

Comparing the Diagnostic Accuracy of Anti-Müllerian Hormone and Follicle Stimulating Hormone in Detecting Premature Ovarian Failure in Iraqi Women by ROC Analysis

Farah Amer Abed*¹, Raya Ezzat Maroof², Ulfat Mohammed Ali Al-Nakkash³

Abstract

Background: In women premature ovarian failure (POF) is a devastating disease impacting women under the age of 40. This involves a significant decrease in a women's quantity and quality of oocytes, or ovarian reserve (OR). POF can result in long-term physical and psychological health consequences. The earlier treatment can occur to manage this disease, the less likely the individual is going to suffer from the potential consequences. Accurate diagnosis is a critical proponent to ensuring immediate care. A traditional diagnostic marker includes follicular stimulating hormone (FSH). This individual test cannot be used to make a diagnosis in isolation due to the large variability in FSH levels among different women, and throughout a women's menstrual cycle. Anti-Müllerian hormone (AMH) is an alternative diagnostic marker for determining a women's OR. Serum levels of AMH have been shown to be associated with the size of the resting primordial follicle pool. When the levels of AMH are low, this is generally considered to be an indicator of a decline in fertility. In this study, we examined the specificity, sensitivity and accuracy of the FSH assay, against the more recently emerged AMH assay for diagnosing and predicting POR via Receiver Operator Characteristic curve (ROC) analysis.

Methods: A total of 60 participants were enrolled in the study. The POF group included 30 infertile women with POF, the infertile control group included 13 women without POF, and the fertile control group included 17 healthy women. Participants were recruited from the Kamal Al-Samarray Hospital in Bagdad city from December 2017 to March 2018. The age of participants ranged from 19-39 years of age. On day 2 of the menstrual cycle, peripheral blood samples were collected from each participant and the serum levels of AMH and FSH were examined using ELISA.

Results: Statistical analysis examining the FSH and AMH assays indicate that measuring AMH levels leads to an increased sensitivity, specificity and accuracy in determining the presence or absence of POF among the control fertile and POF groups. However, when comparing the specificity, sensitivity, and accuracy of AMH to FSH among the POF group and infertile controls, there were no differences among sensitivity, furthermore there was a slight decrease in the accuracy and specificity of AMH compared to FSH.

Conclusions: Our findings indicate that the serum levels of AMH have higher sensitivity, specificity and accuracy in detecting POR than FSH when comparing the POF patients to healthy fertile controls. As the AMH levels have minimal within-menstrual cycle variation they can therefore be assessed whenever necessary, opposed to FSH, in which the levels vary throughout the menstrual cycle. The role of AMH may therefore hold a more useful role in the early diagnosis of POF.

Keywords: Anti-mullerian hormone (AMH), Follicular stimulating hormone (FSH), Premature ovarian failure (POF), Receiver Operator Characteristic (ROC) curve.

Introduction

Premature ovarian failure (POF) is a heterogeneous condition occurs due to a deficiency in ovarian

function that significantly impacts the fertility of women within the normal childbearing age. Clinical

1: Ministry of Science and Technology, Baghdad.

2: College of Health and Medical Technology, Baghdad.

3: Consultant Gynecologist, Director of Elwlyia Maternity Hospital, Baghdad. All authors contributed equally to this work.

*Corresponding author: Farah Amer Abed; Tel: +96 47904774532, Fax: +96 7704341535; E-mail: ahmed.suleiman@uoanbar.edu.iq.

Received: 10 Nov, 2018; Accepted: 5 Dec, 2018

characteristics of POF include at least four months of amenorrhea accompanied by elevated levels of follicle stimulating hormone (FSH) ($\geq 30\text{IU/ml}$) and a reduction in the levels of anti-Müllerian hormone (AMH). The etiology of POF is largely unknown, however, it has been linked to autoimmune-induced ovarian damage or genetic abnormalities. Additionally, iatrogenic factors such as ovarian surgery, radiation, and chemotherapeutic interventions can lead to the development of POF (1).

The status of a women's fertility is assessed through determining ovarian function. Examining ovarian reserve (OR) reflects the quality and quantity of oocytes, and thus the fertility potential of a female. Several markers of OR currently exist, including FSH and AMH. In women, AMH is a dimeric glycoprotein that belongs to the transforming growth factor-beta (TGF β) superfamily produced by the granulosa cells of the ovarian follicles. Expression of AMH increases after puberty and peaks around the age of 24. After this point, as women age, the AMH levels decline and reach their lowest levels at menopause. There is a strong correlation of AMH levels with the number and quality of oocytes (2).

Early follicular phase (basal) measurements of FSH is the traditional endocrine test for evaluating OR. FSH is a member of the glycoprotein hormone family and is produced and secreted via the gonadotrophs of the anterior pituitary gland. FSH binds to receptors on granulosa cells of the ovaries in women. Throughout a women's menstrual cycle, the activity and levels of FSH differs. During the typical menstrual cycle, serum levels of FSH are often measured at days 2-3 to identify a women's OR. At this point in the menstrual cycle, the levels of FSH are expected to be low. The levels of FSH rise as a result of follicle depletion. Therefore, if the levels of FSH are high it indicates a diminished OR (3). Although FSH is used as a means of determining OR, there is a large amount of intra- and inter-cycle variability which creates some uncertainty for its use as an accurate marker in determining OR. The more consistent levels of AMH throughout the menstrual cycle make it a more reliable marker in estimating OR. As POF can result in serious health consequences, such as psychological distress, infertility, osteoporosis, autoimmune disorders, ischemic heart disease, and

an increased risk of mortality it is critical to begin treatment and receive a diagnosis as early on as possible. In identifying a better diagnostic tool, this can help prevent the occurrence of the long-term consequences (4,5). Using receiver operator characteristics (ROC) curve analysis, we compared the accuracy, specificity and, sensitivity of the FSH assay to the AMH assay among POF patients and fertile or nonfertile controls.

Materials and methods

Subjects

In this study, 60 women from the ages of 18-40 years old were assessed. Infertile patients were recruited from Kamal Al-Samarray hospital in Baghdad city from December 2017 to March 2018. The controls without POF were divided into two groups, the first group included 13 infertile women, the second group 17 fertile women. The standard diagnostic criteria of POF (6) included four months of amenorrhoea in women before the age of 40, and at least a 2fold increase in the concentration of FSH $>40\text{ IU/l}$. Patients with autoimmune disorders, endocrinopathies, iatrogenic agents such as, radiation therapy, chemotherapy, and pelvic surgery were excluded from this study.

Hormonal measurements of FSH and AMH

On day 2 of each participant's menstrual cycle, 5ml of peripheral blood was collected and serum was immediately isolated via centrifugation for 6 min at room temperature. The serum AMH and FSH levels were measured for all subjects via ELISA (enzyme-linked immune sorbent assay) technique using the FSH ELISA Test Kit (Monobind, USA) and AMH hormone ELISA Kit (Beckman coulter, USA).

Results

ROC curve analysis was used to compare and predict the specificity, sensitivity, and accuracy of the AMH assay with the FSH assay as a diagnostic test for identifying diminished OR in females suffering from POF.

Table 1 shows the mean FSH levels in the control fertile, control infertile, and POF groups. The means for each group were 10.341 ± 16.843 , 16.936 ± 19.408 and 73.172 ± 99.294 , respectively. The results showed significant differences in the

ROC Analysis of AMH and FSH Diagnostic Accuracy

FSH levels between groups (p value=0.008). The mean levels of AMH of the control fertile, control

infertile and POF group were, 6.494± 11.486; 4.778 ± 5.069; and 0.766 ± 0.631, respectively.

Table 1. Descriptive Statistics of Continuous Variables among the control fertile, infertile and POF groups.

VARIABLES	POF (30)	Control fertile (17)	Control infertile (13)	P-Value
FSH (mIU/ml)	73.172±99.294	10.341±16.843	16.936±19.408	0.008 Highly sign
AMH (mIU/ml)	0.766±0.631	6.494±11.486	4.778±5.069	0.014 sign

Values are mean ± SD. P-value is determined using the ANOVA test. POF = premature ovarian failure; NS = non-significant

Table 2 shows the sensitivity, specificity, and accuracy of the FSH assay when examining the control fertile group and the POF group. The sensitivity, accuracy, and specificity of the FSH assay were, 93.3%, 91.49% and, 88.2%, respectively. The p value of 0.00, indicates statistical significance (p<0.01). The sensitivity, accuracy and, specificity for the AMH assay in determining POF among the control fertile and POF groups were all 100%. The p value, 0.00, indicates statistical significance.

Table 3 shows the sensitivity, accuracy and, specificity of the FSH and AMH assays among the

non-fertile controls and POF group. The sensitivity, accuracy and, specificity of the FSH assay in absence of POF among the control infertile group to the POF group was 86.7%, 81.4% and, 69.2%, respectively. There are highly statistical differences with p value=0.00, determining the presence of POF among the control in fertile group and POF group was 86.7, 83.7% and, 76.9% respectively. The p value=0.00, indicates statistical significance. The sensitivity, accuracy and, specificity of the AMH assay when determining the presence or Figures 1-4 show the ROC curves.

Table 2. ROC curve analysis for the FSH and AMH assays comparing the control fertile and POF groups.

Variable	The area under the curve	P value	Sensitivity	Accuracy	specificity
FSH	0.927	0.00.HS	93.3%	91.49%	88.2%
AMH	1	0.00.HS	100%	100%	100%

HS: highly significant differences.

Table 3. ROC curve analysis for FSH and AMH assays comparing the control infertile and POF groups.

Variable	The area under the curve	P value	Sensitivity	Accuracy	specificity
FSH	0.859	0.00.HS	86.7%	83.72%	76.9%
AMH	0.874	0.00.HS	86.7%	81.4%	69.2%

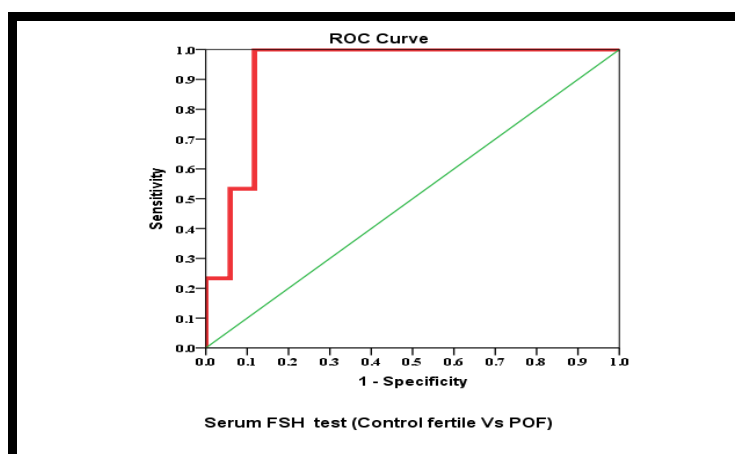


Fig. 1. ROC curve indicating the trade-off between specificity (what is the rate of false positives) and the sensitivity (which is the rate of the true positives) for FSH test between POF and the control fertile groups.

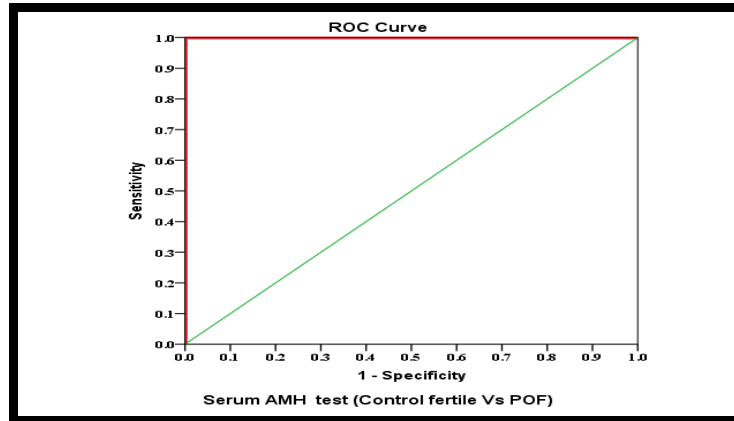


Fig. 2. ROC curve indicating the trade-off between specificity (which is the rate of false positives) and the sensitivity (which is the rate of the true positives) for the AMH test between POF and control fertile groups.

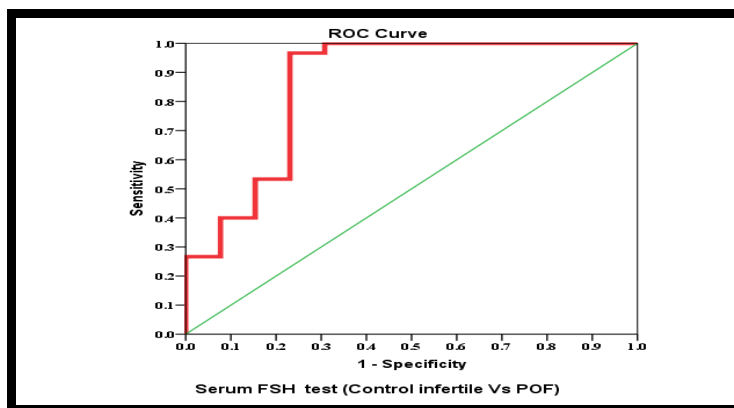


Fig. 3. ROC curve indicating the trade-off between specificity (which is the rate of false positive) and the sensitivity (which is the rate of true positives) for the FSH test between POF and the control infertile groups.

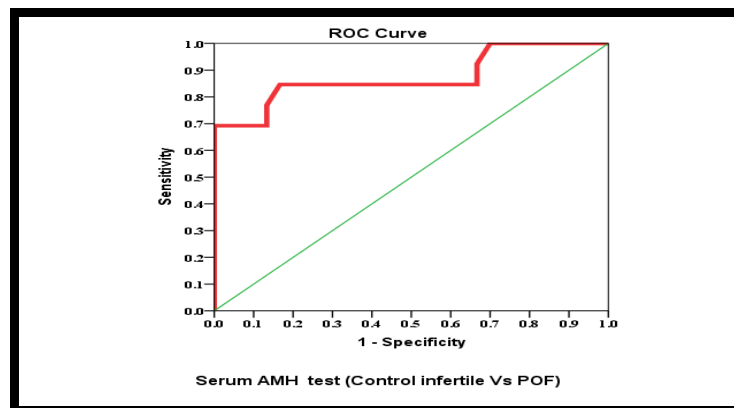


Fig. 4. ROC curve indicating the trade-off between specificity (which is the rate of false positive) and the sensitivity (which is the rate of true positives) for the AMH test between POF and control infertile groups.

Discussion

In females, POF is a devastating disease referring to the loss of ovarian function prior to the age of 40. Common characteristics of POF include the presence of amenorrhea for a period of four months or longer, associated with menopausal levels of FSH. Women with POF have severely compromised fertility (7). Our research shows that when comparing the

detection of POF among fertile female patients and POF patients using either the FSH or AMH assays, the AMH assay is more sensitive, accurate and, specific for determining POF than the commonly used FSH biomarker. When comparing the non-fertile control group to the POF group, the sensitivity of the FSH and AMH assays were the same.

However, the FSH assay was more specific and accurate than the AMH assay. Our findings corroborate previous research (8), that examined the specificity and sensitivity of the AMH assay compared to the FSH assay among women with infertility issues and menstrual disorders. The specificity and sensitivity for the AMH test was 80 % and 78.95%, respectively. The AMH test was observed to be significantly better than the specificity and sensitivity of the FSH assay, which was 28.57 % and 78.65%, respectively. In addition to enhanced sensitivity for POF diagnosis by AMH over FSH when examining fertile controls to POF patients, the diagnostic accuracy of AMH was also significantly improved in comparison to the FSH assay.

Since the concentration of AMH decreases progressively with age and strongly correlates with the size of the primordial follicle pool and number of antral follicles, it is a very good marker for fertility decline and POF. In females with POF, the primordial follicle pool drops below the threshold causing the cessation of follicular activation. The reduction in follicle counts may occur due to deficiencies in the apoptotic mechanisms of oocytes. This may lead to either a reduction in follicular formation, which then leads to a decrease in the number of oocytes formed during ovarian development, or accelerated follicle loss. AMH is a marker of non-cyclic ovarian activity and is considered a stable hormone, regardless of the menstrual cycle stage and demonstrates low variability among menstrual cycles. Since the levels of FSH are variable throughout the menstrual cycle, it has the potential to provide an inaccurate

indication of a women's fertility (9-11).

Our findings in figures 3 and 4 show that the ROC curve for the individuals with POF have a positive serum level for AMH. The ROC curve analysis found the sensitivity of the AMH assay to be high in diagnosing POF.

Infertility is considered a common issue that requires early detection to increase the potential for receiving effective fertility treatments. There are different conventional tests related to OR evaluation involving early antral follicle count via transvaginal ultrasound and basal second day FSH levels. ROC curve analysis has been implemented in predicting and comparing the specificity, sensitivity, and accuracy of the AMH assay with the FSH assay as a diagnostic test for determining OR in infertile females. The area under the curve for FSH was determined to be 0.87 and 0.874 for AMH. These results corroborate previous findings (12) that indicated that the area under the ROC curve for FSH was 0.70 and for AMH was 0.9. In conclusion, our study suggests that the sensitivity of the AMH assay is higher than the FSH assay as predictors in identifying OR among females. Furthermore, the levels of AMH have greater accuracy than those of FSH, with a considerable increase in the area under the curve. This indicates that AMH assay may be a more dependable diagnostic test for evaluating OR in infertile females.

Acknowledgment

Authors acknowledge Dr. Ahmed A. Suleiman, University of Anbar for his assistance in the work and production of this article.

References

1. Arora P and Polson D W. Diagnosis and management of premature ovarian failure. *Obstetrics Gynaecology* . 2011;13(2): 67-72.
2. Visser, JA, De Jong FH, Laven JS and Themmen AP. Anti-Müllerian hormone: new marker for ovarian function. *Journal of reproduction*. 2006;131:1-9.
3. Kwee J, Schats R, McDonnell, Lambalk CB, Schoemaker J. Intercycle variability of ovarian reserve tests: Results of a prospective randomized study. *Human Reproduction*. 2004; 19:590- 95.
4. De Carvalho BR, Sobrinho DBG, Vieira ADD, Resende MPS and Barbosa ACP. Ovarian Reserve Assessment for Infertility Investigation. *ISRN. Obstetrics Gynaecology*. 2012; 576-85.
5. Grynnerup A G A, Lindhard A and Sørensen S. Recent progress in the utility of anti-Müllerian hormone in female infertility. *Current Opinion in Obstetrics and Gynecology*. 2014;26(3):162-167.
6. Qin R, Chen SH, Yao J, Wu W and Xie J. Identification of novel missense mutations of the TGFBR3 gene in Chinese women with premature

ovarian failer. *Reproductive Biomedicine online Journal*. 2011;23: 697-703.

7. Nelson LM. "Primary ovarian insufficiency. *New England Journal of Medicine*. 2009;360:606-614.

8. Alipour F, Rasekhjahromi A, Maalhigh M, Sobhanian S. and Hosseinpoor, M. Comparison of Specificity and Sensitivity of AMH and FSH in Diagnosis of Premature Ovarian Failure, *Disease Markers*. 2015;1-4.

9. Yoon SH, ChoiYM, Hong MA, KimJJ, Lee GH, HwangeKR and MoonSY. Association study of anti-Mullerian hormone and anti-Mullerian hormone type II receptor polymorphisms with idiopathic primary ovarian insufficiency. *Human. Reproduction*. 2013;28(12): 3301-3305.

10. QinC, Yuan Z, Yao J, Zhu W, Wu W and Xie J. AMH and AMHR2 genetic variants in Chinese women with primary ovarian insufficiency at normal menopause *Reproductive Biomedicine Online Journal*. 2014; 29:311-318.

11. Torrealday S, Kodaman P and Pal L. Premature ovarian insufficiency – an update on recent advances in understanding and management". *Faculty Review*. 2017;1(3):1-15.

12. Parveen N, Ashifaq A and Parveen S. Comparison of diagnostic accuracy of anti-mullerian hormone with follicle stimulating hormone for ovarian reserve assessment in infertile women by Roc analysis. *Isra Medical Journal*. 2015; 7:(4):212-215.