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황체기 단축현상을 가진 한국여성의 호르몬 양 (Ⅲ) : 뇨내 Steroid Glucuronides의 변동

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THE HORMONAL LEVELS OF THE SHORT LUTEAL PHASE IN KOREAN WOMEN (III): CHANGE OF STEROID GLUCURONIDES

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월경주기 중 황체기가 짧은 여성 즉 황체기 단축현상을 가진 한국여성(SLP) 의 특성을 조사하고 진단하는 방법을 개발하기 위하여 본 실험을 행하였다.

재료는 정상 월경주기를 가진 12명의 여성에서 26주기와 8명의 황체기 단축현상을 가진 여성에서 17주기를 선택하였다. 기상후 첫 오줌에서 steroid glucuronides의 농도를 측정하여 비교한 결과는 다음과 같았다.

SLP 의 estrone-3-glucuronide (E_1G)의 농도는 초기 여포기와 황체기 동안 정상인에 비하여 현저히 낮았다. 또한 SLP의 전 월경기 중 pregnanediol-3α-glucuronide (P_2G)의 농도 역시 유의성있게 낮았다. 그러나 E_1G/P_2G의 비율은 여포기가 황체기에 비하여 현저히 높았고, SLP 여성의 비율이 정상인 보다 각 월경주기 중 유의성있게 높았다.

위의 결과로 보아 E_1G, P_2G 및 E_1G/P_2G의 농도차로 황체기 단축현상을 가진 여성을 진단 하고 또 치료시 난소의 기능조절에 이용될 수 있을 것으로 사려된다.

INTRODUCTION

It has been reported that luteal phase defect (LPD) is believed to be one of the important causes of female infertilities. LPD is due to the functional defect of corpus luteum and is found in 3.5% - 9% of the infertile group (1-8).

Recently, many clinical studies have been concentrated on the following factors of producing an inadequate luteal phase (LPD). The ovarian factor such as the insufficient progesterone production during the postovulatory period is known to induce an inappropriate endometrial development (9, 10). The aspiration of the Graafian follicle or the induction of ovulation with HMG and HCG also induce LPD (11-13). However, the majority of these inadequately functioning corpus luteum appear to be due to the unbalances of the hypothalamo-pituitary ovarian axis. Those are suboptimal levels of follicle-stimulating hormone (FSH), inadequate lutenizing hormone (LH) surge, sublevel of prolactin, prostaglandins produced by the uterus, and the endometrial progesterone receptor defects (4-6, 14-17).

The short luteal phase (SLP) of the menstrual cycle was first described by Jones (1949) and further characterized as one of the phenomena of luteal phase defect (2, 10, 18-21). On the other hand, this SLP is found in women without fertility (21-22). However, the aethiology and diagnosis for the treatment of these LPD or SLP are not clear yet.

Due to the development of direct radioimmunoassay for urinary steroid glucuronides (23, 24), it can be monitored the ovarian functions or determined the fertile period by the determination of steroid glucuronide concentration in early morning urine (25-27). These results suggest that this simple, reliable method, the RIA determination of steroid glucuronides, can be applied to monitor the ovarian functions of SLP women.

The present study, thereby was designed to determine the changes of urinary steroid glucuronides and to characterize further SLP phenomena during SLP menstrual cycle in Korean women.

MATERIALS AND METHODS

Subjects

A total of women (aged 20 to 28 years) were recruited. They had all experienced previously three regular menstrual cycles with 25-35 days prior to the investigation. Particularly they were all in good health and were not receiving any chronic drug therapy. All were no history or evidence of liver or kidney diseases. 17 cycles from women who had short menstrual cycle (less than 25 days) were studied.

Urine collection

The volunteers started to collect urine at day 1 of the menstrual cycle (first day of bleeding) and continued for each day of a complete cycle.

The first urine obtained after deep sleep was defined as early morning urine (EMU) or overnight urine (ONU). The basal body temperature (BBT), time of collection, and urine volume were recorded. An appropriate aliquot added with 0.1% sodium azide was stored at -30°C prior to analysis. The menstrual cycle was divided into 7 subcategories in order to compare the hormonal values in the ovarian cycle of normal to SLP women. 1) Three subcategories in the follicular period (FP) such as early (EFP, more than -11th days before LH surge), middle (MFP, -10th to -6th days) and late (LFP, -5th to 1st days), 2) peak day of LH surge (0 days), 3) three subcategories of luteal phase period such as early (+1st to 5th after LH surge), middle (MLP, +6th to 10th days), and late luteal phase (LLP, more than +11th days).

Assay procedures for RIA of steroid glucuronides

Antibodies and tracers for the measurement of estrone-3-glucuronide (6,7(n)-^{3}H E_1G, SA 38 Ci/m mol) and pregnanediol-3 -glucuronide (6,7(n)-^{3}H P_2G, SA 42 Ci/mol) were gifts from Professor, A.E. Kellie and Dr. P. Samarajeewa, The Cautauld Institute of Biochemistry, the Middlesex Hospital Medical School, U.K. Iodine - 125 human luteinizing hormones (hLH) were provided by WHO Matched Reagent Programme at monthly interval being labelled by the lactoperoxidase method and were repurified at one week intervals using AcA 44 Ultrogel column chromatography.

Sodium phosphate buffer (0.1 M) containing 8.8g of NaCl, and 0.1g of Merthiolate (Sigma Chem. Co.) was used as a basic buffer. The assay buffer (PB-GEL) for the routine RIA of steroid glucuronides was made as the above buffer containing 0.1% gelatin. The buffer for the peptide hormones was made by adding 0.025M of EDTA and 5 g/ℓ bovine serum albumin (BSA, Sigma, RIA grade) instead of gelatin. Tracer-diluent buffer was added with 0.5 - 1% of normal rabbit serum. Charcoal (10g, Norit A, Amend Co.) and Dextran T-70 (1g, Mol. Wt. 70,000, Pharmacia Fine Chem.) were dissolved in one liter of PB-GEL. Liquid scintillation counting fluid was made as follows: 0.5% (w/v) of 2,5-diphenyloxazole (Sigma) and 0.01% (w/v) of 1,4-bis-(5-diphenyloxazole-2-yl) benzene (Sigma) in a toluene/Triton X-100 mixture (3:1, v/v).

All three metabolites were measured by RIA previously described (28). Cross reaction of E_1G antiserum was estradiol-3-glucuronide 4.7%; estrone 7.6%; estradiol 0.02%; estriol 0.06%. The minimum detectable level of E_1G was 2.5 n mol/ℓ The coefficient of variation (CV) of precision was 6.7%. Cross reaction of P_2G was negligible to all steroid glucuronides but pregnanediol, 4.6%. The lowest detectable amount of P_2G assay were 0.5 μ mol/ℓ and 7.4% respectively.

The urine samples were diluted to give 1/200 dilution (v/v) for the determination of E_1G and 1/200 - 1/1,000 for the P_2G.

One hundred microlitre of suitably diluted sample or standard series mixed thoroughly with 100 $\mu\ell$ of tritiated radioligands and with 100 $\mu\ell$ of antiserum. Total 300 $\mu\ell$ mixture was incubated for 1 hour at 37°C. The free steroid glucuronides were separated using 2 mg/tube dextran-coated charcoal, after equilibrating for 10 min at 4°C. The supernatant was decanted into a polypropylene minivial containing 3 ml of cocktail fluid and then the radioactivity was determined using β-counter (Packard TriCarb 2450 or 4530).

Assay procedures for urinary LH.

Urine was concentrated 5 folds. Aliquot was acidified with glacial acetic acid to pH 4.5. Two volumes of acetone were then added and the mixture refrigerated overnight at 4°C. The mixture was then centrifuged at 10,000 rpm for 30 min at 4°C. After the supernatant was discarded and the precipitate dried, resuspended in appropriate amounts of assay buffer and recentrifuged.

The LH concentrations in the extracted urine samples were measured by a double antibody RIA, of which reagents were provided by Matched Reagent Programme of WHO (15).

Assays for urinary creatinine

The colorimetric measurement of urinary creatinine was based on the Joffe reaction using Autoanalyzer MT II system. Creatinine was reacted with picric acid (0.03 mol/ℓ).

The assay was carried out on 3 ml of an appropriately diluted urine. To each tube 1.0 ml of 0.036 mol/ℓ picric acid was added and mixed thoroughly. And then 0.5 ml of 1.4 mol/ℓ

sodium hydroxide was added. Exactly 15 min after adding the alkali, the absorbance against the reagent blank was read at 500 nm using double beam spectrophotometer.

The absorbance of unknown/standard was multiplied by a factor 0.88 to calculate mg creatinine/ml urine.

Statistical analysis

Student t-test was mainly applied to determine the significances between the two means.

RESULTS

Some characteristics of the short menstrual cycles are summarized in Table 1.

The total lengths of 17 short mesnstrual cycles from 8 women were significantly shorter than those of 26 normal cycle from 12 women. The duration of luteal phase in short menstrual cycle women was significantly shorter than those in normal cycle. This result suggests that this 17 short menstrual cycles are short luteal phase (SLP) cycles.

The cyclic patterns of E_1G in the serial samples of early morning urine in normal or SLP cycle are shown in Fig 1.

Table 1. Some characters of the menstrual cycle with normal and short luteal phase in Korean women

Group	Number of cycle	Length of cycle	Duration of follicular phase	Duration of luteal phase
Normal (NLP)	26	28.3 ± 0.4	14.6 ± 1.2	13.4 ± 0.8
Short (SLP)	17	24.3 ± 2.3	26.3 ± 1.3	8.3 ± 1.5

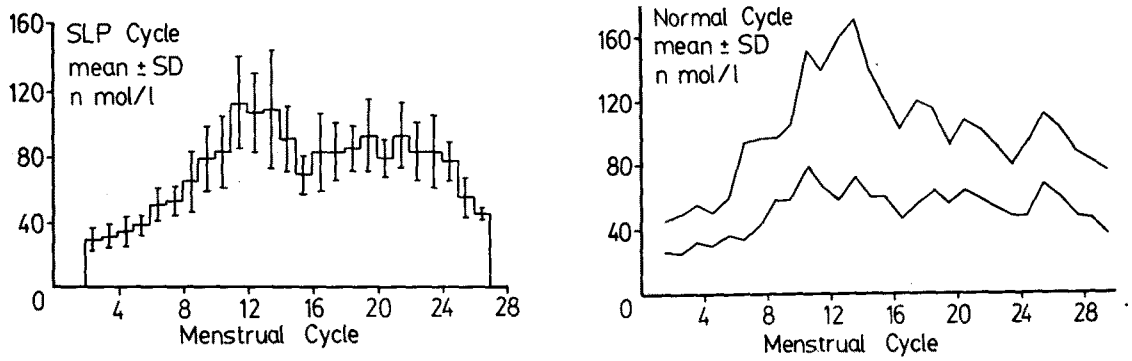

Figure 1. The concentration of estrone-3-glucuronide in serial samples of early morning urine during menstrual cycle of normal and short luteal phase women.

The E_1G level was determined in the serial samples of the complete menstrual cycles from 26 cycles of normal cycle women. The standard deviations are indicated as the vertical bars on the average means in SLP cycle and as shaded areas in normal cycle.

This figure depicts that the peak value of E_1G is shown on 13-14th days in both normal or SLP cycle. The E_1G concentration of early follicular phase was in the range of 21-65 nmol/l in both cycle and raised continuously until 14th day of the cycles. The level of E_1G was gradually and continuously decreased until bleeding period.

The concentrations of P_2G in early morning urine during the menstrual cycle are shwon in Fig. 2.

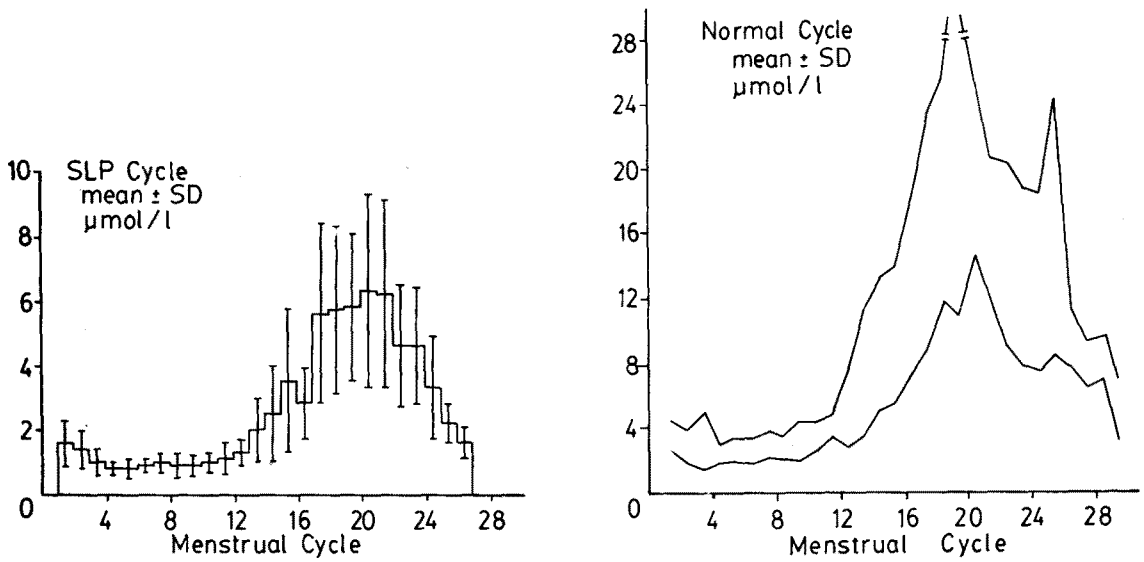

Figure 2. The concentration of pregnanediol-3 alpha- glucuronide in serial samples of early morning urine during menstrual cycles of normal and short luteal phase (SLP) women.

The legends are the same as those in Figure 1.

The cyclic secretory pattern of P_2G was similar from the analysis in both cycles. The tonic levels during follicular phase of normal and SLP cycle were 3.4 μ mol/ and 2.1 μ mol/ respectively. The significant rises of P_2G were observed after 14-16th days in both cycles.

The concentrations of E_1G in EMU at defined phases of the menstrual cycles of normal and SLP women are shown in Table 2.

Table 2. The concentration of estrone-3-glucuronide in serial samples of early morning urine throughout the menstrual cycles of normal or short luteal phase women.

Unit: n mol/l

Phase of ovarian cycle	Normal cycle	Short luteal phase cycle
Follicular phase (FP)		
early (< -11)	40.25 ± 2.22	34.42 ± 1.57 a
middle (-6 – -10)	56.67 ± 3.72	51.73 ± 2.36
late (-1 – -5)	110.46 ± 5.57	91.92 ± 5.85 a
Midcycle day (0)	118.35 ± 4.29	105.14 ± 4.4 1
Luteal phase (LP)		
early (1 -5)	70.84 ± 3. 60	58.53 ± 2.79 b
middle (6 - 10)	83.73 ± 3.88	63.38 ± 2.44 c
late (11 <)	64.95 ± 3.33	-----

Data are expressed as the mean ± SEM of 26 cycles from 12 normal women (NLP) or of 17 cycles from 8 women who have short luteal phase cycles (SLP). The data obtained in individual subjects were grouped according to the day of LH surge. Student's t-test was used to compare the results NLP vs SLP a = $p < 0.05$ b = $p < 0.01$ c = 0.001

The E_1G levels during early follicular and luteal phase in SLP women were significantly lower than those in normal women.

The concentrations of pregnanediol 3α-glucuronide in EMU at defined stages of the menstrual cycles are summerized in Table 3.

Table 3. The concentration of pregnanediol-3a-glucuronide in serial sample of early morning urine throughout the menstrual cycles of normal or short luteal phase women

Unit: μ mol/l

Phase of ovarian cycle	Normal cycle	Short luteal phase cycle
Follicular phase (FP)		
early (<-11)	+ 3.61 ± 0.23	1.51 ± 0.19
middle (-6 – -10)	2.69 ± 0.14	1.99 ± 0.11
late (-1 – -5)	3.83 ± 0.29	2.89 ± 0.14 b
Midcycle day (0)	5.91 ± 0.42	3.97 ± 0.21 c
Luteal phase (LP)		
early (1 – 5)	25.44 ± 1.20	6.91 ± 0.32
middle (6 – 10)	17.95 ± 1.09	6.65 ± 0.51 c
late (11 <)	9.05 ± 0.45	----

The legends are same as those in Table 2. The numerals in the parentheses represent the deviated days from LH surge day (0) of midcycle.

The mean values of 7 stages of the ovarian cycle of SLP women were significnalty lower than those of normal women.

The mean ratio of concentration of E_1G (n mol/1) to that of P_2G (μmol/1) in daily samples of EMU throughout the ovarian cycle is shown in Table 4.

Table 4. The ratios of mean value for estrone-3-glucuronide/pregnanediol-3a-glucuronide in early morning urin during the menstrual cycle of normal or short luteal phase women.

Phase of ovarian cycle	Normal cycle	Short luteal phase cycle
Follicular phase		
early (< -11)	11.15	22.79
middle (-6 – -10)	21.07	25.99
late (-1 – -5)	28.84	31.08
Midcycle day (0)	20.02	26.48
Luteal phase		
early (1 – 5)	4.59	8.47
middle (6 – 10)	4.66	9.53
late (11 <)	7.18	–

For the calculation of the mean ratio, the concentration of E_1G (n mol/ℓ) was devided by that of P_2G (μ mol/1) in daily sample during the menstrual cycle of normal and SLP women.

This ratio was increased during the follicular phase and preceded the LH surge day in both cases. The ratios in the luteal phase were significantly lower than those in the follicular phase of both cycle. This ratio may be of value to indicate the menstrual phase and fertile period of the ovarian cycle. This ratio in the menstrual cycle of SLP women was higher than that of normal cycle throughout the cycle.

DISCUSSION

The present study shows two important indicies for the diagnoses of short luteal phase during the menstrual cycle. One is the subnormal level of E_1G and P_2G in EMU, especially of luteal phase. The other one is the higher ratio of E_1G/P_2G in SLP women.

It has been well known that the production rate of steroid hormone is correlated well with the concentration in peripheral plasma (23-27) Estradiol-17β is produced at the level 60-350 μg/24 hr and secreted into peripheral blood (50 to 300 pg/ml) and then into urine. Most important urinary metabolites of estradiol is E_1G. Progesterone production rates range from 0.75 to 2.5 mg per day during follicular phase (up to 90% originating from adrenal cortex) and from 15 to 50 mg per day during luteal phase (less than 1 mg from extra-ovarian sources)25. The concentration of urinary P_2G, major metabolite of progesterone, is also cor-

related with that of progesterone in blood. Therefore, the secretory pattern of steroid glucuronides is used as an index of ovarian functions (23-29). Therefore, the present findings strongly suggest that the steroid glucuronides in EMU can be used as an index to monitor the ovarian function during the menstrual cycle of short luteal phase women.

The present data for the normal values of two steroid glucuronides in early morning urine are well agreed with those of the several workers (23-27, 29-31).

The present data show that the lower secretory level of P_2G of SLP women seems to be correlated with that the lower production of progesterone by corpus luteum. The previous results (15) have demonstrated that the serum progesterone level in SLP is lower than that of normal women. Thus, the present study suggests that the lower level of P_2G in EMU of SLP women is not influenced by the metabolic clearance rate.

The present data also show that the advantages of urinary E_1G and P_2G measurements over plasma steroid hormone assays are great, Patient suitability, rapid evaluation by performing daily assay. These advantages are greater when the measurement of steroid glucuronides is done in the urine of pregnant women in the preliminary study to predict the pregnancy as Mendizab et al (1984) did (32).

The data of the ratio of E_1G to P_2G in the serial samples of early morning urine are known to be used to predict the start and finish of the fertile period (25-27, 29-31). The present data also suggest that this ratio in eary morning urine may be used as an index for the detect of short luteal phase cycle and to monitor the treatment of SLP women and for measuring the effect of the drugs on ovarian function of short luteal phase women (33).

In the present study, conclusion on the absolute value of steroid glucuronides in early morning urine could not be suggested because of insufficient members of experiment subjects. However, the aim of this study of demonstrate the change of urinary steroid glucuronides in EMU and to develop for characterizing the SLP phenomena seems to be successful.

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