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ORIGINAL ARTICLE

Elevated anti-Mullerian hormone in lean women may not indicate polycystic ovarian syndrome

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Background: Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder with clinical features shared with functional hypogonadotrophic hypogonadism (FHH).

Aim: To investigate the usefulness of an elevated (>40 pmol/L) anti-Mullerian hormone (AMH) in identifying PCOS and distinguishing PCOS from FHH.

Materials and methods: 141 patients with an elevated AMH and body mass index either <20 kg/m^2 (lean) or >30 kg/m^2 (obese) were selected and three subgroups analysed – obese, lean, lean with suspected FHH. FHH was diagnosed clinically, incorporating diet, weight and exercise history; confirmatory tests included pituitary MRIs, progestin challenges and endometrial thickness measurements. PCOS features of oligo/anovulation, polycystic ovarian morphology (PCOm) and hyperandrogenism were determined by clinical history, pelvic ultrasound, free androgen index and physical examination, respectively. Features of PCOS and blood levels of AMH, follicle-stimulating hormone, luteinising hormone, sex hormone binding globulin (SHBG) and testosterone were compared between subgroups.

Results: Of 141 patients with elevated AMH, 76 were obese and 65 lean. Greater than one-third of lean women had the clinical picture of FHH. Elevated AMH predicted PCOm and menstrual irregularity across all subgroups but uniquely associated with hyperandrogenism in the obese. Median AMH levels were similar among FHH and non-FHH women. Median SHBG levels were significantly higher (111 ± 73 vs 56 ± 31, $P < 0.001$) in lean women with FHH compared to those without FHH.

Conclusions: PCOS and FHH share common features of elevated AMH levels, oligo-anovulation and polycystic ovarian morphology. AMH did not assist in differentiating FHH from PCOS. A higher SHBG level shows promise as a discriminatory finding in FHH.

KEYWORDS

anti-Mullerian hormone, functional hypothalamic amenorrhoea, polycystic ovarian syndrome

INTRODUCTION

Menstrual disturbances and/or sub-fertility are common concerns among reproductive-aged women presenting to endocrine/infertility clinics. Differential diagnoses vary widely.[1] Healthy reproductive function is directly related to optimal body weight range and energy availability. Both obesity and eating disorders can impact on hypothalamic-pituitary-ovarian (HPO) axis activity[2]

and are frequent features of polycystic ovarian syndrome (PCOS) and functional hypogonadotrophic hypogonadism (FHH) respectively. Classically, PCOS and FHH are considered mutually exclusive, but recent studies suggest overlapping features of the two conditions, namely polycystic ovarian morphology (PCOm).[3]

Given the lack of consensus on how to diagnose FHH, concern arises that FHH could be misdiagnosed as PCOS among lean women. Anti-Mullerian hormone (AMH), a dimeric glycoprotein produced by developing follicles,[4] has gained interest both as a diagnostic marker of PCOS and PCOS 'severity'.[5,6] We compared the inter-relationship between elevated AMH and PCOS characteristics (clinical/biochemical/imaging) among lean and obese women in order to identify the PCOS phenotype at most risk of concealing FHH.

MATERIALS AND METHODS

Participants

This is a retrospective study of women with elevated AMH levels (>40 pmol/L; normal range: 14–30 pmol/L) who attended our clinic for assessment. The AMH >40 pmol/L cut off was chosen based on a recent study that showed 40 pmol/L corresponded to the 95th percentile of controls using our manual assay.[7] Women were included into the study if they had a body mass index (BMI) <20 kg/m^2 (lean group) or >30 kg/m^2 (obese group).

Clinical, hormonal and radiological findings over a three-year period (2012–2014 inclusive) were retrieved from our database. Individual files were reviewed to obtain information about menstrual cycle regularity, clinical hyperandrogenism (acne, hirsutism or alopecia), medical history, ethnicity and lifestyle characteristics. Menstrual cycles were considered irregular if the patient had a history of oligomenorrhoea (mean cycle length > 35 days) or amenorrhoea (mean cycle length > 180 days).

All women in the study had a transvaginal pelvic ultrasound within 12 months of presentation. PCOm was recorded from the ultrasound report as per the attending ultrasonographer.

Patients suspected of FHH

Given a lack of universally accepted criteria for diagnosing FHH, FHH diagnosis was made on strong clinical suspicion. Lean patients (BMI < 20 kg/m^2) were included in the FHH group if they admitted at least one of the following: an eating disorder (anorexia or bulimia nervosa), pre-occupation with weight (daily weighs, overly concerned about any gains), involvement in extreme exercise practices (at least five days of weight/cardiovascular training per week) and reliance on calorie counting. All amenorrhoeic women suspected of FHH had an endometrial thickness of <4 mm confirmed on ultrasound. Some, but not all women, had a confirmatory progestin challenge test or pituitary magnetic resonance imaging. All women with prolactin levels > 1½ × upper limit of normal were excluded from the study (Fig. S1).

Identifying PCOS and clinical phenotypes

Polycystic ovarian syndrome was diagnosed in accordance with the Rotterdam Criteria (2003), when two of the three criteria were present: oligo- or anovulation, hyperandrogenism (free androgen index (FAI) > 4.0% and/or clinical evidence of acne, hirsutism or alopecia) and PCOm[8] and/or the National Institutes of Health (NIH)-Criteria if they had a history of menstrual irregularity and hyperandrogenism.[9]

Patients were categorised into one of four phenotypic groups in accordance with the Rotterdam Criteria. Group I: hyperandrogenism, chronic anovulation and polycystic ovaries (H-CA-PCOm), Group II: chronic anovulation and polycystic ovaries (CA-PCOm), Group III: hyperandrogenism and chronic anovulation (H-CA), and Group IV: hyperandrogenism and polycystic ovaries (H-PCOm).

Clinical and biochemical measurements

Anti-Mullerian hormone was measured using manual plate-based enzyme-linked immunosorbent assays (EIAs). All but two samples were measured using EIA AMH/MIS (Müllerian-inhibiting substance) manual assay (A11893 Immunotech, Beckman Coulter Inc., Marseilles, France). Two samples were measured using Gen II assay (A79765, Beckman Coulter, Brea, CA, USA) in 2014 and thus comparable to the EIA AMH/MIS assay.[6]

Serum sex hormone binding globulin (SHBG) (reference range 30–110 nmol/L) was measured using Elecsys® SHBG immunoassay (Roche Diagnostics, Mannheim, Germany) analysed on a Roche Modular E170 immunoanalyser, coefficient of variation (CV) < 4%. Testosterone (reference range 0.2–1.8 nmol/L) was measured on a Roche Modular E170 immunoanalyser using Testosterone II Cobas E601® immunoassay (Roche Diagnostics) (CV < 5%). FAI was calculated as Testosterone/SHBG × 100 (reference range 0.3–4.0%). Blood samples for follicle-stimulating hormone (FSH) and luteinising hormone (LH) measurements were taken during the early follicular phase of the menstrual cycle. FSH and LH were determined by chemiluminescent immunoassays (ADVIA Centaur and ADVIA Centaur XP Systems; Siemens Medical Solutions).

Statistical analysis

Statistical analysis was undertaken using SPSS 23.0 software (IBM, Armonk, NY, USA). Descriptive statistics were computed for all study variables. The Shapiro–Wilk normality test was used to verify normality of distribution of continuous variables. Data are expressed as mean ± standard deviation and median (interquartile range) for normally distributed and non-normally distributed variables, respectively. Comparisons between groups were made using Fisher's exact test for categorical covariates. Differences in normally distributed continuous variables between groups were analysed by one-way analysis of variance with Tukey's multiple comparisons test. For variables which are not normally distributed, comparisons were made using Kruskal–Wallis test with

Dunn's multiple comparisons test. Spearman *r* was computed for correlations between hormonal variables. *P* values of <0.05 were considered statistically significant.

Ethical approval

The study was approved by the Human Research Ethics Committee (HREC) of the Western Sydney Local Health District (WSLHD).

RESULTS

Clinical and hormonal profile of BMI/FHH subgroups

One hundred and forty-one patients with AMH levels above 40 pmol/L met selection criteria for BMI and were included in the final analysis. Table 1 summarises patient characteristics stratified to BMI and lifestyle practices. One-third of the lean women were suspected of FHH by clinical/biochemical criteria specified *a priori*. Women with FHH were significantly younger than the obese but of similar age to the remaining lean group.

By design, AMH levels were greater than normal range in all patients but did not differ between subgroups (Table 1). There was no correlation ($r = 0.03$, $P = 0.75$) between AMH and BMI across the group as a whole, or within each of the three subgroups (data not shown). As expected, SHBG was significantly lower ($P < 0.001$) and calculated FAI higher ($P < 0.001$) in obese compared to lean women, particularly those with FHH. Median SHBG was two-fold higher in lean women with suspected FHH compared to lean women without FHH. While follicular phase LH was significantly lower in lean women with FHH ($P < 0.001$), median LH and LH/FSH ratio did not differ between obese and lean women without FHH. Half of the obese patients were taking metformin prior to their review. Women with suspected FHH were almost exclusively Caucasian, whereas the remainder of lean women were predominantly from a non-Caucasian background (Indian, Chinese, Japanese and South-East Asian – data not shown).

Elevated AMH as a marker of PCOS

Figure 1 compares the prevalence of PCOS components: (i) PCOm; (ii) menstrual irregularity; and (iii) biochemical/clinical hyperandrogenism among obese and lean women with or without FHH. An elevated AMH was associated with PCOm across all three subgroups, ranging from 83% (obese) to 95% (lean with suspected FHH). Obese and lean women without FHH tended to present with either regular cycles or oligomenorrhoea. In contrast, amenorrhoea occurred almost exclusively in lean women with FHH. Hyperandrogenism, clinical and/or biochemical, was four times more prevalent among the obese compared to lean women regardless of FHH history. Hyperandrogenism was not a feature of any lean women with FHH.

Potential for PCOS misdiagnosis

Prevalence of PCOS between subgroups was assessed prior to any knowledge of diet/exercise history to assess the potential for misdiagnosis (Table 2). PCOS was diagnosed in 76% of obese and 59% oflean patients without FHH. Eighty-six per cent of lean patients with suspected FHH would have met the PCOS criteria if FHH had not been identified (Table 2A). None of the latter group met NIH criteria for PCOS diagnosis regardless of FHH recognition. Among phenotypes, Group II (chronic anovulation and PCO morphology) was most prevalent among lean women, particularly

TABLE 1 Clinical and hormonal comparison of obese and lean women presenting to a fertility clinic with elevated AMH levels

	Obese	Lean (non-FHH)	Lean (FHH)	*P*-value†
Number	76	43	22	
AMH (pmol/L)	55.0 (35.5)	60.8 (27.2)	76.0 (60.0)	0.39
Age (years)	33.4 ± 5.1‡§	31.2 ± 4.1	29.7 ± 4.8	0.002
BMI (kg/m^2)	32.8 (3.1)‡§	19.0 (1.5)	19.2 (1.6)	<0.001
SHBG (nmol/L) (30–110)	29 (22)‡§	56 (31)‡	111 (73)	<0.001
FAI (%) (0.3–4.0)	6.0 (5.9)‡§	1.6 (1.4)	0.7 (0.9)	<0.001
FSH (IU/L) (1.5–10)	5.2 ± 1.6§	6.4 ± 1.8‡	4.2 ± 2.6	0.003
LH (IU/L) (2.0–12)	6.0 (5.0)‡	5.5 (3.0)‡	1.6 (4.0)	<0.001
LH/FSH	1.2 (1.0)‡	1.0 (0.7)	0.5 (0.7)	<0.001
Metformin use	53%‡§	7%	0%	<0.01
Caucasian	63%‡§	35%‡	91%	<0.001

One third of women with BMI < 20 kg/m^2 were identified as having FHH. The remaining lean women shared numerous clinical trends with AMH-matched obese women including; higher LH levels, lower SHBG and/or higher ethnic variability. Results are mean ± SD or median (inter-quartile range). AMH, anti-Mullerian hormone; BMI, body mass index; FAI, free androgen index; FHH, functional hypogonadotrophic hypogonadism; FSH, follicle stimulating hormone; LH, luteinising hormone; SHBG, sex hormone binding globulin.

†*P*-value indicates if differences between means/medians significant across groups.

‡$P < 0.05$ compared to lean (FHH).

§$P < 0.05$ compared to lean (non-FHH).

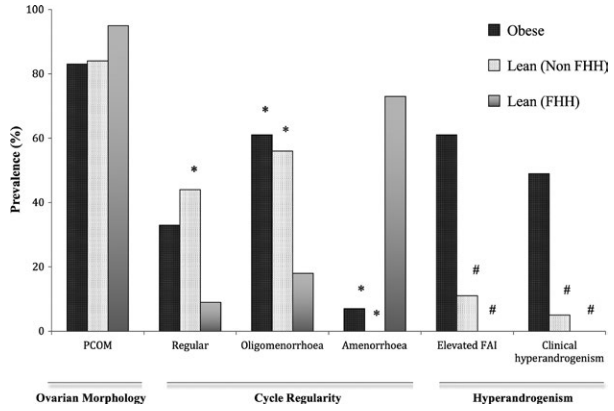

FIGURE 1 Components of polycystic ovarian syndrome (PCOS). Prevalence of polycystic ovarian morphology (PCOm), menstrual irregularity and hyperandrogenism (defined as free androgen index (FAI) > 4% or clinically) among elevated anti-Mullerian hormone-matched lean and obese women. *P < 0.05 compared with lean (functional hypogonadotrophic hypogonadism (FHH)); #P < 0.05 compared with obese.

those with FHH (Table 2B). Among the obese, hyperandrogenic phenotypes dominated, with 71% of patients demonstrating all three PCOS components. Lean women without FHH had the highest prevalence of regular cycles (Fig. 1) and were the least likely to be diagnosed with PCOS (Table 2).

DISCUSSION

In this study, over 40% of lean women with elevated AMH levels and pre-existing 'PCOS' exhibited clinical histories indicative of FHH. The more inclusive Rotterdam Criteria that permits PCOS diagnosis based on PCOm and irregular cycles misdiagnosed four out of five FHH women with the so-called 'lean PCOS phenotype'. Our findings call for a reappraisal on lean PCOS diagnoses, or at the very least, careful consideration when based on AMH levels and/or PCOm features.

PCOm *per se* does not confer metabolic risk in asymptomatic women.[10] It occurs in up to 30% of asymptomatic women with regular cycles[10] and does not predict PCOS progression.[11] Hence, the significance of PCOm needs to be carefully considered with each presentation. There is gathering interest in serum AMH replacing ultrasound ovarian morphology criterion in the diagnosis of PCOS[7] but concerns remain on using AMH as a pathognomonic diagnostic marker.[12] In this study, an AMH level > 40 pmol/L was a good indicator of PCOm (83–95%) in all women regardless of BMI. Its association with hyperandrogenism among obese women meant AMH was also a reliable predictor of PCOS in this group, an association not shared with many lean women. Of concern was the strong positive correlation between AMH and amenorrhoea in lean women with FHH. Consequently, we are of the opinion if AMH were to replace ultrasound in the diagnosis of PCOS, there would be a high likelihood of mislabelling FHH as PCOS in lean women.

The issue lies not with the Rotterdam definition *per se* which clearly stipulates PCOS remains a diagnosis of exclusion,[8] but rather with the clinician's ability to first suspect and second, diagnose FHH. A recent survey showed that despite 83.7% of Australian and New Zealand fertility specialists agreeing it was important to screen for eating disorders during preconception assessments, only 37.5% felt confident in their ability to recognise symptoms of an eating disorder and as many as 65% of fertility specialists did not routinely screen.[13] The overwhelming majority of patients with eating disorders (76.4–100%)[14,15] do not disclose eating disorder history. If a detailed diet and exercise history had not been sought, 86% of lean women with suspected FHH would have been labelled with PCOS. They would have universally fallen into the phenotype of PCOm and chronic anovulation, arguably the least understood (and mildest) of the four Rotterdam phenotypes.[16]

Functional hypogonadotrophic hypogonadism is a condition characterised by oligo/amenorrhoea and oestrogen deficiency due to suppression or insufficient stimulation of the HPO axis, in which no anatomic or organic pathology can be identified.[17] Chronic hypoestrogenism leads to endometrium thinning and a negative

TABLE 2 Prevalence of PCOS and its clinical phenotypes in obese and lean women with AMH levels > 40 pmol/L (A) Prevalence PCOS as per Rotterdam and NIH criteria if diet/exercise history not known and FHH status not identified and (B) PCOS phenotype

	Obese, %	Lean (non-FHH), %	Lean (FHH), %
A			
PCOS as per Rotterdam	76	59	86
PCOS as per NIH	54	9	0
PCOS not identified	24	41	14
B			
Group I (H-CA-PCOm)	71	25	0
Group II (CA-PCOm)	13	75	100
Group III (H-CA)	4	0	0
Group IV (H-PCOm)	13	0	0

AMH, anti-Mullerian hormone; CA, chronic anovulation; H, hyperandrogenism (clinical or biochemical); NIH, National Institutes of Health; PCOm, polycystic ovarian morphology.

progestin challenge. Three main underlying causes of FHH have been recognised, weight loss, and/or vigorous exercise and/or stress, although the aetiology invariably involves a combination of synergistic factors. There is no consensus on what constitutes restrictive nutrition, excessive exercise, or indeed, extreme psychogenic or stress factors, nor is there a reliable clinical investigative or biochemical diagnostic test. A state of energy deficit can occur independent of body weight changes, as is often the case with bulimia nervosa[18] meaning a woman's BMI may not raise the level of suspicion. Regardless of the trigger, FHH is characterised by abnormalities in gonadotropin-releasing hormone (GnRH) secretion or dynamics. Measuring GnRH pulsatility to aid diagnosis[19] is not practical in a clinical setting and while LH levels may be low in women with FHH (as encountered in this study), they are often normal.[20]

Functional hypogonadotrophic hypogonadism and PCOS are the two most common causes of hormonal infertility and may co-exist. A retrospective study comparing FHH women with and without concurrent PCOS demonstrated the latter had a higher incidence of hyperandrogenism and higher mean LH levels.[21] Dual diagnoses are unlikely in our FHH-identified women as none demonstrated hyperandrogenism and all but one had low LH levels. We recognise concurrent PCOS and FHH cannot be excluded in the remaining lean women, potentially resulting in under-reporting of FHH prevalence among lean women with high AMH levels. It is unknown whether lean women with FHH who have both PCOm and elevated AMH levels are at higher risk of progression to PCOS. We appreciate lean women with FHH in this study could represent a 'suppressed' form of PCOS and that weight gain and hypogonadotrophic-pituitary recovery following caloric reconstitution may uncover underlying dysmetabolism.

Sex hormone binding globulin was nearly two times higher in lean patients with suspected FHH compared to BMI and phenotypically matched women with PCOS, and on average, higher than normal. The difference is likely explained by the injudicious dieting, weight gain restriction and resistance training that is intrinsic to FHH.[22] Given the clinical dilemma of differentiating FHH and PCOS in lean women, we propose that a high SHBG could help identify FHH among lean women with PCOm and/or high AMH levels. The relatively lower SHBG seen in both lean women without FHH and obese women suggests possible overlapping metabolic features, namely insulin resistance, which is intrinsic to PCOS.[23]

Knowledge about eating disorders in the context of fertility treatment is important. Ovulation induction using clomiphene citrate is unlikely to be recommended (due to their chronic hypoestrogenic state);[24] exogenous gonadotrophin therapy is likely to require both FSH and LH stimulation[25] and the patient needs to be educated about heightened risk of ovarian hyperstimulation syndrome (OHSS) owing to their dual risk factors of low weight and increased sensitivity to gonadotrophic stimulation.[26] Metformin is not a treatment for FHH and pre/post-pregnancy oral glucose tolerance tests are unnecessary. Finally, obstetric risks such as fetal loss, small for gestational age babies, preterm labour and delivery by caesarean section, are likely to be higher in those women with FHH[27] compared with lean PCOS. Beyond fertility, both bone and mental health screens should be considered.

This pragmatic study has several limitations. First, it is a retrospective analysis from a single fertility practice. Second, most women presented with fertility issues and results may not be generalisable to all women with elevated AMH. Finally, FHH was in part diagnosed on diet, weight and exercise history that could have resulted in inconsistent reporting. However, the findings should stimulate a carefully conducted prospective study with uniform nutrition/exercise questionnaires and more precise adiposity measurements, such as waist circumference measurement and/or dual-energy X-ray absorptiometry scanning.

In summary, PCOS and functional hypogonadotrophic hypogonadism are common diagnoses for women with menstrual disturbances and sub-fertility. Yet, both share common features of oligo-anovulation with PCOm and distinguishing these diagnoses is challenging. We observed a close correlation between elevated AMH levels and PCOm, that AMH appears not to be a hyperandrogenism or PCOS severity marker in lean women and does not assist in differentiating FHH from PCOS. However, SHBG in lean women appears higher in clinically diagnosed FHH than in PCOS, thus unveiling an important and novel area for future reproductive endocrine research.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. Flowchart of patient selection for study.