Prostate-Specific Antigen and Human Glandular Kallikrein 2 Are Markedly Elevated in Urine of Patients with Polycystic Ovary Syndrome

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ABSTRACT

Prostate-specific antigen (PSA) is a well-established tumor marker of prostatic adenocarcinoma. Human glandular kallikrein 2 (hK2), another serine protease closely related to PSA, is also gaining ground as a promising diagnostic tool in prostate cancer. The expression of these 2 proteases is known to be regulated by androgens and progestins in hormonally responsive tissues, such as the male prostate and the female breast. Previously, we have shown that serum PSA levels in normal women are very low but still detectable by ultrasensitive PSA immunoassays. We have also demonstrated that some women with hyperandrogenic syndromes have elevated serum PSA levels. In this study, we have measured urinary PSA and urinary hK2 levels in 35 polycystic ovary syndrome (PCOS) patients and compared them to those of 41 age-matched controls. We found that urinary PSA levels were significantly higher (P < 0.0001) in PCOS patients (mean ± SE = 820 ± 344 ng/L) than in the controls (mean ± SE = 4.3 ± 1.8 ng/L). Similarly, the difference between urinary hK2 of patients (mean ± SE = 8.2 ± 3.1 ng/L) and controls (0.5 ± 0.3 ng/L) was also significant (P < 0.001). A weak correlation was observed between urinary PSA and serum 3α-androstanediol glucuronide (r = 0.42, P = 0.03) as well as between urinary PSA and serum testosterone (r = 0.40, P = 0.04). The results of this study indicate that urinary PSA, and possibly urinary hK2, are promising markers of hyperandrogenism in females suffering from PCOS. (J Clin Endocrinol Metab 86: 1558–1561, 2001)

POLYCYSTIC OVARY SYNDROME (PCOS) is a disorder characterized by hyperandrogenism and chronic anovulation (1). Though the occurrence of polycystic ovaries is common in the general population, as indicated by ultrasound examinations (2), only a fraction of these women suffer from PCOS. Although the pathogenesis of this syndrome has not, as yet, been defined, abnormalities in insulin and insulin-like growth factor I (IGF-I) levels have been implicated as the underlying cause (3, 4). The apparent physical outcome of this syndrome is the failure of a dominant follicle to emerge during folliculogenesis, resulting in the accumulation of small, antral follicles (5). Because IGF-I and insulin have been shown to emerge during folliculogenesis, resulting in the accumulation of small, antral follicles (5), the production of which was previously thought to be confined exclusively to the male prostate (12). PSA is the most valuable marker for diagnosis and treatment of prostate cancer (13). With the development of ultrasensitive immunoassays, it was shown that PSA was also produced by a wide variety of female tissues (14–16), most notably in normal and malignant breast tissue. This led to some important implications in the field of breast cancer diagnosis and prognosis (17, 18). Another protein known as human glandular kallikrein 2 (hK2) is very closely related to PSA. Its use in conjunction with PSA has been shown to be useful in the differentiation of prostate cancer from benign prostatic hyperplasia (19). In addition, hK2 has been found to be expressed in many fluids from females, including breast cyst fluid and breast milk (20).

PSA and hK2 are known to be up-regulated by androgens and progestins, which has been demonstrated in cell culture studies (21) as well as in breast tumors (18, 22) and in breast tissues obtained from androgenized females (23). Elevated serum levels of PSA in response to androgenic therapy in female-to-male transsexuals has also been demonstrated (24), whereas serum PSA and hK2 levels in normal females not receiving steroid hormones are too low to be of clinical use. Slightly elevated serum PSA levels were noted in hirsute women (25, 26). In addition, a clear up-regulation of urinary PSA in response to androgens has been shown in female-to-male transsexuals undergoing long-term testosterone treatment (27). The source of urinary PSA seems to be the...
URINARY PSA AND hK2 IN PCOS

Subjects and Methods

Urine samples

First morning urine voids were collected, after informed consent, and before initiation of any treatment, from 35 females with PCOS diagnosis clinically established and 41 age-matched controls. PCOS diagnosis was established by absence of ovulation and elevated serum testosterone levels. The Ferriman-Gallway (FG) score for the PCOS patients ranged from 5–14, whereas all control subjects had normal scores (<8) and regular ovulatory cycles. The FG score was established as described by Hatch et al. (30). Samples were stored and shipped at −20 C. Before performance of immunological analyses, urine samples were thawed and centrifuged for 5 min at 14,000 rpm. An aliquot (1 mL) of the resulting supernatant was withdrawn for analysis. Our study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Review Board of the University of Southern California.

Immunological assays

PSA was measured in 96-well microtiter plates, as described elsewhere (31), in undiluted urine, using a one-step time-resolved fluorometric immunoassay with an established lower detection limit of 1 ng/L and upper limit of 10,000 ng/L. Half of the microtiter plates were loaded with PCOS urine samples in duplicates, while the other half, with the controls, to eliminate possible interassay differences between the two groups.

hK2 was measured in duplicate in undiluted urine, using a two-step time-resolved immunofluorometric assay developed by our group. This assay, with a lower and upper detection limit of 6 ng/L and 10,000 ng/L, respectively, was performed as described elsewhere (32). Microtiter plates were loaded with samples in the same way as for PSA analysis.

Serum 3α-androstanediol glucuronide (3α-diol G) and total testosterone were measured using commercial kits from Diagnostic Systems Laboratories, Inc., Webster, TX. The day-to-day precision obtained with these kits was within the specifications of the manufacturer (<10% coefficients of variation).

Statistical analysis

Because the distributions of the resulting data were non-Gaussian, the analyses of differences between PSA and hK2 concentrations for the two groups were performed with the nonparametric Mann-Whitney U test. Association of PSA and hK2 in urine with the other continuous parameters was examined using the Spearman correlation. All other statistical analyses were performed with SAS statistical software (SAS Institute, Inc., Cary, NC).

Results

PSA and hK2 were measured in 76 urine samples obtained from 35 patients with PCOS and 41 females without PCOS (controls). In Figs. 1 and 2, we present the distributions for these variables. The ages of the PCOS patients ranged from 24–39 yr, with a median of 33 yr. The ages of the controls ranged from 23–38 yr, with a median of 32 yr, close to that of the PCOS patients. Total serum testosterone levels for the controls ranged from 22–56 ng/dL (median = 32) and from 70–115 ng/dL (median = 90) for the PCOS patients. The reference range for the method used is 20–60 ng/dL. FG scores (range = 5–14, median = 10) and serum 3α-diol G concentrations (range = 0.5–10.8 ng/L, median = 3.4) were available for 28 PCOS patients.

Total urinary PSA values ranged from undetectable (<1 ng/L) to 10,289 ng/L in PCOS patients, with a mean ± se of 820 ± 344 ng/L. In controls, the range was 0–46 ng/L, with the mean ± se value being 4.3 ± 1.8 ng/L. A statistically highly significant difference (P < 0.0001) was found for PSA concentrations between the patient and the control group. Urinary hK2 levels ranged from 0–8 ng/L (mean ± se = 0.5 ± 0.3 ng/L) and from 0–87 ng/L (mean ± se = 8.2 ± 3.1 ng/L) in controls and patients with PCOS, respectively (P < 0.001).

For the data collected on patients comprising the PCOS group, a positive correlation was found between urinary PSA and hK2 concentrations (Spearman correlation coefficient rs = 0.48, P = 0.003) (Table 1). Urinary PSA and serum 3α-diol G concentrations also correlated (rs = 0.42, P = 0.03). Similarly, a correlation was noted between urinary PSA and serum testosterone levels (rs = 0.40, P = 0.04). However, FG score was not found to correlate with either urinary PSA, urinary hK2, or serum 3α-diol G in PCOS patients.

The distributions of urinary PSA concentrations in the 2 groups are presented (Fig. 1). With the detection limit of 1 ng/L as the cut-off value, 27 (77%) patients could have been detected (sensitivity = 77%), whereas 7 (17%) of the controls would have tested as false positives (specificity = 83%). In Fig. 2, the distribution of hK2 concentrations in the same 2 populations is shown. At a cut-off level of the detection limit of the method (6 ng/L), 18 (51%) of the patients could have been detected (sensitivity = 51%), whereas only 3 (7%) of the controls are false positives (specificity = 93%). The positive and negative predictive values for the 2 tests, for this patient population, are 79% and 81% for PSA and 86% and 70% for per urethral glands, an observation that is supported by the fact that these glands in females are highly homologous to the male prostate (28, 29).

In this study, we attempted to determine whether urinary PSA and hK2 levels are elevated in response to the high levels of androgens endogenously present in PCOS patients. If this holds true, urinary PSA and/or hK2 may represent useful markers of hyperandrogenism in PCOS patients.
The mean values are indicated by parentheses above or below the detection limit (6 ng/L).

hK2, respectively. However, when these tests are performed as screening tests in the general population, the predictive values will depend on disease prevalence.

Discussion

In vitro breast carcinoma cell line tissue culture systems have unequivocally shown that PSA and hK2 proteins are up-regulated by androgens and progestins (21,33). PSA and hK2 can now be measured with excellent sensitivity and specificity using well-established immunofluorometric procedures (31,32). Administration of androgens or progestins to patients causes significant elevations of PSA in urine (24,27), serum, and tissues (18,22,23). Recent studies have already demonstrated that women suffering from hyperandrogenism usually have elevated serum PSA (25,26). Our preliminary clinical studies have also indicated that women treated with testosterone, over prolonged periods of time, significantly increased serum PSA and, to a higher extent, their urinary PSA concentration (24). Thus, we hypothesized that urinary PSA and urinary hK2 concentrations may be elevated in women with high levels of endogenous androgens. Women with PCOS usually suffer from hyperandrogenism and were selected to test our hypothesis in this study.

It is clear from our results (Figs. 1 and 2) that both urinary PSA and urinary hK2 are highly elevated in a subset of women with PCOS. Furthermore, we found a weak correlation between urinary PSA and serum 3α-diol G, which is a dihydrotestosterone metabolite and is a relatively good index of peripheral androgen action in hyperandrogenic women. Similar correlations were noted between urinary PSA and serum total testosterone (Table 1).

Although we did not establish the source of urinary PSA and urinary hK2 in these women, we speculate that these proteins may be secreted into the urine from the periurethral glands. These small, androgen-responsive glands have previously been shown to have the capacity of producing PSA, and they are considered the female equivalent of the male prostate (28,29).

We did not find any association between the Ferriman-Gallwey score and levels of either urinary PSA, urinary hK2, or serum 3α-diol G (Table 1). This result is consistent with the fact that, although androgen-regulated PSA and hK2 reflect the levels of circulatory androgens in the same way as the dihydrotestosterone metabolite, 3α-diol G, the Ferriman-Gallwey score indicates hirsutism, which may be attributable to either circulating or local androgens produced and acting in the skin. Thus, although hirsutism is common in PCOS patients, the prevalence is only about 75% (34). It is also known that not all PCOS patients are hirsute, even with elevated circulatory androgens. Also, 3α-diol G seems to be a better indicator of hirsutism, rather than being an indicator of PCOS (35). Testosterone seems to be a better indicator of PCOS-related symptoms, such as infertility and menstrual cycle disturbances (36). As seen in Table 1, we found a weak correlation between serum testosterone levels and urinary PSA.

In conclusion, we here present evidence that women with PCOS have highly elevated urinary levels of PSA and hK2. These data suggest that measurement of the two serine proteases in urine may aid in the diagnosis of such patients. Because neither the sensitivity nor the specificity of these tests is 100%, other measures of hyperandrogenism and clinical information should be combined to accurately diagnose and treat this common syndrome.

References

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<tr>
<th>Variable</th>
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<tr>
<td></td>
<td>Testosterone</td>
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<tr>
<td>Age</td>
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<td>3α-diol G</td>
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<tr>
<td>Testosterone</td>
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NS, Statistically nonsignificant correlation.

a PCOS. A positive correlation between urinary PSA and hK2 (r = 0.48; P = 0.003) was found in samples of PCOS patients.
b FG score; 3α-diol G. Data from 28 patients was available.
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