ANTI-MULLERIAN HORMONE AS A MARKER FOR DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME AND ITS FUNCTION AFTER LAPAROSCOPIC OVARIAN DIATHERMY

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ABSTRACT

Background: Although the polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women of reproductive age, its diagnosis remains one of the most challenging issues in endocrinology, gynecology, and reproductive medicine. The elevated levels of Anti-mullerian hormone (AMH) in the women of PCOS strongly suggested that serum AMH levels may be used in the diagnosis of this condition. Laparoscopic ovarian diathermy (LOD) frequently induces ovulation in patients with polycystic ovary syndrome (PCOS). The mechanism by which this effect occurs remains largely unexplained.

The aim of this study is to assess whether AMH could play a role in the diagnosis of PCOS and to measure changes in (AMH) production in response to (LOD) to see whether this could explain the mechanism of action of (LOD).

Methods: This prospective study included 30 anovulatory women with PCOS and 10 ovulatory women as a control. All PCOS women underwent LOD. Blood samples were collected before and after LOD to measure plasma concentrations of Anti-Mullerian Hormone (AMH), estradiol (E2), gonadotrophins (FSH and LH), and testosterone. The number of early (at day 3 of the cycle) antral follicles (2-9mm in diameter) were estimated by ultrasound scanning.

Results: The mean serum AMH level was about 3-folds higher in PCOS patients than in controls (P < 0.001). Likewise, the 2- to 9-mm follicular numbers per ovary (FNPO) at U/S were 3-fold higher in PCOS patients than in controls (P < 0.001). It was significantly related to the serum AMH levels, both in controls (r = 0.370; P< 0.022) and in patients (r = 0.670; P < 0.0001). AMH was also positively related to the serum testosterone level in cases in comparison to controls (r = 0.454 and P < 0.0004 in cases; versus r = 0.163 and P = 0.288 in controls). No significant correlation was observed between FSH, E2, BMI and AMH levels in PCOS patients and controls. In PCOS patients, the mean serum level of AMH differed significantly by ANOVA (P < 0.05) between those presenting either with amenorrhea (n = 10), oligoamenorrhea (n = 10) or regular cycles (n = 10); the former and the latter having the highest and the lowest values, respectively(8.17ng/ml and16.8 ng/ml). As expected, the 2- to 9mm follicular number per ovary (FNPO)followed the same trend in these three subgroups (P < 0.05), whereas age did not exert a statistically significant effect. Our data suggest that, high androgen (HA) is associated with higher serum AMH levels in PCOS patients with irregular cycles. Following LOD, there was a statistically significant reduction of mean plasma concentrations of AMH from the pre-operative values in the overall group of 30 women with PCOS [8.2ng/ml versus12.2ng /ml]. Women who ovulated after LOD (n = 10) had a higher mean pre-LOD AMH level [15.5 ng/ml] compared with that [10.5 ng/ml] of patients (n = 20) who did not respond. After LOD there was a statistically significant reduction in LH: FSH ratio and the plasma concentrations of LH and testosterone. Plasma concentrations of FSH did not change after LOD.

Conclusions: Anti-Mullerian Hormone could be a valuable marker for diagnosis of polycystic ovary syndrome and improvement of its function after laparoscopic ovarian diathermy. The dramatic and significant reduction of Anti-Mullerian hormone after LOD especially in responders makes it likely that this hormone has a role to play in the mechanism of action of LOD.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting 5-10% of women of reproductive age [1], accounting for at least 75% of cases with anovulatory infertility[2]. It is characterized by a heterogeneous group of disorders that occur in varied combinations including clinical [oligomenorrhoea /anovulation, hirsutism, acne and elevated body mass index (BMI)], biochemical (elevated circulating androgens and/or LH and evidence of insulin resistance) and/or ultrasound features of polycystic ovaries (increased ovarian volume >10 ml and/or number of small follicles >12[3])

Although the polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women of reproductive age, its diagnosis remains one of the most challenging issues in endocrinology, gynecology, and reproductive medicine [4].

The mechanism(s) leading to anovulation is (are) still poorly understood. For many years the excess in intraovarian androgens has been suspected to disturb folliculogenesis, through a proatretic effect on growing follicles[5]. However, more recent experimental data in rhesus monkeys strongly suggest that in fact, intraovarian androgens promote granulosa cell (GC) proliferation and inhibit apoptosis, especially in small follicles whose GCs are the richest in androgen receptors[6]. Accordingly, polycystic ovaries (PCOs) are characterized by an excessive number of growing follicles (2- to 3-fold that of normal ovaries), up to the stage of 2-5 mm in size (small antral follicles)[7]. This phenomenon is thought to result from an impaired action of FSH on the follicle cohort, whose mechanism(s) is (are) unclear [2].

AMH, also known as Mullerian inhibiting substance (MIS), has been mainly studied for its regulatory role in male sex differentiation. AMH, produced by the Sertoli cells of the fetal testis, induces the regression of the Mullerian ducts, the anlagen of the female reproductive tract[8]. However, after birth, this sex-dimorphic expression pattern is lost and AMH is also expressed in granulosa cells of growing follicles in the ovary.

Anti-Mullerian hormone AMH is an ovarian product, a marker of ovarian reserve [9,10] and is a local inhibitor of FSH action [11]. Ovarian production of AMH is higher in PCOS patients compared with women with regular menstrual cycles [4,12], probably due to an excess of antral follicles [13].

Ovarian surgery for ovulation induction has been used in the management of clomiphene citrate-resistant anovulatory women with PCOS. Various types of ovarian surgery have been employed (wedge resection, electrocautery, laser vaporization, multiple ovarian biopsies and others) and all procedures result in an altered endocrine profile after surgery. The mechanism behind the reversal of endocrinological dysfunction in PCOS after ovarian surgery remains incompletely understood [14].

To establish the exact role of AMH in the pathophysiology of PCOS, we measured the plasma concentrations of AMH in a series of PCOS women before and after LOD. the aim of the present study was to assess: 1) whether AMH could play a role in the diagnosis of PCOS, and 2) to assess any change of Anti-Mullerian Hormone after LOD and if this hormone has any role to play in the mechanism of action of LOD. To the best of our knowledge, no studies have been published about the possible AMH changes after ovarian surgery in PCOS patients [14].

MATERIALS & METHODS

Subjects:

Thirty women with anovulatory infertility associated with PCOS who were scheduled for LOD were prospectively included in this study between 2005 and 2006. All women had polycystic ovaries According to The Rotterdam criteria, 2003[15]. The

diagnosis of PCOS was based on the association of at least two of the three following criteria: 1) ovulatory disturbance, mainly oligomenorrhea or amenorrhea, 2) hyperandrogenism (IIA) as defined either clinically by hirsutism or severe acne/seborrhea, and/or biologically by a testosterone serum level greater than 0.7 ng/ml, and 3) more than 12 follicles in the 2- to 9-mm range in each ovary at U/S and/or an ovarian volume higher than 10 ml. All women had previously failed to respond to incremental doses of clomifene citrate. All women gave informed consent for laparoscopic ovarian drilling using diathermy. U/S measurements were taken in real time, according to a standardized protocol, as previously described [13].

Controls:

The control population consisted of 10 women who were infertile due to tubal and/or male infertility. No clinical biochemical or US evidences of PCOS.

Collection of blood samples:

Blood samples were taken from PCOS patients shortly before LOD at a random time in amenorrhoec women and early in the follicular phase (menstrual cycle day 2-4) in menstruating women. Further blood samples were obtained 1 week after surgery.

Hormonal assays:

The hormone assays for FSH, LH, testosterone and progesterone were performed using the electrochemiluminescence immunoassay " ECLTA" (eleisys 1010) Roch Diagnostics Corporation ,Manheim, Germany .

Serum AMH levels were assessed using the second-generation enzyme immunoassay AMH-EIA (reference A16507) provided by Immunotech (a Beckman Coulter company from Marseille, France) according to the supplier's instructions. A recombinant human AMH was used as a calibration standard to build a standard curve ranging from 0-12 ng/ml. Intra- and interassay coefficients of variation were less than 12.3 and 14.2%, respectively.

LOD

The techniques of laparoscopic ovarian drilling

used in this study have previously been published [16-18]. A specially designed monopolar electrocautery probe, was used to penetrate the ovarian capsule at several points with the aid of a short burst of monopolar diathermy. The electrosurgical unit used was the Force 2 Valleylab electrosurgical generator (Valley lab Inc., Boulder, CO, USA). A monopolar coagulating current was used to make three to four punctures per ovary at a power setting of 40 W applied for 4 s per puncture.

Post-operative monitoring for ovulation

Following LOD, patients were followed for 6 weeks for evidence of ovulation. Ovulation was diagnosed by serum progesterone (25ng/l) or by US monitoring of ovulation.

Statistical analysis

Plasma AMH values did not follow a normal distribution and were therefore compared using non-parametric statistical tests (Mann-Whitney U test, Spearman's correlation coefficient and ANOVA test). Other continuous data were normally distributed and were therefore compared using t-test. $P < 0.05$ was considered the minimum level of significance.

RESULTS

The characteristics of 30 women with anovulatory infertility associated with polycystic ovary syndrome (PCOS) are shown in table I. The main clinical, biological, and U/S differences between PCO cases and controls are shown in table II. As expected, patients with PCOS had higher body mass index values, higher mean ovarian volume and higher serum levels of LH and testosterone, than controls. There was a trend toward lower FSH values in PCOS patients than in controls. The mean serum AMH level was about 3-fold higher in PCOS patients than in controls (P _ 0.001). Likewise, the 2- to 9-mm FNPO at U/S were about 3-fold higher in PCOS patients than in controls ($P < 0.001$). It was significantly related to the serum AMH levels, both in controls

(r=0.370; P=0.022) and in patients (r=0.670; P=0.0001). AMH was also positively related to the serum testosterone level in cases in comparison to control (r=0.454 and P=0.0004 in cases; versus r=0.163 and P=0.288 in control) (table III). No significant correlation was observed between FSH, E2, BMI and AMH levels in PCOS patients and controls, see table III.

In PCOS patients, the mean serum level of AMH differed significantly by ANOVA (P<0.05) between those presenting either with amenorrhea (n=10), oligoamenorrhea (n=10) or regular cycles (n=10); the former and the latter having the highest and the lowest values, respectively, (table IV). As expected, the 2- to 9mm FNPO followed the same in these three subgroups (ANOVA, P<0.05), whereas age did not exert a statistically significant effect (table IV). When the PCOS patients with abnormal cycles were gathered together (n=20), we observed that the AMH levels varied significantly (p=0.03) according to the absence (n=10) or the presence (n=20) of HA, as

defined by a serum level of testosterone above 0.7 ng/ml and/ or clinically by hirsutism. Therefore, these data suggest that HA is associated with higher serum AMH levels in PCOS patients with irregular cycles, (table V).

Laparoscopic ovarian diathermy and AMH in PCOS:

Following LOD, there was statistically significant reduction (p <0.001) of mean plasma concentrations of AMH from the pre-operative values in the overall group of 30 women with PCOS [(8.2ng/ml) versus (12.2ng /ml)] (table VI). Women who ovulated after LOD (n = 10) had a significantly higher mean pre-LOD AMH level [15.5 ng/ml] compared with that [10.5 ng/ml] of Women (n = 20) who did not ovulate. Table V, summarizes other endocrine changes after LOD. The results show a statistically significant reduction in LH: FSH ratio and the plasma concentrations of LH and testosterone. Plasma concentrations of FSH did not change after LOD.

Table I : Characteristics of 30 women with anovulatory infertility associated with polycystic ovary syndrome (PCOS).

Characteristic	Mean ± SEM
— Age (years)	29.0 ± 0.6
— Body mass index (BMI) (kg/m2)	27.0 ± 0.7
— LH (IU/l)	8.0 ± 0.9
— FSH (IU/l)	5.6 ± 0.2
— LH: FSH ratio	1.4 ± 0.2
— Testosterone (ng)	0.73 ± 0.2
— Ovarian volume	12.5 ± 1.1
Menstrual cycle pattern	n (%)
Regular	10 (33)
Oligomenorrhoea:	10 (33)
Amenorrhoea:	10 (33)
Hirsutism Yes No	 20 (67) 10 (34)
Acne Yes No	 17 (57) 13 (43)
Infertility 1ry 2ndry	 25 5

TABLE II: Main clinical, hormonal, and ultrasonographic differences between controls and patients with PCOS.

	Controls n_10)	PCOS patients (n_10)	P value
Age (yr)	28 (22-35)	29 (22-36.4)	NS
BMI (kg/m2)	23.4 (18.2 - 31.8)	27 (19 - 39)	– 0.01
LH (IU/liter)	3.7 (2.1-5.9)	8 (3.3 - 14.9)	– 0.001
FSH (IU/liter)	5.8 (4.5-7.3)	5.6 (4.0 - 7.4)	– 0.01
Testosterone (ng/ml)	0.3 (0.17 - 0.45)	0.53 (0.25 - 0.83)	– 0.001
AMH (ng/ml)	2.9 ng (2.3 - 3.8)	6.6 ng (7.3 - 18)	0/001
Ovarian volume	8.2	12.05 ± 1.1	– 0.001
2- to 9- mm follicle no.	6.8 (3.3 - 12.0)	18.7 (7.8 - 44.3)	– 0.001
E2 (pg/ml)	30 (20 - 52)	32 (20 - 47)	– 0.36

Values are expressed as the mean with the 5th-95th percentile range in parentheses. NS, Non significant.

TABLE III: Relationships between the AMH plasma level and BMI or other biological data (serum levels) in patients with PCOS and in controls.

	PCO (N = 30)		Controls (N = 10)	
	Correlation coefficient	P	Correlation coefficient	P
AMH, 2-9mm FN	0.670	0.0001	0.370	0.022
AMH, FSH	0.313	0.018	0.311	0.037
AMH, testosterone	0.454	0.0004	0.163	0.288
AMH, E2	0.083	0.54	0.072	0.64
AMH, BMI	0.061	0.66	0.280	0.048

TABLE IV: AMH levels in patients with PCOS according to their menstrual status.

Menstrual status	Regular cycles	Oligoamenorrhea	Amenorrhea	Pvalue
No. of patients	10	10	10	—
Age (yr)	29.4 (4.1)	29.0 (4.0)	28.7 (4.8)	– NS
AMH (ng/ml)	8.17 (1.1)	11.70 (1.5)	16.80 (1.8)	– 0.05
2- to 9-mm FNPO	15.2 (4.9)	18.6 (9.4)	25.9 (13.3)	– 0.05

Values are expressed as the mean with the (SD).
NS, Non significant.

Table V: Endocrine changes after laparoscopic ovarian diathermy (LOD) in 30 women with anovulatory infertility associated with polycystic ovary syndrome (PCOS). Data presented as mean ± SEM.

Plasma	Before LOD	After LOD	P
LH (IU/l)	12.2 ± 0.9	9.1 ± 0.8	< 0.01
FSH (IU/l)	4.8 ± 0.2	4.7 ± 0.2	0.496
LH: FSH ratio	2.6 ± 0.2	0.9 ± 0.2	< 0.001
Testosterone (ng/ml)	2.7 ± 0.2	2.2 ± 0.1	< 0.001
AMH (ng/ml)	12.2 ± 0.3	8.2 ± 0.2	< 0.001

DISCUSSION

PCOS encompasses a broad spectrum of clinical and biochemical characteristics, and, although the mechanisms leading to PCOS are still poorly understood, the common denominator is a disturbance in the selection of the dominant follicle resulting in anovulation. The defective selection mechanism results in an accumulation of small antral follicles, which contribute significantly to the production of AMH [3].

In the present study, we have measured the circulating concentrations of AMH in 30 women with anovulatory infertility associated with PCOS and studied its association with the clinical, biological, and U/S features of this population and also, the effect of LOD on these levels.

The data showed that most PCOS women in this study as expected, had higher body mass index values and higher serum levels of LH, testosterone, than controls. There was a trend toward lower FSH values in PCOS patients than in controls.

The mean serum AMH level was about 3-fold higher in PCOS patients than controls and than the reported level in normal women [19]. Also, the mean 2- to 9-mm FNPO at U/S was 3-fold higher in PCOS patients than in control women. It was also significantly related to the serum AMH levels, both in controls and in patients.

In 2003, Fanchin et al; demonstrated that the antral follicle count was closely related to the serum AMH level on cycle d 3 in infertile women, in a stronger way than those obtained with other hormonal markers such as inhibin B, estradiol, or FSH [20].

Later, several teams reported on a strong increase (2- to 4-fold) of serum AMH levels in PCOS patients, in close relationship with the increase in the small antral follicular number(FN) [19,21].

Moreover, it was recently demonstrated that AMH levels remain relatively constant during the follicular phase and entire menstrual cycle [12,22] and the measurement of serum AMH levels was highly reproducible from one cycle to another, a fact that underlines its robustness as a biological marker of the ovarian follicle status [23].

Thus, all these data led to the conclusion that AMH could be the best available biological marker of the ovarian early antral FN, both in fertile and infertile normoovulatory women and in PCOS patients.

In this study AMH was also positively related to the serum testosterone level in both groups (r=0.225 and P=0.03 in controls; r=0.360 and P=0.003 in patients). No significant correlation was observed between FSH, E2, BMI and AMH levels in PCOS patients and controls.

This is in agreement with the previous report of

(Eldar-Geva et.al.)[24] that follicle number and testosterone levels independently correlated with AMH serum levels. These results suggest that the non visible pool of follicles may be further increased in the presence of increased androgen level. However, the mechanism behind the positive association between androgens and AMH in PCOS women requires further studies [25].

This lends support to the hypothesis of Jonard in 2004[26] that AMH may be involved in the ovulatory disorder of PCOS patients and may have effects distinct from HA, through inhibition of the FSH-dependant cyclic recruitment[27] and/or aromatase activity [28].

Moreover, we also showed in our study that, in patients with irregular cycles, the presence of HA was associated with a further increase of the AMH values.

This is in agreement with Laven et al.(2004)[21] who also reported a positive correlation between the cycle duration, the serum androgen levels, and AMH in PCOS patients. Our results also agree with those of La Marca et al; 2004[22] showing that amenorrheic women with PCOS had a higher mean serum AMH levels than their oligomenorrheic patients.

In conclusion, it can be proposed from the present study that single measurement of AMH in the early follicular phase in women with HA and/or oligo-anovulation could indicate to the clinicians the presence of PCO when reliable U/S is not available. However, it must be kept in mind that neither a high value of AMH nor a high FNPO is per se is sufficient to ascertain PCOS. Both criteria need to be incorporated into the PCO diagnosis.

In the present study, no significant correlation was observed between FSH, E2, BMI and AMH levels in PCOS patients and controls.

AMH and BMI:

In this study, no significant correlation was observed between AMH and BMI. This comes in

agreement with the studies of Pigny et al. (2003), and Laven et al. (2004) that showed that serum AMH levels do not seem to correlate with BMI and insulin levels[19,21].

In contrast, in a small study, La Marca et al. (2004)[22] observed a positive correlation between serum AMH levels and the HOMA index, an insulin resistance index calculated from fasting insulin and fasting glucose levels.

AMH and FSH and E2.

In this study, no significant correlation was observed between AMH and FSH and E2 .

Durlinger et al. [1] found the absence of regulation of AMH by gonadotropins that was shown in both rodents and man, suggesting that AMH acts as a paracrine rather than a systemic factor, and thus is not part of a negative feedback loop with involvement of gonadotropins.

In a study done by Fanchin et al (2003)[20], they found that AMH correlated more intensely with early antral follicular counts than did the other hormonal markers of follicular status (inhibin B, E2, FSH and LH); the reasons for this striking phenomenon were unclear.

The impact of LOD in PCOS:

A-The impact of LOD on LH: FSH, LH and testosterone:

In this study, the results show a statistically significant reduction in LH: FSH ratio and the plasma concentrations of LH and testosterone after LOD

Van Santbrink et al.(1997) stated that chronically elevated LH concentrations are common and characteristic of women with PCOS and are (partly) responsible for the problems associated with this syndrome[29].

The reduction of testosterone and LH observed after LOD in this study, is consistent with several other previous studies [30]. Others showed that LH levels decreased day after ovarian surgery and

remained low. Some studies showed that the decline is more pronounced in responders compared with non-responders. A few studies found unchanged LH levels weeks to months after surgery [14].

B-The impact of LOD on FSH:

In this study, the results show that Plasma concentrations of FSH did not change after LOD.

Anovulation in PCOS is thought to be partly due to a relative intrinsic inhibition of FSH action[21].

Kamel et al.(2004) and Api et al.(2005) stated that in the first weeks after surgery, FSH concentrations declined to levels comparable to baseline values before ovarian surgery[30,31].

Some studies found elevated FSH in PCO responders, in non-responders.

C-The impact of LOD on AMH (The prognostic value of AMH in women undergoing LOD):

In this study, we have demonstrated that Following LOD, there were statistically significant reduction of mean plasma concentrations of AMH from the pre-operative values in the overall group of 30 women with PCOS (8.2ng/ml) versus controls (12.2ng /ml).Women who ovulated after LOD (n = 10) had a higher mean pre-LOD AMH level (15.5 ng/ml) compared with that (10.5 ng/ml) of patients (n = 20) who did not respond. The results show a statistically significant reduction in LH: FSH ratio and the plasma concentrations of LH and testosterone. Plasma concentrations of FSH did not change after LOD.

The cause for reversal of the endocrinological dysfunction after ovarian surgery in PCOS is unclear. Many theories have been proposed for the cause of the re-establishment of menstrual cycles after ovarian surgery. Originally, the removal of a mechanical barrier allowing gonadotrophins to act more effectively. Others have suggested that surgery may cause increased blood flow to the ovaries. Others suggested reduction of local ovarian androgens with restoration of feedback to the hypothalamus and

pituitary. More recent theories include gonadotrophin surge attenuating factor (GnSAF, also called gonadotrophin surge inhibiting factor: GnSIF). GnSAF is a hormone produced by the ovaries, and its function lies in regulating and suppressing LH secretion by reducing pituitary sensitivity. Deficiency of GnSAF in PCOS patients has been hypothesized. Another suggested reduced inhibin levels after ovarian surgery leading to increased FSH levels. Less is known about the importance of Anti-Mullarian hormone (AMH) regarding ovarian surgery in PCOS [14].

Basic research data obtained from the adult ovary indicate that AMH is mainly expressed in pre-antral and early antral follicles and has either direct or indirect roles in various phases of folliculogenesis[28]. AMH acts as a paracrine rather than a systemic factor, and thus is not part of a negative feedback loop with involvement of gonadotropins. Thus, AMH is not influenced by the gonadotropic status and reflects only the follicle population.

Studies in mice showed that AMH lowers the sensitivity of follicles to FSH[11], possibly contributing to deranged follicle selection. It has been suggested that aromatase activity in PCOS patients might be decreased because follicles from PCOS women do not produce large amounts of E2. AMH also inhibits aromatase activity suggesting that AMH contributes to the severity of PCOS [32].

In 2003, Fanchin et al; demonstrated that the antral follicle count was closely related to the serum AMH level on cycle d 3 in infertile women, in a stronger way than those obtained with other hormonal markers such as inhibin B, estradiol, or FSH[20].

Several teams reported on a strong increase (2- to 4-fold) of serum AMH levels in PCOS patients, in close relationship with the increase in the small antral follicular number(FN) [19,21].

On the bases of these studies we can suggest that the effect of LOD could be through the reduction of number of small antral follicles leading to systemic

and local reduction of AMH leading to increased local sensitivity to the action of FSH on the follicles, resulting in the stimulation of follicular growth and production of a dominant follicle. We recommend wide scale of research on this point

INCONCLUSION

Because AMH levels are strongly correlated with the size of the follicle pool, and because of the lack of cycle variations, serum levels of AMH are a good candidate for inclusion in standard diagnostic procedures to assess, ovarian dysfunctions, PCO. Knowledge of the serum AMH levels in such conditions might provide more insight into the possible cause or effect of altered AMH levels after LOD.

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