Extended Abstract

Effect of past hormonal contraceptive use on blood, salivary, and urinary progesterone levels in young women

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Background

Salivary and urinary hormones are widely used in population studies based on the assumption that they reflect the blood levels. However, several studies suggest that the salivary or urinary progesterone levels can differ between populations even when the blood levels do not. While dietary intake may account for some of the population difference in urinary excretion of the hormone, factors that cause different salivary excretion at the population level are not known.

Corticosteroid Binding Globulin (CBG) levels are increased when women use oral contraceptives.[1,2] It is not known whether such increase of CBG persists after discontinuing oral contraceptive use. If the increase of CBG levels does persist among the previous-users of hormonal contraceptives, the proportion of CBG-bound P4 would increase and the proportion of free-P4 in blood would decrease. Since salivary progesterone reflects the free fraction of the hormone in blood, past users of hormonal contraceptives are hypothesized to show lower salivary progesterone compared to never-users.

The aim of this study was to examine the effect of previous hormonal contraceptive use on progesterone levels in blood, saliva, and urine.

Methods

Study participants were recruited in Seattle, Washington, using flyers and advertisements on the web, and in newspapers and magazines. Women aged 18-34 years who had not been on hormonal contraceptives or hormonal medications within the past 3 months and who were not currently pregnant or breastfeeding were eligible to participate. In order to examine the ethnic differences in urinary and salivary excretion of progesterone, the participants were limited to women of either Caucasian or Japanese ethnicity.

At the intake interview, the participants underwent anthropometric measurements, filled out a questionnaire on hormonal contraceptive use, and gave a matched set of dried blood spots, saliva, and urine samples. After the intake, matched specimens were collected for each participant once a week for the following three weeks. Blood specimens were collected on a filter paper (Whatman, #903) by pricking a finger with a disposable micro-lancet. Blood spots were dried at room temperature for 4-18 hours and stored at -20C° until assay. Saliva and urine specimens were collected into plastic vials and stored at -20C°.

The blood and saliva specimens were assayed for progesterone using ELISA. The urine specimens were assayed for pregnanediol glucuronide (PDG), a major metabolite of progesterone in urine, using ELISA. Urinary PDG levels were adjusted for specific gravity of urine to control for hydration status.[3]

The participants were stratified into 3 groups according to the past hormonal contraceptive use. Never users (N=31), previous-users A--ever users who discontinued use ≤2 years before the

enrollment (N=20), or previous-users B--ever users who discontinued use ≥5 years before the enrollment (N=7). There were no participants who discontinued use between 2 and 5 years before the enrollment. According to cycle day counted from first day of menstruation, all the samples were categorized into 3 menstrual phases. Follicular--cycle day 1-14, late-follicular to early-luteal--cycle day 15-23, and luteal--cycle day 24+. Geometric means of the progesterone levels were calculated for each group of the past hormonal contraceptive use. The hormone values were log transformed and compared between the groups using ANOVA. The hormone concentrations were further adjusted for age, BMI, ethnicity, menstrual cycle phase, and repeated measures using a mixed-effects model. The hormone levels were log transformed for the statistical analysis and transformed back for the data presentations. All statistical analysis was conducted using R 2.9.0. The level of statistical significance was defined as $p<0.05$.

Results

Sixty-one women were enrolled in the study and 58 women completed 4 sampling sessions, yielding a total of 232 specimens (blood, saliva, and urine). Demographic characteristics of the participants are shown in Table 1. Thirty-one (53%) women had never used hormonal contraceptives (never-users), while 20 (34%) women discontinued contraceptive use less than 2 years before the study enrollment (previous-users A) and 7 (12%) women discontinued use more than 5 years ago (previous-users B). There were no women who discontinued use between 2 and 5 years before the enrollment.

The geometric mean of the progesterone levels by the past hormonal contraceptive use is summarized in Table 2. Neither the blood P4 ($p=0.361$) nor the urinary PDG levels ($p=0.632$) showed significant differences between the groups, while there was significant between-group differences in the salivary P4 levels ($p<0.001$). The geometric means of salivary P4 levels were 0.35 ng/mL for the never-users, 0.26 ng/mL for the previous-users A, and 0.22 ng/mL for the previous-users B.

The results of the mixed-effects model analysis are summarized in Table 3. Compared to the never-users, the previous-users A showed slightly lower blood P4 levels, whereas the previous-users B showed slightly higher blood P4 levels without statistical significance ($p=0.302$ and 0.178, respectively) after adjusting for the covariates. Salivary P4 levels were lower among women in both previous-users A and B, compared to the never-users ($p=0.059$ and $p=0.017$, respectively). Urinary PDG did not differ among the groups ($p=0.979$ for never-users vs. previous-users A; $p=0.639$ for never-users vs. previous-users B). Progesterone levels by timing of discontinuing hormonal contraceptive use estimated with the mixed-effects models are illustrated in Figure 1.

Discussion

Our P4 assay cross-reacts with both free and bound progesterone, which are both present in blood. Only free progesterone is present in saliva. Therefore, we hypothesize that the lower salivary progesterone of women in the previous-users, which is not reflected in the blood progesterone data, indicates that they have a higher free fraction of the hormone in blood, caused by higher concentration of CBG and/or albumin in blood.

A number of studies[1,4,5] have shown that use of oral contraceptives increases CBG production in women, although the consistent effect after discontinuing use was not examined. Only one study [6], as far as we know, showed the effect of previous oral contraceptive use on reproductive hormone levels. Chan et al. [6] showed that among postmenopausal women past oral contraceptive users had significantly lower endogenous estradiol, estrone, and sex hormone-binding globulin concentrations compared with the never users. They did not quantify CBG nor progesterone levels in their study, but their findings suggest that the effect of past oral contraceptive on endogenous sex hormones and the binding protein concentrations may persist years after discontinuing use. The present result may reflect the persistent change of endogenous progesterone and/or binding protein levels after discontinuing hormonal contraceptive use.

Salivary progesterone is frequently used in population research because of the ease of saliva collection and storage. However, the current finding suggests that salivary progesterone levels might differ according to past contraceptive use, even when the blood levels are not different between populations.

Conclusion

When applying salivary progesterone in population research, it may be necessary to adjust for the past hormonal contraceptive use.

References

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Table 1. Demographic characteristics of the participants (n=58)

Attributes	Mean ± SD (range) or proportion (%) and (N)
Age	24.0 ± 4.4 (18-33)
BMI	22.4 ± 3.7 (15.9-38.8)
Ethnicity	Caucasian: 74 % (N=43)
	Japanese: 26% (N=15)
Parity	0: 95% (N=55)
	1: 5% (N=3)
Past hormonal contraceptive use*	Never-users: 53% (N=31)
	Previous-users A[§]: 34% (N=20)
	Previous-users B[¶]: 12% (N=7)

* There were no participants who discontinued hormonal contraceptive use between 2 and 5 years ago.

[§] Discontinued hormonal contraceptive use ≤2 years ago

[¶] Discontinued hormonal contraceptive use ≥5 years ago

Table 2. Geometric means (and 95% CIs) of progesterone concentrations among participant women by past hormonal contraceptive use

		Past hormonal contraceptive use			
Progesterone levels	N	Never-users	Previous-users A	Previous-users B	P-value*
Blood P4, ng/mL	232	25.9 (8.9-75.7)	23.8 (11.5-49.3)	31.4 (12.4-79.0)	0.361
Salivary P4, ng/mL	179	0.35 (0.12-0.97)	0.26 (0.09-0.75)	0.22 (0.10-0.51)	**<0.001**
Urinary PDG[§], ng/mL	232	4105 (640-26318)	4068 (633-26134)	4666 (557-39105)	0.632

* ANOVA

[§] Adjusted for specific gravity of urine to control for hydration status

Table 3. Association of progesterone levels (in blood, saliva, or urine) with age, BMI, ethnicity, past hormonal contraceptive use, and menstrual cycle phase by mixed-effects models

Covariates		e^{β} (95% CI)	p-value
Blood P4 (ng/mL)			
Age		0.97 (0.95-0.99)	0.013
BMI		1.02 (0.99-1.05)	0.242
Ethnicity (vs. Caucasian)	Japanese	1.03 (0.81-1.31)	0.797
Past hormonal contraceptive use (vs. never-users)	Previous-users A	0.90 (0.73-1.10)	0.302
	Previous-users B	1.24 (0.90-1.71)	0.178
Menstrual cycle day (vs. day 1-14)	day 15-23	**1.32 (1.20-1.45)**	**<0.001**
	day 24+	**1.50 (1.35-1.65)**	**<0.001**
Salivary P4 (ng/mL)			
Age		0.99 (0.96-1.02)	0.629
BMI		1.02 (0.99-1.06)	0.182
Ethnicity (vs. Caucasian)	Japanese	0.89 (0.66-1.19)	0.415
Past hormonal contraceptive use (vs. never-users)	Previous-users A	0.78 (0.61-1.01)	0.059
	Previous-users B	**0.62 (0.42-0.91)**	**0.017**
Menstrual cycle day (vs. day 1-14)	day 15-23	**1.18 (1.03-1.34)**	**0.013**
	day 24+	**1.43 (1.25-1.64)**	**<0.001**
*Urinary PDG (ng/mL)**			
Age		1.01 (0.97-1.05)	0.639
BMI		0.99 (0.95-1.03)	0.626
Ethnicity (vs. Caucasian)	Japanese	1.41 (0.97-2.05)	0.072
Past hormonal contraceptive use (vs. never-users)	Previous-users A	1.00 (0.73-1.39)	0.979
	Previous-users B	1.13 (0.68-1.86)	0.639
Menstrual cycle day (vs. day 1-14)	day 15-23	**2.14 (1.70-2.68)**	**<0.001**
	day 24+	**3.48 (2.74-4.40)**	**<0.001**

* Adjusted for specific gravity of urine to control for hydration status

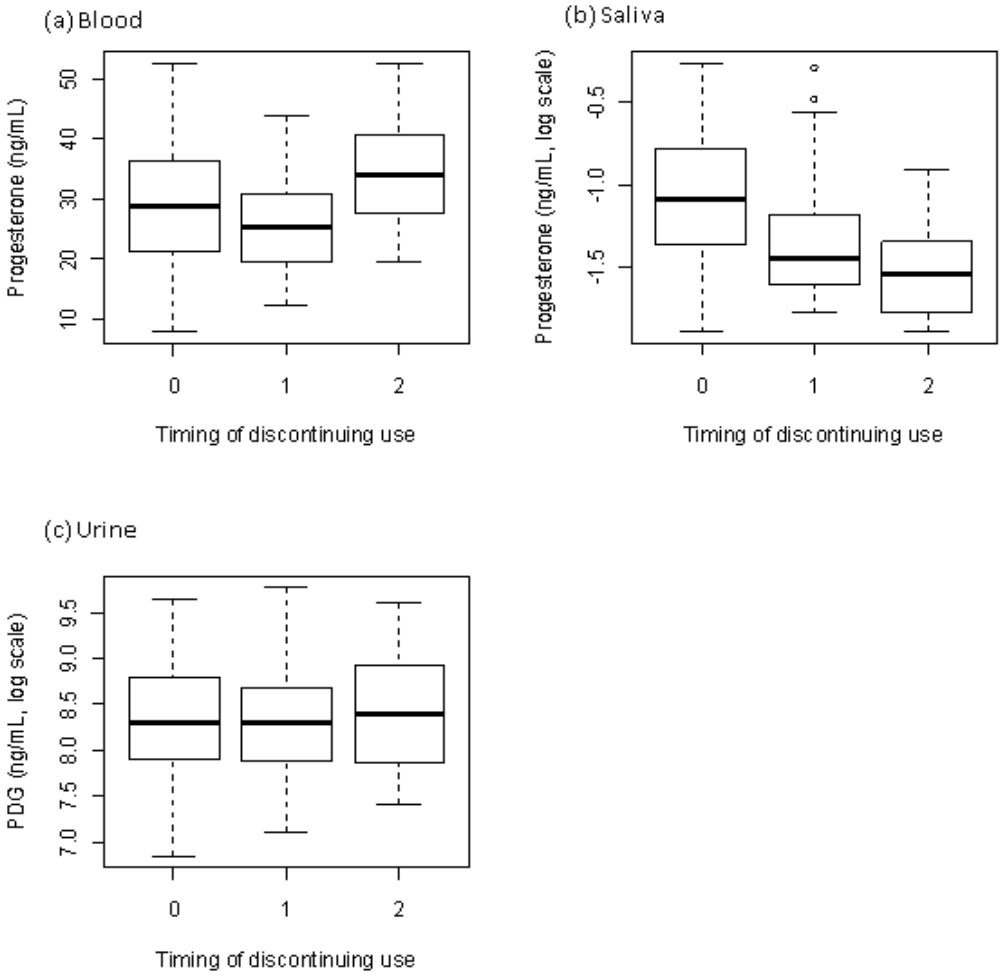

Figure 1. Progesterone levels in (a) blood, (b) saliva, and (c) urine by timing of discontinuing hormonal contraceptive use [Never-users (N=124), Previous-users A--discontinued use ≤2 years ago (N=80), Previous-users B--discontinued use ≥5 years ago (N=28)]. Progesterone levels are adjusted for age, BMI, ethnicity, menstrual cycle phase, and repeated measures using a mixed-effects model.