Changes of PSA Concentrations in Serum and Saliva of Healthy Women during the Menstrual Cycle

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Abstract. This study investigated the changes of prostate specific antigen (PSA) concentrations in serum and saliva of women during the menstrual cycle. Thirty healthy volunteers (age 23-35 yr) were enrolled in the study. During the menstrual cycle, serum and saliva PSA concentrations on days 9 (follicular phase) and 14 (mid-cycle) were significantly higher than on days 4 (early follicular phase) and 21 (luteal phase). The expected changes in gonadal hormones were seen, as evidenced by significantly higher serum estradiol and progesterone concentrations during the midcycle and luteal phase, compared to the other phases of the cycle. Serum PSA concentrations were positively correlated with salivary PSA concentrations at all 4 times (days 4, 9, 14, and 21) of the menstrual cycle, but not with the serum progesterone or estrogen concentrations. This study suggests that salivary PSA, rather than being produced in the salivary gland, may reflect the serum PSA during the normal menstrual cycle. (received 24 August 2001, accepted 13 October 2001)

Keywords: prostate specific antigen (PSA); saliva; menstrual cycle

Introduction

Prostate specific antigen (PSA) was discovered in seminal plasma in 1971 and isolated from prostatic tissue in 1979 [1]. PSA, a single chain glycoprotein (mol wt ~33 kD), is a kallikrein-like serine protease that is thought to be exclusively produced by the epithelial cells that line the acini and ducts of the prostate gland. After ejaculation of semen, PSA induces the liquefaction of the seminal coagulum. PSA is present in normal and malignant prostatic tissue, benign prostatic hyperplasia, and metastatic prostatic carcinoma. Assay of serum PSA is widely used for the early detection and follow-up of prostate carcinoma. PSA has generally been considered a specific marker for prostate epithelial cells [2].

Recently, PSA immunoreactivity has been found in amniotic fluid of normal and abnormal pregnancies and serum PSA been shown to be higher in pregnant women than in healthy nonpregnant women [3,4]. Studies indicate that PSA is expressed in various physiological and pathological conditions and has limited organ- or sex-specificity [3-5]. In female and male breast tumors, close association has been reported between the levels of PSA and progesterone receptors [6]. PSA expression in a human prostatic adenocarcinoma cell line is strongly upregulated by androgen and progesterone [7]. Therefore, it seems possible that progesterone receptors may mediate low levels of PSA expression in nonprostatic glandular cells.

A dynamic relationship exists between pituitary secretion of gonadotropin and ovarian secretion of steroids during the menstrual cycle. In the early follicular phase, serum estradiol and progesterone levels are lower than in the late follicular phase. At the middle of the follicular phase, serum estradiol increases rapidly and then, at midcycle, lutinizing hormone (LH) surges and progesterone becomes elevated. On the relationship of the menstrual cycle with PSA, Zarghami et al [8] found that the serum PSA level peaks during the mid- to late-follicular phase. In that study, the difference between the PSA and progesterone peaks was approximately 10-12 days, suggesting that PSA may be regulated by corpus luteum steroids. Escobar-Morreale et al [9]...
found that serum PSA levels were increased in most women with hirsutism. Clements and Mukhtar [10] showed that PSA is expressed in the human endometrium and may have a regulatory role in uterine function. Mannello et al [11] have demonstrated that PSA is found in the saliva of healthy women and that the salivary PSA concentrations were correlated with PSA concentrations in plasma.

Insofar as the authors can ascertain, no previous study has investigated the relationship of serum and saliva PSA concentrations during the menstrual cycle. The aim of this study was to elucidate the fluctuations of serum PSA that occur during the menstrual cycle in a large series of healthy women and to explore the relationship of PSA concentrations in serum and saliva samples taken at the same time.

Materials and Methods

Subjects. Thirty healthy female volunteers (age 23-35 yr), with regular menstrual cycles of 28 ± 2 da during the last 6 mo, were enrolled in the study. The study was approved by the local medical ethics committee and informed consent was obtained from all volunteers. The subjects were not taking any medications, including oral contraceptives. Blood samples were collected at 0800-0900, after a 12 hr overnight fast, on day 4 (early follicular phase) from the beginning of menstruation and then on day 9 (follicular phase), 14 (mid-cycle), and 21 (luteal phase) for determinations of PSA, estradiol, and progesterone. At the same times, saliva samples were also obtained. Each subject rinsed her mouth with water for 5 min before the saliva was collected. The samples of blood and saliva were centrifuged at 3500 g for 10 min at 4°C; the cell-free supernatant serum and saliva specimens were aspirated and stored at -80°C until assayed.

Assays. PSA concentrations in serum and saliva were determined by an ultrasensitive chemiluminescent enzyme immunoassay (Immulite Third Generation PSA, Diagnostic Product Corporation, Los Angeles, CA). As serum PSA levels in women are usually very low, near the limit of detection of the assay [12], all serum and saliva samples were analyzed for PSA within a single assay to avoid interassay variations. The results were expressed as ng/ml. The lower limit of detection was 0.003 ng/ml, and the intraassay coefficients of variation (CV) for 0.005, 0.015, and 0.030 ng/ml samples were determined as 9, 5, and 7 %, respectively. The calibrators, controls, and patient samples were all assayed in duplicate.

Progesterone and estradiol measurements were performed by a competitive immunometric assay based on enhanced luminescence (Immulite, Los Angeles, CA). The detection limits for serum progesterone and estradiol were 0.6 nmol/L and 44 pmol/L, respectively.

Statistical Analysis. Changes of serum estrogen and progesterone concentrations and serum and salivary PSA concentrations during the menstrual cycle were analyzed by one way ANOVA; differences among groups were evaluated by the least significant difference (LSD) test. Spearman's rank correlation test was used to assess relationships of serum PSA to serum estradiol, progesterone, and saliva PSA.

Results

The results are summarized in Table 1. In the 30 healthy women, PSA concentrations were nondetectable in 9 serum and 10 saliva samples in the early follicular phase, 5 serum and 7 saliva samples in the follicular phase, 6 serum and 7 saliva samples at mid-cycle, and 8 serum and 10 saliva sample in the luteal phase. Serum and saliva PSA concentrations in the follicular phase and at midcycle were significantly higher than in the early follicular and luteal phase. (Figs. 1, 2).

The anticipated changes of gonadal hormones were observed, as evidenced by significantly higher serum estradiol and progesterone concentrations at the midcycle and during the luteal phase than during the other phases of the menstrual cycle. Serum PSA concentrations were positively correlated with the paired salivary PSA concentrations at all phases of the menstrual cycle, but were not significantly correlated with age or with the serum progesterone or estradiol concentrations (Table 2).
Fig. 1. Changes of PSA concentrations in serum of healthy women at the early follicular phase, follicular phase, mid-cycle, and luteal phase of the menstrual cycle.

Fig. 2. Changes of PSA concentrations in saliva of healthy women at the early follicular phase, follicular phase, mid-cycle, and luteal phase of the menstrual cycle.
Table 1. Concentrations of PSA, estradiol, and progesterone in serum, and PSA in saliva of healthy women during the phases of the menstrual cycle. Values are means ± SD, with n in parentheses.

<table>
<thead>
<tr>
<th>Time of cycle</th>
<th>Serum PSA (ng/mL)</th>
<th>Saliva PSA (ng/mL)</th>
<th>Estradiol (pmol/L)</th>
<th>Progesterone (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4 (early follicular phase)</td>
<td>0.009±0.008 (n=21)</td>
<td>0.007±0.004 (n=20)</td>
<td>131.3±61.6 (n=30)</td>
<td>2.35±1.46 (n=30)</td>
</tr>
<tr>
<td>Day 9 (follicular phase)</td>
<td>0.032±0.014a (n=25)</td>
<td>0.024±0.011a (n=23)</td>
<td>212.7±107.4d (n=30)</td>
<td>2.28±1.33 (n=30)</td>
</tr>
<tr>
<td>Day 14 (mid-cycle)</td>
<td>0.035±0.015a (n=24)</td>
<td>0.029±0.013a (n=23)</td>
<td>495.4±189.3a,b (n=30)</td>
<td>6.61±8.89 (n=30)</td>
</tr>
<tr>
<td>Day 21 (Luteal phase)</td>
<td>0.007±0.005b,c (n=22)</td>
<td>0.008±0.003b,c (n=20)</td>
<td>278.9±141.2a,c (n=30)</td>
<td>42.96±26.39a,b,c (n=30)</td>
</tr>
</tbody>
</table>

a p<0.001 vs day 4.
b p<0.001 vs day 9.
c p<0.001 vs day 14.
d p<0.05 vs day 4.

Table 2. Correlation of baseline serum PSA concentrations with saliva PSA concentrations and serum gonadal hormone concentrations in healthy women at all phases of the menstrual cycle (r = Spearman’s rank correlation).

<table>
<thead>
<tr>
<th></th>
<th>Early follicular phase</th>
<th>Follicular phase</th>
<th>Mid-cycle</th>
<th>Luteal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Saliva PSA</td>
<td>0.77</td>
<td>0.0008</td>
<td>0.59</td>
<td>0.005</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.19</td>
<td>0.41</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.10</td>
<td>0.64</td>
<td>0.18</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Discussion

PSA was formerly considered one of the most specific biochemical markers and was believed to be produced exclusively by epithelial cells of the prostate [2]. During the past few years, investigators have challenged the tissue specificity of PSA, since it has been detected in apocrine sweat gland carcinoma, salivary gland neoplasms, periurethral and perianal glands, and breast tumors [6,13-17].

In the prostate, PSA production is regulated by androgenic steroids, which bind to androgen receptors and upregulate transcription of the PSA gene [2]. Since many of the tumors that produce PSA contain steroid hormone receptors, it could be speculated that any tissue with steroid hormone receptors might be able to produce PSA, provided the cognate steroid hormones are available. Experiments with female breast cancer cell lines have shown PSA production in these cells lines to be mediated by progesterone, androgen, mineralocorticoid, and glucocorticoid receptors, but not by the estrogen receptor [17].

Clements and Mukhtar [10] reported that PSA is present in normal endometrial tissue; they proposed that PSA may be a local regulator of uterine function. They also suggested that PSA is a ubiquitous enzyme, and that breast cancer and endometrial cells are not the only female tissues that can produce it. Yu et al [18] found that the normal female breast contains readily detectable amounts of PSA in subjects receiving progestin-containing oral contraceptives.

During the post-pregnancy period, the normal breast produces PSA and secretes it into milk [19]. The high serum PSA concentrations in pregnant women, compared to healthy nonpregnant women, suggested that steroid hormones are nongenetic factors that affect PSA metabolism. Zarghami et al [8] studied 7 informative menstrual cycles from three different patients and demonstrated that serum PSA concentrations are highest during the mid- to latefollicular phase and are lowest during the mid- to late-luteal phase. We confirmed this finding. Zarghami et al also examined whether serum specimens obtained during the menstrual cycle could stimulate PSA production in a breast carcinoma cell line (T-Line 47D). By measuring PSA protein and PSA mRNA levels, they showed that the ability of serum to induce PSA production in the tumor cells parallels the serum progesterone levels, the greatest stimulation occurring with the serum containing the most progesterone (day 24 of the menstrual cycle). When serum was collected during the luteal phase, PSA mRNA expression was markedly increased.

The present study shows a significant correlation between serum and salivary PSA concentrations at the 4 phases of the menstrual cycle. Mannello et al [11] observed that PSA levels in saliva of normal healthy women correlated with their plasma PSA concentrations. In women taking progestin-containing oral contraceptives, the salivary PSA concentrations were significantly higher than the plasma PSA concentrations. Progesterone receptors in the human salivary gland may provide a possible mechanism for increased stimulation of PSA expression in saliva of women receiving oral hormonal contraceptives.

In the study of Mannello et al [11], salivary PSA was measured by two-site IRMA assay with a detection limit of 0.02 ng/ml. In contrast, the two-site chemiluminescent enzyme immunometric assay (IMMULITE) used in the present study has a detection limit 0.003 ng/ml. Interestingly, Mannello et al [11] found detectable amounts of PSA—even higher values than our results—in all samples of serum and saliva from healthy women. On the other hand, in a study of the relationship between serum PSA and hirsutism, Escober-Morreale et al [9], with the same kit and instrument used in the present study, reported that serum PSA was detectable in only 4 of 11 normal menstruating women.

In a study by Tazawa et al [20], PSA expression was observed in the major salivary gland of men and women, but not in the minor salivary glands; immunoreactive sex hormone receptors were not identified in the salivary gland specimens. Tazawa et al [20] suggested that the PSA-like immunoactivity in small-sized duct epithelial cells of the major salivary gland and in tumors may be due to cross-reactivity of the PSA antiserum with a kallikrein-like substance.

Yousef et al [21] reported PSA gene expression to be low in salivary glands, and Lewis et al [22]
detected progesterone receptors in only 1 of 24 salivary duct carcinomas.

From the results of the present study, we speculate that PSA in saliva, rather than being produced in the salivary gland, may reflect the serum PSA during the normal menstrual cycle. Since salivary PSA levels paralleled serum PSA levels throughout the menstrual cycle, this cyclic variation should be taken into consideration in future research on serum PSA levels in women.

References