ABSTRACT

OBJECTIVE: To compare the diagnostic accuracy of Anti-Mullerian hormone with follicle stimulating hormone for ovarian reserve assessment in infertile women by Receiver Operator Characteristic curve analysis.

STUDY DESIGN: A Cross Sectional study.

PLACE AND DURATION OF STUDY: At Institute of Basic Medical Sciences, University of Health Sciences in collaboration with Gynecology and Obstetrics Unit-II, Civil Hospital, Karachi from 2nd October 2011 to 15th October 2012.

METHODOLOGY: Fifty two infertile women between 20-35 years of age were selected from the out-patient. Out of 52 females, 25 women presented with primary infertility (no history of previous pregnancy) and 27 were secondary sub-fertile (history of previous pregnancy). Blood samples were obtained for follicle stimulating hormone and Anti-mullerian hormone levels.

RESULTS: Descriptive statistics for Anti-mullerian hormone and follicle stimulating hormone levels was taken as mean ± SD (26 ±4.02, 1.6± 0.62 and 8.98± 0.701) respectively. The sensitivity, specificity and accuracy of serum Anti-mullerian hormone levels and follicle stimulating hormone were evaluated by receiver operating characteristic curve (ROC curve). Area under the curve was 0.80 (AUC: 0.92) with standard error of 0.044 which was significant (P<0.05). Sensitivity and specificity was 79% and 88% respectively. Area under the curve for follicle stimulating hormone was 0.70 (AUC: 0.70) which was significant (P<0.05). Sensitivity and specificity was 65% and 76% respectively.

CONCLUSION: Anti-mullerian hormone assay has shown greater sensitivity so Anti-mullerian hormone assay can be used as a reliable diagnostic test for ovarian reserve assessment in infertile women.

KEYWORDS: Ovarian Reserve, Anti-Mullerian Hormone, Follicle Stimulating Hormone, Infertility.

INTRODUCTION

In recent decades, infertility has become a major global issue with medical, economic and psychosocial impact on infertile couples. Large number of infertile population is very anxious and eager to be treated. The prevalence of infertility worldwide is approximately 10-15% whereas in Asia it is around 8-12%. Infertility rate in Pakistan is about 21.9%1,2. Ovarian reserve is the amount of oocytes present in both ovaries. In order to determine an individual’s ovarian reserve, third day serum follicle-stimulating hormone levels are measured. The level of FSH is unstable throughout the menstrual cycle because it is affected by the negative feedback from the hypothalamus and pituitary gland. On the other hand, serum AMH levels show minimal fluctuation due to the continuous non-cyclic growth of small follicles3. Anti-Mullerian hormone is a protein hormone and belongs to the group of transforming growth factor (TGF- β) super family. It is mainly produced and secreted by the granulosa cells of ovarian follicles. The levels of serum AMH are hardly detectable at birth, but remains stable throughout the reproductive period. With advancing age, AMH levels start declining. According to recent studies, serum AMH levels represent the quantitative aspect of ovarian reserve. Moreover, its levels strongly correlate with the size of the early growing follicle pool, and due to lack of inter-cycle variability, it can be regarded as a diagnostic marker to assess ovarian reserve4. The ovary secretes AMH directly into the blood; hence it is easy to measure AMH in serum. Follicle stimulating hormone (FSH) is one of the major reproductive hormones in humans. It is a glycoprotein which is produced and secreted by the anterior pituitary gland. Since, it is a gonadotropin hormone; it acts by binding to specific receptors in the gonads. At puberty, it causes development and maturation of gonads. Moreover, during the fertile period it is necessary for the production of gametes5. Basal FSH on third day of the menstrual cycle is considered as an important marker of ovarian reserve. It reflects the qualitative aspect of the follicles during different phases of folliculogenesis6. There is an increase in FSH levels with depletion of follicles7,8. It is an easily accessible and inexpensive marker and is useful in pretreatment evaluation of infertile women. Reduced chances for pregnancy have been observed in younger infertile women when the levels of FSH exceed 8 IU/L9.

Follicle stimulating hormone shows increased variability from cycle to cycle and its measurement in women with irregular or scanty cycles is difficult due to its timely sample collection. Together these factors limit the use of FSH as a measure of ovarian reserve. On the other hand, AMH does not show any variability between the menstrual periods and can be measured
on any day irrespective of the menstrual cycle. Thus, serum AMH levels are being increasingly used in the assessment of ovarian reserve along with FSH. Moreover, AMH would be more convenient to assess the ovarian reserve status of infertile patients coming from the remote areas or interior parts of the country, since AMH measurements are not time bound. The major aim of this study is to compare the diagnostic accuracy of AMH assay with the FSH assay in infertile women with diminished ovarian reserve by determining the sensitivity, specificity, cut-off values and area under the curve by ROC analysis.

**METHODOLOGY**

This cross-sectional study was conducted at Institute of Basic Medical Sciences, Dow University of Health Sciences in collaboration with Gynecology & Obstetrics Unit-II, Civil Hospital, Karachi from 2nd October 2011 to 15th October 2012. Patients were selected from outpatient department and bench work was carried out at the Dow Diagnostic Research and Reference Lab, Ojha Campus. 52 infertile females were taken between the age range of 20-35 years. Primary infertile women (no history of previous pregnancy) with normal semen analysis of their husbands and patent fallopian tubes on the basis of hysterosalpingography were included in the study. Secondary sub-fertile females (history of previous pregnancy), with normal report of husband’s semen analysis, patent fallopian tubes and no history of pelvic inflammatory disease or endometriosis were also included in the study. Infertile women > 35 years and having history of either blocked fallopian tubes, ovarian malignancy, previous pelvic surgeries, drugs interfering with fertility like estrogen antagonists and male infertility factor were excluded from the study.

For estimation of serum AMH and FSH levels, all selected subject’s blood samples were drawn by venipuncture in serum separator tubes. Blood samples were taken from the infertile group for AMH levels on any day of the menstrual cycle. Approximately, 3ml of blood was collected by venipuncture in separate gel tubes, centrifuged and serum collected and frozen in aliquots at -200°C. Samples were stored temporarily at the Dow Collection Point and were then transferred to the Microbiology Lab at the Dow Diagnostic Research and Reference laboratory for further storage. Serum AMH levels were determined by enzyme linked immunosorbent assay, using Human AMH Elisa kit (CDN-E 1350) at Dow Diagnostic Research and Reference Lab. About 2 ml of blood was drawn to determine basal FSH levels on day 3 of menstrual cycle. Serum FSH levels were determined by using an automated chemiluminescence assay (Cobas-E 114) at the Dow Lab, Civil Hospital Karachi.

Descriptive statistics of AMH and FSH were taken as mean and standard deviation. Sensitivity, specificity and the area under the roc curve (AUC) which is a quantitative measure for accuracy were evaluated by receiver operating characteristic curve for AMH and FSH.

**RESULTS**

Mean AMH and FSH levels were taken as mean ± SD (26 ±4.02, 1.6± 0.62 and 8.98±0.701 respectively). ROC analysis was conducted to predict and compare the accuracy, sensitivity and specificity of AMH assay with FSH assay used as diagnostic tests for diminished ovarian reserve in infertile women. Infertile patients were divided into normal ovarian reserve group and diminished ovarian reserve group on the basis of cut off value of AMH and FSH.

Optimal cut-off value for AMH was 1.2ng/ml and for FSH, it was 8.5 mIU/ml. Area under the curve for AMH was 0.92 (AUC: 0.92) with standard error of 0.044 which was significant (P<0.05). Sensitivity and specificity for AMH was 79% and 88% respectively. Area under the curve for FSH was 0.70 (AUC: 0.70) which was significant (P<0.05) with sensitivity and specificity of 65% and 76% respectively.

**TABLE-I: DESCRIPTIVE STATISTICS OF CONTINUOUS VARIABLES**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH(ng/ml)</td>
<td>2.9</td>
<td>0.05</td>
<td>1.6 ± 0.62</td>
</tr>
<tr>
<td>FSH(mIU/ml)</td>
<td>18.60</td>
<td>2.30</td>
<td>8.98±0.701</td>
</tr>
</tbody>
</table>

AMH: Anti-Mullerian hormone
FSH: follicle stimulating hormone.
SD: standard deviation

**TABLE - II: ROC CURVE ANALYSIS FOR AMH & FSH:**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Area under the curve</th>
<th>Std. Error</th>
<th>P value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH</td>
<td>0.921</td>
<td>0.04</td>
<td>0.001**</td>
<td>0.79</td>
<td>0.88</td>
</tr>
<tr>
<td>FSH</td>
<td>0.707</td>
<td>0.076</td>
<td>0.018*</td>
<td>0.65</td>
<td>0.76</td>
</tr>
</tbody>
</table>

**P value is significant at <0.05**
Infertility is a global issue and needs to be assessed at an earlier stage for better options of fertility treatments. There are various conventional tests available for ovarian reserve assessment which includes the basal third day FSH levels and early antral follicle count by transvaginal ultrasound. Apart from these tests, AMH has also been proved as most reliable marker for ovarian reserve assessment. This study was designed to determine the use of AMH levels as a significant marker during the infertility workup in Pakistani population. Mean serum AMH was 1.6 ng/ml in infertile females. These findings were consistent with the Gleicher N study in which mean AMH was 1.59 ± 0.12 ng/ml. Mean serum FSH was 8.98±0.70 which was comparable with mean FSH 7.96 IU/l±2.6 S.D in Nardo et al study. Comparison of sensitivity and specificity of AMH and FSH for diagnosis of diminished ovarian reserve in infertile women were evaluated by receiver operating characteristic curve. AMH showed greater sensitivity (79% versus 65%) and greater specificity ( 88% versus76%) compared to FSH. These findings were close to the study conducted by Alipour F et al in which AMH had more sensitivity (80% versus 28.57%) and almost equal specificity (78.89% versus 78.65%) compared with FSH in diagnosis of premature ovarian failure. Area under the curve for AMH was 0.92 and this was consistent with the findings of Pigny et al and Choi et al study, who found that area under the receiver operating characteristic curve for the AMH assay was 0.851. However, a contrast study conducted by Riggs et al 2008 established sensitivity and specificity of AMH as 83-84% and 67-79% respectively. Our study showed that AMH levels with an optimal cut-off value of =1.2ng/ml and FSH levels with a cut-off value of = 8.5 mIU/ml predicts diminished ovarian reserve. This was in agreement with study conducted by Negm SM et al study in which serum AMH levels with an optimal cut-off value of 1.2 ng/ml and serum basal FSH levels with an optimal cut-off value of 8.9 IU/ml was a reliable predictor of poor ovarian response.

The present study concluded that AMH assay has shown greater sensitivity than the FSH assay as predictors in determining the diminished ovarian reserve in infertile women. Moreover, serum AMH levels have shown greater accuracy than FSH, with significant area under the curve. This suggests that AMH assay can be used as a reliable diagnostic test for ovarian reserve assessment in infertile women.

REFERENCES