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Research Article

Comparison of antral follicle count, antimullerian hormone and day 2 follicle stimulating hormone as predictor of ovarian response and clinical pregnancy rate in patient with an abnormal ovarian reserve test

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ABSTRACT

Background: Patients having abnormal ovarian reserve test are likely to have poor response to controlled ovarian stimulation (COS) in artificial reproduction technique, where large number of follicles is desirable. Although direct measurement of the primordial follicle pool is impossible, it has been shown that the number of antral follicles in the ovaries is proportionally related to the size of primordial follicle stock from which they were recruited. Therefore, the antral follicle count (AFC) is believed to represent the quantitative aspect of ovarian aging. The aim of the study was to To compare the day two Antral follicle count, antimullerian hormone and Follicle stimulating hormone levels as a predictor of ovarian response among the patients undergoing controlled ovarian stimulation using GnRH antagonist and its implications in clinical pregnancy rate.

Methods: A prospective study was conducted in KJK Hospital Trivandrum on 119 patients having abnormal ovarian reserve test undergoing controlled ovarian stimulation (COS) with GnRH antagonist protocol from January 2010-December 2015. Patients AFC, AMH and FSH levels were measured and their association in predicting the ovarian reserve in terms of oocyte maturation, fertilization and embryo cleavage and their pregnancy rate.

Results: AFC had the highest accuracy for predicting ovarian response in patient with abnormal ovarian reserve test and was statistically significant (number of oocyte aspirated p value <0.001) than AMH (p value 0.06) and FSH (p value 0.212) in predicting ovarian response. For prediction of poor ovarian response a model including AFC+AMH was found to be almost similar to that of (p value 0.001) using AFC alone. However AFC (p value 0.458), AMH (p value 0.267) and FSH (p value 0.486) did not predict pregnancy rate in patient with abnormal ovarian reserve test and it was statistically not significant.

Conclusions: This study indicates that AFC is the most useful marker in predicting the ovarian response. Doing AFC assessment alone would be more cost effective for predicting the ovarian response in patients undergoing controlled ovarian stimulation with GnRH antagonist.

Keywords: AFC, AMH, FSH, Controlled ovarian hyperstimulation, Gonadotropin releasing hormone antagonist

INTRODUCTION

Antral follicles are highly responsive to gonadotrophin stimulation and the measure of ovarian reserve is defined as the total number of follicles, which can be stimulated to grow under maximal stimulation of GnRH. The primordial follicles usually decreases gradually as the age increases.[1,2] Age and follicle stimulating hormone (FSH) levels in the early follicular phase were considered as markers for assessing the ovarian reserve.[3,4] Tests such as, GnRH stimulation test (G-test) and ovarian stimulation test (OST) have been used to predict the ovarian response to gonadotrophin stimulation.[5,6] The principle of these tests is to stimulate ovarian hormone

(oestradiol) secretion by increasing the gonadotrophins in circulation. These tests give an indication of ovarian function directly compared with the measurement of FSH, which is an indirect measure of ovarian hormone feedback on the pituitary. Although direct measurement of the primordial follicle pool is impossible, it has been shown that the number of antral follicles in the ovaries is proportionally related to the size of primordial follicle stock from which they were recruited. Therefore, the antral follicle count (AFC) is believed to represent the quantitative aspect of ovarian aging.[7]

Ovarian response to ovarian hyperstimulation in IVF is another way in which the quantitative ovarian reserve may come to expression. But poor response is still a concern, factors like under dosing in obesity and FSH receptor polymorphism contribute to it. So by assessing the true nature of a poor ovarian response, will help us in planning the appropriate treatment for the patient [8]. For all patients before entering the IVF programme they should be identified as whether they are poor respondents or not, so that it would help in proper management regarding gonadotropin dosing and denial of treatment. Currently for this purpose, the tests of choice which is readily available are the AFC or basal FSH.[9]

Anti-mullerian hormone, a member of the transforming growth factor β family, is produced in the granulosa cells. The highest level of AMH expression is present in granulosa cells of secondary, pre-antral, and small antral follicles up to 6 mm in diameter, whereas in follicles growing into dominance, this expression ceases.[10] Secreted from pre antral and early antral follicles, AMH regulates ovarian activity and follicular steroidogenesis. Animal studies have revealed that not only does AMH decrease aromatase activity of FSH-stimulated granulosa cells, but it also decreases the number of luteinizing hormone (LH) receptors, and regulates testosterone production in theca cells.[11]

Serum AMH levels have a strong positive correlation with the number of antral follicles and it is found to be cycle independent.[12] From several reviews, AMH was considered to be a predictor of ovarian response to hyperstimulation which invariably gives a chance of becoming pregnant after IVF.[13]

In India as such very few studies had been conducted to determine the role of AFC, AMH and day 2 FSH for predicting ovarian response and clinical pregnancy rate. So the present study was undertaken to measure these hormones among the patients undergoing controlled ovarian stimulation using GnRH antagonist.

Aim of the study was to compare the day two Antral follicle count, Antimullerian hormone and Follicle stimulating hormone levels as a predictor of ovarian response among the patients undergoing controlled ovarian stimulation using GnRH antagonist and its implications in clinical pregnancy rate.

METHODS

A prospective study was conducted in KJK Hospital Trivandrum on 119 patients having abnormal ovarian reserve test undergoing controlled ovarian stimulation (COS) with GnRH antagonist protocol from January 2010- December 2015, fulfilling the following inclusion criteria (two of the following three features should be present)

1. Patients with advanced maternal age (AMA) ≥35 years
2. Patients having low AMH (≤ 1.1 ng/ml) or low AFC (<5-7)
3. Patients with poor ovarian response (POR) in previous attempts ≤ 3 oocytes aspirated.

Patients with associated male factor infertility were excluded from the study.

Procedure

Oral contraceptive pill pretreatment was given for 14 days in a month prior to stimulation. On day 2 of menses serum estradiol (E2), leutinising hormone (LH) and progesterone level was measured and trans vaginal sonography (TVS) was done to measure AFC. All patient stimulation was started with recombinant follicle stimulating hormone (r FSH,Gonal-F;Merck Serono, Germany) injection with a fixed dose of 300 IU. All patients were subjected to GnRH antagonist protocol (Cetrolix 0.25 mg/day,Cetrotide; Merck Serono,Germany), in which GnRH antagonist was started once the follicle was ≥12 mm in size and was continued till the day before ovum pick up (OPU). When follicle was ≥ 17 mm in size trigger was given with human chorionic gonadotropin (HCG, Pregnyl) injection with dose of 10,000 IU intramuscularly. E2, LH and progesterone level and endometrial thickness was measured on the day of trigger. OPU was done 35-36 hour after HCG trigger. A poor response was defined when ≤ 3 oocytes were aspirated (14). For luteal phase support (LPS) estradiol valerate tablets were given orally and micronized progesterone vaginal pessary was given from day of OPU until the day of pregnancy test (17 days). Day 3 embryo transfer (ET) was done, usually 2 or 3 good quality embryos were transferred in most patient and if remaining embryos were left they were frozen for transfer in frozen embryo transfer cycle (FET). Serum beta HCG levels were measured 14 days after ET.

Measurement of AFC, AMH and FSH

To determine AFC, eligible subjects underwent transvaginal 2-Dimensional ultrasound on day 2 of their cycle. To decrease the intra observer variability the ultrasound was done by one trained senior doctor. Total AFC included all follicles of 2-10 mm diameter in both ovaries as previously defined 14.

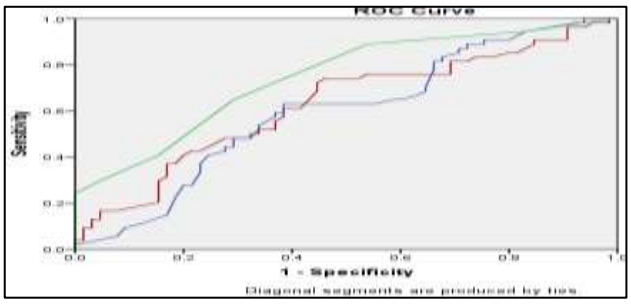
Eligible subjects had 2 ml of blood drawn on day 2 of their menstrual cycle and just prior to FSH stimulation for determination of AMH and FSH levels. Serum separation was done within half hour after blood collection. Serum AMH levels were determined using AMH Gen II assay (Beckman Coulter, Texas, USA; lowest detection limit 0.08ng/ml). FSH level was determined using the enzyme linked immunoassay (Vidas).

Statistical analysis

Data were entered in Microsoft excel and data analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).Quantitative data was described by mean and standard deviation (SD). Qualitative data was described by percentage distribution. Between group comparision of quantitative data was performed by student t test and that of qualitative data was performed by Pearson chi-square test. A p value o < 0.05 was taken as level of significance. Reciever operating characteristic (ROC) curve is used to predict the cut off value of AMH, AFC for predicting poor ovarian response with area under curve (AUC) and 95% confidence interval (CI).

RESULTS

The hormonal levels and their association with the oocyte and embryo were shown in Table 2. It is inferred from the table that there was a strong association between the AFC levels and the oocyte retrieved, matured and fertilised and the embryo cleavage. As the AFC count decreases the oocyte matured, fertilised and the embryo cleavage was also reduced and as the AFC count is high the oocyte and embryo cleavage was also high and the difference was found to be statistically significant (P <.01) but this difference was not seen with AMH and FSH (P >.05). Demographic characteristics and clinical data at baseline including day 2 AFC, AMH and AFC are shown in Table 1. The mean age of patient undergoing the study was 38±4.5 years. Majority of the patients were diagnosed with low AMH (73.9%) as the cause for infertility and almost 70% of the patients were receiving COS (controlled ovarian stimulation) for the first time.



Green line - AFC, Red line - AMH, Blue line - FSH.

Figure 1: Reciever operator curve for AFC, AMH and FSH.

Table 1: Demographic characteristics and the hormonal levels among the study population.

Characteristic	Mean±SD
AGE (years)	38±4.5
BMI (kg/m^2)	25.9±4.12
Duration of infertility (years)	8.7±4.76
AMH (ng/ml)	0.85±0.63
AFC	4.69±2.25
Day 2 FSH (IU/L)	7.9±4.1
Cause for infertility n (%)	
Increase age	8 (6.7%)
Low AFC	14 (11.8%)
Low AMH	88 (73.9%)
POR	9 (7.6%)
Previous pelvic surgery n (%)	
No	37 (31%)
Yes	82 (68.9%)
Controlled ovarian stimulation (COS) n (%)	
1st attempt	83 (69.7%)
2nd attempt	36 (30.3%)

Table 2: Association between AFC, FSH and AMH with oocyte and embryo parameter.

Hormone levels	Oocytes aspirated		Oocytes matured		Oocytes fertilised		Embryo cleaved	
	≤3	**>3**	**≤3**	**>3**	**≤3**	**>3**	**≤3**	**>3**
AFC	65 (61.3%)	41 (38.7%)	69 (65.1%)	37 (34.9%)	76 (71.7%)	30 (28.3%)	81 (76.4%)	25 (23.6%)
≤7	-	13 (100%)	1 (7.7%)	12 (92.3%)	1 (7.7%)	12 (92.3%)	1 (7.7%)	12 (92.3%)
>7								
P value	<0.001		<0.001		<0.001		<0.001	
Day 2 FSH	48 (51.6%)	45 (48.4%)	53 (57%)	40 (43%)	60 (64.5%)	33 (35.5%)	64 (68.8%)	29 (31.2%)
≤10	17 (65.4%)	9 (34.6%)	17 (65.4%)	9 (34.6%)	17 (65.4%)	9 (34.6%)	18 (69.2%)	8 (30.8%)
>10								
P value	0.212		0.442		0.935		0.968	
AMH ≤1.1	49 (60.5%)	32 (39.5%)	51 (63%)	30 (37%)	54 (66.7%)	27 (33.3%)	59 (72.8%)	22 (27.2%)
	16 (42%)	22 (57.9%)	19 (50%)	19 (50%)	23 (60.5%)	15 (39.5%)	23 (60.5%)	15 (39.5%)
>1.1								
P value	0.060		0.18		0.513		0.176	

P value derived by applying chi-square test

Among the patients who had both low AFC (≤7) and low AMH (≤ 1.1ng/ml) about 67.6% of patients had ≤ 3 oocytes retrieved and for 32.4% of the patients it was >3 oocytes retrieved on day of oocyte pick up (OPU) and the difference was found to be statistically significant (p value 0.001). Similar type of results was also observed for oocyte maturity, oocyte fertilisation and cleavage of the embryo. Whereas for the patients with low AFC of ≤7 and high FSH >10 ng/dl, and also for patients with low AMH of <1.1 ng/ml and high FSH >10 ng/dl there was no statistical significant difference in the numbers of oocyte aspirated, matured and fertilised, and also in the number of embryo cleavage (Table 2).

Table 3: Association between AFC+AMH, AFC+FSH and AMH+ FSH with oocyte and embryo parameter.

Hormonal levels	Oocyte aspirated		Oocyte matured		Oocyte fertilised		Embryo cleaved	
	≤3	>3	≤3	>3	≤3	>3	≤3	>3
AFC≤7 and AMH ≤1.1	46 (67.6%)	22 (32.4%)	48 (70.6%)	20 (29.4%)	51 (75%)	17 (25%)	56 (82.4%)	12 (17.6%)
P value	0.001		0.003		0.007		0.001	
AFC ≤7 and FSH >10	17 (70.8%)	7 (29.2%)	17 (70.8%)	7 (29.2%)	17 (70.8%)	7 (29.2%)	18 (75%)	6 (25%)
P value	0.074		0.181		0.482		0.47	
AMH ≤1.1 and FSH >10	11 (73.3%)	4 (26.7%)	11 (73.3%)	4 (26.7%)	11 (73.3%)	4 (26.7%)	12 (80%)	3 (20%)
P value	0.119		0.222		0.455		0.321	

P value derived by applying chi-square test

Table 4: Association between the hormonal levels and the pregnancy results.

Hormone levels		Pregnancy result		Chi-square value	P value
		Positive	Negative		
AFC	<7 ng/dl	16 (15.1%)	90 (84.9%)	0.554	0.458
	>7 ng/dl	3 (23.1%)	10 (76.9%)		
AMH	<1.1 ng/dl	15 (18.5%)	66 (81.5%)	1.231	0.267
	>1.1 ng/dl	4 (10.5%)	34 (89.5%)		
Basal FSH	<10 ng/dl	16 (17.2%)	77 (82.8%)	0.486	0.486
	>10 ng/dl	3 (11.5%)	23 (88.5%)		

Area under the curve					
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig. (P value)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
FSH (ng/ ml)	0.596	0.052	0.071	0.494	0.699
AFC	0.745	0.045	0.000	0.657	0.834
AMH	0.627	0.052	0.017	0.526	0.729

Among the various hormonal assays measured among the females undergoing infertility treatment, the AFC levels of >7, AMH levels of >1.1 ng/dl and the basal FSH of <10 ng/dl showed increase number of positive pregnancy results that the AFC <7, AMH <1.1ng/dl and FSH >10 ng/dl but the difference was not found to be statistically significant (Table 3). To identify the best parameter and the threshold value ROC curves was used for AFC, AMH and for FSH levels.

Among the three parameters day 2 AFC levels showed the highest sensitivity and specificity with the area under curve of 0.745 (P <0.001), followed by AMH with AUC = 0.627 (P=0.017) and for FSH the area under curve is 0.596 (P=0.071). So from the ROC it is interpreted the 2 day AFC found to be the best predictor of ovarian response when compared to AMH and FSH (Figure 1).

DISCUSSION

The results of this prospective study demonstrated that, overall AFC alone is the best predictor of ovarian response than AMH and FSH alone in patients undergoing COS using a GnRH antagonist protocol having abnormal ovarian reserve test. AFC and AMH combination also had a high predictive value in determining ovarian response to controlled ovarian stimulation in patients having abnormal ovarian reserve test in comparison of using AFC and FSH or AMH and FSH as combination. But none of these markers of

ovarian reserve test was able to predict the pregnancy rate in patients having abnormal ovarian reserve test. Our findings was different from other studies which had shown that AMH is the good predictor of ovarian response in GnRH antagonist cycles, but according to our results AFC was found to be a better predictor of ovarian response in patients undergoing GnRH antagonist cycle for having abnormal ovarian reserve.[15-19] A study done among the Vietnamese women had also shown AMH as the best predictor of ovarian response in GnRH antagonist cycle.[20]

In the present study we found that the combination of biomarker particularly AFC+AMH was found to have statistical significant association in predicting the ovarian response, whereas the other biomarker combinations like AFC+FSH and AMH+FSH did not had a significant association in the ovarian response prediction. This report was contradicting with the result showed by TNL Vuong et al, where he quoted that there was no value of adding AFC to AMH in prediction models.[20] This difference might be due to the difference in the selection of the patients where they had selected only patients with high responders and also the patients belonging to a different ethnic group.

A study done by Polyzos et al reported that keeping the AFC cut-off of 8 it had the sensitivity of 72.2% and specificity of 84.6%, and the AMH cut off value as 3.52 ng/ml for which the sensitivity and specificity was 89.5% in predicting the ovarian response.[18] In our study we kept the AFC cut off value as 7 where it had the sensitivity as 100% but specificity was only 24.1%, and for AMH the cut off value was kept as 1.1 ng/ml for which the sensitivity and specificity was 75.4% and 40.7% respectively. We made the cut-off values based on the ESHRE (European Society of Human reproduction and Embryology) guidelines 2015. Few of the studies done by Arce et al and TNL Vuong et al have found that FSH was significantly less useful than AFC and AMH as a predictor of ovarian response and this was almost in par with our study, where among the poor responders in our study the FSH had the lowest predictive value when compared to AFC and AMH in predicting ovarian response.[16,20] While AFC and AMH are good predictors of ovarian response, they appear to have less value in predicting live birth rate. There are number of factors that determine the chance of pregnancy other than ovarian response including embryo quality, transfer technique and endometrial receptivity, which may be why tests for ovarian response may not be sensitive enough to predict pregnancy outcome after IVF/ICSI.[21] The patients who were found to have only with low AMH value were advised for donor egg, whereas patient with low AFC count usually conceive by their own eggs. Patient with very poor ovarian reserve still become pregnant, and those with high ovarian reserve might not achieve pregnancy. The inclusion of data on oocyte quality would have added value to this study.

CONCLUSION

This study indicates that AFC is the most useful marker in predicting the ovarian response, than that of AMH and FSH. Combination of biomarkers, AFC+AMH is also highly predictive of ovarian response in terms of oocyte matured, fertilised and embryo cleavage and none of the biomarker was able to predict the pregnancy rate. So doing AFC assessment alone would be more cost effective for predicting the ovarian response in patients undergoing controlled ovarian stimulation with GnRH antagonist.

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