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Study of association of poor responders with serum Inhibin B levels in ovulation induction

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Abstract
Objective: The aim of this study is to assess the association of poor ovarian response with serum inhibin B levels.
Material and Methods: This study was a prospective observational study. A A total of 100 infertile patients attending the infertility clinic at Heritage Institute of Medical Sciences, Varanasi from june 2018-2019 were included in the study. Blood samples were drawn on day 3 of menstrual cycle for assessment of serum Inhibin-B levels. Ovulation induction was done with clomephene citrate 100mg 1OD from day 3 to day 7 and patients followed with serial USG measurements until at least one leading follicle was ≥ 20 mm. Number of dominant follicles (>or =14mm) at the time of HCG administration was counted to analyse the result of ovulation induction. Patients with 3 or more follicles in 1 st cycle were taken in group 1.Patients with less than 3 follicles in 1st cycle were taken as group 2. Patients with no follicles formation in 1 st cycle had their cycle cancelled. Patients of group 2 who did not conceive or had their cycles cancelled were subjected to 2nd cycle of ovulation induction. In the second cycle ovulation induction was done with clomephene citrate 100mg from day3 for five days with inj HMG 150 IU given i.m. on day 8 and then every other day until HCG 10,000 IU was administered as a single I.M injection to trigger ovulation when at least one leading follicle was ≥ 20 mm. Patients with more than 3 or more follicles after 2nd cycle of induction were included in group 2a. Patients developing less than <3 follicles at end of second cycle were considered as poor responders and included in Group 2b.
Results: Out of the100 patients, 55(group 1 + group 2 a) turned out to be good responders while remaining 45(group 2b) were poor responders. Good response rate of those having S. Inhibin values <12.45 pg/ml was lower (13.3%) as compared to that of patients having S. Inhibin 12.45-43.47 pg/ml (54.3%) and >43.47pg/ml (87.5%). Statistically, this association was significant too (p< 0.001). Mean S. Inhibin was higher among good responders (55.4±17.2) as compared to poor responders (17.2±15.5) and the difference was significant statistically too (p< 0.001).
By Receiver operator curve analysis for poor outcome during entire study for selected cut-off value of 21.43 pg/ml, S. Inhibin B had specificity of 81% and sensitivity of 80.3%.
Conclusion: Inhibin B proves to be a novel and promising marker for assessment of poor ovarian reserve. The cut off value of inhibin B to identify poor responders was found to be 21.43pg/ml. Levels of serum inhibin B lower than <21.43pg/ml were associated with higher chances of development of poor response.

Keywords: Poor ovarian response, Inhibin B, Ovarian reserve, ART, Ovulation induction

Introduction
In this modern era of urbanization infertility has emerged as a serious health problem in India. The trend of maternity postponement has become very common. According to the census reports of India 2011, 2001, 1991, 1981 Institute of Population Sciences, Mumbai infertility in India has risen by 50% since 1981 [2]. This has led to an increasing demand for assisted reproduction technologies. Thus, the need for evaluation of functional ovarian reserve has arisen. Estimation of ovarian reserve would help physicians and reproduction specialists to give better advice to infertile couples. It would also help in guiding individualized stimulation protocols & will lead to reduction of the emotional and financial burden of hard and stressful therapeutic procedures to the infertile couples.
These assisted reproductive technologies involve various ovulation induction protocols. Failure to respond adequately to standard stimulation protocol is called poor ovarian response[3]. This result in decreased oocyte production, cycle cancellation and is overall associated with significantly diminished probability of pregnancy.
The Rotterdam ESHRE/American Society for Reproduction Medicine (ASRM) sponsored

PCOS consensus workshop group 2004, a consensus was reached on minimal criteria to define poor ovarian response

1. Either advanced age more than 40 years
2. Less than 3 oocytes retrieved with conventional stimulation protocol
3. Abnormal ovarian reserve test (i.e. AFC < 5-7 follicles or AMH < 0.5-1.1 ng/ml).

Two episodes of poor ovarian response after maximal stimulation were sufficient to define patient as a poor responder [1].

Various markers have been to assess the ovarian reserve. Commonly used markers are S.FSH, S.LH, S.AMH, Antral follicle count and ovarian volume.

Inhibin-B is a glycoprotein hormone of the super family of transforming growth factor-beta secreted by granulosa and theca cells. Inhibin–B inhibits pituitary FSH secretion and paracrine action on developing follicles. The aim of this study is to assess the association of poor ovarian response with serum inhibin B levels.

Material and Methods

This study was a prospective observational study. A A total of 100 infertile patients attending the infertility clinic at Heritage Institute of Medical Sciences, Varanasi from june 2018-2019 were included in the study. Inclusion criteria. (i) Apparently healthy infertile women having ovulatory factor infertility willing to participate in the study with written informed consent. (ii) Age less 40 years. Exclusive Unwillingness to participate in the study. Patients suffering from acute infections, PID, Endometriosis, active tuberculosis, acute liver disease, hypersensitivity to the drugs used, immunocompromised individuals, h/o ovarian surgery, genetic causes of infertility for example Turners syndrome.

On day three of the menstrual cycle, 5 ml of venous blood was withdrawn from the cubital vein in plain vacuutainers and stored at -4 degree temperature in the refridgerator and transported within 1 hour to the pathology lab, HIMS. There the sample was centrifuged at 3500 rpm and stored at -20 degree C. Assay of s. Inhibin B was done in a single batch by human Inhibin-B ELISA kit.

Transvaginal ultrasound was done on day 3.antral follicle count was measured.

First cycle of ovulation induction done with standard stimulation protocol of clomephene citrate 100mg 1OD from day 3 to day 7 and patients followed with serial USG measurements until at least one leading follicle was ≥ 20 mm. All patients were followed up by follicular monitoring with vaginal ultrasonography starting on the 8th day of the cycle and then every other day until HCG 10,000 IU was administered as a single I.M injection to trigger ovulation when at least one leading follicle was ≥ 20 mm. Number of dominant follicles(>or =14mm) at the time of HCG administration was counted to analyse the result of ovulation induction.

Patients with 3 or more follicles in 1 st cycle were taken in group 1.Patients with less than 3 follicles in 1st cycle were taken as group 2. Patients with no follicles formation in 1 st cycle had their cycle cancelled. Patients of group 2 who did not conceive or had their cycles cancelled were subjected to 2nd cycle of ovulation induction. In the second cycle ovulation induction was done with clomephene citrate 100mg from day 2/day3 for five days with inj HMG 150 IU given i.m. on day 8 and then every other day until HCG 10,000 IU was administered as a single I.M injection to trigger ovulation when at least one leading follicle was ≥ 20 mm.Patients with more than 3 or more follicles after 2nd cycle of induction were included in group 2a. Patients developing less than <3 follicles at end of second cycle were considered as poor responders and included in Group 2b.

Results

Out of a total 100 patients enrolled in the study and evaluated for outcome at first cycle, 30 (27.6%) showed good response and classified as group 1 and 70 (72.4%) showed poor response classified as group 2.Group 1 was excluded from second cycle observations and hence second cycle observations were made in 70 patients of group 2 only. Out of these 70 patients, 25 (35.2%) turned out to be good responders while remaining 45(64.8%) were poor responders

Good response rate of those having S. Inhibin values <12.45 pg/ml was lower (13.3%) as compared to that of patients having S. Inhibin 12.45-43.47 pg/ml (54.3%) and >43.47pg/ml (87.5%). Statistically, this association was significant too ($p< 0.001$). Mean S. Inhibin was higher among good responders (55.4±17.2) as compared to poor responders (17.2±15.5) and the difference was significant statistically too ($p< 0.001$). Refer table 1.

By Receiver operator curve analysis for poor outcome during entire study for selected cut-off value of 21.43 pg/ml, S. Inhibin B had specificity of 81% and sensitivity of 80.3%.

Table 1: Association of Serum inhibin B with Pattern of Response in Entire Study Period (n=100)

S. Inhibin B level (in pg/ml)	Good responders(group 1 +group 2a) ie. having more than 3 follicles	Poor responders(group 2 b) ie. Having less than 3 follicles	Significance of difference
First tertile (<12.45) (n=31)	5 (13.3%)	26 (86.7%)	z=3.549; $p< 0.001$ (Mann-Whitney U test)
Second tertile (12.45-43.47) (n=37)	21 (54.3%)	16 (45.7%)	
Third tertile (>43.47) (n=32)	29 (87.5%)	3 (12.5%)	
Mean±SD (n=100)	55.4±17.2 pg/ml (n=55)	17.2±15.5pg/ml (n=45)	t=6.139; $p< 0.001$

Discussion

Ovarian response is dependent on ovarian reserve. It determines the capacity of the ovary to provide eggs that are capable of fertilisation resulting in a healthy and successful pregnancy [2]. Since true ovarian reserve cannot be determined directly as it would entail performing ovarian biopsy to establish the number of primordial follicles, markers of ovarian reserve that can estimate the reserve have evolved.

Inhibin B is a glycoprotein hormone of the transforming growth factor β (TGF-β) family. It is secreted by the granulosa cells of the growing follicles, and is selectively responsible for pituitary inhibition of FSH secretion. Thus serum inhibin B is a direct measure of ovarian reserve**.**

In 1998, Hall *et al.* [5]. conducted a study to test the hypothesis that dimeric inhibin A and/or inhibin B concentrations represent improved markers of in-vitro fertilization (IVF) outcome over follicle stimulating hormone (FSH), 78 women who achieved pregnancy within three assisted reproduction treatment cycles were matched to 78 women who underwent at least three assisted reproductive treatment cycles and failed to achieve pregnancy. They concluded that in patients undergoing assisted reproductive technology, of the parameters available prior to

cycle initiation, a combination of lower FSH and higher inhibin B was associated with a greater chance for a successful outcome but an absolute cut-off could not be defined; and during ovarian stimulation, higher concentrations of inhibin A and inhibin B in serum are associated with successful IVF and marked ovarian reserve as a measure of oocyte number and quality.

Hofmann *et al.* [6]. in 1998 conducted a study to determine inhibin-B concentrations during ovarian reserve screening in women with normal and diminished ovarian reserve as determined by the clomiphene citrate challenge test. Nineteen patients with normal ovarian reserve and 15 with diminished ovarian reserve had serum inhibin-B concentrations determined during ovarian reserve screening. For all patients, day 10 inhibin-B concentrations were higher than day 3. Women with normal ovarian reserve had higher inhibin-B concentrations on both days 3 and 10 than women with diminished ovarian reserve. Inhibin-B concentrations demonstrated a negative correlation with FSH levels on both cycle days 3 and 10 and a positive correlation with E2 on cycle day 10th. Thus they concluded that women with diminished ovarian reserve during ovarian reserve screening had reduced granulosa cell inhibin-B production compared with women with normal ovarian reserve. The lower inhibin-B concentrations may be responsible for the elevated FSH concentrations and may be indicative of the aging follicular apparatus.

Corson *et al.* [3] in 1999 conducted a study to compare a standard clomiphene citrate challenge test with inhibin-B serum concentrations also obtained on cycle days 3 and 10 as a negative predictor of pregnancy in a group of 106 women at risk for compromised ovarian function. Mean duration of follow-up was 8.25 months in 95 patients with 30 pregnancies recorded (plus one biochemical). Inhibin-B concentrations on cycle days 3 and 10 were correlated only with each other and not with serum oestradiol, follicle stimulating hormone (FSH) and/or pregnancy rates. Pregnancy occurred in 34.5% (10/29) of all patients with inhibin-B values ≥45 pg/ml on cycle day 3 and in 31.8% (21/66) of those with values <45 pg/ml. Seifer *et al.* reported higher cycle cancellation rates and lower pregnancy rates in women with low (<45pg/ml) day 3 serum inhibin B concentrations [4]. According to the study of Seifer *et al.* the value of inhibin B of 45pg/ml was considered as a cut off to identify poor responders. Muttukrishna *et al.* in 2004 in their study on the role of AMH in predicting ovarian response to gonadotrophin stimulation found a significant role of AMH in identifying poor responders [7]. Serum AMH was found to have a strong correlation with serum inhibin B.

In our study the mean value of serum inhibin B has been found to be 55.4±17.2 pg/ml in the good responders as compared to the poor responders in which the mean value is 17.2±15.5pg/ml. By ROC analysis the cut off value of inhibin B to identify poor responders was found to be 21.43pg/ml. Table 2.

Table 2: ROC Analysis for Poor Outcome during overall study period

Test Result Variable(s)	Area	Std. Error(a)	Asymptotic Sig.(b)	Selected cut-off	Predicted Sensitivity	Predicted specificity
SINHIB	.831	.039	.000	<21.75	80.3%	81.0%

a - Under the nonparametric assumption, b - Null hypothesis: true area = 0.5

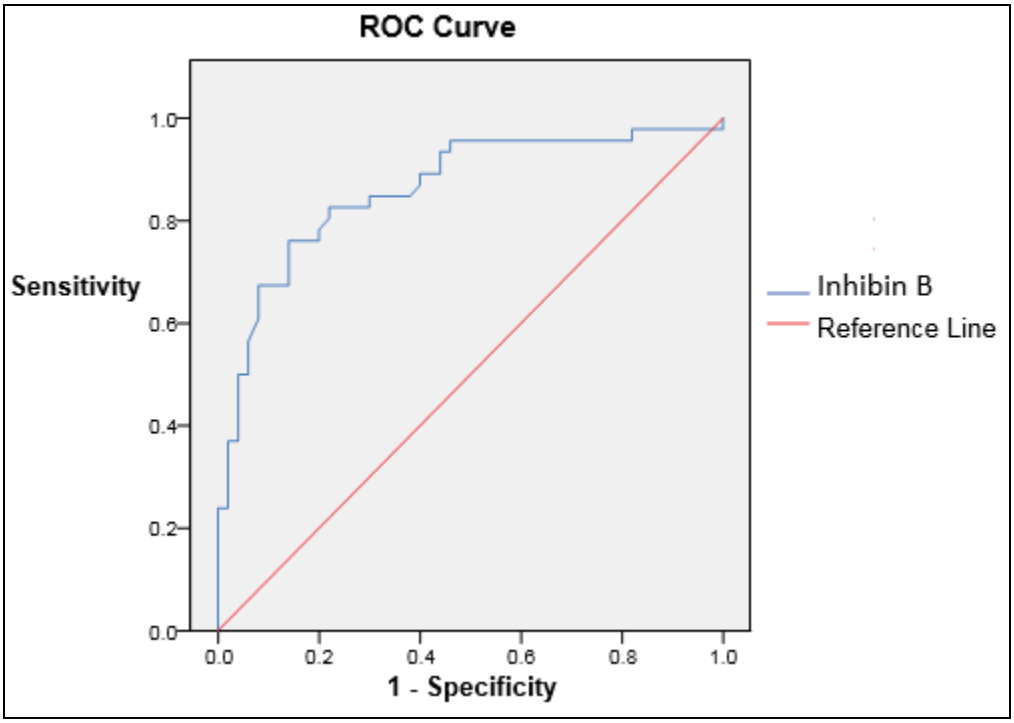

Fig 1: showing ROC Analysis for inhibin B for poor outcome during overall study period

Conclusion

Assessment of ovarian reserve in artificial reproductive technology is beneficial to plan the appropriate stimulation protocol and aids in counseling of patients with poor ovarian reserve. Inhibin B proves to be a novel and promising marker for assessment of poor ovarian reserve. The cut off value of inhibin B to identify poor responders was found to be 21.43pg/ml. Levels of serum inhibin B lower than <21.43pg/ml were associated with higher chances of development of poor response.

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