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Markers of Ovarian Function: Anti-Mullerian Hormone (Amh) Vs. Others

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Abstract

Aim: the aim of this study is to determine day 3 serum AMH, FSH, LH, Estradiol (E_2), Inhibin B levels, ovarian volume and antral follicle count to assess ovarian function.

Methods: The study was conducted on 130 infertile women between age 18 and 43 years. Day 3 serum AMH level was estimated by sandwich enzyme immunoassay; serum FSH, serum LH, serum E_2 by solid phase two site chemiluminescent immunometric assay; Inhibin B by ELISA; and ovarian volume and AFC by transvaginal ultrasonography.

Results: With advancing age, serum AMH level ($p<0.0001$), AFC ($p<0.05$), ovarian volume ($p>0.05$), and Inhibin B ($p>0.05$) were decreased and serum FSH ($p<0.05$), LH ($p>0.05$) and E_2 ($p<0.05$) were increased. Serum AMH level was 4-6.8 mg/ml with optimal fertility in 26.15% cases and 2.2-4.0 mg/ml with satisfactory fertility in 53.85% cases. Serum AMH levels were more strongly correlated with AFC ($p<0.0001$) and ovarian volume ($p<0.0001$).

Conclusion: Serum AMH levels were more robustly correlated with AFC than FSH, LH, E_2 and Inhibition B on day 3 of the cycle. This suggests that serum AMH might be taken as single test to reflect ovarian reserve.

Keywords: Anti-Mullerian hormone, antral follicle count, follicle stimulating hormone, luteinizing hormone, Estradiol.

1. Introduction

The ovarian reserve constitutes one of the most important factors affecting ovulation. By “ovarian reserve”, we basically mean the size of ovarian follicle pool and the quantity of the oocytes therein. Many efforts have been made since the beginning to assess the ovarian reserve. Previously, a composite test consisting of early follicular serum level of FSH, Inhibin B and Estradiol (E_2) was used. Inhibin B and E_2 are produced by early antral follicles in response to FSH, having the classical feedback loop of pituitary gonadal axis. With the decline of the follicle pool, serum levels of Inhibin B and E_2 decrease leading to a rise in serum FSH level. Because these factors are part of feedback system, their serum levels are not independent of each other, and hence, they have to be measured collectively. Separately, their levels are poor predictors of ovarian reserve because their levels vary widely by assay, laboratory population and reproductive aging.

So far, AFC (follicles of 2-10 mm size), which quantifies the number of antral follicles in the ovary by ultrasonography on the day 3 of menstrual cycle, best

predicts the quantitative aspect of ovarian reserve. However, it might be sometimes difficult for the patient to get ultrasound done on a specific day; additionally, it requires for the patient measurements of the AFC by additional transvaginal ultrasound examination during early follicular phase.

Therefore, in search of a better, time dependent parameter, serum Anti-Mullerian hormone (AMH) emerged as a promising test to assess the ovarian reserve. AMH or Mullerian Inhibiting substance (MIS) is a glycoprotein hormone, with a molecular weight of 140 kDa, and produced by granulosa cells in ovarian follicles from 36 weeks of gestation until menopause. It is first made in the primordial follicle stage but the highest production of is in the preantral and small antral stages (<4mm diameter) of folliculogenesis. During these stages, follicles are microscopic and can't be seen by ultrasonography, thus limiting their ability to be counted by ultrasound. Production of AMH gradually decreases as the follicle reaches 8mm diameter. AMH levels do not change significantly throughout the menstrual cycle. Normal serum AMH level ranges 2-6.6 mg/ml (14.28-48.55p mol/l) in any phase of the cycle. In recent years, accumulated data indicate that serum AMH may fulfill the requirements to be the best test to predict ovarian reserve.

2. Materials and Methods

The study was conducted on 130 infertile patients, aged between 18 and 43 years, over a period of 12 months. The infertile women who had regular menstrual cycles of 21-35 days with no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion; a body mass index (BMI) of 18-27 kg/m^2, on no current therapy; and adequate visualization of ovaries during transvaginal ultrasound scanning. Complete hemogram, blood sugar estimation, thyroid profile, liver function test, kidney function test, VDRL, HIV1 and 2, HBsAg urine routine and microscopy examination and Montoux test were done. Hysterosalpingography was done on day 3 of menstrual cycle to measure ovarian volume and for antral follicles count. The cases with endocrinological disorders, abnormal liver and kidney function tests were excluded from the study.

2.1 Study protocol

Serum AMH; FSH, LH, E_2 and Inhibin B were estimated for venous samples on day 3 of menstrual cycle at ~9:00 h. serum AMH estimation was done by sandwich enzyme immunoassay for in vitro quantitative measurements in human serum. Normal level, corresponding to normal ovarian reserve, was 2.0-6.8mg/ml. serum site, chemiluminescent immunometric assay. Patient test results were determined automatically by the system software using the smooting 'spline' math model, Inhibin B was tested by enzyme-linked immunosorbant assay (ELISA) method. Normal values for Inhibin B was >45pg/ml. normal values in serum considered were for FSH 1.4-9.9mIU/ml, LH 1.0-90.0 mIU/ml, and E_2 30-4—pg/ml. Transvaginal sonography was done on day 3 of menstrual cycle using the 7.5 MHz transvaginal probe, to assess antral follicular count and total ovarian volume. Antral follicle count was done by scanning the ovary from the outer to inner margin. All follicles measuring 2-10 mm size were counted in both the ovaries. The sum of both counts was "Antral Follicular Count". Normal AFC was taken if it was more than 12. The volume of the ovaries was assessed by measuring the diameter of the contour in three perpendicular directions and applying the equation of volume of an ellipsoid (D_1 X D_2 X D_3 X 0.523). Total ovarian volume was then obtained by sum of the volumes of the left and right ovary. Normal reference level was 9-11 cm^3. Finally correlations of day 3 "serum AMH" and day 3 "serum FSH,LH, E_2 and Inhibin B" levels were found out with antral follicular count and ovarian volume.

The correlation was found to be statically significant, if on analysis p-value was <0.05.

3. Result

The cases were divided into four groups according to age. The maximum infertile women were of age between 21 to 30 years (Table-1). Age dependent loss of fertility has been described due to decreasing follicle pool. However, this fact may be variable individually. Hence, for the assessment of the follicle pool, among the biochemical markers, serum FSH and serum E_2 showed statically significant variations (p-value <0.05). Serum level of FSH at day 3 increased with advancing age. Serum E_2 level was also found to increase with the increasing age. Though the results may look to be contradicting the fact of negative feedback loop, the fact, however is that serum E_2 level basically depicts follicular growth rather than the number of antral follicles. Elevation in the FSH and decrease in Inhibin B results in advanced follicular growth at the end of preceding luteal phase, in response to which day 3 serum E_2 levels are typically higher in older women with advanced reproductive aging but serum AMH level had highly significant reduction with increasing age (<0.0001), even more than correlation of AFC with advancing age (p-value <0.05) as depicted in Table-2.

34 patients were having serum AMH level 4.0-6.8 mg/ml and 70 patients had 2.2-4.0 mg/ml. All of them ovulated on giving ovulation induction medication. Hence, it can be reemphasized that serum AMH ranging between 2.2 and 6.8 mg/ml predicts optimal to satisfactory ovarian reserve patients. Four cases had high level of AMH, and their ultrasound revealed polycystic ovaries. Inspite of having higher levels of AMH, these patients had low fertility. This mandates ultrasonographic picture of the ovary in the work up of ovarian reserve. PCOS patients had large number of antral follicles secreting high amount of AMH. Subfertility in this group of patients is due to other coexisting hormonal imbalances. In each case where AMH level was < 0.3 mg/ml, the fertility was said to be very low (Table-3).

A noticeable reduction in the number of early antral follicles characterizes the decline of ovarian function that results from relative follicular attrition. Antral follicle count is so far the best predictor of the ovarian reserve.

The strength of correlation between the number of early antral follicles and other biochemical markers of the ovarian reserve were compared. As the number of AFC increased, ovarian volume also increased (p-value < 0.05), serum FSH decreased (p-value < 0.001), serum LH decreased (p-value < 0.001), serum Inhibin B increased (p-value < 0.05), serum E_2 decreased (p-value < 0.05), serum AMH increased (p-value < 0.001). All the parameters assessed in this study showed statically significant correlation, but serum AMH level was the most strongly correlated with antral follicle count (p-value < 0.001) (Table-4).

4. Discussion

The present study was designed to evaluate the direct relationship between peripheral AMH levels and the ovarian follicular status on day 3 of menstruation and to compare the strength of correlation between the number of follicles and hormonal parameters implicated directly or indirectly in the eventual stage of folliculogenesis. It was observed that serum AMH levels are closely related to early antral follicle count with a relationship that was remarkably more intense than those obtained with serum levels of Inhibin B, E_2, FSH and LH. These results are not only corroborating to but also expand clinical data reported previously by other investigators. Outcome of the present study revealed that with the assessment of ovarian function and reserve, we can identify those patients who are destined to fail. Age, as on ovarian function marker, was found to have prognostic value in in general infertile

population because age showed strong correlation with all markers of ovarian function like AMH, AFC, E_2 and FSH. AMH can screen the present status of ovarian function in general subfertile population because it has a role in both the processes of initial and critical recruitments as well as in women entering controlled ovarian stimulation and ART program. AMH better reflected the continuous decline of follicle pool with age than the other markers; it appears to be the best marker of gradual dwindling of follicle numbers and ovarian volume. AMH gives the most reliable reflection of individual reproductive aging. In accordance with the present study, Van Rooij et al [1] studied the relationship between AMH level and ovarian response during ovarian stimulation for IVF on 130 patients. They found that serum AMH levels were highly correlated with the number of antral follicles ($r=0.77$, $p < 0.01$) and a number of oocytes retrieved ($r=0.57$, $p < 0.01$). A negative association was found between AMH levels and poor ovarian response (fewer than 4 oocytes or cycle cancellation or 0.82, 95% CI 0.75-0.90, $p < 0.01$). the post GnRH rise in FSH and LH levels did not influence AMH values and concluded that poor response in IVF, indicative of a diminished ovarian reserve, associated with reduced baseline serum AMH concentrations. Fanchin et al.[2] studied day 3 serum levels of AMH, Inhibin B, E_2, FSH, LH and the number of early antral follicles estimated to compare the strengths of hormonal-follicular correlations; they also found that serum AMH levels were more strongly correlated with follicular count than were with other markers. Ernest et al. [3] found that AFC was the ovarian reserve marker that was significantly different among different age groups. Muttukrishna et al [4] found that the patients, who had concealed IVF treatment cycle, had AMH levels lower than the detection limits, while FSH levels were significantly high and Inhibin B was 50 folds lower compared with the patients who completed treatment. Muttukrishna et al [5] postulated that there was no significant change in AMH levels on ovarian stimulation with gonadotrophins, referring to AMH levels as independent marker, and not affected by FSH or LH even in normal menstrual cycle. Van Rooij et al [6] found that serum AMH concentrations showed the best consistency, with AFC. The FSH and Inhibin B showed only modest consistency, whereas E_2 showed no consistency at all, and it was concluded that serum AMH represented the best endocrine marker to assess the age related decline of reproductive capacity. La Marca et al [7] investigated that AMH exhibits a relatively stable expression during the menstrual cycle, making it an attractive determinant of ovarian activity. Knauff et al [8] concluded that in comparison with Inhibin B and AFC, AMH was more consistently correlated with the clinical degree of follicle pool depletion in young women presenting with evaluated FSH level.

5. Conclusion

Serum AMH levels were more robustly correlated with antral follicle count than serum FSH, LH, Inhibin B and E_2 at day 3 of cycle. Serum AMH is superior marker of ovarian reserve because it is highly associated with the number of antral follicle and has little cycle variability and decline throughout the reproductive life span. This suggests that AMH a new master marker reflects ovarian function better than usual hormone markers.

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TABLES

Table-1 Age Distribution

Sr. No.	Group	Age (in years)	No. of cases	%age of cases	Mean±SD
1.	1	<20	19	14.62	18.33±0.594
2.	2	21-30	76	58.46	26.07±2.25
3.	3	31-40	22	16.92	42.66±1.11
4.	4	>40	13	10.00	27.67±2.04
Total			130	100	

Table-2 Correlation of various parameters with different age groups

Sr no.	characteristics	Group 1	Group 2	Group 3	Group 4	p-value
1	Antral follicle count	16.22±4.00	13.31±3.00	9.26±1.17	6.21±0.92	<0.05
2	Ovarian volume (ml)	8.5±1.08	7.31±0.93	7.21±0.96	7.41±0.46	>0.05
3	FSH(MIU/ml)	4.77±0.53	6.09±0.59	6.48±0.28	6.80±0.23	<0.05
4	LH(MIU/ml)	3.86±0.59	5.54±0.59	5.59±0.33	6.58±0.36	>0.05
5	Inhibin B (pg/ml)	81.98±1.20	77.9±5.58	77.34±0.95	75.43±0.71	>0.05
6	E_2 (pg/ml)	81.48±3.14	39.72±11.84	54.20±3.57	59.96±4.61	<0.05
7	AMH (mg/ml)	5.56±0.99	3.25±0.70	2.61±1.20	0.20±0.11	<0.00001

Table-3 Serum AMH levels and Infertility

Sr. no.	AMH level (mg/dl)	No. of cases	%age of cases
1	High level (>6.8)	04	3.08
2	Optimal fertility (4-6.8)	34	26.15
3	Satisfactory fertility (2.2-4.0)	70	53.85
4	Low fertility (0.3-2.2)	14	10.77
5	Very low(undetectable)(0.0-0.3)	08	6.15
Total		130	100

Table - 4 Correlation of AFC with other parameters

Sr. no.	AFC	Mean ± SD	No. of cases	Ovarian volume(ml)	S.FSH (MIU/ml)	S.LH (MIU/ml)	S.Inhibin B (pg/ml)	S. E_2 (pg/ml)	S.AMH (mg/ml)
1.	<4	2.82±0.73	8	5.95±3.85	6.67±0.90	6.1±0.64	43.81±35.3	59.77±13.65	1.26±1.79
2.	4-7	6.34±0.76	15	6.10±2.98	7.02±2.09	5.73±1.30	75.61±39.87	57.12±14.03	1.49±1.64
3.	8-12	9.19±1.58	82	7.56±1.66	6.20±0.84	5.47±0.75	56.10±34.49	55.35±24.97	4.78±2.52
4.	>12	16.32±1.79	25	7.88±0.68	5.16±1.32	4.28±1.15	78.23±25.32	26.84±16.27	3.15±0.98
p- value				<0.05	<0.001	<0.001	<0.05	<0.05	<0.0001