



Association between FSH, E1, and E2 levels in urine and serum in premenopausal and postmenopausal women

Yoko Onizuka^a, Kazue Nagai^a, Yuki Ideno^b, Yoshikazu Kitahara^c, Akira Iwase^c, Toshiyuki Yasui^d, Junko Nakajima-Shimada^a, Kunihiro Hayashi^{a,*}

^a Graduate School of Health Sciences, Gunma University, 3-39-22 Showa-machi, Maebashi, Gunma 371-8514, Japan

^b Center for Mathematics and Data Science, Gunma University, Maebashi, Japan

^c Graduate School of Medicine, Gunma University, Maebashi, Japan

^d Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Japan

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ABSTRACT

Objective: We aimed to establish correlations for the levels of follicle-stimulating hormone (FSH), estrone (E1) and estradiol (E2) between urine and serum in premenopausal and postmenopausal women using immunoassays.

Methods: In this study of 92 women (61 postmenopausal, 31 premenopausal), both urine and blood specimens were collected on the same day and stored at 4 °C for analysis by chemiluminescent immunoassay, radioimmunoassay and/or electrochemiluminescent immunoassay.

Results: There were correlations in the levels of FSH, E1 and E2 between urine and serum in both postmenopausal ($r = 0.96$ for FSH, $r = 0.91$ for E1, $r = 0.80$ for E2) and premenopausal ($r = 0.98$ for FSH, $r = 0.92$ for E1, $r = 0.90$ for E2) women. It is indicated that the correlations were stronger in the premenopausal group compared with the postmenopausal group, especially for FSH.

Conclusion: The levels of FSH, E1 and E2 in urine correlated with those in the serum in premenopausal and postmenopausal women. Urine samples could be used instead of serum samples to measure hormone levels, which would reduce the difficulty of conducting large survey studies.

1. Introduction

Follicle-stimulating hormone (FSH), one of the gonadotropic hormones, is released by the pituitary gland into the bloodstream. The most important functions of FSH are to develop premature follicles in the ovary and promote the secretion of estrogens. The two forms of estrogen that are important indicators of menopausal status are estradiol (E2) and estrone (E1) [1]. As menopause progresses, the levels of E2 decrease significantly and remain low, whereas the levels of FSH increase and remain high [2,3]. According to one report, the trajectory of FSH in postmenopausal women can be divided into three stages [4], which could be an important indicator for conditions that are affected by endogenous estrogen such as cardiovascular disease [5,6]. For example, higher FSH levels were associated with an increased frequency of hot flashes and night sweats [7], an increased progression of asymptomatic atherosclerosis in middle-aged women [8] and a lower

prevalence of depression and depressive symptoms [9]. FSH was shown to cause an increase in the circulating low-density-lipoprotein (LDL) cholesterol levels by reducing the LDL receptor level [10]. However, lower FSH levels were significantly associated with prediabetes and diabetes [11] and negatively associated with non-alcoholic fatty liver disease in women over 55 years of age [12]. Additionally, the probability of metabolic syndrome was two times higher when the FSH level increased or decreased by one standard deviation [13]. Therefore, it is important that we have a clear picture of the expression patterns of FSH, E1 and E2 in the premenopausal and postmenopausal periods, especially the postmenopausal FSH level.

Because these hormones are often measured using serum, the potential for large-scale survey studies has been limited by costs and the requirement of facilities such as hospitals that employ personnel who are permitted to collect blood. Urine sampling, however, offers a non-invasive collection method for study participants. In previous reports,

* Corresponding author.

E-mail addresses: yokoi@gunma-u.ac.jp (Y. Onizuka), kazue-nagai@gunma-u.ac.jp (K. Nagai), y-iden@gunma-u.ac.jp (Y. Ideno), kitahara@gunma-u.ac.jp (Y. Kitahara), akiwase@gunma-u.ac.jp (A. Iwase), tosyasui@tokushima-u.ac.jp (T. Yasui), jshimada@gunma-u.ac.jp (J. Nakajima-Shimada), khayashi@gunma-u.ac.jp (K. Hayashi).

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the correlation between serum and urinary FSH was examined using radioimmunoassay (RIA) or sandwich immunoenzymometric assays (IEMAs), but only in specimens from premenopausal women [14,15].

With the cooperation of participants from the Gunma Nurses' Health Study (GNHS) [16] and the Japan Nurses' Health Study (JNHS) [17], we measured FSH, E1 and E2 levels in urine and blood samples from premenopausal and postmenopausal women using three different immunoassays. Our aim was to establish the correlations in the levels of FSH, E1 and E2 between urine and serum in premenopausal and postmenopausal women.

2. Material and methods

2.1. Samples

This study enrolled 92 women (61 postmenopausal and 31 premenopausal) who agreed to participate in this research and were among the original participants of the GNHS and the JNHS, excluding one woman whose urine sample volume was too small to detect sex hormones. GNHS is an ongoing cohort study of 699 female nurses aged 20 and over that was initiated in 1999 [16]. JNHS is also an ongoing cohort study that was established in 2001, and the participants consisted of female nurses in Japan. Participants were recruited from 2001 to 2007; a total of 15,019 women responded to the baseline survey and provided written informed consent to be followed up. Women with a duration of < 1 year from their last menstrual period were defined as premenopausal women, and those whose menstrual periods had ceased for > 1 year were defined as postmenopausal. All samples were collected on November 24, 2018, February 6, 2019, February 13, 2019, and February 23, 2019. The ethics committee of Gunma University reviewed and approved the study.

2.2. Urine and serum assays

Blood samples were collected in plain tubes that included coagulation activators (Terumo, Tokyo, Japan) followed by centrifugation at 1720g for 10 min; serum was collected and stored at 4 °C until the assay was performed within 48 h after sample collection. Urine specimens were collected in the morning on the same day as blood collection. Urine specimens were subjected to centrifugation at 430g for 10 min; supernatants were collected and stored at 4 °C until the assay was performed, which was within 48 h after the sample collection. Urinary and serum FSH levels were measured by chemiluminescent immunoassay (CLIA) [18], urine and serum E1 and urine E2 levels were measured by radioimmunoassay (RIA) [19] and serum E2 levels were also measured by electrochemiluminescent immunoassay (ECLIA) [20–22]. All measurements were performed by SRL, Inc. (Tokyo, Japan). The creatinine-corrected urinary FSH, E1 and E2 were calculated to indicate the excretion amount per 1 g of urinary creatinine (Cr), assuming that 1 g of urinary Cr is discharged per day.

2.3. Data analysis

We examined the correlations between FSH, E1 and E2 in urine and serum using the Pearson product-moment correlation coefficient in a linear regression model without an intercept. The limits of detection (LODs) for serum E2 and urine E2 were 5.0 ng/L and 0.20 mg/L, respectively. Women for whom no E2 was detected were assigned to the E2 value of the LODs/2 (serum, 2.5 ng/L; urine, 0.10 mg/L). $P < .05$ was considered statistically significant. We used SAS 9.4 for Windows for statistical analysis (SAS Institute Inc., Cary, NC, USA).

3. Results

The levels of FSH, E1 and E2 in postmenopausal and premenopausal women are shown in Table 1. Serum E1 and E2 levels were higher (E1;

$p = .0001$, E2; $p = .0002$) while the serum FSH levels were lower ($p < .0001$) in premenopausal women compared with postmenopausal women. The urinary measurements of these hormones showed similar tendencies.

There were significant correlations between the urine and serum levels of all three hormones in postmenopausal women: FSH ($r = 0.96$, $p < .0001$), E1 ($r = 0.91$, $p < .0001$) and E2 ($r = 0.80$, $p < .0001$) (Fig. 1). In premenopausal women, the positive correlations between the urine and serum levels of E1, E2 and FSH were even stronger: ($r = 0.98$, $p < .0001$), E1 ($r = 0.92$, $p < .0001$) and E2 ($r = 0.90$, $p < .0001$) (Fig. 1).

4. Discussion

In this study, we showed that the correlations in the relative concentrations of FSH, E1 and E2 between urine and serum were valid in both premenopausal and postmenopausal women. There was a particularly strong correlation between serum and urine FSH in premenopausal women ($r = 0.98$), which is consistent with a previous report [23]. In addition, the correlations in E1 and E2 between serum and urine in premenopausal women were within the reference values despite the fact that spot urine samples were used for the analysis (E1: 1.00–8.00 µg/day; E2: 0.50–5.00 µg/day). In the clinical setting, RIA is typically performed using 24-h urine collection, and this process is considered to be complex because the compounds need to be extracted from the urine specimens. However, in this study we were able to obtain significant results using a simplified sample preparation of early morning urine, which is considered to be suitable for analysis in large cohorts. Previous studies measured the correlation between serum and urinary FSH in premenopausal specimens [14,15], but there are few reports that mention the analysis of this hormone in postmenopausal women. The Study of Women's Health Across the Nation (SWAN) identified three trajectories of changes in FSH during the menopausal transition: low, high and medium rise patterns. After the final menstrual period, FSH continued changing slightly, but leveled off. The approximate plateau values for the FSH trajectory groups were 40 IU/L for the low group, 85 IU/L for the medium group and 120 IU/L for the high group [4]. Because strong correlations were found between blood and urinary excretion levels of FSH in this study, we calculated the urinary excretion of FSH corresponding to the FSH value for each of the SWAN group categories. The urinary excretion of the low FSH group was 61.1 IU/g-Cr, the medium FSH group was 129.7 IU/g-Cr and the high FSH group was 183.2 IU/g-Cr (data not shown). These results indicate that the measurement of even just one time point of FSH in urine could be used to analyze FSH levels in postmenopausal women. We clarified the correlation between urine and serum not only for FSH but also for E1 and E2, using samples from postmenopausal women. These results indicate that urine samples could be used to determine the relative hormones level within a population and thereby support clinical and epidemiological studies that are conducted in perimenopausal and postmenopausal women.

A limitation of this study is that serum FSH, E1 and E2 values were indicative of serum levels at the time of measurement, while urinary FSH, E1 and E2 values reflected urinary excretion over a certain time period.

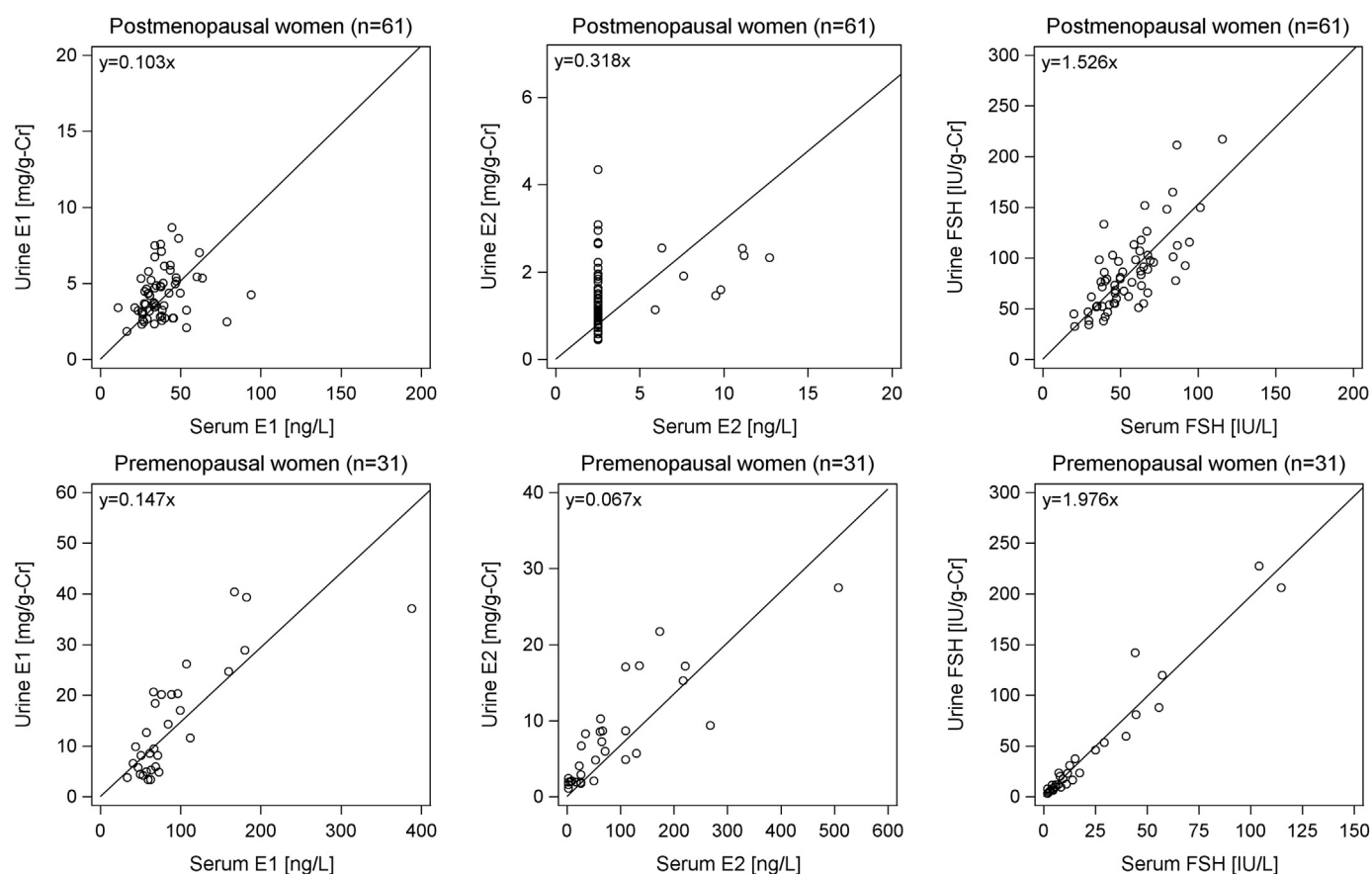
In conclusion, we showed that the urinary measurements of FSH, E1 and E2 levels were similar to those of serum in both postmenopausal as well as premenopausal women. Before this study, the correlations for FSH levels between urine and serum in premenopausal and postmenopausal women were not well established. Using the CLIA method, this study revealed a strong correlation for FSH between urine and serum. The use of urine specimens could make it possible to determine the levels of FSH, E1 and E2 without undue burden on the study participants, and contribute to the establishment of large-scale surveys.

Table 1
Levels of E1, E2 and FSH in premenopausal and postmenopausal women.

	Postmenopausal (n = 61)	Premenopausal (n = 31)	p-value for t-test
Age (years)	62.8 ± 6.8	48.0 ± 4.4	< 0.0001
BMI (kg/m ²)	21.8 ± 2.9	21.7 ± 3.4	0.9107
Urine E1 (mg/g-Cr)	4.29 ± 1.61	14.5 ± 11.0	< 0.0001
(μmol/mol-Cr)	1.80 ± 0.67	6.06 ± 4.60	< 0.0001
Urine E2 (mg/g-Cr)	1.48 ± 0.75	7.51 ± 6.73	< 0.0001
(μmol/mol-Cr)	0.61 ± 0.31	3.12 ± 2.80	< 0.0001
Urine FSH (IU/g-Cr)	86.0 ± 38.8	43.0 ± 57.9	0.0005
(IU/mol-Cr)	9733.3 ± 4388.7	9083.7 ± 4841.2	0.0005
Serum E1 (ng/L)	37.9 ± 13.8	91.4 ± 68.2	0.0001
(pmol/L)	140.4 ± 50.9	338.1 ± 252.3	0.0001
Serum E2 (ng/L)	3.39 ± 2.45	85.1 ± 105.4	0.0002
(pmol/L)	12.4 ± 9.0	312.4 ± 386.8	0.0002
Serum FSH (IU/L)	55.7 ± 20.4	21.8 ± 28.4	< 0.0001

Data are expressed as mean ± standard deviation.

Cr: creatinine, E1: estrone, E2: estradiol, FSH: follicle-stimulating hormone.



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Fig. 1. Correlations in E1, E2 and FSH concentrations between urine and serum samples in postmenopausal and premenopausal women.

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