Assessment of Ovarian Reserve by Hormonal and Ultrasound Markers among Fertile and Infertile Women

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ABSTRACT

Background: Infertility of an ovarian origin is one of the most distressing conditions to both the gynecologist and the partners which necessitate the use of markers that can predict the ovarian reserve and the response for infertility treatment.

Objective: To assess the ovarian reserve in the infertile women by means of hormonal and ultrasound markers and compare them with fertile women.

Methods: A prospective case control study was carried out on 60 infertile women (study group) and 60 healthy fertile women (control group) for a period of one year from January 2012 till January 2013 at Al Yarmouk teaching hospital in Baghdad. We compared the ultrasound and hormonal markers as predictors of ovarian reserve in both groups.

Results: The mean serum inhibin B level was lower in the study group in comparison to the control group. The difference was statistically significant. There was no significant difference in the mean serum estradiol level in the study group in comparison to the control group. The mean serum follicle stimulating hormone level was significantly higher in the study group. The ovarian volume was larger in the control group than the study group. The difference was statistically significant. The mean number of large size antral follicles was significantly higher in the control group than the study group. The mean number of small size antral follicles was significantly higher in the control group than the study group.

Conclusion: This study suggests that higher number of the small size antral follicles, higher serum inhibin B and lower serum follicle stimulating hormone might be considered as good predictors of ovarian reserve.

Keywords: Ovarian reserve, Ultrasound and hormonal markers.

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Tests for ovarian reserve (OR) have been used to predict the ovarian response to stimulation as part of infertility treatment. Observational studies have demonstrated an association between factors such as serum follicle-stimulating hormone (FSH), serum inhibin, anti-mullarian hormone (AMH), antral follicles count (AFC) and subsequent ovarian response to stimulation[1]. Measurement of FSH levels in the early follicular phase is a simple predictor of ovarian reserve, a value greater than 10 mIU/mL indicates poor ovarian reserve and necessitates a thorough further evaluation and possibly higher treatment doses[2,3].

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Early follicular phase serum inhibin B may be a suitable marker of ovarian follicle reserve and fertility potential[4]. The use of transvaginal sonography to measure ovarian volume and obtain an early follicular phase antral follicle count and size is one of the best validated tests to detect ovarian reserve. The number of small antral follicles reflects the size of the resting follicular pool, less than 10 antral follicles predict poor response to gonadotropin stimulation[5].

Studies demonstrated that the number of pre-antral or small antral follicles (2-6 mm) declined with age and the number of larger follicles (7-10 mm) remained constant, suggesting that the number of small AFCs represents the functional OR[6].

AMH is an early and reliable predictor of ovarian reserve, it represents the quantity of the ovarian

follicle pool and has been found to indicate a problem before an increase in baseline follicle-stimulating hormone (FSH) is seen[7,8].

From the review analysis it was clear that the AFC and AMH are superior to the other tests of ovarian reserve[9].

Methods

A prospective case control study was carried out in the Infertility Department at Al-Yarmouk Teaching Hospital in Baghdad, Iraq for a period of one year from January 2012 till January 2013. A total of 120 participants were enrolled in this study, the study group (60 infertile women) were collected from the infertility clinic while the control group (60 fertile women) were collected from the outpatient clinic. A detailed history was obtained and physical examination was done to all women in this work.

The inclusion criteria for the study group were: Women with primary or secondary infertility, both ovaries are present, no current or past diseases affecting the ovaries or gonadotropin or sex steroid secretion or excretion, adequate visualization of the ovaries by transvaginal ultrasound scan and ovarian cause of infertility. All the patients agreed to participate in this study and verbal consents were obtained from all infertile and fertile women before enrolling them in the study.

Each woman had been given an appointment to come in the early follicular phase of menstrual cycle (day 3 of the cycle) underwent blood sampling for serum inhibin B, estradiol (E2) and FSH hormones and transvaginal ultrasound scan for measurement of the size and number of the antral follicles in both ovaries and the size of both ovaries. Collected blood by venipuncture was allowed to clot, and the serum had been separated by centrifugation at room temperature. The serum then frozen at -80C^0 by liquid nitrogen prior to assay by enzyme-linked immunosorbent assay (ELISA) tests. Using inhibin B, E2 kits each one contains 60 strips and the results were expressed by (pg/mL) and FSH kit contains 60 strips and the results were expressed by mIU/ml in the laboratory of Al-Yarmouk Teaching Hospital.

Results

The current study showed the mean women's age was 31.45±6.39 (years) for the study group and 32.07±6.66 (years) for the control group, this difference was not significant statistically since P value was 0.094, there was a larger ovarian volume in the control group than the study group and this was statistically significant since p value was 0.0001, (Table 1).

The mean number of large size antral follicles in both ovaries in the study and the control groups was 0.70 ± 0.62 and 1.08 ± 0.70 respectively and this was statistically significant as P value was 0.007, the mean number of small size antral follicles in both ovaries in the study and the control groups was 7.48 ± 1.69 and 11.17± 2.58, respectively and this was statistically significant as P value was 0.0001, (Table 2).

Concerning the hormonal markers, the mean serum inhibin B for the study group was 62.34 ± 9.68 (pg/ml) which was lower than the control group which was 76.40 ± 8.11 and the difference was statistically significant since the p value was 0.0001, the mean serum E_2 for the study group was 40.37 ± 6.28 compared with the mean serum E_2 in control group which was 42.24 ± 4.68, this difference was statistically not significant since the p value was 0.067, the mean serum FSH for the study group was 8.54 ± 1.30 (mIU/ml) which was significantly higher than the control group which was 6.27 ± 1.20 since the p value was 0.0001, (Table 3).

Serum inhibin B was inversely correlated with women's age in the study and the control groups, it was directly correlated with the number of the large size and small size antral follicles in both ovaries in the study and the control groups, it was directly correlated with the serum E_2 and inversely

correlated with the serum FSH in the study and the control groups, (Table 4).

The current study showed that serum FSH was directly correlated with the women's age in the study and the control groups, it was inversely correlated with the number of the large size and small size antral follicles in both ovaries in the study and the control groups. However, it was inversely correlated with the serum inhibin B and serum E2 in the study and the control groups, (Table 5).

Table 1: The distribution of women's age, volume of both ovaries in the study and the control groups.

		Study group		Control group		P value
		No.	%	No.	%	
Age (years)	20-24	10	16.7	9	15.0	
	25-29	8	13.3	12	20.0	
	30-34	26	43.3	15	25.0	
	35-39	8	13.3	18	30.0	
	≥40	8	13.3	6	10.0	
	Mean ±SD (range)	31.45±6.39 (20-42)		32.07±6.66 (19-41)		0.094
Rt ovary volume	Small (<2.5x2x2)	14	23.3	15	25.0	0.0001*
	Medium (2.5- <3x2x2)	44	73.3	25	41.7	
	Large (≥3x2x2)	2	3.3	20	33.3	
Lt ovary volume	Small (<2.5x2x2)	3	5.0	10	16.7	0.0001*
	Medium (2.5- <3x2x2)	45	75.0	21	35.0	
	Large (≥3x2x2)	12	20.0	29	48.3	

*Significant using Pearson Chi-square test at 0.05 level of significance

Table 2: The distribution of number of large and small size antral follicles in both ovaries for the study and the control groups.

		Study group		Control group		P value
		No.	%	No.	%	
Large size antral follicle(≥7mm) number in both ovaries	0	23	38.3	12	20.0	0.007*
	1	32	53.3	31	51.7	
	2	5	8.3	17	28.3	
	3	-	-	-	-	
	Mean ±SD (Range)	0.70±0.62 (0-2)		1.08±0.70 (0-2)		
Small size antral follicle (2-6 mm) number in both ovaries	5-6	22	36.7	3	5.0	0.0001*
	7-8	17	28.3	10	16.7	
	9-10	21	35.0	7	11.7	
	11-12	-	-	19	31.7	
	≥13	-	-	21	35.0	
	Mean ±SD (Range)	7.48±1.69 (5-10)		11.17±2.58(5-15)		

*Significant using Pearson Chi-square test at 0.05 level of significance

Table 3: The mean serum inhibin B, serum E_2 and serum FSH for both the study and the control groups.

	Study group	Control group	P value
	Mean ±SD (Range)	Mean ±SD (Range)	
Serum Inhibin B (pg/ml)	62.34±9.68 (47.10-73.60)	76.40±8.11 (63.20-90.40)	0.0001*
Serum Estradiol (pg/ml)	40.37±6.28 (30.10-49.40)	42.24±4.68 (31.10-50.20)	0.067
Serum FSH (mIU/ml)	8.54±1.30 (7.10-10.50)	6.27±1.20 (4.00-8.10)	0.0001*

*Significant using Students-t-test for difference between two independent means at 0.05 level of significance.

Table 4: The correlation of serum inhibin B with the women's age, ultrasound and hormonal markers in the study and the control groups.

		Serum Inhibin B (pg/ml)	
		Study group	Control group
Age (years)	r	-0.783**	-0.637**
	P	0.0001	0.0001
Large size antral follicle number in both ovaries	r	0.892**	0.529**
	P	0.0001	0.0001
Small size antral follicle number in both ovaries	r	0.830**	0.727**
	P	0.0001	0.0001
Serum estradiol (pg/ml)	r	0.955**	0.363**
	P	0.0001	0.004
Serum FSH (mIU/ml)	r	-0.965**	-0.814**
	P	0.0001	0.0001

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Table 5: The correlation of serum FSH with the women's age, ultrasound and hormonal markers in the study and the control groups.

		Serum FSH (MIU/ml)	
		Study group	Control group
Age (years)	r	0.758**	0.750**
	P	0.0001	0.000
Large size antral follicle number in both ovaries	r	-0.889**	-0.219
	P	0.0001	0.093
Small size antral follicle number in both ovaries	r	-0.810**	-0.846**
	P	0.0001	0.000
Serum inhibin B (pg/ml)	r	-0.965**	-0.814**
	P	0.0001	0.000
Serum estradiol (pg/ml)	r	-0.947**	-0.079
	P	0.0001	0.548

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Discussion

A spectrum of prognostic markers of OR were validated in the infertile population. These included biochemical markers (FSH, estradiol (E_2), inhibin B, anti-Mullerian hormone, FSH-LH ratio)[10] and ovarian morphometric markers like ovarian volume, antral follicles count (small and large size antral follicles) and mean ovarian diameter that were assessed in the early follicular phase of the menstrual cycle[11]. Studies showed that the lower antral follicles count (less than five per ovary) at base line, low ovarian volume (less than 2 ml), low base line serum E_2, elevated serum FSH and low serum inhibin B levels were all associated with diminished ovarian reserve (DOR)[12].

The goal of ovarian reserve testing is to add more prognostic information to the counseling and planning process so as to help couples choose among treatment options[13].

The current study showed that the mean serum inhibin B was significantly higher in the control group than the study group. The mean E_2 was not significantly different in the study and the control groups. Mean FSH was significantly higher in the study group as compared with the control group. Ultrasound markers showed that the control group had a larger ovarian volume than the study group. These results agreed with the study done by Stacea Bowen et al who showed that there is a statistically significant decrease in three ovarian size parameters accompanied advancing age (ovarian width, ovarian length and the mean overall ovarian diameter). Levels of FSH demonstrated a significant and linear correlation with age, increasing levels of FSH (and hence declining OR) were associated with a statistically significant decline in the mean ovarian width, length, and overall mean ovarian diameter[11].

In consistent with the current study, Shachi Shukla et al found that with increasing age (> 35 yrs) hormonal markers of ovarian reserve i.e., s.FSH increases while sonographic markers of ovarian reserve i.e., AFC and ovarian volume decreases thus decreasing the chances of conception and there was a highly significant negative correlation between s.FSH and ovarian volume and s.FSH and AFC[14].

Frattarelli et al demonstrated that mean ovarian diameter (length + width/2) provided a comparable degree of predictability of OR as the ovarian volume in the infertile women undergoing assisted reproductive technology[15].

There is a study demonstrated that the number of pre-antral or small antral follicles (2-6 mm) declined with age and the number of larger follicles (7-10 mm) remained constant, suggesting that the number of small AFCs represents the functional OR[6].

And this is consistent with Grynberg et al who reported that large follicles showed much poorer correlation to FSH and Inhibin B than smaller antral follicles[16].

In another study done by Yomna Islam et al to determine the value of mean ovarian volume, AFC, maternal age, FSH and AMH in infertile patients undergoing ovulation induction or IVF cycles showed that the total AFC and AMH are found to correlate significantly with the ovarian response with p values < 0.001 and 0.03 respectively, indicating that they are good predictors of ovarian reserve. The basal FSH and ovarian volume did not correlate with the ovarian response indicating their poor value as predictors of ovarian reserve[17].

The current study did not agree with Marzieh et al who stated that neither follicular phase FSH nor estradiol concentrations are reliable predictors of ovarian reserve[18] and Maheshwari et al stated that the available tests for ovarian reserve do not have enough predictive power to justify their routine clinical use[19].

Yarali et al considered poor ovarian reserve and poor responders as those patients who have a day 3 FSH > 10 mIU/ml, day 3 E > 60 pg/ml or bilateral antral follicle count <6[20].

In conclusion; ovarian reserve can be assessed by hormonal and ultrasound marker (FSH, serum inhibin, serum estrogen, ovarian volume, AFC and size) to predict ovarian response to stimulation as part of infertility treatment.

We recommend further prospective studies with larger sample size to confirm these finding. Also, further studies required to know the role of anti mullarin hormone (AMH) as marker of ovarian reserve.

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