European Journal of Obstetrics & Gynecology and Reproductive Biology 177 (2014) 121–125

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Serum AMH levels in the differential diagnosis of hyperandrogenemic conditions

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ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 5 March 2014
Accepted 19 March 2014

Keywords:
AMH
PCOS
LOCAH
Hyperandrogenism
Hirsutism

ABSTRACT

Objective: To investigate the diagnostic potential of anti-Müllerian hormone (AMH) in the differential diagnosis of various hyperandrogenemic conditions.
Study design: Among 2241 consecutive women of reproductive age who were seen at a tertiary care university hospital with complaints of acne, hirsutism, androgenetic alopecia, and menstrual dysfunction (oligomenorrhea and/or amenorrhea), 107 patients with serum 17α-hydroxyprogesterone (17α-OHP) levels higher than 2 ng/ml were recruited for this study. An ACTH stimulation test was performed, and basal serum hormonal parameters and AMH levels were measured for all patients.
Results: 25 patients were diagnosed with late-onset congenital adrenal hyperplasia (LOCAH), and 59 patients with polycystic ovary syndrome (PCOS) had significantly higher serum AMH levels than all other groups.
Conclusion: Among hyperandrogenemic patients with serum 17α-OHP levels >2 ng/ml, serum AMH levels might be introduced as a marker to be utilized clinically in the differential diagnosis of hyperandrogenemic patients, especially for identifying patients with PCOS.

Introduction

Polycystic ovary syndrome (PCOS), late-onset congenital adrenal hyperplasia (LOCAH), idiopathic hyperandrogenism (IHA) and idiopathic hirsutism (IH) are the most common diseases which result in hyperandrogenemia in women and all these disorders share common clinical features and laboratory findings [1]. In PCOS, most of the circulating androgens are produced in the ovaries but the adrenal gland also contributes to the hyperandrogenism in these patients [2,3]. Having similarities with PCOS, idiopathic hyperandrogenism is also characterized with an excess amount of ovarian androgens. In contrast to both these conditions, LOCAH is an adrenal gland disorder [4,5], and adrenal androgens constitute the majority of the circulating androgens. Idiopathic hirsutism, on the other hand, presents clinically with increased male-type hair growth, but circulating androgen levels are precisely between normal limits in this particular group of patients. One hypothesis on the pathophysiology of idiopathic hirsutism puts the emphasis on increased activity of the 5-α-reductase enzyme in the skin [6].

Anti-Müllerian hormone (AMH), a member of the transforming growth factor-β (TGF-β) superfamily, is secreted specifically from the granulosa cells of early developing pre-antral and antral follicles [7]. AMH is documented to have an inhibitory role in the ovary, and increased levels of serum AMH may contribute to diminished follicular development [8]. AMH is suggested to be a useful marker of ovarian reserve since it indicates the quantity of the ovarian follicle pool [9]. Serum [10] and follicular fluid [11] AMH levels are documented to be increased in women with PCOS compared to women with normal ovaries. In addition, the serum concentration of AMH correlates with the severity of symptoms [12].

Comparison of serum AMH levels between patients with different hyperandrogenemic diseases has never been investigated so far. Hyperandrogenism may be the symptom of ovarian or adrenal gland pathology, while in some cases both organs may contribute to the elevated levels of circulating androgens. In addition, in some patients the hyperandrogenism may be classified as idiopathic. As a well-known ovarian reserve marker, serum AMH levels may differ in various hyperandrogenemic diseases due to the

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http://dx.doi.org/10.1016/j.ejogrb.2014.03.016
0301-2115/

varying extent of the ovarian contribution. Therefore we hypothesized that serum AMH levels might be a useful marker for the differential diagnosis of hyperandrogenemic conditions.

Materials and methods

The medical records of 2241 consecutive women of reproductive age who were seen at the Department of Gynecology and Obstetrics, Cerrahpasa School of Medicine, Istanbul, Turkey, between January 2009 and June 2012 with acne, hirsutism, androgenetic alopecia (hereafter termed alopecia) and menstrual dysfunction (oligomenorrhea and/or amenorrhea) complaints were reviewed retrospectively. Approval from the Human Ethics Committee of Istanbul University was obtained. Age, records of clinical examinations and sonographic evaluations, body mass index (BMI) values, serum levels of hormones measured on the third day of the menstrual cycle (AMH, follicle stimulating hormone (FSH), luteinizing hormone (LH) estradiol (E2), prolactin (PRL), thyroid stimulating hormone (TSH), 17-alpha-hyrdoxyprogesterone (17α-OHP), dehydroepiandrosterone sulphate (DHEAS), total testosterone, free testosterone, and 1–4 androstenedione) were recorded.

A group of 107 patients with serum 17α-hydroxyprogesterone (17α-OHP) levels higher than 2 ng/ml were recruited in this study. All the patients presented with the features of clinical hyperandrogenism. Acne was described as the presence of comedones on the face, neck, upper chest, upper back, or upper arms. Hirsutism was considered when a woman had a score of ≥8 on the Ferriman-Gallwey scale [13]. Women with androgenetic alopecia who normally exhibited diffuse hair thinning over the top of the scalp were defined according to Ludwig classification system [14]. Oligomenorrhea and amenorrhea were described as menstrual cycles longer than 40 days and the absence of a menstrual period for three consecutive months, respectively. Body mass index (BMI) was calculated as kg/m^2. Criteria for inclusion were as follows: age between 15 and 40 years, BMI values of 18–30 kg/m^2, normal serum PRL and TSH values, normal gynecological sonographic examination and negative cervical smear test result.

All patients were examined by transvaginal ultrasonography with a 7-MHz transvaginal transducer (Sonoline Elegra; Siemens SAS, Saint-Denis, France) on the 3rd or 4th day of the menstrual cycle. Sonographic evaluations were performed by experienced sonographers. None of the patients had a history of any medication which could have influenced the hormonal parameters for the past six months. Patients with known systemic illnesses such as hypothalamic, pituitary and adrenal gland disorders were excluded from the study. Informed consent was obtained from all women who participated in the study.

As the first step of the evaluation process, an ACTH stimulation test was performed on all women in the fasting state and in the supine position between 08.00 and 09.00 am of the 3rd–5th day of their cycle. A heparin lock was placed in the forearm prior to any venous blood sampling. Venous blood samples were collected at baseline and at 60 min after the intravenous injection of 0.25 mg synthetic ACTH (Synacthen, Ciba-Geigy, Basel, Switzerland). Serum was separated afterwards and stored at −20 °C until it was assayed for 17α-OHP levels. An ACTH-stimulated 17-OHP level >10.0 ng/ml was considered as a criterion for non-classical or 'late-onset' CAH (LOCAH). Twenty-five patients out of 107 were diagnosed with LOCAH.

Fifty-nine of remaining 82 patients were diagnosed with PCOS according to the Rotterdam criteria, due to presence of at least two of the following features: oligo/anovulation, clinical or biochemical hyperandrogenism, and presence of polycystic ovarian morphology (PCOM) on ultrasonographic evaluation. PCOM was defined as the presence of ≥12 follicles of 2–9 mm in diameter and/or ovarian volume ≥10 ml in at least one ovary [15]. Ten patients were diagnosed with idiopathic hyperandrogenism (IHA), based on increased serum androgen levels (free $T > 4.2$ pg/ml, total $T > 5.82$ ng/ml, 1–4 androstenedione >3 ng/ml in follicular phase: the accepted normal range of DHEAS levels varied between ages) with the presence of normal ovulatory cycles, and normal ovaries on ultrasonographic evaluation [16]. Thirteen patients were diagnosed with idiopathic hirsutism (IH), with the presence of normal serum androgen levels (T, free T, 1,4-androstenedione and DHEAS), regular menstrual cycles and no signs of any ovarian abnormality on ultrasonographic evaluation [17].

Measurements of AMH were determined in duplicate using the AMH/MIS enzyme-linked immunosorbent assay kit (Diagnostic Systems Lab, Webster, TX, USA). The sensitivity of the assay was 0.017 ng/ml. The intra- and inter-assay variations were 5% and 8%, respectively. All samples were assayed for 17α-OHP in competitive immunoenzymatic colorimetric method (Dia Metra S.r.l., Milano, Italy). The sensitivity of the assay was 0.009 ng/mL. The intra- and inter-assay variations were <7.4% and <13%, respectively. Reference values are between 0.2 and 1.3 ng/ml in follicular phase in women. DHEAS, free testosterone and total testosterone values were assayed with competitive immunoenzymatic colorimetric method (Dia Metra S.r.l., Milano, Italy). Serum FSH, LH, TSH, E2 and PRL levels were measured by Chemiluminescent Microparticle Immunoassay (Architect Abbott Lab, IL, USA), and 1–4 androstenodione levels were measured by enzyme immunoassay with commercial kits (Biosource, Nivelles, Belgium).

Statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). For the statistical analyses,

Table 1
Comparison of demographical and clinical parameters in LOCAH, PCOS, IHA and IH patients.

	LOCAH *n*: 25	PCOS *n*: 59	IHA *n*: 10	IH *n*: 13
%	23.4	55.1	9.3	12.1
Age (years)	21.08 ± 3.62	21.49 ± 4.18	20.90 ± 2.23	21.56 ± 3.11
BMI (kg/m^2)	25.20 ± 4.16	26.02 ± 4.77	26.40 ± 3.86	24.49 ± 4.40
Cycle length (days)	47.12 ± 23.95[a]	72.12 ± 27.46[b]	30.50 ± 2.83	30.60 ± 3.30
Menstrual Irregularity n/N (%)	14/25(56%)[c,d]	52/59(88.1)[c]	0/10(0)	0/13(0)
Acne, n/N (%)	12/25(48)	27/59(45.7)	2/10(20)	4/13(30.7)
Hirsutism, n/N (%)	21/25(84)	47/59(79.6)	10/10(100)	13/13(100)
Alopecia, n/N (%)	4/25(16)	4/59(6.7)	1/10(10)	1/13(7.6)
PCO PCOM	12/25(48)	50/59(84.7)[e]	0/10(0)	0/13(0)

PCOS: polycystic ovary syndrome; LOCAH: late-onset congenital adrenal hyperplasia, IHA: idiopathic hyperandrogenism, IH: idiopathic hirsutism; BMI: body mass index; PCOM: polycystic ovarian morphology

[a] Tukey: compared to PCOS (*p*: 0.001).
[b] Tukey: compared to IHA, IH (*p*: 0.001).
[c] Chi-square test: compared to IHA, IH (*p*: 0.001).
[d] Chi-square test: compared to IHA, IH (*p*: 0.001).
[e] Chi-square test: compared to PCOS (*p*: 0.001).