmenopausal women, the accuracy of the pelvic MRI in diagnosing VOT was 82% ($P=0.01$).

PET-CT was performed on five women (one VOT and four OH) and was able to detect the ovarian lesion in the single VOT patient and was negative in all four women diagnosed with OH.

Discussion

Hyperandrogenism in postmenopausal women is a rare condition that encompasses a broad spectrum of entities. The differential diagnosis of androgen excess syndrome in postmenopausal women remains very challenging, because these diseases may present several similarities in the clinical manifestations and hormonal profile.

Clinical features and the hormonal profile of 22 postmenopausal women with biochemical hyperandrogenism were reported by Sarfati *et al.* (13). Although, the etiology of hyperandrogenism in these women was extremely heterogeneous, including benign and malignant diseases of adrenal and ovarian origin,

Table 5 Pelvic transvaginal ultrasound and magnetic resonance comparison and correlation with anatomopathology of patients with hyperthecosis.

Pt	Pelvic ultrasound		Magnetic resonance of pelvis		AP	
	RO	LO	RO (cm ³)	LO (cm ³)	RO	LO
14	6.5 (solid nodule, hypovasc, 1.1 cm)	4.2	7.6	11.5 (solid nodule, 0.8 cm)	OH	OH
15	16	Not visualized	19.0 (simple cysts)	19.6 (simple cysts)	OH	OH
16	1.2	1	–	–	OH	OH
17	10.3	9.8	–	–	OH	OH
18	7.9	8.6	–	–	OH	OH
19	2.4	4	–	–	OH	OH
20	5.2	10.3	7.4	4.3	OH	OH
21	12.4 solid nodule, hypovasc, 2.1 cm)	7.2 (solid nodule, hypervasc, 1.2 cm)	–	–	OH	OH
22	3.6 (solid nodule, hypovasc, 1.5 cm)	3.2 (solid nodule, hypovasc, 1.4 cm)	–	–	OH	OH
23	Not visualized	4.5	NM (simple cyst, 1.2 cm)	Not visualized	OH	OH
24	15	16	5.3	8.1	OH	OH
25	10.0 (solid nodule, hypervasc, 3.0 cm)	11.1 (solid nodule, normovasc, 3.1 cm)	–	–	OH	OH
26	1.4	2.5	–	–	OH	OH
27	5.3 (multiple anechoic cysts)	3.4 (multiple anechoic cysts)	4.1 (simple cysts)	2.7 (simple cysts)	OH	OH
28	11.8 (solid nodule, hyperechoic, with peripheral calcifications)	12.7 (solid nodule, hyperechoic, with peripheral calcifications)	15.9	18.1	OH	OH
29	6.1	Not visualized	–	–	OH	OH
30	2.7 (solid nodule, hypovasc)	1.5	–	–	OH	OH
31	Not visualized	0.9	–	–	OH	OH
32	2.8	Not visualized	Not visualized	Not visualized	OH	OH
33	Not visualized	Not visualized	1.1	5.6	OH	OH
34	PSR	30.4 (solid nodule with peripheral calcifications)	–	–	PSR	OH

Pt, patients; RO, right ovary; LO, left ovary; AP, anatomopathology; OH, ovary hyperthecosis; NM, not measured; Hypovasc, hypovascularized; Hypervasc, hypervascularized; Normovasc, normovascularized; PSR, previous surgical removal.

it was suggested that testosterone and FSH levels could be useful parameters in the discrimination between androgen-secreting tumors and other diseases evaluated in the study.

Clinical data of 34 postmenopausal women, including the age at diagnosis, the age of onset of symptoms, the elapsed time from the onset of symptoms until diagnosis, or the rate of disease progression were not different between the VOT and OH groups of patients, which was a similar finding in the study by Sarfati *et al.* These findings ruled out the notion that the time to onset of symptoms and the rapidly worsening signs of virilization must be considered as important predictive factors of virilizing ovarian tumors (14, 31).

A prior history of a normal menstrual cycle and normal fertility in our patients were antagonistic features to the hypothesis that the origin of the postmenopausal OH might be an evolved form of PCOS (27). A few reports described women with premenopausal OH and the presence of menstrual disturbances (28).

Signs of virilization in postmenopausal women are uncommon, and the presence of these features should prompt an immediate investigation of a tumor source of androgen excess. Moreover, in virilized postmenopausal women, the OH must be considered an important differential diagnosis (12, 32). The comparative data of clinical features in our cohort showed that the signs of virilization were more prevalent in VOT than those in OH patients. Although, voice deepening and muscular hypertrophy were more prevalent in the VOT group than those in the OH group, these signs were subjectively assessed and slight variations in these parameters could not be identified.

The excess of androgen have been associated with increased advanced glycation end-products (AGEs), metabolic syndrome and increased risk of cardiovascular disease (33). However, these findings were not confirmed in recent studies (34, 35, 36). In our study, 61.7% of postmenopausal women with hyperandrogenism had DM2, but there was no statistically significant difference

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in fasting glucose and insulin values between the VOT and OH groups.

Differences were also described regarding the hormonal profile in postmenopausal women with tumor and non-tumor disease. Sarfati *et al.* reported that T and E₂ serum levels were significantly higher in women with virilizing tumor disease, whereas gonadotropin levels were significantly lower in these patients (13).

We identified this same hormonal pattern in the comparative study between the groups of VOT and OH patients. Women with a diagnosis of VOT had higher T levels than the OH patients, and the gonadotropins levels were lower in the VOT group than those in the OH group. However, an important overlap was observed between the T levels and the gonadotropin levels in both groups of patients.

In the literature, a total testosterone level >200 ng/dL has been advocated as a cut-off level for increased VOT suspicion (31, 37). However, this cut-off level has revealed a low diagnostic accuracy (31). Moreover, it is not clear if this cut-off value could be used in the postmenopausal women group, considering that few studies have been conducted in women in this age group with hyperandrogenic conditions. In the group of patients with OH, we identified seven (33%) women that presented with T levels higher than 200 ng/dL, which is an unlikely finding.

In our study, the best discriminating criteria for the presence of tumor were the levels of T > 312.5 ng/dL (Se=76.9%, Sp=90.5%) and LH < 10.8 IU/L (Se=76.9%, Sp=90.5%), followed by FSH < 22.3 IU/L (Se=77%, Sp=85.7%).

The GnRHa suppression test could be useful in differentiating an adrenal from an ovarian source of hyperandrogenemia (12), although it has not been a useful *tool* in the differentiation between VOT and OH, as both diseases present with the suppression of the gonadal axis (7, 11, 12, 19). All fifteen patients tested in this study showed a decrease in LH and FSH levels after the GnRHa test, and a minimum of 50% reduction in T levels was observed in all of these patients. Two women in the VOT group had suppressed gonadotropin basal levels, which were not observed in the OH group. These findings suggest the involvement of gonadotropins in the pathophysiological mechanism of these two conditions, and an incomplete autonomy of VOT cells.

Despite the fact of mean testosterone (T = 182 ± 89.5 ng/dL) and mean FSH (FSH = 45 ± 24.8 IU/L) levels in the group

of OH patients were elevated, the estradiol levels were only discretely increased (E₂ = 26 ± 8.7 pg/mL; postmenopausal women NV < 25 pg/mL). This condition may be explained because in the postmenopausal women the ovarian aromatase activity is decreased when compared to women during the reproductive years (38, 39). The activity of aromatase in non-gonadal tissues, that has an important role in estrogen synthesis in postmenopausal women, is not dependent of the FSH levels (39).

Conventional radiological criteria have established that the identification of a significant asymmetry between the ovarian volumes or the presence of a nodule in one of the ovaries may indicate the presence of an ovarian tumor, whereas the bilateral increased ovarian volume is characteristic of the presence of OH. However, some cases reported in the literature have demonstrated the presence of unilateral OH, as well as the presence of bilateral tumors (22, 40, 41). The absence of nodules or complex cysts does not exclude the presence of small tumors, and the presence of an ovarian nodule does not confirm the presence of a tumor; some of these conditions were identified in our patients.

Unlike the transvaginal US, the pelvic MRI showed good sensitivity (83%), specificity (80%) and accuracy (82%) in the diagnosis of VOT in this group of hyperandrogenic women. The characteristic image of the VOT observed in the pelvic MRI was a solid nodule with a hypointense signal on T1 with enhancement after contrast. Differently, in patients with OH, the ovarian characteristic MRI image was a bilateral enlargement of the ovary with a hypointense signal on T2 and T1 without enhancement after contrast.

The experience with PET-CT in virilizing syndromes in postmenopausal women is limited (42). Although the result was positive in our single patient, the literature is controversial (20, 43, 44). Moreover, this is the first report about the employment of PET-CT in women with hyperthecosis and suggests that this method is a compromised one in discriminating between VOT and OH, at least in postmenopausal women. However, this result needs to be confirmed by other studies.

The differential diagnosis between these two conditions is often difficult and bilateral oophorectomy is still the treatment of choice in postmenopausal women with ovarian hyperandrogenism. Clinical, hormonal and pelvic MRI findings suggestive of OH may help consider the option of GnRH analog treatment for women who refuse surgery or have high-risk surgery (33).

Conclusion

In this group of patients, the main features contributing to the differential diagnosis between VOT and OH were serum levels of testosterone and gonadotropins and the presence of an ovarian nodule on the pelvic MRI. Although the association of clinical, hormonal and radiological features contributes to the differential diagnosis between these two conditions, the histopathological analysis remains the gold standard for the differential diagnosis of ovarian hyperandrogenism in postmenopausal women.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality in this study.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPq (grant numbers 158332/2014-8);

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Received 8 February 2017

Revised version received 31 March 2017

Accepted 21 April 2017