Original Article

Serum AMH Level to Predict the Hyper Response in Women with PCOS and Non-PCOS Undergoing Controlled Ovarian Stimulation in ART

Radha Vembu, Nellepalli Sanjeeva Reddy

Department of Reproductive Medicine & Surgery, Sri Ramachandra Medical College & Research Institute, Sri Ramachandra University, Chennai, Tamilnadu, India

ABSTRACT

Background: It is essential to determine the cut-off value of serum anti-Mullerian hormone (AMH) to predict the hyper response in assisted reproductive technology (ART). There are few studies mentioning the cut-off value for the hyper response in infertile women but not specifically for polycystic ovary syndrome (PCOS) and non-PCOS groups. With this in background, this study was conducted. **Aim:** To determine the cut-off value of serum AMH to predict the hyper response in women with PCOS and non-PCOS undergoing a controlled ovarian stimulation (COS) in ART. **Objective:** To compare the outcome of stimulation in PCOS and non-PCOS groups. **Materials and Methods:** All 246 women enrolled for Intra Cytoplasmic Sperm Injection (ICSI) fulfilling the selection criteria were recruited. On the day 3 of the cycle, the serum AMH, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), estradiol and antral follicle count (AFC) were measured. They underwent COS as per the unit protocol. They were divided into PCOS and non-PCOS groups as per the Rotterdam's criteria. The mean age, duration of infertility, Body Mass Index (BMI), Ovarian reserve markers and outcome of stimulation were compared. Using the Statistical Package for the Social Sciences version 16.0 software, the significant difference was measured by multivariate analysis, as well as a one-way analysis of variance with Tukey's post-hoc test was used. **Results:** Among 246 women, 31.3% were in PCOS group, and 68.7% were in non-PCOS group. Comparison of PCOS and non-PCOS groups showed a significant difference in the age with the mean age being 29.2 and 31.5 years, respectively. The mean AMH and AFC were 2-fold higher in PCOS group. The mean number of follicles, oocytes retrieved, MII and oocytes fertilised were significantly higher in PCOS group. The pregnancy rate was 52.6% in PCOS and 30.9% in non-PCOS group. In the PCOS group, 22.1% had ovarian hyper stimulation syndrome (OHSS), and only 4.7% had OHSS in non-PCOS group ($P = 0.0005$). Receiving Operator Curve (ROC) curve was plotted to predict the hyper response, which showed a cut-off value of 6.85 ng/ml with a sensitivity of 66.7% and a specificity of 68.7% for PCOS group and 4.85 ng/ml with a sensitivity of 85.7% and a specificity of 89.7% in non-PCOS group. **Conclusion:** The cut-off value of serum AMH to predict the hyper response in PCOS group is 6.85 ng/ml and in non-PCOS group is 4.85 ng/ml.

KEYWORDS: *Anti-Mullerian hormone, controlled ovarian stimulation, hyper response, non-PCOS, PCOS*

INTRODUCTION

The ovarian response to gonadotropins in a controlled ovarian stimulation (COS) is associated with an inter individual variability. To improve the safety and efficacy

Address for correspondence: Dr. V. Radha, MBBS, DGO, DNB, FICOG, PhD, Associate Professor, Department of Reproductive Medicine & Surgery, Sri Ramachandra Medical College & Research Institute, Sri Ramachandra University, Chennai 600116, Tamilnadu, India. E-mail: ganesh_radha@yahoo.in

Access this article online

Quick Response Code:

Website:
www.jhrsonline.org

DOI:
10.4103/jhrs.JHRS_15_16

of COS, it is better to individualise the initial dose of gonadotropins based on the ovarian reserve markers. Among all the markers, anti-Mullerian hormone (AMH) and antral follicle count (AFC) are showing promising results.[1,2] By definition, the hyper response includes a number of oocytes retrieved above a certain threshold, the development of ovarian hyper stimulation syndrome (OHSS) or cycle cancellation due to the hyper response or a combination of these three entities.[3] There are studies to predict the cut-off value of AMH to predict the hyper response but not specifically in the polycystic ovary syndrome (PCOS) and non-PCOS groups. Hence, a study was conducted to determine the cut-off value of serum AMH to predict the hyper response in women with PCOS and non-PCOS and to compare the outcome of COS among the women with PCOS and non-PCOS.

MATERIALS AND METHODS

This was a prospective cohort study conducted in the Department of Reproductive Medicine at a tertiary care centre from January 2011 to August 2013. A total of 246 women enrolled for Intra Cytoplasmic Sperm Injection (ICSI) were included in the study. Women in the age group of 20–45 years with bilateral ovaries were included in the study. Women who were <20 years and more than 45 years, with hypogonadotropic hypogonadism were excluded. Informed consent was taken from all the participants, and Institutional Ethics Committee (IEC) approval (IEC – NI/10/June/17/17) was obtained to conduct the study.

A detailed history and a physical examination were performed on all the participants. On day 3 of the cycle, serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Estradiol were assayed by Chemi Luminescent Immuno Assay (CLIA), and on the same day with the same sample, serum sample for AMH assay was separated within one hour of venipuncture and was stored at −20°C. The samples were later assayed by using AMH gen II method, the analytical sensitivity was 0.14 ng/ml, intra-assay and inter-assay CVS were <12.3 and <14.2% respectively. These patients underwent COS within three months as per the unit protocol. The dose of gonadotropins was individualised based on the age and ovarian reserve markers, and the starting dose did not exceed 225 IU in the hyper responders. On the basis of the Rotterdam's criteria, they were divided into PCOS and non-PCOS groups,[4] and on the basis of the number of oocytes retrieved, response was considered as poor if ≤3 oocytes were retrieved, normal if 4–19 oocytes were retrieved and hyper if ≥20 oocytes were retrieved.[5]

Statistical analysis

The data obtained were analysed using the Statistical Package for the Social Sciences version 16.0 software (SPSS Inc., Chicago, IL, United States). To describe the data, descriptive analysis, mean and standard deviation were used. To find the significant difference in the multi-variate analysis, one-way analysis of variance with Tukey's post-hoc test was used.

RESULTS

All the 246 women enrolled were analysed. Among them, 77 (31.3%) were in the PCOS group, and the remaining 169 (68.7%) were in the non-PCOS group. Table 1 depicts the baseline characteristics of the study population, and Table 2 depicts the mean ovarian reserve markers in both the groups.

In our study, 67.5% had an agonist protocol, and the remaining 22.5% had an antagonist protocol with no significant difference in the protocol among the PCOS and non-PCOS groups ($P = 0.549$). Among the PCOS group, 32.5% and in non-PCOS group, 28.5% had agonist protocol, and there was no significant difference in the protocol in both the groups. The protocol was decided based on age, Body Mass Index (BMI) and ovarian reserve markers as per the institutional protocol.

Table 1: Baseline characteristics

Characteristics	PCOS (N = 77)	Non-PCOS (N = 169)	P value
Age distribution (years)	29.2 ± 3.8	31.5 ± 4.6	0.0005[**]
Duration of infertility (years)	6.8 ± 3.5	7.3 ± 4.2	0.417[#]
BMI (kg/m²)	26.6 ± 5.1	26.4 ± 4.5	0.825[#]

[**]$P < 0.01$ is highly significant. [#]Not significant.

Table 2: Ovarian reserve markers

Ovarian reserve markers	PCOS (N = 77)	Non-PCOS (N = 169)	P value
FSH (IU/L)	6.1 ± 1.4	7.6 ± 2.5	0.0005[**]
AMH (ng/ml)	7.8 ± 3.3	2.9 ± 1.8	0.0005[**]
AFC	19.3 ± 4.2	9.8 ± 4.0	0.0005[**]

[**]$P < 0.01$ is highly significant.

The dose of gonadotropins required was significantly more in the non-PCOS group, (mean value being 2700 IU versus 3300 IU) ($P = 0.0005$) with no significant difference in the number of days of stimulation ($P = 0.406$) and with the mean number of days being 12 days. The outcome of stimulation is shown in Table 3.

In PCOS group, there were no patients with poor response; in that, 41.6% had normal response, and 58.4% had hyper response, whereas in non-PCOS group, 20.1% had poor response, 71.6% had normal response and 8.3% had hyper response.

The pregnancy rate was 52.6% in PCOS group and 30.9% in non-PCOS group, which was statistically significant ($P = 0.0005$).

Among the complications, 5.3% of the cycles were cancelled in the non-PCOS group due to poor follicular growth. According to Schenker and Weinstein classification, OHSS was observed in 22.1% of PCOS group, and in 4.7% of non-PCOS group, this was statistically significant ($P = 0.0005$) with mild OHSS being common.

To determine the cut-off value of AMH to predict the hyper response in both the groups, Receiving Operator Curve (ROC) curve was plotted, and the Area under the curve (AUC) was recorded as shown in Table 4.

DISCUSSION

PCOS is a common endocrine disorder affecting 10% of the women of reproductive age group.[6] To our knowledge, our study is the first of its kind to predict the cut-off value of AMH to predict the hyper response in both PCOS and non-PCOS groups. In an assisted reproductive technology (ART) cycle, excessive response to gonadotropins introduces the risk of OHSS[7] and can decrease the chances of pregnancy.[8] In the view of these drawbacks, prediction of the hyper response is very essential to improve the success rate and to avoid complications.

In a meta-analysis, both AMH and AFC were found to be the accurate predictors of the hyper response, and both have a clinical value.[9] However, AFC has to be measured in the early follicular phase, requires high-end machine, trained personal and shows inter-observer variability. The advantage of measuring AMH is that it can be assayed on any day of the menstrual cycle with negligible inter-cycle variability.

In our study, even though age distribution was found significant among the PCOS and non-PCOS groups, we did not observe any significant difference in the subgroups of poor, normal and hyper response in each group. We observed a significantly high AMH and AFC in PCOS group when compared to non-PCOS group. This correlates with the respective studies of Broekmans et al.[10] and La Marca et al.[11] This might be due to the more number of small antral follicles producing more AMH.

Even though the follicles recruited are more in the PCOS group, the number of oocytes retrieved and fertilised is comparatively less. This might be due to the detrimental effects of supra-physiological hormone levels on oocytes and embryo quality,[12,13] and affects the orderly proliferation and subsequent luteinization of the endometrium and its receptivity.[14] However, the pregnancy rate was more in the PCOS group in our study, probably due to the decreased dose of gonadotropins requirement in the PCOS group, due to individualisation of the initial dose and availability of more better quality embryos to choose.

In our study, we did not observe any significant difference in the type of stimulation protocol used ($P = 0.549$) either between the two groups or within the groups.

In our study, to predict the hyper response, we observed a cut-off value of AMH of 6.85 ng/ml in PCOS group, which had a sensitivity and specificity of 66.7 and 68.7%, respectively. In non-PCOS group, a value

Table 3: Outcome of stimulation

Parameters	PCOS (N = 77)	Non-PCOS (N = 160)	P value
Number of follicles	22.2 ± 6.5	12.9 ± 5.9	0.0005[**]
Oocytes retrieved	21.4 ± 7.8	10.0 ± 6.2	0.0005[**]
MII	17.1 ± 6.9	8.6 ± 5.6	0.0005[**]
Oocytes fertilised	13.4 ± 6.2	7.0 ± 4.7	0.0005[**]

[**]$P < 0.01$ is highly significant.

Table 4: AUC for AMH to predict the hyper response

AMH	Cut-off value (ng/ml)	AUC	95% CI	Sensitivity (%)	Specificity (%)
PCOS	6.85	0.722	0.602–0.842	66.7	68.7
Non-PCOS	4.85	0.959	0.927–0.992	85.7	89.7

of 4.85 ng/ml had a sensitivity and specificity of 85.7 and 89.7%, respectively. These values are high as the AMH assay is by Gen II assay unlike older assays being used in other studies. There are not many studies to describe the cut-off value of AMH to predict the hyper response in PCOS. Dewailly *et al.*[15] have observed a threshold value of serum AMH >35 pmol/L or >5 ng/ml for the diagnosis of PCOS, but they have not identified the cut-off value for predicting the hyper response in PCOS. Nardo *et al.*[16] observed a cut-off value of AMH of 3.5 ng/ml with a sensitivity and specificity of 88 and 70%, respectively. They included both PCOS and non-PCOS together unlike our study, where we determined separately in both the groups, and they assayed serum AMH by the older assay methods, which was 40% lower than the generation II assay. Aflatoonian *et al.*[17] observed a cut-off value of 4.83 ng/ml with a sensitivity and specificity of 93 and 78%, respectively to predict the hyper response. They had included both PCOS and non-PCOS patients together and their cut-off for the hyper response was ≥15 oocytes retrieved unlike our study, even though the AMH assay method remains the same. The main strength of our study is the prospective study design with no dropout of the subjects during the study. However, multicentric trials may be essential to confirm the AMH cut-off values in different ethnic groups.

CONCLUSION

For the women undergoing COS in ART, the cut-off value of AMH to predict the hyper response in PCOS group is 6.85 ng/ml, and for non-PCOS group, it is 4.85 ng/ml.

Acknowledgements

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

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