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Elevated LH levels draw a stronger distinction than AMH in premature ovarian insufficiency

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Key words: PREMATURE OVARIAN FAILURE, ANTI-MÜLLERIAN HORMONE, ELEVATED LH, ELEVATED FSH

ABSTRACT

Objectives  A significant number of individuals have high serum follicle stimulating hormone (FSH) levels but do not meet the criteria for diagnosis of premature ovarian insufficiency (POI) due to ongoing menstruation. We compared a group of women with elevated FSH levels and POI with a control group in terms of biochemical markers.

Methods  In this cross-sectional retrospective study, 38 POI cases and 48 cases of elevated FSH were compared to 89 individuals in a control arm in terms of biochemical markers. The receiver operating characteristics curve was calculated to assess the utility of anti-Müllerian hormone (AMH) levels to discriminate women with elevated FSH levels accompanied by POI from those women with elevated FSH levels but not definable as having POI.

Results  A multiple regression analysis revealed that only the AMH level was significantly different for the discrimination between the control and elevated FSH groups. AMH and estradiol levels were found to be statistically significant for the discrimination between control and POI cases. However, only luteinizing hormone (LH) was found to be significant for distinguishing between women with elevated FSH and POI, interestingly excluding the serum AMH level in this context.

Conclusions  AMH was the most important and superior marker to differentiate both POI cases and patients with an elevated FSH level from the controls; however, it did not show the same resolution for differentiating POI cases from those with elevated FSH. Moreover, we conclude that the serum LH level is the most useful marker for differentiating POI cases from women with elevated FSH levels.

INTRODUCTION

Premature ovarian insufficiency (POI) is the most extreme phenotype of diminished ovarian reserve at a young age. POI occurs at an age less than two standard deviations below the mean established for the reference population, but in practice is defined as menopause before the age of 40 years. POI affects 1% of women < 40 years old and 0.1% of women < 30 years old. Incipient ovarian failure, or late reproductive aging according to the Stages of Reproductive Aging Workshop classification, describes another subgroup characterized by elevated follicle stimulating hormone (FSH) levels in the follicular phase along with a regular menstrual cycle. Incipient ovarian failure precedes the onset of cycle irregularity and hence the menopausal transition by 3–10 years and may be considered as an early sign of advanced ovarian aging in young women.

Various markers such as FSH levels or antral follicle count have been used to measure ovarian insufficiency. Serum levels of FSH, estradiol or inhibin B are of limited value for predicting the presence of an ovarian reserve in patients with POI, as most of these markers only indicate advanced ovarian senescence. Women with POI may develop follicles up to the antral stage and the serum anti-Müllerian hormone (AMH) level might be a good indicator of follicular presence. Serum AMH is negatively correlated with age and positively correlated with antral follicle count on ultrasound. Serum AMH levels show an age-related decrease after 30 years, whereas other markers associated with ovarian aging do not change during this time.
Women in Western society are delaying having children until later in life\(^1\). As a result, significant numbers of women present with elevated FSH levels suggestive of decreased ovarian reserve, with or without cycle abnormalities\(^11\). We have encountered many women who have elevated serum FSH levels but who do not fulfil the definition of POI. One study compared patients with secondary amenorrhea and controls and identified a high percentage of very low AMH levels in patients with POI\(^9\). Another small study identified low AMH levels as a marker of diminished ovarian reserve in patients with incipient ovarian failure with consistently elevated FSH levels\(^12\). There is insufficient evidence as to whether serum AMH measurements are sufficient for distinguishing between POI cases and women with only elevated FSH. In light of this, we hypothesized that the determination of serum AMH level and other serum markers might differentiate the patient with elevated serum FSH levels from POI patients.

**METHODS**

**Participants**

The study was conducted at the Reproductive Endocrinology and Infertility Clinic of Department of Obstetrics and Gynecology, Cerrahpasa School of Medicine, Istanbul University between January 2008 and January 2010. The files were reviewed retrospectively to evaluate the utility of serum AMH levels in women with elevated serum FSH levels and in a POI group. Inclusion criteria for POI were: age 25–39 years, secondary amenorrhea, concomitant vaso-motor symptoms, normal karyotype, no endocrinopathy and no history of radiotherapy, chemotherapy or ovarian surgery. The spontaneous absence of menses for at least 4 months in combination with FSH levels > 40 IU/l before age 40 years is accepted as a definition of POI\(^1\). The POI group included 38 patients. The group with elevated FSH levels included 48 patients who had elevated FSH levels (above 10.2 IU/l and below 40 IU/l) but who did not fulfil the criteria for the POI definition. The control group included 89 patients in the same age group who were admitted to our department as routine controls. The control group had regular menstrual cycles, no signs of hyperandrogenemia, and normal sonographic appearance of the ovaries. Potential participants were excluded if they were smokers, pregnant, breastfeeding or had a history of cardiovascular, liver, kidney or respiratory disease, uncontrolled hypertension, diabetes or malignancy. None of the study participants reported the use of any medications in the previous 3 months that could have interfered with the normal functioning of the hypothalamic–pituitary–gonadal axis. Informed consent was obtained from all women, and approval from the Human Ethics Committee of Istanbul University was obtained. All study procedures were performed in accordance with the Declaration of Helsinki.

**Biochemical measurements**

Blood samples in women with regular menstrual cycles (26–32 days) were collected during the early follicular phase of the menstrual cycle, whereas samples were obtained at random in the same laboratory using the same assays from women without a regular cycle. Serum levels of AMH, FSH, LH and estradiol were analyzed in each group. All blood samples for AMH measurement were collected in a lithium-heparin tube. Serum AMH concentrations were measured with an enzymatically amplified two-sided immunoassay (DSL-10-14400 Active Müllerian inhibiting substance/AMH enzyme-linked immunosorbent assay (ELISA) kit, Diagnostic Systems Laboratories (DSL), Webster, TX, USA). The theoretical sensitivity of the method was 0.006 ng/ml, the intra-assay coefficient of variation for low values was 3.3%, and the inter-assay coefficient of variation for high values was 6.7%. The levels of serum FSH, LH, and estradiol were measured with the Roche E-170 automated immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). The inter-batch coefficient of variation for these assays was 10%.

**Statistical analyses**

The data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Whether the distributions of continuous variables were normal or not was determined by the Kolmogorov–Smirnov test. Data for continuous variables are shown as mean ± standard deviation or median (range), where applicable. Mean differences between groups were compared by Student’s \(t\)-test. The Mann–Whitney \(U\)-test was applied for comparisons of the median values between the case and control groups. The Kruskal–Wallis test was used to assess differences in median values among groups when the number of independent groups was more than two. When the \(p\)-value from the Kruskal–Wallis test was statistically significant, Conover’s non-parametric multiple comparison test was used to identify which group differed from the others. The optimal cut-off points for levels of AMH, FSH, LH and estradiol to distinguish among the groups were evaluated by receiver operating characteristic (ROC) analyses after calculating the area under the curve (AUC) given the maximum sum of sensitivity and specificity (i.e. Youden Index) for the significance test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each clinical measurement at the best cut-off points. Multiple logistic regression analyses were used to identify the predictive ability of the clinical measurements after adjustment. Odds ratios (OR) and 95% confidence intervals (CIs) were also calculated for each independent variable. A \(p\)-value < 0.05 was considered significant.

**RESULTS**

No differences were found for age or body mass index (BMI) between the groups (Table 1). Serum AMH and estradiol...
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levels were significantly lower in the elevated FSH and POI groups than in the control group. FSH and LH levels were significantly higher in the elevated FSH and POI groups compared to controls \( (p < 0.001) \). The serum LH levels (with the serum FSH) were significantly higher in the POI group than in the elevated FSH group \( (p < 0.001) \).

The AUCs for serum AMH, FSH, LH and estradiol significantly discriminated between controls and the elevated FSH group. The highest AUC value was for AMH \( (0.963; 95\% \text{ CI } 0.936–0.989) \) and the lowest AUC value was for estradiol \( (0.769; 95\% \text{ CI } 0.676–0.862) \) (Table 2, Figure 1). The best cut-off point was 0.955 ng/ml for the elevated FSH group. AMH had the highest sensitivity (97.9%) for discriminating between the two groups and FSH had the highest specificity (91.0%) among the four biochemical markers.

The AUCs for serum AMH, FSH, LH and estradiol were significantly discriminated between the POI group and controls (Figure 2). The highest AUC value was for LH in the analysis of the POI group vs. controls \( (0.999; 95\% \text{ CI } 0.997–1.001) \) and the lowest AUC value was for estradiol \( (0.860; 95\% \text{ CI } 0.777–0.944) \) (Figure 2). The best cut-off point for AMH was 0.945 ng/ml and it was 12.85 IU/l in the POI group. Among four biochemical markers, LH had the highest sensitivity (97.4%), specificity (100%), positive predictive (100%) and negative predictive (98.9%) values for discriminating between the POI group and controls.

The AUCs for LH and estradiol significantly discriminated between the elevated FSH and POI groups (Table 2, Figure 3). That for AMH was not significant. The ROC analysis for FSH

### Table 1

Demographic and clinical parameters. Data are given as mean ± standard deviation or median (range)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls ( (n = 89) )</th>
<th>Elevated FSH ( (FSH &lt; 40 \text{ IU/l}) ) ( (n = 48) )</th>
<th>POI ( (FSH &gt; 40 \text{ IU/l}) ) ( (n = 38) )</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.7 ± 5.5</td>
<td>33.0 ± 5.5</td>
<td>30.4 ± 6.5</td>
<td>0.055</td>
</tr>
<tr>
<td>Body mass index ( (\text{kg/m}^2) )</td>
<td>26.4 ± 4.9</td>
<td>27.4 ± 5.7</td>
<td>24.4 ± 3.5</td>
<td>0.208</td>
</tr>
<tr>
<td>AMH</td>
<td>2.1 ( (0.04–11.0) ), †</td>
<td>0.1 ( (0.01–1.1) )</td>
<td>0.03 ( (0.01–2.0) ), †</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FSH</td>
<td>6.2 ( (0.1–10.1) ), †</td>
<td>22.2 ( (10.2–39.0) )</td>
<td>67.0 ( (41.0–162.0) ), †</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LH</td>
<td>2.9 ( (0.1–12.7) ), †</td>
<td>6.7 ( (1.6–24.0) )</td>
<td>31.5 ( (8.4–120.0) )</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Estradiol</td>
<td>39.0 ( (9.0–108.0) ), †</td>
<td>22.5 ( (8.0–130.0) )</td>
<td>16.5 ( (4.0–110.0) )</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\( , \) difference between controls and the group with elevated FSH levels was statistically significant \( (p < 0.001) \);
\( † , \) difference between controls and the group with POI was statistically significant \( (p < 0.001) \);
\( ‡ , \) difference between the group with elevated FSH levels and the group with POI was statistically significant \( (p < 0.001) \).

FSH, follicle stimulating hormone; POI, premature ovarian insufficiency; AMH, anti-Müllerian hormone; LH, luteinizing hormone

### Table 2

Results of receiver operating curve analysis for clinical measurement discrimination between women with elevated follicle stimulating hormone (FSH) levels and women with premature ovarian insufficiency

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Definitions</th>
<th>AMH</th>
<th>LH</th>
<th>Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>0.527</td>
<td>0.970</td>
<td>0.633</td>
<td></td>
</tr>
<tr>
<td>95% CI for AUC</td>
<td>0.401–0.653</td>
<td>0.941–1.000</td>
<td>0.514–0.752</td>
<td></td>
</tr>
<tr>
<td>( p )-Value</td>
<td>0.664</td>
<td>&lt; 0.001</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>The best cut-off point</td>
<td>–</td>
<td>&gt; 16.18</td>
<td>&lt; 21.5</td>
<td></td>
</tr>
<tr>
<td>Number of cases</td>
<td>86</td>
<td>86</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>TP/(TP + FN)</td>
<td>34/38 ( (89.5%) )</td>
<td>27/38 ( (71.1%) )</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>TN/(TN + FN)</td>
<td>45/48 ( (93.8%) )</td>
<td>26/48 ( (54.2%) )</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>TP/(TP + FP)</td>
<td>34/37 ( (91.9%) )</td>
<td>27/49 ( (55.1%) )</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>TN/(TN + FN)</td>
<td>45/49 ( (91.8%) )</td>
<td>26/37 ( (70.3%) )</td>
<td></td>
</tr>
<tr>
<td>OR ( (95% \text{ CI}) )</td>
<td>127.500</td>
<td>2.901</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI for OR</td>
<td>2.6744–607.839</td>
<td>1.177–7.150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LH, luteinizing hormone; AMH, anti-Müllerian hormone; AUC, area under the curve; CI, confidence interval; TP, true positive; FN, false negative; TN, true negative; FP, false positive; PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio
could not be performed because the groups were segregated according to FSH level.

Only serum AMH and FSH levels significantly discriminated between the elevated FSH group and controls in multiple logistic regression analyses. The AMH level was more statistically decisive than FSH (Table 3). On the other hand, AMH and estradiol significantly discriminated between controls and POI patients, with AMH being statistically decisive (Table 3). FSH and LH could not be included in the regression models, because the odds ratios could not be calculated for the best cut-off values of these markers. Only the serum LH level was statistically significant when serum LH and estradiol levels were evaluated together for discriminating between the elevated FSH and POI groups (Table 3).

DISCUSSION

POI is a common condition that is of growing concern in the general population due to the increasing trend of delaying pregnancy. Women with POI are at risk for not only infertility but also urogenital problems, low bone density, earlier onset of osteoporosis and fractures, earlier onset of coronary heart disease and increased cardiovascular mortality. The causes of POI are largely unknown and it is generally irreversible. After a diagnosis of POI, patients have limited options. Unfortunately, for most patients, oocyte donation, embryo donation or adoption seem to be the only options for such patients to have children. Therefore, the anticipatory guidance of these patients and the clinical approaches are critical.

The most frequently applied definition of POI is the spontaneous absence of menses for at least 4 months in combination with FSH levels >40 IU/l before the age of 40 years. Although often used as synonyms, POI and menopause are not the same entities as many patients with POI can exhibit intermittent ovarian function and ovulation. Moreover, 5–10% may conceive. The only feature of patient history that is helpful in determining the etiologic of ovarian failure is a positive family history.

Figure 1  Receiver operating characteristic curve analysis of anti-Müllerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol for clinical measurement discrimination of the group with elevated FSH and the control group.
Early predictors of ovarian aging are challenging to identify. So far, no biological markers have been identified that are able to distinguish between women with elevated FSH and women with POI. But we have demonstrated that the serum LH level is the most important parameter and AMH did not show any superiority in differentiating between those two groups. In this sense, our results are very important, indicating the status of women with ovarian aging.

Several ovarian reserve tests have been used to predict ovarian aging or poor ovarian reserve. The main purpose of these tests is to determine reduced fertility at a stage when appropriate treatment can be pre-emptively applied. AMH levels decrease, while other factors associated with perimenopausal status such as FSH, inhibin B or estradiol levels do not change significantly in aging females. Serum levels of FSH, estradiol or inhibin B are of limited value for predicting the presence of an ovarian reserve in patients with POI. An elevated serum level of FSH is an undeniable hormonal hallmark of reproductive aging; however, many studies have shown that it is a relatively late predictor of completed menopausal transition. In contrast, AMH is reported to be a useful marker of the ovarian follicular pool. AMH appears in serum after birth, increases until puberty, and progressively decreases in parallel with ovarian aging. It has been suggested that POI and early ovarian aging are associated with very low or undetectable serum AMH levels. It was demonstrated that, when AMH levels fall to undetectable levels, menopause will be observed within 5 years. Several studies have demonstrated that the serum AMH level is a better marker of ovarian reserve than age alone or other markers such as basal serum FSH, estradiol and inhibin B levels. The main challenge is to diagnose the condition early enough to identify an ‘ongoing’ or rather insidious menopausal transition. From this point of view, serum levels of AMH seem to be the best marker for declining ovarian reserve. AMH measurements have practical advantages with respect to other methods that are used for follicular evaluation. One of the most appealing advantages of using serum levels of AMH is that it is stable under various influences such as hormonal contraception, the menstrual cycle, and pregnancy, and measurements can therefore be made at any time during the menstrual cycle.

Figure 2  Receiver operating characteristic curve analysis of anti-Müllerian hormone (AMH), luteinizing hormone (LH) and estradiol for clinical measurement discrimination of the group with premature ovarian insufficiency and the control group.
Serum LH measurement is a commonly used diagnostic test; however, knowledge on its practical implications is limited. The two-cell/two-gonadotropin model states that LH stimulates the conversion of cholesterol into androgens in theca cells and, in parallel, FSH stimulates the aromatization of androgens into estrogen in granulosa cells. The lack of stimulating gonadotropins or the lack of response of the target ovaries results in hypogonadism. While in some patients only FSH is elevated, in some others elevated LH levels, low serum estradiol levels and symptoms of estrogen deprivation accompanied by secondary amenorrhea, may be observed. In this study, multiple regression analysis revealed that the serum AMH level was the most prominent parameter in differentiating the POF and the elevated FSH groups from the control group, whereas the serum LH level was the most important marker in demarcating the POF cases from women with elevated FSH. Considering the two-cell/two-gonadotropin model and that a healthy functioning ovary needs the collaboration of the theca and granulosa cells, we hypothesize that the group with elevated FSH may represent an early stage of POI when there are insufficient granulosa cells and which progresses as the LH level increases due to malfunctioning of the theca cells; it is then definable as ‘POI’. Data regarding the significance of serum LH levels in patients with POI are very limited. The value of LH measurements for discriminating between POI and elevated FSH cases has not been evaluated in any study, so far.

The most important limiting factor in our study was the small number of cases. Due to the low prevalence of POI in society, published POI studies include relatively few patients. Studies that include a larger number of patients must be conducted in multicenters and even internationally so as to achieve higher statistical power for the results.

AMH is a promising tool for many fields of gynecology. Our findings suggest that, among biochemical parameters, AMH is the most important and superior marker for distinguishing between controls and patients with both elevated FSH and POI; however, AMH is no more superior than other

Table 3  Results of multiple logistic regression analyses

<table>
<thead>
<tr>
<th>Models</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>Wald</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs. elevated FSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMH&lt;0.955</td>
<td>62.672 (6.582–596.734)</td>
<td>&lt;0.001</td>
<td>12.952</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH&gt;11.8</td>
<td>11.059 (2.236–54.695)</td>
<td>0.003</td>
<td>8.683</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH&gt;4.35</td>
<td>1.090 (0.203–5.846)</td>
<td>0.920</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol&lt;30.5</td>
<td>3.673 (0.814–16.576)</td>
<td>0.091</td>
<td>2.863</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. POI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMH&lt;0.945</td>
<td>46.846 (10.828–202.678)</td>
<td>&lt;0.001</td>
<td>26.496</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol&lt;28.5</td>
<td>14.803 (3.944–55.566)</td>
<td>&lt;0.001</td>
<td>15.944</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated FSH vs. POI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH&gt;16.18</td>
<td>144.712 (26.276–796.985)</td>
<td>&lt;0.001</td>
<td>32.661</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol&lt;21.5</td>
<td>4.028 (0.717–22.646)</td>
<td>0.114</td>
<td>2.502</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; FSH, follicle stimulating hormone; POI, premature ovarian insufficiency; AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone.
markers in discriminating women with POI from those with elevated FSH levels. In this situation, we found the serum LH level to be the most important parameter for discriminating between POI and elevated FSH. To the best of our knowledge, this is the first study which states the significance of LH for discriminating between POI and cases of elevated FSH. Our conclusions draw attention to the significance of LH for evaluating and characterizing cases with seemingly limited ovarian reserve who are more likely to be ‘POI’. However, due to the low number of cases in this study, we cannot conclusively recommend LH as a marker for distinguishing women with POI from women with elevated FSH. Further studies with larger patient numbers will probably clarify our hypothesis.

Conflict of interest  The authors report no conflicts of interest. The authors alone are responsible for the conduct and writing of this study.

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