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Medical Journal of Obstetrics and Gynecology

Research Article

Letrozole Resistance in Women with Polycystic Ovary Syndrome-The Impact of Anti-Mullerian Hormone

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Submitted: 09 April 2015

Accepted: 20 May 2015

Published: 28 May 2015

ISSN: 2333-6439

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OPEN ACCESS

Keywords

- Letrozole
- Anti-mullerian hormone
- Polycystic ovarian syndrome

Abstract

Objective: To study the level of Anti-Mullerian Hormone (AMH) in women with PCOS who failed to develop a mature mid-cycle follicle during ovulation induction with letrozole (LZ).

Design: Retrospective chart review.

Setting: The University of Louisville, Louisville, KY, USA.

Patients: Forty-nine infertile women with PCOS.

Interventions: Baseline serum AMH concentrations were compared between participants who did and did not develop a mid-cycle mature follicle after attempting ovulation induction with LZ. Main Outcome Measure: The presence of a mature follicle.

Results: Using a ROC curve analysis, serum AMH concentration had an area under the curve of 0.69 (95% CI 0.51 – 0.86). The optimal cutoff value of 4.53 ng/mL had a sensitivity of 67% and specificity of 71% in predicting the absence of a mature follicle on mid-cycle ultrasonography. In patients diagnosed with PCOS, baseline AMH serum levels ≥ 4.53 ng/mL were less likely to develop a mature follicle compared to women with AMH levels < 4.53 ng/ML. There was a gradient increase of serum AMH levels with increasing dose of LZ required to develop a mature follicle, p=0.002.

Conclusions: PCOS women with serum AMH levels ≥ 4.53 ng/mL were found to be less likely to develop a mature follicle after attempting ovulation induction with LZ.

ABBREVIATIONS

AMH: Anti-Mullerian Hormone; PCOS: Polycystic Ovary Syndrome; LZ: Letrozole; ROC: Receiver Operating Characteristic; CC: Clomiphene Citrate; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is the most common syndrome associated with anovulation in reproductive-age women. It is diagnosed by the presence of hyperandrogenism, the sonographic appearance of polycystic ovaries, and oligo-ovulation with associated oligomenorrhea [1]. Infertility caused by anovulation in women with PCOS has been effectively treated by several ovulation inducing agents, most commonly clomiphene citrate (CC) and LZ. Whether one is superior to the other in successfully producing mature follicles and increasing pregnancy rates in patients is debated [2]; nevertheless, some women with PCOS fail to ovulate with either LZ or CC for reasons that are not clear [3,4].

There are several candidate hormones that may be responsible for an ovulation and CC or LZ resistance in PCOS, including low follicle stimulation hormone (FSH) and excess luteinizing hormone (LH), androgens, insulin, and anti-Mullerian hormone (AMH) [5-7]. AMH is a glycoprotein [8] produced by granulosa cells of preantral and small antral follicles [9]. Evidence suggests that it limits the number of primordial follicles

recruited in the initial phase of the menstrual cycle by decreasing the sensitivity of ovarian follicles to FSH so that only the follicle with an adequate number of FSH receptors is able to develop into a mature, dominant follicle [10,11]. In the absence of AMH as a gatekeeper, mice experience early follicular depletion [12].

Elevated serum levels of AMH may prevent normal follicular development. A recent study investigated the impact of circulating AMH levels on ovulation rates after the administration of CC in women with PCOS. Results indicated a negative correlation between serum AMH levels and ovulation rates with a threshold predictive value of 3.4 ng/mL [13]. The two to three-fold increase in mean serum AMH levels in women with PCOS [14] is in proportion to the increase in small growing follicles that never reach dominance and from increased production of AMH from individual granulosa cells [15].

Our literature search did not reveal any studies that investigated the predictive value of serum AMH levels in women with PCOS receiving LZ. Since LZ is the current first-line infertility treatment in women with PCOS [2], our objective in this pilot study was to evaluate serum AMH levels in PCOS patients who failed to develop a mature mid-cycle follicle during ovulation induction with LZ.

MATERIALS AND METHODS

A total of 49 infertile women 18 to 39 years of age with PCOS participated in this retrospective cohort study conducted at the University of Louisville Health Care Outpatient Center from January 2013 to April 2014. Patients who were included in the study had either been diagnosed with PCOS previously by a referring physician or were diagnosed as part of our institutional infertility evaluation. All patients included in the study met the Rotterdam criteria for PCOS regardless of referral status. All participating women had at least two of three characteristics: ovulatory dysfunction, hyperandrogenism (on the basis of hirsutism or an elevated free or total testosterone level), and polycystic ovaries (defined by an increased number of small antral follicles [≥12 follicles that were <10 mm in diameter in each ovary]. Exclusion criteria were women with increased serum levels of FSH, TSH, 17-OH progesterone, and prolactin. Additionally, women were also excluded if their BMI ≥ 35 or if they were taking metformin and developed a mature follicle.

Outcome

The primary outcome measure was the presence or absence of a mature follicle (≥17 mm seen by transvaginal ultrasound) at mid-cycle.

Study design

Patients received LZ per standard protocol: a starting dose of LZ 5.0 mg daily in the early follicular phase (cycle day 3 to 7) for 5 days. Mean follicular size in both ovaries in the late follicular phase (cycle day 10 to 14) was assessed via transvaginal ultrasonography. The maximum dose of LZ used in any cycle was 7.5 mg. The study design was approved by the University of Louisville's Institutional Board of Review (#14.0057).

Serum AMH analysis

Prior to treatment with LZ a venous blood sample was collected to measure baseline serum concentrations of AMH. All assays were performed and validated at Quest Diagnostics in Louisville, KY. Serum samples were handled, processed, and stored in accordance to the sample collection and preparation protocol provided by the AnshLite Bovine AMH CLIA assay kit package insert. Serum samples were assayed using the AnshLite Bovine AMH CLIA quantitative three-step sandwich type immunoassay. Total imprecision ranges from 3.34-4.75% with an analytical measurable range of 0.06 – 14.2 ng/ML. The minimum detectable level of human AMH by the kit is approximately 23 pg/mL. The assay is marketed as both sensitive and reliable.

Sample size calculation

Based on the estimates used by *Mahran et al* to investigate the predictive value of circulating AMH hormone levels in women with PCOS receiving CC, 46 patients (with 19 expected to have a high AMH level) were needed to achieve a power of 90% and 5% significance level. Therefore, we reviewed the charts of 49 patients (all that were available in our electronic medical record) with PCOS to participate in our study.

Statistical analysis

Baseline characteristics of women with PCOS who developed a mature follicle and those who did not were compared using the independent samples *t*-test, the Mann-Whitney U test, or the chi-square test of independence when appropriate. An independent samples *t*-test was used to compare serum AMH levels between patients who developed a mature follicle and those that did not after receiving LZ 5.0 mg. Logistic regression was used to predict the odds of developing a mature follicle in the presence of increasing serum AMH levels. A receiver operating characteristics (ROC) curve analysis was used to evaluate the predictive value of serum AMH concentration levels. Logistic regression was also used to evaluate whether a serum AMH (above and below our assigned cut-off) and BMI (<30 *vs.* ≥ 30) interaction existed. Using analysis of variance, a test for linear trend assessed if the mean serum concentration of AMH increased between PCOS patients who responded to different LZ doses. Five PCOS patients who did not respond to LZ 5.0 mg elected to receive in vitro fertilization instead of ovulation induction with a higher dose of LZ. They were excluded from this analysis. A Chi-square Test for Linear Trend of Proportions was performed to assess whether the percentage of those who developed a mature follicle increased with age. Data was analyzed using SPSS version 22.0. A p value <0.05 was considered statistically significant.

RESULTS

This study evaluated 49 infertile women with PCOS. Table 1 illustrates a comparison of baseline characteristics between patients who developed a mature follicle in each cycle in response to LZ 5.0mg (responders) to patients who did not (non-responders). The only significant demographic difference between the two groups was age in years (32.2± 3.9 *vs.* 30.1± 2.2, p=0.020). Mean serum AMH levels significantly differed between responders and non-responders (4.05± 2.52 *vs.* 7.20± 4.80, p=0.017).

A test of linear trend was performed using analysis of

Table 1: Baseline characteristics of responders (developed a mature follicle in every cycle after attempting ovulation induction with LZ 5.0 mg) *vs.* non-responders (did not develop a mature follicle in every cycle after attempting ovulation induction with LZ 5.0 mg). Numerical values are presented as mean and standard deviation (SD), and categorical values are given as frequency (percentage).

Characteristic	Non-responders (n=18)	Responders (n=31)	P Value
Age, years	30.1 (2.2)	32.2 (3.9)	0.02
BMI (kg/m^2)	24.8 (4.4)	25.2 (3.7)	0.745
Duration of infertility, months	28.9 (28.2)	28.0 (45.1)	0.94
Total T, nmol/L	43.3 (21.6)	36.0 (23.2)	0.383
Free T, nmol/L	6.9 (10.7)	15.4 (56.8)	0.646
Serum LH, IU/L	10.2 (4.8)	12.4 (7.8)	0.418
Serum FSH, IU/L	6.6 (2.4)	8.5 (10.7)	0.561
Serum AMH, ng/ML	7.2 (4.8)	4.1 (2.5)	0.017
Number of antral follicles	28.1 (12.0)	28.9 (13.0)	0.822
Hirsutism			0.707
Present	6 (33%)	12 (39%)	
Absent	12 (67%)	19 (61%)	
Antral Follicles			0.375
Present	1 (6%)	0 (0%)	
Absent	17 (94%)	30 (100%)	
Gravidity			0.722
0	13 (72%)	18 (58%)	
1	3 (17%)	7 (23%)	
2+	2 (11%)	6 (19%)	
Parity			0.78
0	15 (83%)	22 (71%)	
1	3 (17%)	6 (19%)	
2+	2 (11%)	6 (19%)	
Race/Ethnicity			0.784
White	15 (83%)	27 (87%)	
African American	0 (0)	1 (3%)	
Asian	3 (17%)	3 (10%)	

Abbreviations: PCOS: Polycstic Ovary Syndrome; LZ: Letrozole; BMI: Body Mass Index; T: Testosterone; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; AMH: Anti-Mullerian Hormone

variance to determine if a LZ dose response exists for serum AMH levels in women with PCOS. Dose responses were derived from women who developed a mature follicle in each cycle after ovulation induction with either 5.0 mg or 7.5 mg of LZ, compared to those who did not develop a mature follicle after the highest dose, 7.5 mg of LZ. Among the 3 groups the response to LZ varied significantly depending on serum AMH levels [F (1,41) = 11.43, p=0.002]. Figure 1 depicts this linear trend.

Using the ROC curve analysis serum AMH concentration had an area under the curve of 0.69 (95% CI 0.51 – 0.86). The optimal cutoff value of 4.53 ng/mL had a sensitivity of 67% and specificity of 71% in predicting the absence of a mature follicle on mid-cycle ultrasonography.

Table 2 also illustrates a comparison of baseline characteristics between patients with low (<4.53 ng/mL) versus high (≥ 4.53 ng/mL) serum AMH levels. The results show that both groups were similar. The chi-square test of linear trend illustrated that as women with PCOS aged the percentage that developed mature follicles increased (p=0.015) (Figure 2).

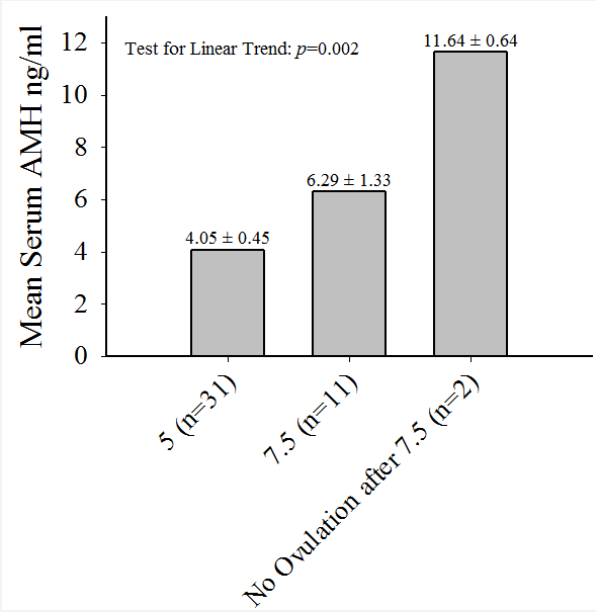

Figure 1 Mean serum AMH concentrations in women with PCOS receiving different doses of LZ. Values presented represent Mean±standard Error. Test for linear trend shows a significant dose response (p=0.002).

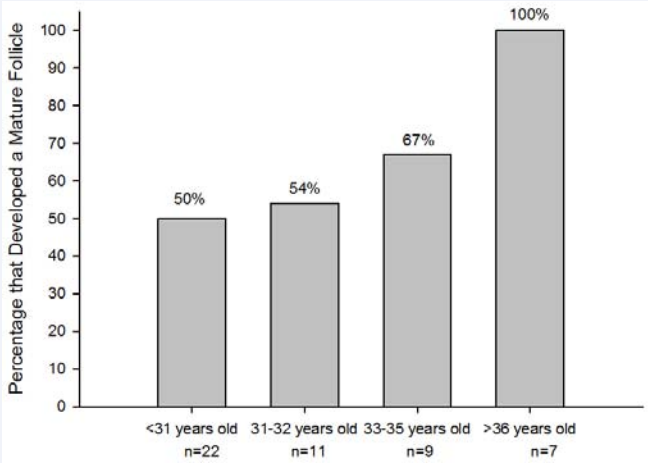

Figure 2 Linear Trend analysis assessing the association of age with the presence of a mature follicle in women with PCOS who received LZ ovulation induction. Age was stratified into 4 different groups: <31 years, 31-32 year, 33-35, and >36 years. The percentage of women with PCOS whom developed a mature follicle increased with age (p=0.015).

Table 2: Comparison of patient with PCOS who received LZ ovulation induction with a serum AMH level below the cutoff value of 4.53 ng/ml *vs.* at or above the cutoff value of 4.53 ng/ml. Numerical values are presented as mean and standard deviation (SD), and categorical values are given as frequency (percentage).

Characteristic	Patients with AMH < 4.53 ng/ml (N = 28)	Patients with AMH ≥ 4.53 ng/ml (N = 21)	P Value
Age, years	31.8 (3.7)	31.0 (3.3)	0.399
BMI (kg/m^2)	25.1 (3.9)	25.1 (4.0)	0.983
Duration of infertility, months	32.1 (47.3)	23.2 (25.8)	0.44
Total T, nmol/L	38.5 (25.7)	39.0 (20.3)	0.953
Free T, nmol/L	22.9 (67.1)	3.1 (1.5)	0.273
Serum LH, IU/L	10.5 (7.4)	12.6 (3.7)	0.466
Serum FSH, IU/L	8.5 (11.2)	6.8 (3.4)	0.585
Number of antral follicles	28.1 (12.0)	28.9 (13.0)	0.822
Mature follicle			0.01
Present	22 (79%)	9 (43%)	
Absent	6 (21%)	12 (57%)	
Hirsutism			0.864
Present	10 (56%)	8 (44%)	
Absent	18 (58%)	13 (42%)	
Antral Follicles			0.417
Present	28 (60%)	19 (40%)	
Absent	0 (0%)	1 (100%)	
Gravidity			0.722
0	17 (55%)	14 (45%)	
1	6 (60%)	4 (40%)	
2+	5 (63%)	3 (38%)	
Parity			0.78
0	21 (57%)	16 (43%)	
1	4 (44%)	5 (56%)	
2+	3 (100%)	0 (0%)	
Race/Ethnicity			0.819
White	23 (55%)	19 (45%)	
African American	1 (100%)	0 (0%)	
Asian	4 (67%)	2 (33%)	

Abbreviations: PCOS: Polycystic Ovary Syndrome; LZ: Letrozole; BMI: Body Mass Index; T: Testosterone; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; AMH: Anti-Mullerian Hormone

DISCUSSION

Our primary objective in this observational, retrospective pilot study was to see if baseline serum AMH levels predicted the development of mature follicles after LZ therapy in infertile PCOS patients. In this study women with PCOS and AMH levels ≥ 4.53 ng/mL were less likely to develop a mature follicle after attempting ovulation induction with LZ. The odds of not developing a mature follicle increased by 27% for every unit increase in serum AMH level. There was also a gradient increase in the effective dose of LZ with increasing serum AMH levels.

Our ROC curve seems to suggest that women with PCOS and higher serum AMH levels are less likely to develop a mature follicle after attempting ovulation induction with LZ. The sensitivity and specificity of our ROC curve was consistent with an adequate assessment model. Our findings are also similar to those of *Mahran et. al*, who reported a negative correlation between serum AMH levels and ovulatory rates in the background of serum AMH concentrations above 3.4 ng/mL in PCOS patients treated with CC [13]. They also reported a gradient increase in the effective dose of CC with rising serum AMH levels.

The negative association between serum AMH levels and the presence of a mature follicle may possibly reflect the inhibitory effect of AMH on FSH-stimulated follicular growth. *In vitro* and *in vivo* mice models have demonstrated that preantral follicles cultured in the presence of FSH and AMH have significantly smaller diameters and ovarian weight than preantral follicles cultured in the presence of FSH alone [11]. AMH-knockout mice are depleted of primordial follicles sooner than expected, demonstrating its role in controlled recruitment of follicles during the menstrual cycle [10].

Interestingly, our linear trend showed a greater chance of developing a mature follicle with increasing age. This may be explained by the natural history of the PCOS phenotype, which becomes less severe with age [16]. Women with and without PCOS demonstrate a decrease in androgens, including testosterone, androstenedione, and dehydroepiandrostenedione sulfate (DHEAS) as they age [16]. A 20-year longitudinal study demonstrated decreases in testosterone by 25%, DHEAS by 30%, and ovarian size by 20% [17]. Anti-Mullerian hormone serum levels also decline in controls and in women with PCOS as they become older. When levels fall to less than 5 ng/mL 60% are more likely to ovulate spontaneously; at <4 ng/mL, women with PCOS become ovulatory [18]. This is consistent with our data demonstrating an increased chance of a positive response to LZ when AMH values were less than 4.53 ng/mL.

While there are advantages in performing a retrospective study, such as decreased costs, the chance of selection bias is increased; thus, this is one of the limitations of our study. We reviewed all records of women diagnosed with PCOS in our electronic medical records (EMR). Patients diagnosed with PCOS prior to the establishment of our EMR system were not included in the study.

Since a literature search did not reveal any studies comparing serum AMH levels and the development of a mature follicle in women with PCOS undergoing LZ ovulation induction, our power analysis was based on a similar study using CC. It is possible that their study may not be directly applicable to ours.

CONCLUSION

In conclusion, women with PCOS and an AMH ≥ 4.53 ng/mL are less likely to develop a mature follicle after attempting ovulation induction with LZ. There is also a gradient increase in the effective dose of LZ with serum AMH levels. Starting at a higher dose of LZ may be warranted when AMH levels are elevated.

ACKNOWLEDGEMENTS

The University Of Louisville Department Of Obstetrics, Gynecology, and Women's Health; and Reproductive Endocrinology and Infertility. The University of Louisville funded the collection and interpretation of data.

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Cite this article

Torres D, Delaney AA, Ziegler CH, Salyer CV, Nakajima ST, et al. (2015) Letrozole Resistance in Women with Polycystic Ovary Syndrome-the Impact of Anti-Mullerian Hormone. Med J Obstet Gynecol 3(3): 1060.