Diagnostic accuracy of ovarian reserve markers in the differentiation between responders and non responders after laparoscopic ovarian drilling

Haitham M. Badran*, Ahmed E. Abd El Tawwab*, Abd Elsamie A. Abd Elsamie*, Mohammed S. Bakry*
*Department of Obstetrics and Gynaecology, Faculty of Medicine – Fayoum University
Haitham M. Badran, MD
Assistant Professor of Obstetrics and Gynaecology, Faculty of Medicine – Fayoum University
Ahmed E. Abd El Tawwab, MSc
Assistant lecturer of Obstetrics and Gynaecology, Faculty of Medicine – Fayoum University
-Abdelsamie A. Abdelsamie, MD
Professor of Obstetrics and Gynaecology, Faculty of Medicine - Fayoum University
Mohammed S. Bakry, MD
Assistant Professor of Obstetrics and Gynaecology, Faculty of Medicine – Fayoum University

Corresponding author:
Ahmed E. Abd El Tawwab
Department of Obstetrics and Gynaecology, Faculty of Medicine – Fayoum University
Assistant lecturer of Obstetrics and Gynaecology, Faculty of Medicine – Fayoum University
Email: aea11@fayoum.edu.eg
01003520960

Abstract

Background: Polycystic ovary syndrome results in many endocrinological derangements leading to infertility. This warrants the search for proper solutions to achieve ovulation. However, the prediction of ovarian response represents a significant challenge.

Aim: To evaluate the diagnostic accuracy of ovarian reserve markers to differentiate between responders and non-responders among women with clomiphene resistance PCOS after doing LOD.

Methods: This prospective cohort study was conducted at the obstetrics and gynecology department at Fayoum university from May 2018 to February 2020. The study recruited 50 women diagnosed with PCOS who had clomiphene citrate resistance. Transvaginal sonography was done to confirm the diagnosis of PCOS, measure the ovarian volume, and assess the mean antral follicle count (AFC) in both ovaries. A venous blood sample was obtained for AMH measurement. LOD was done early in the follicular phase under general anesthesia. Ovulation induction was prescribed using clomiphene citrate 100-150 mg from day 2 of the cycle.

Results: AMH had excellent discriminative power for response (AUC 0.995, the optimal cut-off point was 7.65 which yielded sensitivity 93.5%, specificity 100.0%, PPV 100.0% NPV 90.4% and accuracy 95.9%). AFC had good discriminative power for response (AUC 0.741, the optimal cut-off point was 16.5which yielded sensitivity 51.6%, specificity 84.2%, PPV 84.2% NPV 51.6%and accuracy 63.9%). OV had poor discriminative power (AUC 0.540, the optimal cut-off point was 13.75which yielded sensitivity 64.5%, specificity 52.6%, PPV 68.9% NPV 47.6%and accuracy 60.0%).

Conclusions: Serum AMH and AFC represent potent markers for predicting ovarian response in women with PCOS.

Keywords: PCOS; response; ovulation; AMH; AFC; ovarian volume.

Introduction

Polycystic ovary syndrome (PCOS), a common cause of female infertility, is characterized by insulin resistance and hormonal derangement (1, 2). It is diagnosed by two out of three criteria: oligo/anovulation, clinical or laboratory evidence of hyperandrogenism, and polycystic appearance of the ovaries (3). Patients present with a wide range of symptoms such as acne, hirsutism, irregular cycles, and infertility (4). Induction of ovulation is the mainstay treatment for women with infertility. Clomiphene citrate is the first-line treatment. When clomiphene resistance developed, other treatment options were introduced as the use of gonadotropins or laparoscopic ovarian drilling (LOD) (5, 6). However, the latter would impact the ovarian reserve, and the response after LOD is not guaranteed, dramatically impacting the patients psychologically (7). This study was conducted to evaluate the diagnostic accuracy of ovarian reserve markers to differentiate between responders and non-responders among women with clomiphene resistance PCOS after doing LOD.

Methods

This prospective cohort study was conducted at the obstetrics and gynecology department at Fayoum university from May 2018 to February 2020. The study recruited 50 women diagnosed with PCOS who had clomiphene citrate resistance. Clomiphene resistance was defined as the inability to detect ovulation after a daily dose of 150 mg and a thinned endometrium < 5mm for 2-3 cycles (8). All participants signed an informed written consent after explaining the aim of the study. Patients were recruited according to the following inclusion and exclusion criteria. **Inclusion criteria:** a) Age ranged from 18 – 35 y, b) PCOS according to Rotterdam criteria (3), c) clomiphene citrate resistance, d) average semen analysis, and e) normal Hysterosalpingography (HSG). **Exclusion criteria:** a) apparent cause of infertility rather than PCOS, b) hyperandrogenism due to any other endocrinal disorder, c) women refusing to participate in the study, d) the previous history of ovarian surgery, e) patients with endometriosis and/or fibroid, f) patients with chronic illness as diabetes mellitus and liver diseases, and g) hyperprolactinemia.

Women eligible for the study were subjected to detailed history and examination. Transvaginal sonography with a probe 7.5 MHz (China Philips HD11 C8-4v) was done to confirm the diagnosis of PCOS, exclude ovarian or adnexal pathology, measure the ovarian volume, and assess the mean antral follicle count (AFC) in both ovaries. Ultrasound was done two times, the first was before LOD, and the second was three months after the procedure. The same sonographer performed the ultrasound for all cases at the early follicular phase, days 3-5.

AFC is defined as counting all echo lucent rounded follicles measuring (2-10mm) present in the ovary's substance (9). The ovarian volume was evaluated with the ovary at its longitudinal axis, and the widest longitudinal and transverse diameters were measured on a frozen image. Rotation of the probe 90 degrees to get a transverse section of the ovary where the anteroposterior diameter was measured in this view. The ovarian volume was calculated using this equation: Ovarian volume = length X width X thickness X 0.5 (10).

A venous blood sample was obtained for AMH measurement. Samples were collected into EDTA-containing tubes, centrifuged for 20 min, and stored at -70 ºC. AMH was measured using an enzyme-linked immunosorbent assay technique (Immunotech, Beckman-Coulter UK Ltd, High Wycombe, Buckinghamshire, UK) following the manufacturer's protocol (11).

LOD was done early in the follicular phase under general anesthesia. Introduction of Verres needle, inflation of the abdomen,

and introduction of primary and secondary trocars were done. Fixation of one ovary away from the intestine by grasping the ovarian ligament with the traumatic grasper was done. A drilling needle was introduced and connected by monopolar current, held against the ovarian surface for 4 seconds using a power of 40 watts; four punctures were done in each ovary, putting into consideration that the puncture must be not superficial and it must go deep through the main substance of the ovary. Cooling of the ovary by lactated ringer's solution, finally removal of all instruments under vision after exclusion of any complication (12).

Follow-up of all patients was done and focused on AFC and Ovarian volume after three months from laparoscopy, AMH estimation after three months from laparoscopy, resumption of ovulation, and pregnancy rates. Ovulation induction was prescribed using clomiphene citrate 100- 150 mg from day 2 of the cycle.

Statistical analysis:

The collected data were organized, tabulated, and statistically analyzed using SPSS software statistical computer package version 22 (SPSS Inc, USA). The **mean, standard deviation (SD)**, and range were calculated **for quantitative data. A paired t-test** was performed to compare the **AMH, AFC**, and **Ovarian volume** values before and after the intervention. An **Independent t-test** was used to compare responders and non-responders regarding **study parameters**. Regarding Qualitative data were presented as **numbers** and **percentages**. For interpretation of results of significance tests, significance was adopted at **P< 0.05**. The receive operating characteristic **(ROC)** curve was used to determine the discrimination value of **AMH, AFC**, and **Ovarian volume** for response and to define optimal cut-points for sensitivity, specificity, and positive and negative predictive values (PPV, NPV).

Results

There were no statistically significant differences between responder and non-responder as regards age, BMI, and duration of subfertility (years) (p >0.05), as shown in table (1).

ROC curves were drawn to look at maximum sensitivity and specificity for indices (AMH, AFC, and OV) before laparoscopy in the diagnosis of responder and non-responder. AMH had excellent discriminative power for response (AUC 0.995, the optimal cut-off point was 7.65which yielded sensitivity 93.5%, specificity 100.0%, PPV 100.0% NPV 90.4%and accuracy 95.9%). AFC had good discriminative power for response (AUC 0.741, the optimal cut-off point was 16.5which yielded sensitivity 51.6%, specificity 84.2%, PPV 84.2% NPV 51.6%and accuracy 63.9%). OV had poor discriminative power (AUC 0.540, the optimal cut-off point was 13.75which yielded sensitivity 64.5%, specificity 52.6%, PPV 68.9% NPV 47.6%and accuracy 60.0%) (Table 3).

Discussion

Currently, the determination of patients at risk for exaggerated response with induction of ovulation medications is essential. This is commonly performed using factors with low predictive values, such as patients' age, body weight, and the presence of polycystic ovaries (13). Accordingly, the use of robust predictive markers is essential.

There was no difference between responders and non-responders in the primary demographic data. This agreed with a previous study where there was no difference in age. BMI, years and etiology of infertility, and basal laboratory levels (14).

Our result proved that AMH had excellent discriminative power for response with an optimal cut-off point of 7.65, which yielded a sensitivity of 93.5%, specificity 100.0%,

PPV 100.0%, NPV 90.4%and accuracy of 95.9%. Another study reported a cut-off value of 3.01ng/ml; however, this was used for fertilization rate prediction in patients undergoing assisted reproduction (15). Variable results were reported regarding the AMH cut-off value for the prediction of ovarian response, together with variable sensitivity and specificity. This ranged from 3.5- 7ng/ml and 40-95% and 31- 96% respectively (16). This predictive role is rendered to producing AMH from the small and intermediate follicles representing the common follicular pool (17). Variability in the results would be rendered to different sample sizes, the nature of recruited patients, different outcomes, and different assay techniques (18).

We proved that AFC had good discriminative power for response with an optimal cut-off point of 16.5, which yielded a sensitivity of 51.6%, specificity 84.2%, PPV 84.2%, NPV 51.6%, and accuracy 63.9%. An earlier study reported similar results with an AFC of 16 had a sensitivity of 89% and a specificity of 92% (14). Variable results were reported regarding the AFC cut-off value for the prediction of ovarian response, together with variable sensitivity and specificity. This ranged from 9- 18 and 20-94% and 33- 98%, respectively (16). This difference would be rendered to their use of small AFC rather than the total AFC, which is vulnerable to inter-cycle, interobserver, and intra-observer variability (19). Additionally, 3D ultrasound yielded more validity and reliability than the 2D ultrasound used in the current study (20).

Ovarian volume had poor discriminative power with an optimal cut-off point 13.75which yielded sensitivity 64.5%, specificity 52.6%, PPV 68.9% NPV 47.6% and accuracy 60.0%. A previous study reported an insignificant difference in ovarian volume between pregnant and non-pregnant participants. It did not correlate with the number of oocytes retrieved or the AFC (21). Also, earlier studies reported that the number of oocytes retrieved decreased, and the dose of drugs used for ovulation induction increased in women with an ovarian volume <3cm3 (22, 23). Another reported an insignificant difference in the ovarian volume between responders and non-responders to clomiphene citrate and had no predictive value (24).

Strength and limitation

This study used reliable markers to predict ovarian response. However, the small sample size is a limitation. Evaluation of the predictive role of these markers in combination would be more informative. Evaluation of the predictive role of these markers in fertility rates was not done.

Conclusion

Serum AMH and AFC represent potent markers for predicting ovarian response in women with PCOS.

Conflict of interest: None.

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Table (1): Comparison between responder and non-responder as regards age, BMI, and duration of subfertility.

		Groups		Significant test	P value
		Responder N= 31	Non-Responder N= 19		
Age	Range	18-35	18-35	t = 0.021	0.718[a]
	Mean± SD	26.6±3.3	27±4.2		
BMI	Range	25-35	25-35	t = 0.684	0.497[a]
	Mean± SD	30.5±3.20	29.8±3.3		
Duration of subfertility (years)	Range	2-7	2-7	**Mann-Whitney** = 221.000	0.140[b]
	Median (IQR)	4.00 (2.00-5.00)	5.00 (3.00-7.00)		

[a] Independent T-test [b] Mann- Whitney test

Table (2): Sensitivity and Specificity of AMH, AFC, and OV level before laparoscopy in the diagnosis of responder and non-responders.

	AUC	P-value	Cut-off point	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
AMH	**0.995**	**<0.0001**	**7.65**	**93.5**	**100.0**	**100.0**	**90.4**	**95.9**
AFC	**0.741**	**0.005**	**16.5**	**51.6**	**84.2**	**84.2**	**51.6**	**63.9**
Ovarian volume	**0.540**	**0.639**	**13.75**	**64.5**	**52.6**	**68.9**	**47.6**	**60.0**