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RESEARCH ARTICLE

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Age-group-specific reference intervals for anti-Müllerian hormone and its diagnostic performance for polycystic ovary syndrome in a Korean population

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Abstract

Background: We established age-group-specific reference intervals for serum anti-Müllerian hormone (AMH) levels in a Korean population and investigated the effectiveness of AMH assay for polycystic ovary syndrome (PCOS) diagnosis.

Methods: We analyzed serum levels of AMH, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) from 1540 Korean women. Subjects were divided into three groups: healthy, benign gynecologic diseases, and PCOS. Age-group-specific reference intervals and AMH diagnostic performance were estimated.

Results: The PCOS group had a median AMH level of 7.0 μg/L, which was higher than for the healthy (1.8 μg/L) and the benign gynecologic diseases (2.7 μg/L) groups. The upper 97.5% reference limits for age groups 12–20 years, 21–34 years, and 35–46 years were 13.2 μg/L, 15.8 μg/L, and 6.6 μg/L, respectively. The area under the curve (AUC) values to estimate AMH ability to discriminate PCOS from healthy women for each age group were 0.741, 0.785, and 0.789, respectively. AUCs for LH/FSH were 0.719, 0.672, and 0.590.

Conclusions: The better diagnostic ability of AMH over LH/FSH in women of late childbearing ages indicates that age and other clinical characteristics should be considered when interpreting these test results.

KEYWORDS
age-group-specific, anti-Müllerian hormone, diagnostic performance, polycystic ovary syndrome, reference interval

1 | INTRODUCTION

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a member of the transforming growth factor-β family. AMH is secreted by Sertoli cells of male fetuses and induces regression of Müllerian ducts during male differentiation.[1] After birth, its regulatory role in sex differentiation is lost and AMH begins to be produced by granulosa cells of preantral and small antral follicles in the ovaries.[2] Serum AMH concentration is an excellent candidate marker of ovarian reserve because AMH is secreted from all developing follicles, and both serum AMH levels and number of antral follicles decline with age.

In addition to be as a marker for ovarian reserve in healthy women, serum AMH level can also serve as a marker for ovarian pathophysiology, including polycystic ovary syndrome (PCOS). PCOS is the most common cause of chronic anovulation and hyperandrogenism in young women. Both result in substantial psychological, economic, and social consequences.[3] In women suffering from PCOS, increased AMH production per follicle, as well as higher number of preantral and small antral follicles, contribute to increased serum AMH levels.[4] Moreover, a strong correlation has been observed between ultrasonographic antral follicular counts (AFC) and serum AMH levels.[5] Thus, serum AMH can be a surrogate for AFC as a diagnostic marker for PCOS.

However, it is questionable whether serum AMH alone can provide sufficient diagnostic value for PCOS. Serum AMH can be influenced by multiple factors. An association between obesity and significantly lower serum AMH has been reported, where AMH levels were 34%–65% lower in obese women compared with non-obese women.[6,7] Furthermore, serum AMH is predominantly dependent on age. Various cutoff values for serum AMH have been proposed for discriminating PCOS women from healthy women.[8] However, few studies have evaluated age-related or age-specific reference intervals of serum AMH, especially for data generated from automated assays.[9–12] Moreover, PCOS prevalence and characteristics of the control population would influence diagnostic performance in a real-world setting. Additionally, ethnicity, which is strongly associated with anthropometric characteristics, risk for other benign gynecologic conditions, and the age range of the subject population, can all affect determination of appropriate cutoff values and overall accuracy of an AMH test.

We aimed to establish age-group-specific reference intervals of serum AMH levels in a healthy Korean population. We also compared the diagnostic performance of the AMH age-group-specific reference intervals estimated in this study to those from previous studies. Finally, we analyzed the diagnostic performance of serum AMH compared with the LH-to-FSH ratio (LH/FSH) to investigate the clinical utility of AMH when applied to various age groups and clinical settings.

2 | MATERIALS AND METHODS

2.1 | Subject characteristics

Between May 2017 and January 2019, serum AMH, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2) assays were simultaneously requested for 1631 females, ages 12–70 years, at Severance Hospital. The serum samples had been requested for hormonal assays on unspecified day of the menstrual cycle. Subjects' medical records between 180 days before and 90 days after laboratory test administration were requested and reviewed. After excluding patients aged 52–70 years and patients diagnosed with any kind of cancers including ovarian carcinoma, or patients with premature ovarian failure and those who are pregnant or were pregnant until recently, 1540 subjects were included in the analyses.

Study subjects were divided into three groups: (i) apparently healthy women without any diagnosed gynecologic diseases, (ii) patients with benign gynecologic diseases or symptoms, and (iii) patients with PCOS. The subject groups were defined mainly based on the registered diagnosis by clinicians in medical records. PCOS diagnosis was made according to the Rotterdam Consensus, as previously described.[13] Diagnosis of benign gynecologic diseases (Table S1) was made based on ultrasonography, laboratory test results, and clinical manifestations. Expert endocrinologists and gynecologists performed comprehensive evaluations and made final diagnoses for all study patients.

2.2 | Assays

Serum samples were tested immediately upon arrival in the laboratory. AMH levels were measured by the Elecsys AMH assay utilizing electrochemiluminescence immunoassay principle with the cobas e 602 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum concentrations of LH, FSH, and E2 were assayed with the chemiluminescence-based UniCel™ DxI 800 Access Immunoassay System (Beckman Coulter Inc.,). All assay procedures were performed following the manufacturers' instructions.

2.3 | Statistical methods

Statistical analyses were performed using Analyse-it Software v5.65 (Analyse-it Software Ltd,). Values are presented as means and standard deviations or medians and 1st to 3rd quartiles. The Steel–Dwass–Critchlow–Fligner test was conducted to detect differences between the medians of all pairs of groups to compensate for alpha errors from multiple comparisons. Age-group-specific reference intervals were calculated using a bootstrapped nonparametric method for 95% central distribution (2.5–97.5 percentiles). Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic parameter performance, including serum AMH level. Youden's index was calculated to determine the optimal cutoff value of serum AMH for each age group. *p*-Values less than 0.05 were considered significant.

2.4 | Ethical approval

This study protocol was reviewed and approved by the Institutional Review Board of Gangnam Severance Hospital in Seoul, Korea (No. 3-2020-0367). As this study is a retrospective cross-sectional case-control study only utilizing medical records that does not include the patients' personal information, informed consents were waived by the IRB.

3 | RESULTS

3.1 | Patient characteristics

Among the 1540 subjects, 347 (22.5%) were assigned to the healthy group, 753 (48.9%) to the benign diseases group, and 440 (28.6%) to the PCOS group. Age, body mass index (BMI), and measured hormone levels are summarized for each group in Table 1. BMI in the PCOS group was significantly higher compared with the healthy population (p = 0.0025) and was insignificantly higher than the benign diseases group. AMH, FSH, LH, and LH/FSH levels were all significantly higher in the PCOS group than in the healthy or benign diseases groups (p < 0.0001 for all comparisons). Also, AMH levels were higher in the benign diseases group than the healthy group (p = 0.0063).

3.2 | Establishment of AMH reference intervals

The healthy population was defined as the reference group here. The reference intervals were set as nonparametric 2.5–97.5 percentiles. The reference intervals along with the mean, median, and upper 95 percentile reference limits of AMH are presented for each age group in Table 2. Upper 97.5 percentile limits of reference intervals for the 12–20, 21–34, and 35–46 age groups were 13.2, 15.8, and 6.6, respectively. These age group values were employed in subsequent analyses of diagnostic performance.

3.3 | Diagnostic performance of AMH for PCOS with different age-related reference intervals

We compared the diagnostic utility of the reference intervals calculated in this study to those of various age-specific or age-related reference limits suggested by recent studies[9,10] or the assay manufacturer. Age-specific or age-related reference limits (Table S2) were applied to each age or age group to estimate overall diagnostic performance characteristics (Table 3). We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each set of reference limits. The set of age-specific reference limits presented by Evliyaoglu et al[10] showed relatively high sensitivity of 0.663 and had a Youden's index of 0.430.

TABLE 1 Study group characteristics and hormone levels

Parameters	Healthy population[a] (n = 347)	Benign gynecologic diseases (n = 753)	p-Value[b]	PCOS (n = 440)	p-Value[c]	p-Value[d]
Mean age (years)	32.5 ± 8.9	31.1 ± 9.0	0.0758	26.2 ± 6.3	<0.0001	<0.0001
BMI (kg/m^2)	21.2 (18.9–23.8)	21.5 (19.3–25.0)	0.2215	22.4 (19.7–26.3)	0.0025	0.0520
AMH (μg/L)	1.8 (0.4–4.4)	2.7 (0.7–5.3)	0.0063	7.0 (4.4–10.4)	<0.0001	<0.0001
Estradiol (ng/L)	51.0 (31.0–99.0)	49.0 (28.0–80.0)	0.7344	50.5 (34.0–76.6)	0.3431	0.7042
FSH (IU/L)	7.1 (5.1–10.5)	7.2 (5.4–9.6)	0.9306	6.4 (5.3–7.7)	<0.0001	0.0001
LH (IU/L)	5.8 (3.2–10.6)	5.5 (3.3–10.7)	0.9399	8.7 (4.7–14.0)	<0.0001	<0.0001
LH/FSH	0.72 (0.41–1.24)	0.68 (0.43–1.18)	0.9629	1.39 (0.75–2.12)	<0.0001	<0.0001

Note: Data are shown as means ± standard deviations or medians (1st to 3rd quartiles).

Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.

[a]Apparently healthy women without any diagnosed gynecologic diseases.

[b]Healthy population versus benign gynecologic diseases.

[c]Healthy population versus PCOS.

[d]Benign gynecologic diseases versus PCOS.

TABLE 2 Age-group-specific reference limits

	AMH (μg/L)				
Age group in years (n)	Mean ± SD	Median (1st to 3rd quartiles)	2.5 percentile reference limit (90% CI)	97.5 percentile reference limit (90% CI)	95 percentile reference limit (90% CI)
12–20 (42)	3.8 ± 3.9	2.8 (0.0–5.7)	0.01 (0.01–0.01)	13.2 (10.5–14.2)	11.8 (9.6–14.2)
21–34 (161)	4.2 ± 3.8	3.5 (1.5–5.6)	0.02 (0.01–0.06)	15.8 (12.5–18.3)	12.4 (10.6–15.4)
35–46 (126)	1.5 ± 1.8	1.0 (0.2–2.1)	0.01 (0.01–0.01)	6.6 (5.2–9.2)	5.3 (4.5–6.7)

Abbreviations: AMH, anti-Müllerian hormone; CI, confidence interval; SD, standard deviation.

TABLE 3 AMH diagnostic performance when age-related reference limits from different references were applied individually by age to each study subject

Reference	Subject ethnicity	Age range applied (*n*)	Percentile of upper reference limit	Youden's index	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Evliyaoglu et al (2020)	Caucasian (German)	14–51 (1520)	97.5	0.430	0.663 (0.617–0.706)	0.768 (0.741–0.792)	0.533 (0.501–0.564)	0.851 (0.832–0.867)
Li et al (2020)	Asian (Hong Kongese)	20–44 (1266)	97	0.217	0.287 (0.243–0.335)	0.930 (0.911–0.945)	0.621 (0.551–0.686)	0.765 (0.753–0.777)
Manufacturer	Caucasian	20–50 (1336)	95	0.265	0.357 (0.310–0.407)	0.908 (0.888–0.925)	0.609 (0.550–0.664)	0.779 (0.765–0.793)
			97.5	0.232	0.305 (0.260–0.354)	0.927 (0.909–0.942)	0.625 (0.559–0.686)	0.769 (0.757–0.781)
The present study	Asian (Korean)	12–46 (1501)	95	0.145	0.200 (0.165–0.240)	0.945 (0.930–0.957)	0.594 (0.517–0.667)	0.747 (0.738–0.756)
			97.5	0.095	0.123 (0.095–0.157)	0.973 (0.961–0.981)	0.642 (0.537–0.736)	0.735 (0.728–0.742)

Abbreviations: AMH, anti-Müllerian hormone; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

3.4 | Determination of AMH cutoff values

We conducted ROC analysis to obtain optimal cutoff values for each age group with better discriminative power than the reference limits. The healthy and benign diseases groups were used as control groups individually and collectively. Areas under curve (AUC), cutoff values, Youden's indexes, and diagnostic performance parameters for all age groups and control groups are summarized in Table 4. The AUC values compared with the healthy group as the control were 0.741, 0.785, and 0.789 for age groups of 12–20, 21–34, and 35–46 years old, respectively. When the benign disease group was used as the control, the AUC values were 0.679, 0.719, and 0.778 for the same age groups.

3.5 | Comparison of LH/FSH and AMH diagnostic performance for PCOS

In the initial ROC analysis for all subjects, ages 12–51 years (N = 1540), the AUC values (and 95% confidence intervals and p-values) for AMH, E2, LH, FSH, and LH/FSH were 0.798 (0.775–0.821, $p < 0.0001$), 0.505 (0.474–0.535, $p = 0.7667$), 0.604 (0.574–0.633, $p < 0.0001$), 0.597 (0.569–0.625, $p < 0.0001$), and 0.706 (0.678–0.734, $p < 0.0001$), respectively. The AUC value for E2 was too low to be a diagnostic marker for PCOS, but LH/FSH yielded a significantly higher AUC than LH or FSH alone ($p < 0.0001$). ROC curves for LH/FSH and AMH according to age group and control type are depicted in Figure 1.

4 | DISCUSSION

In this study, we established age-group-specific reference intervals of serum AMH levels in Korean women, and we also estimated the diagnostic utility of AMH for PCOS by applying the aforementioned reference intervals and other possible cutoffs calculated from our data. Both serum AMH levels and the number of antral follicles decline with age in healthy ovulatory women.[14] Furthermore, this age-based decline in AMH levels is also known to be much less pronounced in PCOS women.[15,16] Therefore, it could be more appropriate to use multiple age-related cutoff values instead of a universal cutoff as a diagnostic threshold. Previous studies have reported that age-specific reference limits or multiples of the median (MoM) can significantly increase the diagnostic utility of AMH.[9,10]

Evliyaoglu et al[10] analyzed serum AMH levels together with other hormones in 4712 reproductive-aged women including 1132 PCOS patients and 525 primary ovarian insufficiency (POI) patients. The authors presented AMH reference limits for each age ranging 14–50 years. Their age-specific AMH reference limits showed high discriminatory power for PCOS diagnosis in patients over 18 years old and for POI patients under 38 years old. Li et al[9] also established a set of age-specific reference ranges for serum AMH levels in 3137

TABLE 4 AMH performance characteristics according to age groups

Age group (years)	No. of PCOS cases	Controls (*n*)	AUC_{ROC} (95% CI)	Condition	Cutoff value	Youden's J	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
12–20	87	Healthy (42)	0.741 (0.641–0.840)	At 95% sensitivity	1.76	0.359	0.954 (0.888–0.982)	0.405 (0.270–0.555)	0.769 (0.720–0.811)	0.810 (0.604–0.922)
				Maximum Youden's J	4.12	0.437	0.770 (0.671–0.846)	0.667 (0.516–0.790)	0.827 (0.755–0.882)	0.583 (0.474–0.685)
				At 95% specificity	10.59	0.125	0.172 (0.107–0.265)	0.952 (0.842–0.987)	0.882 (0.642–0.969)	0.357 (0.331–0.385)
		Benign (115)	0.679 (0.606–0.753)	At 95% sensitivity	1.85	0.215	0.954 (0.888–0.982)	0.261 (0.189–0.348)	0.494 (0.465–0.524)	0.882 (0.733–0.953)
				Maximum Youden's J	3.96	0.297	0.793 (0.696–0.865)	0.504 (0.414–0.594)	0.548 (0.494–0.600)	0.763 (0.673–0.835)
				At 95% specificity	11.38	0.106	0.149 (0.089–0.239)	0.957 (0.902–0.981)	0.722 (0.491–0.875)	0.598 (0.574–0.621)
		All (157)	0.696 (0.630–0.762)	At 95% sensitivity	1.85	0.253	0.954 (0.888–0.982)	0.299 (0.233–0.375)	0.430 (0.403–0.458)	0.922 (0.814–0.969)
				Maximum Youden's J	4.16	0.324	0.770 (0.671–0.846)	0.554 (0.476–0.630)	0.489 (0.437–0.541)	0.813 (0.743–0.868)
				At 95% specificity	11.38	0.105	0.149 (0.089–0.239)	0.955 (0.911–0.978)	0.650 (0.435–0.818)	0.670 (0.648–0.690)
21–34	308	Healthy (161)	0.785 (0.740–0.830)	At 95% sensitivity	2.40	0.330	0.951 (0.921–0.970)	0.379 (0.308–0.456)	0.746 (0.721–0.768)	0.803 (0.705–0.874)
				Maximum Youden's J	5.67	0.462	0.692 (0.638–0.741)	0.770 (0.699–0.828)	0.852 (0.811–0.885)	0.566 (0.520–0.612)
				At 95% specificity	11.30	0.178	0.227 (0.184–0.277)	0.950 (0.905–0.975)	0.897 (0.812–0.947)	0.391 (0.375–0.408)
		Benign (331)	0.719 (0.680–0.758)	At 95% sensitivity	2.40	0.238	0.951 (0.921–0.970)	0.287 (0.241–0.338)	0.554 (0.536–0.572)	0.864 (0.790–0.914)
				Maximum Youden's J	5.69	0.356	0.692 (0.638–0.741)	0.665 (0.612–0.713)	0.657 (0.618–0.694)	0.698 (0.658–0.736)
				At 95% specificity	14.03	0.098	0.146 (0.111–0.190)	0.952 (0.923–0.970)	0.738 (0.619–0.830)	0.545 (0.532–0.558)
		All (492)	0.740 (0.706–0.775)	At 95% sensitivity	2.40	0.268	0.951 (0.921–0.970)	0.317 (0.278–0.359)	0.466 (0.450–0.482)	0.912 (0.862–0.945)
				Maximum Youden's J	5.69	0.391	0.692 (0.638–0.741)	0.699 (0.657–0.738)	0.590 (0.552–0.627)	0.784 (0.752 0.812)
				At 95% specificity	13.28	0.114	0.162 (0.125–0.208)	0.951 (0.928–0.967)	0.676 (0.567–0.768)	0.645 (0.632–0.657)

(Continues)

TABLE 4 (Continued)

Age group (years)	No. of PCOS cases	Controls (*n*)	AUC_{ROC} (95% CI)	Condition	Cutoff value	Youden's J	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
35–46	45	Healthy (126)	0.789 (0.704–0.874)	At 95% sensitivity	0.07	0.154	0.956 (0.852–0.988)	0.198 (0.138–0.277)	0.299 (0.277–0.322)	0.926 (0.755–0.981)
				Maximum Youden's J	1.90	0.508	0.778 (0.637–0.875)	0.730 (0.647–0.800)	0.507 (0.426–0.588)	0.902 (0.841–0.941)
				At 95% specificity	5.21	0.397	0.444 (0.309–0.588)	0.952 (0.900–0.978)	0.769 (0.588–0.886)	0.828 (0.787–0.862)
		Benign (286)	0.778 (0.698–0.858)	At 95% sensitivity	0.07	0.158	0.956 (0.852–0.988)	0.203 (0.160–0.253)	0.159 (0.148–0.170)	0.967 (0.880–0.991)
				Maximum Youden's J	3.55	0.471	0.600 (0.455–0.730)	0.871 (0.827–0.905)	0.422 (0.332–0.517)	0.933 (0.906–0.952)
				At 95% specificity	5.93	0.284	0.333 (0.214–0.479)	0.951 (0.920–0.971)	0.517 (0.357–0.674)	0.901 (0.880–0.918)
		All (412)	0.782 (0.702–0.861)	At 95% sensitivity	0.07	0.157	0.956 (0.852–0.988)	0.201 (0.166–0.243)	0.116 (0.108–0.124)	0.976 (0.914–0.994)
				Maximum Youden's J	3.55	0.467	0.600 (0.455–0.730)	0.867 (0.830–0.896)	0.329 (0.258–0.409)	0.952 (0.933–0.966)
				At 95% specificity	5.65	0.329	0.378 (0.251–0.524)	0.951 (0.926–0.968)	0.459 (0.325–0.600)	0.933 (0.918–0.946)

Abbreviations: AMH, anti-Müllerian hormone; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

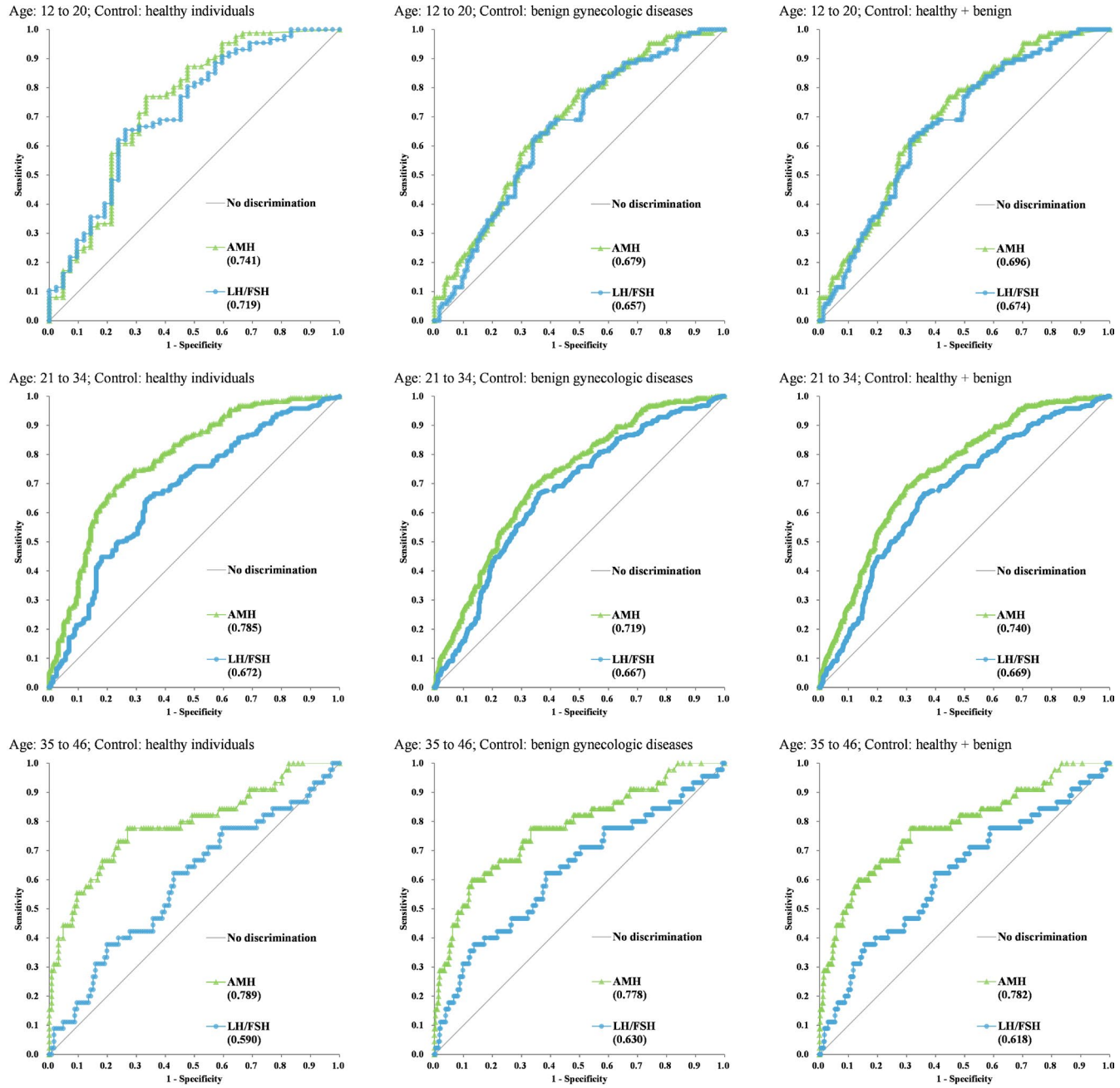

FIGURE 1 Receiver operating characteristics curves of anti-Müllerian hormone (AMH) and the luteinizing hormone to follicle-stimulating hormone ratio (LH/FSH) according to age groups and control types

Chinese women 20–44 years old. The reference intervals derived from healthy women were subsequently applied to a separate cohort of 751 women including 473 PCOS patients. The MoM for AMH in PCOS women was consistently higher than those for controls across all ages. The difference was particularly prominent in women aged 36–40 years.

In this context, we examined the clinical utility of AMH level with age-related reference limits in Korean women. In this study, the AUC value for AMH calculated from all subjects aged 12–51 years was 0.798, which is similar to results from several previous studies.[17] However, the reference interval for each age group in our study was notably higher than that from Evliyaoglu et al.[10] There are several possible explanations for this discrepancy. First, racial/ethnic differences between the study populations could affect AMH levels, that is, genetic and environmental factors could cause the disparity between racial and ethnic groups.[18] For example, the median BMI for our reference group was 21.2, while that of a previous study was 26.1.[10] The relatively low BMI in our subjects might result in higher serum AMH reference limits. In some previous studies, obesity was reported to be related to lower serum AMH levels.[6,7] Previous studies from Asia reported AMH reference intervals and median BMIs similar to those in this study.[11,19] Differences between the assay methods used in the studies and how reference groups were defined may also have affected the different AMH levels.

Meanwhile, the diagnostic performance of AMH for PCOS with age-group-specific reference limits was not entirely satisfactory. Additionally, the age-specific reference limits reported in previous studies[9,10] and the age-group-specific reference limits suggested by the assay manufacturer also only offered limited performance for our study population (Table 3). This may be due to overlaps in the distribution of AMH concentrations from healthy women and PCOS patients in the Korean sample population. Racial and ethnic differences could affect the degree of overlapping in AMH concentration distributions between healthy women and PCOS patients. Consequently, we tried a more elaborate approach to determine optimal AMH cutoff levels for PCOS diagnosis.

In ROC analysis, AUC values were consistently higher in older age groups regardless of control type. This was consistent with previous studies that reported a more modest decline of AMH levels with age in PCOS women than in healthy women.[20,21] Interestingly, the sensitivity was lower, but the specificity was higher at the optimal cutoff point in the older age group (Table 4), whereas the sensitivity at fixed 95% specificity was higher in the older age group. This means that gain of specificity outweighed loss of sensitivity along with increasing cutoff values in the older age group. Additionally, the higher AUC value and smaller variability between the cutoff values determined by 95% sensitivity, maximum Youden index, and 95% specificity were noticeable in the older age group. This would mean that inter-individual variation in AMH levels is smaller in older age groups, particularly among healthy women.

We also calculated AUC values according to age group and control type including healthy women and patients with benign gynecologic diseases. For all age groups, the AUC values were higher when the healthy group alone was used as a control than when the benign diseases group alone or both were applied as a control (Figure 1). This means that careful consideration is needed to assess the actual utility of AMH in routine clinical diagnostic application. Given the context surrounding AMH testing, patients may be suffering from other gynecologic conditions, including anovulation, if not PCOS.[22] Including only normal cycling women in a control group could result in an overestimation of the diagnostic performance of AMH for PCOS.

Meanwhile, the AMH AUC values were significantly higher than those of LH/FSH, except for the 12–20 year age group (Figure 1). Other potential biomarkers for PCOS diagnosis include LH/FSH.[13,23,24] The diagnostic performance of biomarkers would depend on several characteristics of the subject population.[25] Our results show that the age group of a patient is one of the key aspects to consider when selecting an appropriate biomarker for efficient PCOS diagnosis and for interpreting results.

Numerous recent articles have focused on several parameters that can affect the association between AMH and PCOS. It was reported that 8.2%–46.0% of PCOS patients had metabolic syndrome.[26,27] A significant negative correlation between serum AMH and insulin resistance had been observed in PCOS women.[28,29] Abdolhian et al[30] showed that an exercise intervention can significantly decrease AMH levels. AMH levels in PCOS cases could be regulated by a complicated mechanism, because PCOS is a complex reproductive and hormonal disorder that can derange metabolic parameters. Further research into the mechanism of AMH secretion control and its association with metabolic abnormalities in PCOS is necessary.

A major shortcoming of this study is that it was performed retrospectively. However, to minimize potential selection bias, we included all female patients who were requested to undergo a predefined set of hormonal assays conducted within the same day, regardless of their purpose of visit or reason for admission. The second limitation is that the subject groups were classified mainly based on the registered diagnosis in medical records. The clinical/laboratory/ultrasonographic evidences for PCOS patients were hard to be retrieved at once. To make up for this, we additionally examined medical histories for a portion of PCOS patients to estimate the distribution of different phenotypes of PCOS in the present study. Of the 48 sample PCOS patients, 27 (56.3%) were phenotype A [hyperandrogenism (HA) + ovulatory dysfunction (OD) + polycystic ovaries (PCO)], followed by 10 (20.8%) phenotype B (HA + OD), 8 (16.7%) phenotype D (OD + PCO), and 3 (6.3%) phenotype C (HA + PCO) patients. Thirdly, there may have been missed evidences that was not sufficient to support a definite diagnosis, but nevertheless the implication of an underlying disease was present. For example, there might be significant amount of patients with isolated polycystic ovarian morphology, signs of hyperandrogenism, or un-ovulation, in control group. We could not estimate how much this proportion could be, since evidences of the three criteria were not be evaluated directly by the authors. Moreover, this also could attribute to misclassification of PCOS patients into benign gynecologic diseases group. There might be cases of amenorrheic or irregular menstruation patients with pathologically high AFC, for whom the diagnosis of PCOS had yet not registered for some reasons. Those kinds of patients could have been missed through our study design.

A major strength of our study is that we further divided the control group into ostensibly healthy women and patients with benign gynecologic diseases, allowing us to obtain more relevant diagnostic performance characteristics that reflect the clinical situation in which the serum AMH test would be applied. This study also has the advantage of its comparative estimation of AMH diagnostic performance based on reference limits from the literature.

In conclusion, we established the reference intervals of serum AMH levels according to subject age groups and assessed their utility for PCOS diagnosis. Serum AMH assay showed acceptable diagnostic performance for PCOS compared with LH/FSH, particularly in older women of childbearing age. To utilize the AMH test properly and interpret its result appropriately, a thorough consideration of patient age is required.

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CONFLICT OF INTEREST

None of the authors has any conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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