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ORIGINAL

Random urinary gonadotropins as a useful initial test for girls with central precocious puberty

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Abstract. Recent evidence indicates that urinary gonadotropins may be an alternative method for detecting pubertal disorders. The aim of this study was to evaluate the associations of first morning voided (FMV) and random urinary gonadotropins with the pubertal response to a gonadotropin-releasing hormone (GnRH) stimulation test to determine whether random urinary gonadotropins can be used as an alternative method for evaluating central precocious puberty (CPP). In total, 100 girls aged 6.0–8.9 years were enrolled. The subjects were divided into two groups according to their pubertal response to the GnRH stimulation test: a positive group (n = 68) and a negative group (n = 32). Random urinary luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the LH:FSH ratio were significantly positively correlated with FMV urinary LH (r = 0.411, p < 0.001), FMV urinary FSH (r = 0.494, p < 0.001), and the FMV urinary LH:FSH ratio (r = 0.519, p < 0.001). The optimal cutoff values from receiver operating characteristic (ROC) curve analyses were determined to be 0.20 IU/L for random urinary LH (area under the curve (AUC) of 0.812, p < 0.001), 3.03 IU/L for random urinary FSH (AUC of 0.670, p = 0.004) and 0.08 for the random urinary LH:FSH ratio (AUC of 0.784, p < 0.001). No differences were observed between FMV and random urinary LH (p = 0.827), between FMV and random urinary FSH (p = 0.650), or between the FMV and random urinary LH:FSH ratio (p = 0.688) in ROC curve analyses with DeLong's test. Based on our findings, random urinary gonadotropins may be applicable in clinical practice as a useful initial test for girls with CPP.

Key words: Precocious puberty, Urinary gonadotropins, Gonadotropin-releasing hormone stimulation test

PUBERTY refers to the course of maturation from childhood to adulthood and is accompanied by a growth spurt, development of secondary sexual characteristics and, finally, acquisition of an adult reproductive capacity [1]. Puberty is initiated by activation of the hypothalamic-pituitary-gonadal (HPG) axis. Gonadotropin-releasing hormone (GnRH), which is suppressed into relative quiescence during childhood, is reactivated in a pulsatile pattern at the time of pubertal onset; the pulses start at night and are stronger during the nighttime than during the daytime [2]. A pulsatile increase in GnRH in the hypothalamus leads to the production of gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) in the anterior pituitary gland, which then results in gonadal maturation and elevated production of sex steroid hormones [3]. The gradual rise in the levels of sex steroids leads to the development of secondary sexual characteristics [4].

Central precocious puberty (CPP), which results from early activation of the HPG axis, can be defined as pubertal development before 8 years of age in girls and 9 years of age in boys [5]. CPP is considered a medical concern because it can lead to an early age at menarche, a decreased final adult height, and psychological problems [6, 7]. The levels of gonadotropins from a single random serum sample correlate well with the results of the GnRH stimulation test, which is considered the gold standard for diagnosing CPP and is commonly used in clinical settings as an initial screening test for CPP [8, 9]. However, basal gonadotropins and GnRH stimulation tests have been suggested to have some inconvenient features. Basal gonadotropins are somewhat challenging to interpret because they are secreted in a pulsatile manner. The GnRH stimulation test is inconvenient because of its time-consuming nature repeated sampling is required. A convenient alternative test that does not require blood sampling remains in demand.

Recent evidence indicates that first morning voided (FMV) urinary gonadotropins may be an alternative method for detecting pubertal disorders. FMV urinary gonadotropins were closely related to pubertal develop-

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ment in a series of recent studies [10, 11]. Serum gonadotropin levels increase at night during the early stage of pubertal development, and FMV urinary gonadotropins are considered ideal urine samples for CPP screening [12]. However, random urinary gonadotropins may also be applicable for initial CPP screening as random serum gonadotropins are already used successfully in clinical settings.

In the current study, we aimed to evaluate the relationships of FMV and random urinary gonadotropins with parameters from the GnRH stimulation test in girls aged 6.0–8.9 years for a diagnostic workup of CPP. Additionally, we investigated the association between random urinary gonadotropins and FMV urinary gonadotropins to clarify whether random urinary gonadotropins may serve as a viable alternative method for evaluating CPP.

Materials and Methods

Subjects

A total of one hundred girls aged 6.0–8.9 years were enrolled in the current study. All participants visited the Hallym University Medical Center for evaluation of precocious puberty owing to breast development and/or the appearance of pubic hair before the age of 8 years. Subjects with hypothyroidism, organic brain lesions or peripheral precocious puberty were excluded from this study. All participants exhibited a Tanner breast stage ≥2 and an advanced bone age (BA) by >1 year compared with chronological age (CA). The participants underwent the GnRH stimulation test and were divided into two groups depending on whether they had a positive or negative response. The study protocols were approved by the Institutional Review Board of the Hallym University Dongtan Sacred Heart Hospital (IRB No. 2017-03-192). Informed consent was obtained from all subjects and their parents.

Measurements

Anthropometric measurements were performed using standard methods. Height was measured to the nearest 0.1 cm using a Harpenden wall-mounted stadiometer (Holtain Ltd., Crymych, United Kingdom). Weight was measured using an electronic scale (Cas 150A; Cas Co. Ltd., Seoul, Korea) that was accurate to the nearest 0.1 kg. Pubertal stage was determined by two experienced pediatric endocrinologists according to the method of Tanner and Whitehouse [13]. All participants were verified to be in at least Tanner stage 2 of breast and/or pubic hair development. Body mass index (BMI) was calculated as the weight in kilograms/the square of height in meters (kg/m^2). The standard deviation score (SDS) values of height, weight, and BMI were used; these scores were determined using the LMS (lambda for the skew, mu for the median, and sigma for the generalized coefficient of variation) method [SDS = ((measured value/M)^L – 1)/LS] according to the 2007 Korean National Growth Charts [14].

Blood samples were drawn during the GnRH stimulation test. An intravenous cannula was placed in the forearm. Basal serum samples for LH, FSH and estradiol measurement were drawn immediately before administration of 100 μg of GnRH (Relefact; Sanofi-Aventis, Frankfurt, Germany). Blood samples for LH and FSH measurement were collected at 30, 45, 60 and 90 minutes after injection. Serum LH, FSH and estradiol were quantified using an electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, Manheim, Germany). The intra-assay coefficients of variation (CVs) and the interassay CVs were 1.2% and 2.2% for LH, 2.8% and 4.5% for FSH, and 3.3% and 4.7% for estradiol, respectively. The limits of detection for LH, FSH and estradiol were 0.01 mIU/mL, 0.01 mIU/mL and 5.0 pg/mL, respectively. A peak serum LH level ≥5.0 mIU/mL was considered a positive response.

Urine samples were collected two times. FMV (entire nighttime) urine samples were collected on the day of the GnRH stimulation test; the girls had emptied their bladders before going to sleep the previous night, and the FMV urine samples were collected as soon as the subjects had woken up. If the participants voided during the nighttime, they were excluded from this study. Random urine samples were obtained from nontimed voiding urine during a visit to the outpatient clinic of pediatric endocrinology in the same period as the GnRH stimulation test. The volume of each urine sample was required to be more than 10 mL. The urine samples were stored immediately at –20°C and transported to the central laboratory within 5 days. Urinary gonadotropins were measured using dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) hLH-Spec and hFSH-Spec kits (Perkin-Elmer, Turku, Finland). The limits of detection for the hLH-Spec and hFSH-Spec kits were 0.012 IU/L and 0.018 IU/L, respectively. The intra-assay and interassay CVs of urine LH were 7.8 and 8.7%, respectively, and the intra-assay and interassay CVs of urine FSH were 2.3% and 5.2%, respectively.

Statistical analyses

All statistical analyses were performed using R version 3.5.1 (The R Foundation for Statistical Computing, Vienna, Austria). The clinical characteristics of the study population are presented according to the results of the GnRH stimulation test (negative *vs.* positive groups). Continuous variables are reported as the medians [interquartile ranges], and categorical variables are presented

as percentages (%). Differences in clinical characteristics were analyzed using a Mann-Whitney test for continuous variables and a chi-squared test for categorical variables. Correlation coefficients (*r* values) between FMV and random urinary gonadotropins and the parameters of the GnRH stimulation test were assessed using Pearson's correlation analyses. Partial correlation coefficients (*r* values) between FMV and random urinary gonadotropins and the variables of the GnRH stimulation test were determined after adjustment for age and BMI SDSs. In addition, correlation coefficients between random urinary gonadotropins and FMV urinary gonadotropins were generated using Pearson's correlation analysis. Receiver operating characteristic (ROC) curve analysis was conducted, and the area under the curve (AUC) was calculated to assess the diagnostic performance of gonadotropins from FMV and random urine samples. Sensitivity was presented on the y-axis of the ROC curve, and 1-specificity was shown on the x-axis. The optimal cut-off value was determined using the Youden index (which identifies the point nearest to the left upper corner of the curve). DeLong's test for two correlated ROC curves was conducted to evaluate differences in diagnostic performance between FMV urinary gonadotropins (LH, FSH, and the LH:FSH ratio) and random urinary gonadotropins (LH, FSH, and the LH:FSH ratio). In all analyses, differences were considered statistically significant when the *p* value was <0.05.

Results

Clinical characteristics of the children in this study

Of a total of 100 girls in this study, 62 girls exhibited a peak LH ≥5.0 mIU/mL in the GnRH stimulation test (positive group), whereas 38 girls exhibited a peak LH <5.0 mIU/mL in the same test (negative group). The clinical characteristics of the study population are presented in Table 1. In the GnRH stimulation test, the positive group had a significantly higher median basal LH (0.36 mIU/mL *vs.* 0.06 mIU/mL, $p = 0.002$), basal FSH (2.46 mIU/mL *vs.* 1.79 mIU/mL, $p < 0.001$), basal estradiol (14.00 pg/mL *vs.* 10.00 pg/mL, $p = 0.008$), peak LH (11.83 mIU/mL *vs.* 3.20 mIU/mL, $p < 0.001$), and peak estradiol (14.00 pg/mL *vs.* 10.00 pg/mL, $p = 0.002$) than the negative group. In addition, the GnRH-positive group exhibited a significantly greater median FMV urinary LH (1.74 IU/L *vs.* 0.46 IU/L, $p < 0.001$), FMV urinary FSH (11.75 IU/L *vs.* 5.47 IU/L, $p = 0.001$), FMV urinary LH:FSH ratio (0.20 *vs.* 0.09, $p < 0.001$), random urinary LH (0.64 IU/L *vs.* 0.10 IU/L, $p < 0.001$), random urinary FSH (4.75 IU/L *vs.* 2.77 IU/L, $p = 0.004$), and random urinary LH:FSH ratio (0.11 *vs.* 0.04, $p < 0.001$) than the negative group.

Correlations of FMV and random urinary gonadotropins with the results of the GnRH stimulation test

The Pearson's correlation coefficient data (*r* values) between FMV and random urinary gonadotropins and the results of the GnRH stimulation test are shown in Figs. 1 and 2 and Supplementary Table 1, respectively. FMV urinary LH was correlated with the peak LH ($r = 0.259$, $p = 0.009$) and peak estradiol ($r = 0.199$, $p = 0.047$) upon GnRH stimulation. A positive relationship was found between FMV urinary FSH and basal FSH ($r = 0.602$, $p < 0.001$) and peak FSH ($r = 0.242$, $p = 0.015$). A positive relationship was found between the FMV urinary LH:FSH ratio and basal LH ($r = 0.409$, $p < 0.001$), basal FSH ($r = 0.274$, $p = 0.006$), basal estradiol ($r = 0.259$, $p = 0.009$), peak LH ($r = 0.528$, $p < 0.001$), and peak estradiol ($r = 0.228$, $p = 0.022$). In addition, random urinary LH was associated with basal LH ($r = 0.267$, $p = 0.007$), basal FSH ($r = 0.338$, $p = 0.001$), basal estradiol ($r = 0.315$, $p = 0.001$), peak LH ($r = 0.356$, $p < 0.001$), and peak estradiol ($r = 0.302$, $p = 0.002$). A positive relationship was found between random urinary FSH and basal FSH ($r = 0.473$, $p < 0.001$) and peak estradiol ($r = 0.203$, $p = 0.043$). A positive relationship was identified between the random urinary LH:FSH ratio and basal LH ($r = 0.244$, $p = 0.015$), basal estradiol ($r = 0.202$, $p = 0.044$), and peak LH ($r = 0.397$, $p < 0.001$).

Adjusted correlations of FMV and random urinary gonadotropins with the results of the GnRH stimulation test

To evaluate the adjusted relationship between FMV and random urinary gonadotropins and the variables of the GnRH stimulation test, partial Pearson's correlation coefficients were determined after controlling for potential confounding factors such as age and BMI SDSs. The results are presented in Figs. 3 and 4, and Supplementary Table 2, respectively. A positive relationship was found between FMV urinary LH and basal FSH ($r = 0.504$, $p < 0.001$) and peak LH ($r = 0.227$, $p = 0.024$) upon GnRH stimulation. FMV urinary FSH was correlated with basal FSH ($r = 0.593$, $p < 0.001$) and peak FSH ($r = 0.229$, $p = 0.024$). A positive association was identified between the FMV urinary LH:FSH ratio and basal LH ($r = 0.439$, $p < 0.001$), basal FSH ($r = 0.235$, $p = 0.020$), basal estradiol ($r = 0.246$, $p = 0.015$), peak LH ($r = 0.500$, $p < 0.001$), and peak estradiol ($r = 0.216$, $p = 0.033$). On the other hand, random urinary LH was associated with basal LH ($r = 0.281$, $p = 0.005$), basal FSH ($r = 0.313$, $p = 0.002$), basal estradiol ($r = 0.308$, $p < 0.001$), peak LH ($r = 0.336$, $p < 0.001$), and peak estradiol ($r = 0.298$, $p = 0.003$). A positive relationship was observed between random urinary FSH and basal FSH ($r = 0.441$, $p <$

Table 1 Clinical characteristics of the study population (n = 100)

	GnRH stimulation test		p
	Negative n = 38	Positive n = 62	
Age (years)	8.54 [8.16–8.80]	8.60 [8.14–8.84]	0.790
Height SDS	0.94 [0.25–1.57]	0.84 [0.47–1.30]	0.812
Weight SDS	0.59 [0.30–1.17]	0.65 [–0.12–1.32]	0.657
BMI SDS	0.50 [–0.19–1.39]	0.28 [–0.19–1.06]	0.318
Tanner breast stage			0.137
Stage 2 (%)	29 (76.3%)	37 (59.7%)	
Stage 3 (%)	9 (23.7%)	25 (40.3%)	
Bone age (years)	10.25 [10.00–11.00]	10.00 [10.00–11.00]	0.775
Target height (cm)	159.75 [157.50–164.00]	161.00 [158.50–164.00]	0.299
Maternal age at menarche (years)	13.00 [12.00–13.00]	13.00 [12.00–14.00]	0.653
GnRH stimulation test			
Basal LH (mIU/mL)	0.06 [0.05–0.50]	0.36 [0.10–0.50]	0.002
Basal FSH (mIU/mL)	1.79 [1.13–2.35]	2.46 [1.75–3.57]	<0.001
Basal estradiol (pg/mL)	10.00 [10.00–13.00]	14.00 [10.00–20.00]	0.008
Peak LH (mIU/mL)	3.20 [2.27–4.01]	11.83 [7.40–18.14]	<0.001
Peak FSH (mIU/mL)	11.84 [9.62–15.38]	13.51 [10.22–15.56]	0.257
Peak estradiol (pg/mL)	10.00 [10.00–12.00]	14.00 [10.00–20.00]	0.002
First morning voided urine			
LH (IU/L)	0.46 [0.20–1.22]	1.74 [1.06–4.08]	<0.001
FSH (IU/L)	5.47 [2.60–11.70]	11.75 [5.95–19.20]	0.001
LH:FSH ratio	0.09 [0.07–0.12]	0.20 [0.11–0.31]	<0.001
Random urine			
LH (IU/L)	0.10 [0.05–0.28]	0.64 [0.25–1.39]	<0.001
FSH (IU/L)	2.77 [1.62–5.23]	4.75 [2.90–8.01]	0.004
LH:FSH ratio	0.04 [0.02–0.06]	0.11 [0.05–0.24]	<0.001

The data are shown as the medians [interquartile ranges].
Target height was set as the midparental height.
GnRH, gonadotropin-releasing hormone; BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol.

0.001). Additionally, the random urinary LH:FSH ratio was correlated with basal LH (r = 0.246, p = 0.015), basal estradiol (r = 0.206, p = 0.042), peak LH (r = 0.414, p < 0.001), and peak FSH (r = –0.200, p = 0.048).

Correlations between FMV urinary gonadotropins and random urinary gonadotropins

The results of Pearson's correlation analyses linking FMV urinary LH, FSH, and LH:FSH ratio to random urinary LH, FSH, and LH:FSH ratio are shown in Fig. 5 and Supplementary Table 3. A positive association was observed between random urinary LH and FMV urinary LH (r = 0.411, p < 0.001), FMV urinary FSH (r = 0.436, p < 0.001), and the FMV urinary LH:FSH ratio (r = 0.242, p = 0.015). Random urinary FSH was related to FMV urinary LH (r = 0.248, p = 0.013) and FMV urinary FSH (r = 0.494, p < 0.001). A positive correlation was found between the random urinary LH:FSH ratio and FMV urinary LH (r = 0.592, p < 0.001), FMV urinary FSH (r = 0.288, p = 0.004), and the FMV urinary LH:FSH ratio (r = 0.519, p < 0.001).

ROC curve analyses of FMV and random urinary gonadotropins

The diagnostic performance of serum basal and FMV and random urinary gonadotropins was determined *via*

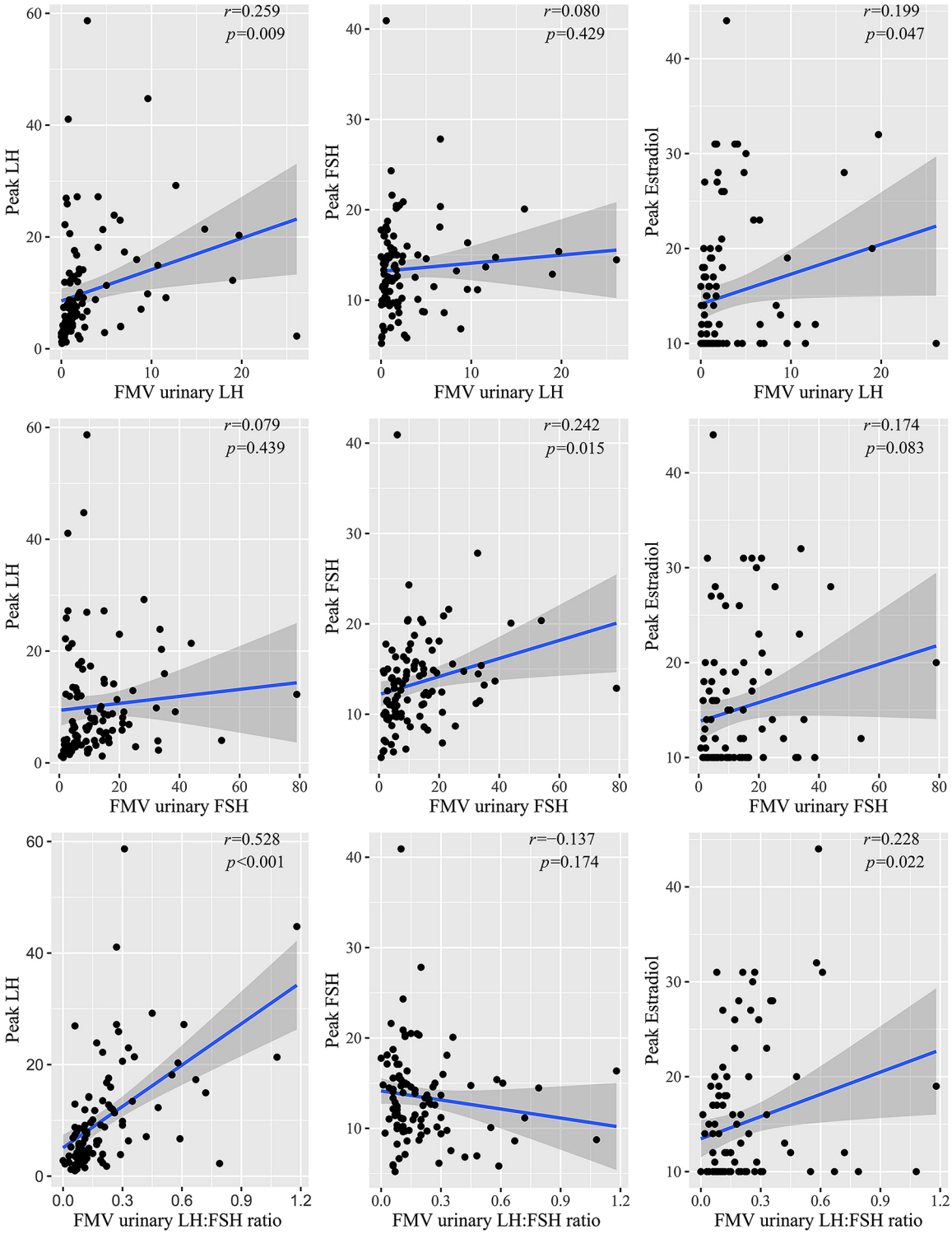

Fig. 1 The relationship between first morning voided (FMV) urinary gonadotropins and the variables of the gonadotropin-releasing hormone (GnRH) stimulation test. FMV, first morning voided; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

ROC curve analysis. The optimal cutoff values and AUCs of serum basal LH, FSH, and LH:FSH ratio and FMV and random urinary LH, FSH, and LH:FSH ratio were determined. The results of the ROC curve analyses of FMV and random urinary gonadotropins are presented in Fig. 6. The optimal cutoff values were determined to be 0.20 mIU/mL for serum basal LH (sensitivity, 74.2%; specificity, 60.5%; and AUC, 0.679 (0.575–0.782); p = 0.026), 2.42 mIU/mL for serum basal FSH (sensitivity, 51.6%; specificity, 84.2%; and AUC, 0.720 (0.616–0.824); $p < 0.001$), and 0.05 for the serum basal LH:FSH ratio (sensitivity, 74.2%; specificity, 55.3%; and AUC,

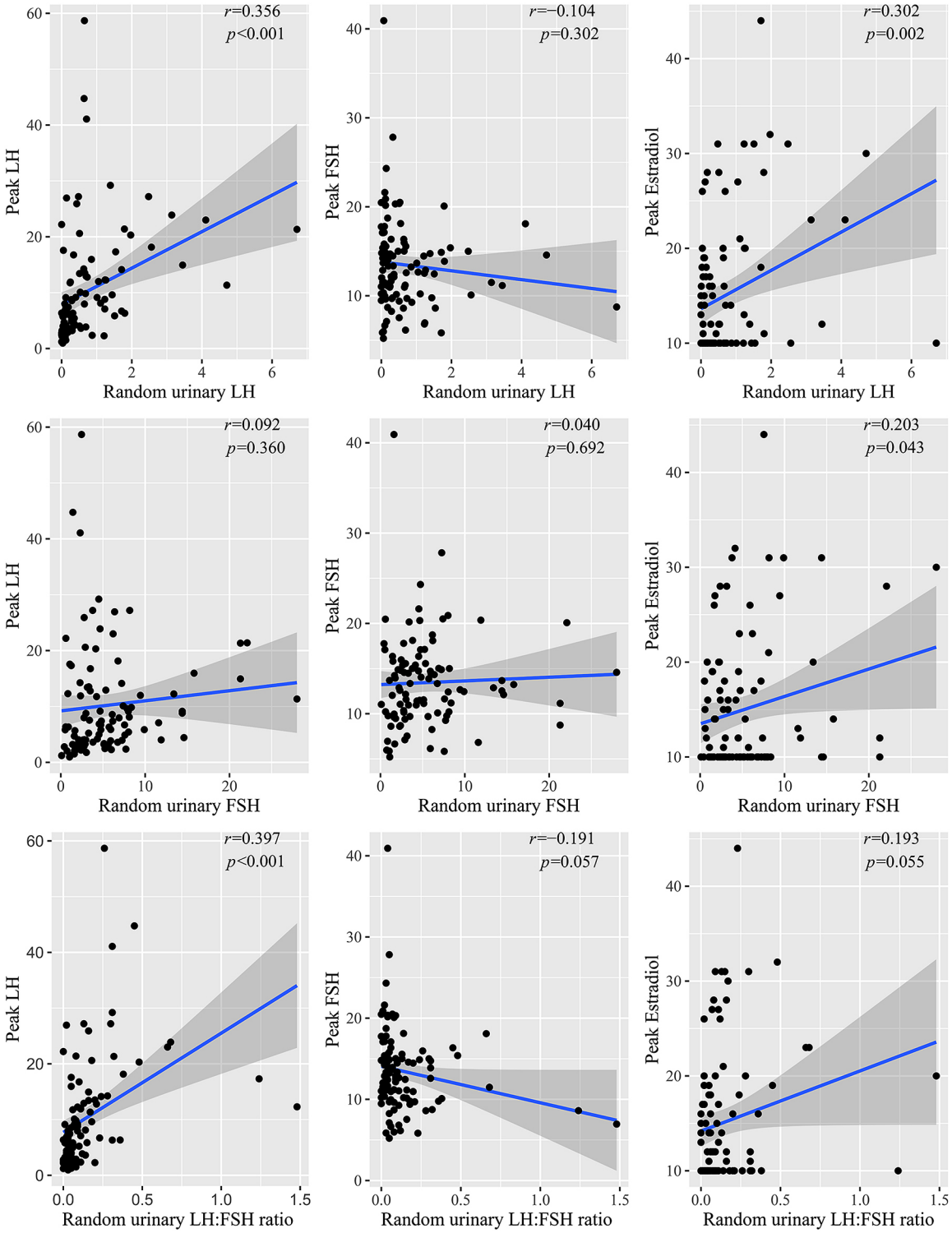

Fig. 2 The relationship between random urinary gonadotropins and the variables of the gonadotropin-releasing hormone (GnRH) stimulation test. LH, luteinizing hormone; FSH, follicle-stimulating hormone.

0.591 (0.470–0.712); $p = 0.005$). The optimal cutoff values were determined to be 0.58 IU/L for FMV urinary LH (sensitivity, 91.9%; specificity, 63.2%; and AUC 0.802 (0.703–0.901); $p < 0.001$), 6.10 IU/L for FMV urinary FSH (sensitivity, 74.2%; specificity, 60.5%; and AUC, 0.696 (0.586–0.806); $p = 0.001$), and 0.13 for the FMV urinary LH:FSH ratio (sensitivity, 67.7%; specificity, 81.6%; and AUC, 0.761 (0.665–0.857); $p < 0.001$). In addition, the optimal cutoff values were determined to be 0.20 IU/L for random urinary LH (sensitivity, 77.4%;

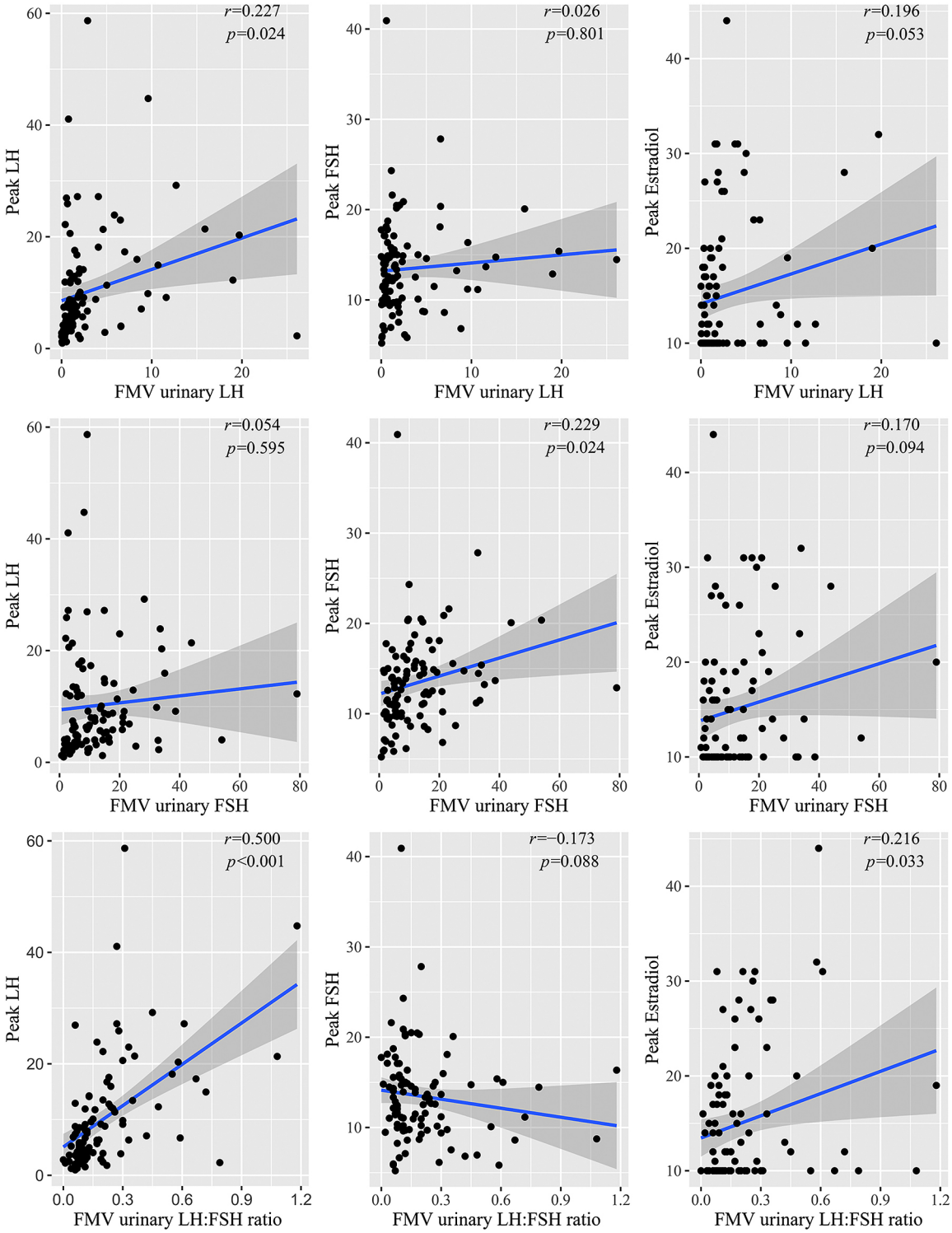

Fig. 3 The adjusted relationship between first morning voided (FMV) urinary gonadotropins and the variables of the gonadotropin-releasing hormone (GnRH) stimulation test after controlling for age and body mass index (BMI) standard deviation scores (SDSs). FMV, first morning voided; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

specificity, 73.7%; and AUC, 0.812 (0.727–0.896); $p < 0.001$), 3.03 IU/L for random urinary FSH (sensitivity, 72.6%; specificity, 60.5%; and AUC, 0.670 (0.562–0.779); $p = 0.004$), and 0.08 for the random urinary LH:FSH ratio (sensitivity, 64.5%; specificity, 89.5%; and AUC, 0.784 (0.696–0.873); $p < 0.001$). In addition, the differences in diagnostic performance between FMV urinary parameters and corresponding random urinary parameters (LH, FSH, and the LH:FSH ratio) were evaluated using DeLong's test. No differences were observed

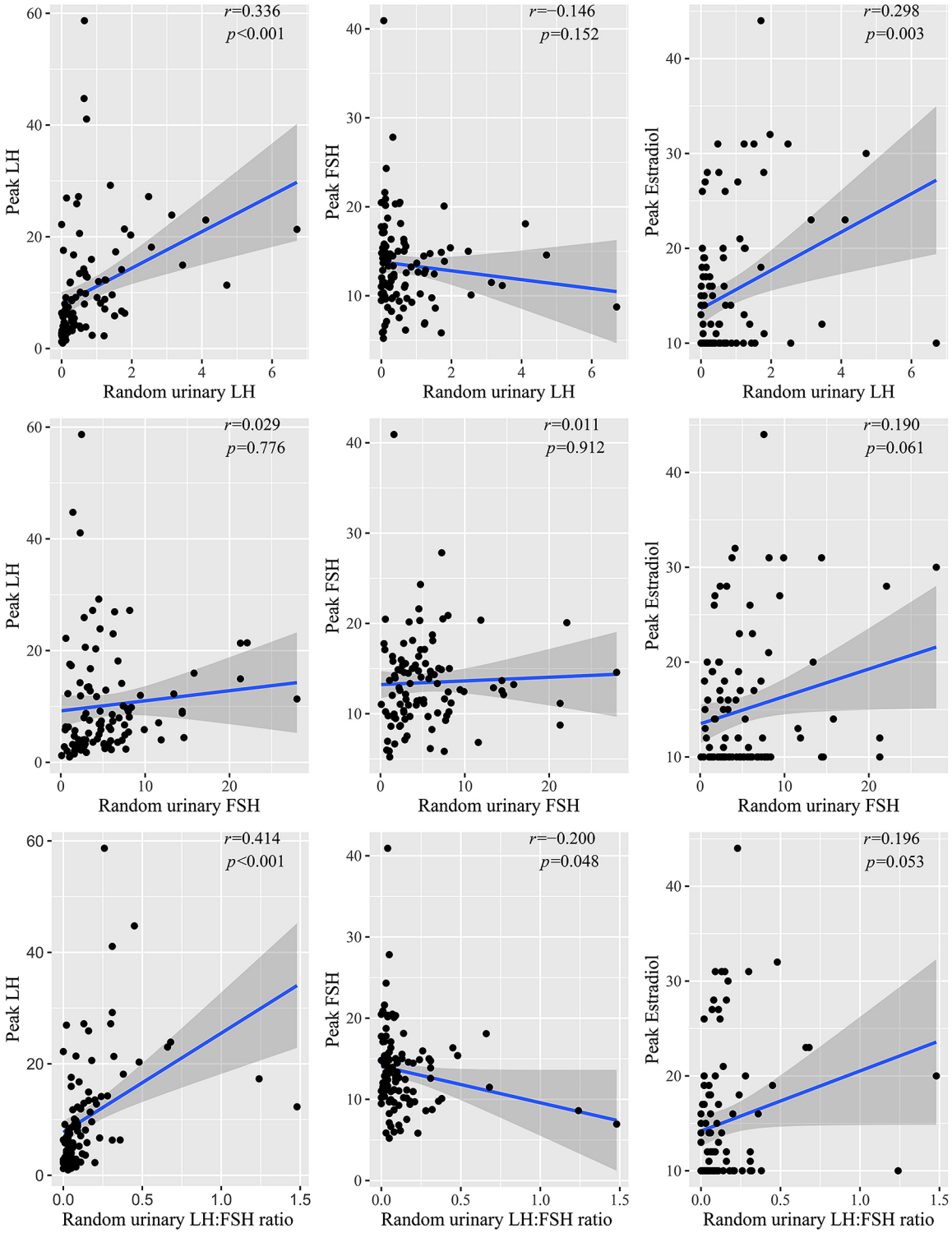

Fig. 4 The adjusted relationship between random urinary gonadotropins and the variables of the gonadotropin-releasing hormone (GnRH) stimulation test after controlling for age and body mass index (BMI) standard deviation scores (SDSs). LH, luteinizing hormone; FSH, follicle-stimulating hormone.

in the ROC curve analysis between FMV and random urinary LH (p = 0.827), between FMV and random urinary FSH (p = 0.650), or between the FMV and random urinary LH:FSH ratios (p = 0.688).

Discussion

In the current study, both FMV and random urinary LH and LH:FSH ratio were observed to be significantly

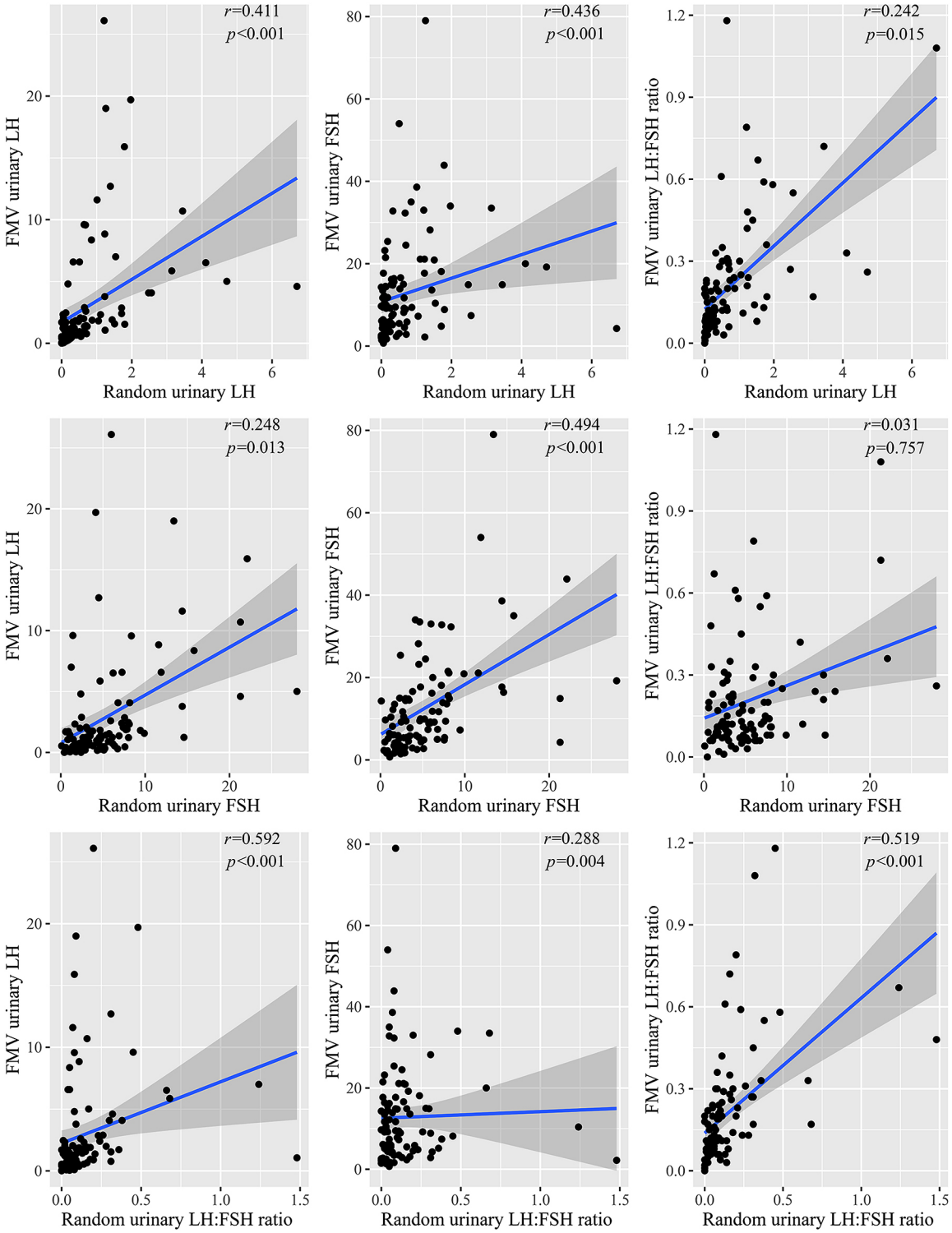

Fig. 5 The relationship between first morning voided (FMV) urinary gonadotropins and the variables of the gonadotropin-releasing hormone (GnRH) stimulation test. FMV, first morning voided; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

positively correlated with the pubertal response to the GnRH stimulation test in Korean girls aged 6–8.9 years. In ROC analyses, both FMV and random urinary LH levels were found to have good diagnostic performance with higher sensitivity than specificity, whereas both FMV and random urinary LH:FSH ratios exhibited fair diagnostic performance with higher specificity than sensitivity. Random urinary gonadotropins were not found to have significantly different diagnostic performance compared to FMV urinary gonadotropins according to the

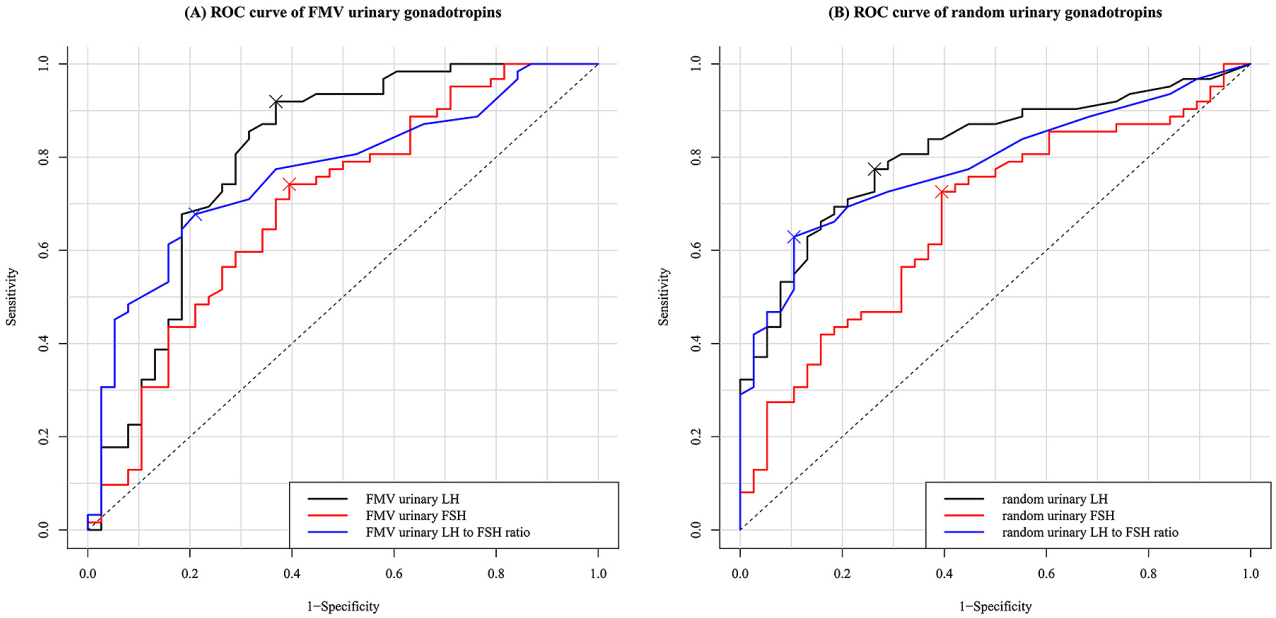

Fig. 6 Receiver operating characteristic (ROC) curve analysis of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and the LH:FSH ratio between first morning voided urine samples (A) and random urinary samples (B) with a positive response on the gonadotropin-releasing hormone (GnRH) stimulation test. Black, LH; red, FSH; blue, LH:FSH ratio. ROC, receiver operating characteristic; FMV, first morning voided; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

ROC curve analyses.

To our knowledge, the current study is the first to examine the relationship of FMV and random urinary gonadotropins with CPP. Previous studies have been conducted regarding the usefulness of FMV urinary gonadotropins, which are considered optimal samples because FMV urinary gonadotropins reflect increased physiologic secretion of gonadotropins during nighttime in early puberty. The levels of urinary gonadotropins, which are determined using an ultrasensitive immunoassay method, have been used in laboratory and clinical settings since the 1980s [15]. A series of studies have examined FMV urinary gonadotropins and pubertal development. Demir *et al.* [10] found that the levels of FMV urinary gonadotropins exhibited age-related changes and observed an approximately 5-fold increase in FSH at the age of 10 years and a 100-fold increase in LH in girls at the age of 9 years. A Finnish study reported that elevated gonadotropin levels preceded the first clinical signs of puberty in both boys and girls [16]. A study of 161 healthy children aged 4–19 years in the United Kingdom reported that the levels of urinary gonadotrophins can be used as an alternative method of tracking pubertal development [17]. Our study is consistent with previous studies regarding the relationship between FMV urinary gonadotropin levels and pubertal development, although the correlation coefficients in this study were inadequate compared to those in previous studies. Nevertheless, FMV urinary LH in the current study exhibited good diagnostic performance as estimated using the AUC in ROC curve analyses.

Some drawbacks, including invasiveness, the relatively high cost, the need for a test drug (a short-acting GnRH agonist), and the time-consuming nature of the procedure, may prevent young children and their parents from cooperating with this test. Many basic and clinical studies seeking a more acceptable and convenient alternative to the GnRH stimulation test have been conducted. FMV urinary gonadotropins have been suggested to be the optimal alternative. A Finnish study demonstrated that the FMV urinary LH level and LH:FSH ratio are strongly correlated with the results of the GnRH stimulation test in identifying early puberty among children presenting with clinical signs of puberty [11]. In that study, the FMV urinary LH level and LH:FSH ratio exhibited good and excellent diagnostic performance, respectively, for distinguishing early puberty from prepuberty compared with the GnRH stimulation test (an AUC of 0.880 for urinary LH and an AUC of 0.925 for the urinary LH:FSH ratio) [11]. A Jewish study showed increased mean levels of LH in FMV urine samples from girls with rapidly progressive CPP *vs.* slowly progressive precocious puberty and prepuberty [18]. Zung *et al.* [18] suggested that urinary LH in FMV urine samples can be used to identify rapidly progressive precocious puberty in girls with CPP and/or prepuberty. In addition, a very recent report from Denmark demonstrated that FMV urinary LH is strongly correlated with basal and peak LH in

the GnRH stimulation test [19]. Based on their study and previous studies, Kolby *et al.* [19] suggested FMV urinary LH as an alternative tool for diagnosing children with CPP. At present, however, the GnRH stimulation test is considered the gold standard for diagnosing CPP [20, 21]. FMV urinary gonadotropins may need some improvements, including international guidelines for measurement and optimal cutoff values, to serve as an alternative to the GnRH stimulation test. Studies regarding urinary gonadotropins have been conducted using inconsistent methods. Some studies reported urinary gonadotropins with adjustment for creatinine [17, 22, 23], although most studies, including the current study, were performed with no adjustment [10, 11, 16, 19]. Future studies can help refine the use of FMV urinary gonadotropins as a diagnostic method for pubertal disorders.

CPP has become a common reason for visits and referrals to pediatric endocrinologists in Korea [24]. In clinical practice, random basal serum gonadotropins are routinely used as an initial screening method for CPP. The levels of random basal serum LH are well correlated with peak LH levels in the GnRH stimulation test [25]. However, most children have a fear of invasive blood sampling. Urinary gonadotropins may provide an opportunity for noninvasive workups in children with pubertal disorders. Because elevated levels of gonadotropins at night are considered to precede elevated secretion of gonadotropins in the daytime during the onset of puberty and early puberty, most studies have focused on FMV urinary gonadotropins, which are considered more effective than random urinary gonadotropins. In our study, however, random urinary gonadotropins were found to have some advantages in clinical settings. Random urinary gonadotropins can be rapidly and conveniently tested and are not bound by time limits, rendering them a more favorable option than FMV urinary gonadotropins for children, their parents and pediatric endocrinologists. Even if random urinary gonadotropin testing is more comfortable than the alternatives, it cannot be used unless precise information can be obtained for a CPP workup in clinical practice. A study in the United Kingdom showed that random urinary LH and FSH adjusted for urinary creatinine are strongly correlated with the peak LH and peak FSH in the GnRH stimulation test [26]. In that study, the optimal cutoff point for untimed spot urinary gonadotropins adjusted for the urinary creatinine ratio exhibited 86% sensitivity, 71% specificity, and 93% positive predictive value for the pubertal response on the GnRH stimulation test [26]. Our study is consistent with a previous study, and the levels of random urinary gonadotropins exhibited good diagnostic performance in this study. In addition, random urinary gonadotropins were not different from FMV urinary gonadotropins in terms of diagnostic performance. Future studies should be conducted to apply random urinary gonadotropins as an initial screening test for the diagnosis of pubertal disorders.

Our study has some potential limitations. First, causality cannot be proven because our study was conducted in a cross-sectional manner. Second, the population in the current study was relatively small, although the age of the study participants was 6.0–8.9 years, which is considered the main target population for CPP. Third, FMV and random urinary gonadotropins were not adjusted by urinary creatinine in our study. Finally, our study did not find strong correlations between FMV or random urinary gonadotropins and the results of the GnRH stimulation test, whereas previous studies reported higher correlation coefficients (r values) between FMV and/or random urinary gonadotropins and the results of the GnRH stimulation test [11, 19]. These relatively weak correlations may be related to the study population. In girls with nonprogressive PP and early-phase PP, daytime gonadotropins may be not elevated compared to nighttime levels because puberty is characterized by nocturnal pulsatile secretion of LH. Urinary gonadotropins in random urinary samples may have lower sensitivity at the optimal cutoff point compared to those in FMV urinary samples in this population. Additionally, a weak relationship between random urinary parameters and the results of the GnRH stimulation test may be observed in girls with nonprogressive PP and early-phase PP. Nevertheless, our study found that both FMV and random urinary LH, as well as the LH:FSH ratio, had good and fair diagnostic performance, respectively, according to ROC curve analyses.

In conclusion, the FMV and random urinary LH levels and LH:FSH ratios were significantly positively correlated with the pubertal response to the GnRH stimulation test in the current cross-sectional study. FMV and random urinary LH exhibited good diagnostic performance with higher sensitivity than specificity, whereas the LH:FSH ratio exhibited fair diagnostic performance according to ROC curve analyses with higher specificity than sensitivity. Random urinary gonadotropins did not exhibit significantly lower diagnostic performance than FMV urinary gonadotropins. Based on our findings, random urinary gonadotropins may be applicable as a useful initial test for girls with CPP in clinical practice.

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Disclosure

The authors have no conflict of interest or industry relationship to disclose.

Supplementary Table 1 Correlation of first morning voided urine and random urine gonadotropin levels with variables in the serum GnRH stimulation test in the study population (n = 100)

	First morning voided urine						Random urine					
	LH (IU/L)		FSH (IU/L)		LH:FSH ratio		LH (IU/L)		FSH (IU/L)		LH:FSH ratio	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
GnRH stimulation test												
Basal LH (mIU/mL)	0.145	0.150	−0.057	0.577	0.409	<0.001	0.268	0.007	−0.027	0.791	0.244	0.015
Basal FSH (mIU/mL)	0.527	<0.001	0.602	<0.001	0.274	0.006	0.338	0.001	0.473	<0.001	0.069	0.494
Basal estradiol (pg/mL)	0.202	0.043	0.141	0.161	0.259	0.009	0.315	0.001	0.189	0.060	0.202	0.044
Peak LH (mIU/mL)	0.259	0.009	0.079	0.439	0.528	<0.001	0.356	<0.001	0.092	0.360	0.397	<0.001
Peak FSH (mIU/mL)	0.080	0.429	0.242	0.015	−0.137	0.174	−0.104	0.302	0.040	0.692	−0.191	0.057
Peak estradiol (pg/mL)	0.199	0.047	0.174	0.083	0.228	0.022	0.302	0.002	0.203	0.043	0.193	0.055

LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone

Supplementary Table 2 Adjusted correlation of first morning voided urine and random urine gonadotropins with variables in the serum GnRH stimulation test in the study population after controlling for age and body mass index (BMI) standard deviation score (SDS) values (n = 100)

	First morning voided urine						Random urine					
	LH (IU/L)		FSH(IU/L)		LH:FSH ratio		LH (IU/L)		FSH(IU/L)		LH:FSH ratio	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
GnRH stimulation test												
Basal LH (mIU/mL)	0.157	0.122	–0.053	0.606	0.439	<0.001	0.281	0.005	–0.010	0.923	0.246	0.015
Basal FSH (mIU/mL)	0.504	<0.001	0.593	<0.001	0.235	0.020	0.313	0.002	0.441	<0.001	0.076	0.459
Basal Estradiol (pg/mL)	0.192	0.058	0.133	0.190	0.246	0.015	0.308	<0.001	0.172	0.090	0.206	0.042
Peak LH (mIU/mL)	0.227	0.024	0.054	0.595	0.500	<0.001	0.336	<0.001	0.029	0.776	0.414	<0.001
Peak FSH (mIU/mL)	0.026	0.801	0.229	0.024	–0.173	0.088	–0.146	0.152	0.011	0.912	–0.200	0.048
Peak Estradiol (pg/mL)	0.196	0.053	0.170	0.094	0.216	0.033	0.298	0.003	0.190	0.061	0.196	0.053

LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone

Supplementary Table 3 Correlation between first morning voided (FMV) urine gonadotropins and random urine gonadotropins in the study population (n = 100)

	Random urine					
	LH (IU/L)		FSH (IU/L)		LH:FSH ratio	
	r	*p*	*r*	*p*	*r*	*p*
First morning voided urine						
LH (IU/L)	0.411	<0.001	0.248	0.013	0.592	<0.001
FSH (IU/L)	0.436	<0.001	0.494	<0.001	0.288	0.004
LH:FSH ratio	0.242	0.015	0.031	0.757	0.519	<0.001

LH, luteinizing hormone; FSH, follicle-stimulating hormone

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