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Prostate-Specific Antigen in Female Serum, a Potential New Marker of Androgen Excess

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ABSTRACT

Prostate-specific antigen (PSA) is present at very low concentrations in female serum, but it can now be measured with highly sensitive immunoassays. We have found that in female tissues the PSA gene is regulated by steroid hormones through the action of steroid hormone receptors. Thus, we examined whether female serum PSA is associated with hyperandrogenic states. Serum PSA levels were compared between 22 hirsute women with a Ferriman-Gallwey score higher than 8 and 50 women without hirsutism. The results show that PSA levels were higher in hirsute women in comparison with controls. In hirsute women, levels of PSA and 3α-androstanediol glucuronide (3α-AG), a specific metabolite of androgen action, showed a significant positive correlation, whereas PSA and 3α-AG showed a significant negative correlation with patient age. Receiver operating characteristic (ROC) analysis revealed that 3α-AG was a slightly better marker of androgen excess than PSA. We conclude that female serum PSA may be a new biochemical marker of androgen action in females. (*J Clin Endocrinol Metab* **82:** 777–780, 1997)

PROSTATE-SPECIFIC antigen (PSA) is a 33-KDa serine protease with chymotrypsin-like enzymatic activity (1). In males, PSA is produced by the prostate gland (2), and it is present in prostatic tissue, seminal plasma, and male serum (3). Small amounts of PSA also can be produced by the periurethral glands, and therefore, urine contains detectable levels of PSA (4). PSA concentration in male serum is a valuable tumor marker for diagnosis and management of prostate cancer (5). PSA production in the prostate is under the control of steroid hormones. Androgens up-regulate the expression of the PSA gene through the androgen receptor (6, 7).

Initially, PSA was believed to be completely absent from all female tissues and fluids. However, PSA has been detected recently in some female tissues (including breast, ovarian, and endometrial tissues) and body fluids (amniotic fluid, milk, and breast cyst fluid) (8). The presence of PSA in these female tissues seems to be associated closely with steroid hormone regulation, especially androgens, glucocorticoids, and progestins (9). This was also demonstrated using a tissue culture system and the breast carcinoma cell line T-47D (10). Indirect *in vivo* evidence was provided by two case reports (11, 12).

Using conventional PSA assays with a detection limit of 0.1–0.01 ng/mL, PSA is detectable in less than 10% of female sera (13), but when a more sensitive PSA assay is used (detection limit 0.001 ng/mL), more than 50% of female sera have detectable PSA levels (14). Among women who have high levels of androgens, relatively high levels of serum PSA should be expected if PSA production in women is under the regulation of androgens.

One of the manifestations of androgen excess in women is idiopathic hirsutism. In most instances, the source of androgen excess in these women is neither adrenal (*e.g.* dehydroepiandrosterone sulfate) nor ovarian (*e.g.* testosterone) but peripheral. The most important peripheral sources of androgen production are sexual and nonsexual skin tissues where testosterone is converted to the potent androgen, dihydrotestosterone (DHT). The latter androgen is responsible for androgen action.

Based on the above considerations, we speculated that serum PSA levels in hirsute females may be high and that the measurement of serum PSA in these women may have some clinical implications. In this study, we measured PSA levels in female serum with a highly sensitive PSA assay and compared the levels between hirsute and apparently healthy women. Because 3α-androstanediol glucuronide (3α-AG) is a major metabolite of DHT and it can be measured easily in serum, we have conducted comparisons between PSA and 3α-AG in serum of hirsute women.

Materials and Methods

We collected, with informed consent, serum specimens from 22 hirsute women and 16 nonhirsute women from the same geographical location in California. The population consisted mainly of Caucasians and Hispanics. Additionally, we collected another 34 serum samples from healthy blood donors in Toronto, Canada. The clinical diagnosis of hirsutism was based on the hirsutism scale of Ferriman and Gallwey, as described elsewhere (15). A score of 8 or more indicates clinical hirsutism. Control subjects had a score less than 8. Of the hirsute women, 20 had a score between 8 and 24 with a mean ± SD of 13 ± 4.8. The score was not available for two hirsute women.

Other available information pertaining to the hirsute women in-

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TABLE 1. The distributions of PSA, age, and 3α-AG in the patients and controls

Patients (N = 22)	Range	Median	Mean (SD)
Age (yr)	17–45	29	30 (6.7)
PSA (pg/mL)	0–579	4	43 (127)
3α-AG (ng/mL)	2.2–12.2	5.5	5.8 (2.4)
Control Group 1 (N = 16)			
Age (yr)	23–41	34	32 (5.5)
PSA (pg/mL)	0–19	2	4 (4.8)
3α-AG (ng/mL)	0.6–4.9	3.0	3.1 (1.4)
Control Group 2 (N = 34)			
Age (yr)	21–40	30	30 (6.5)
PSA (pg/mL)	0–8	1	2 (2.1)
3α-AG (ng/mL)	ND	ND	ND

ND, not done.

cluded: 1) serum levels of total testosterone, which was measured in 14 patients (range: 0.12–1.53 ng/mL; mean ± SD, 0.85 ± 0.44 ng/mL); 2) ovulation, which was available for 21 patients (17 anovulating and 4 ovulating women); and 3) body weight, which was available for 21 patients (11 obese and 10 nonobese women).

PSA and androstanediol glucuronide were measured in serum by previously described methods (16, 17). A sandwich-type time-resolved immunofluorometric assay was used to quantify PSA. The lowest detection limit of the assay is 1 pg/mL, and the coefficient of variation (for between-run precision), at levels of 2 pg/mL or higher, is less than 16%. Each serum sample was measured in triplicate. The DHT metabolite, 3α-AG, was measured by direct RIA using reagents obtained from a commercial ^{125}I-androstanediol glucuronide RIA kit (Diagnostic Systems Laboratories, Webster, Texas). This kit was validated extensively in the laboratory of Dr. F. Stanczyk, Los Angeles, California (17).

The differences in serum PSA and age between cases and controls were compared with the Wilcoxon test and the ANOVA test. The relationships between PSA and age or 3α-AG were examined with the Spearman rank correlation coefficient test when untransformed PSA values were used. The Pearson correlation test was used when logarithmic PSA values were employed. The receiver operating characteristic (ROC) curve was constructed for both PSA and 3α-AG using the cutoff levels at 20%, 50%, 70%, and 90% percentile distributions of their values among all subjects. The start and end points of the curve were hypothetical, *i.e.* assuming the curve starts at 100% specificity and 0% sensitivity and ends at 0% specificity and 100% sensitivity.

Results

Table 1 shows the distributions of age, PSA, and 3α-AG among the three patient groups. The mean ages and median PSA levels between the control groups 1 and 2 were not significantly different ($P = 0.26$ for age and $P = 0.28$ for PSA), and therefore, the two control groups were combined together for comparison with the patient group. The mean ages were not significantly different between patients (29.6 yr) and combined control groups (30.7 yr) ($P = 0.48$).

The distribution of PSA values among all the women studied was positively skewed, but the 3α-AG values were normally distributed with an almost identical mean and median. The average levels of PSA (median) and 3α-AG (mean) were significantly higher in hirsute women than in normal women ($P = 0.001$ for PSA, Wilcoxon Rank Sum test; and $P = 0.002$ for 3α-AG, one-way ANOVA).

PSA values were positively correlated with 3α-AG values (Fig. 1). PSA and 3α-AG values were negatively correlated with patient's age (Table 2, Fig. 2, and data not shown). The regression equation of 3α-AG levels (y) *vs.* age (x) was: $y = -0.22x + 11.6$, $r = -0.58$, $P < 0.001$.

The distributions of logarithmic PSA and 3α-AG between

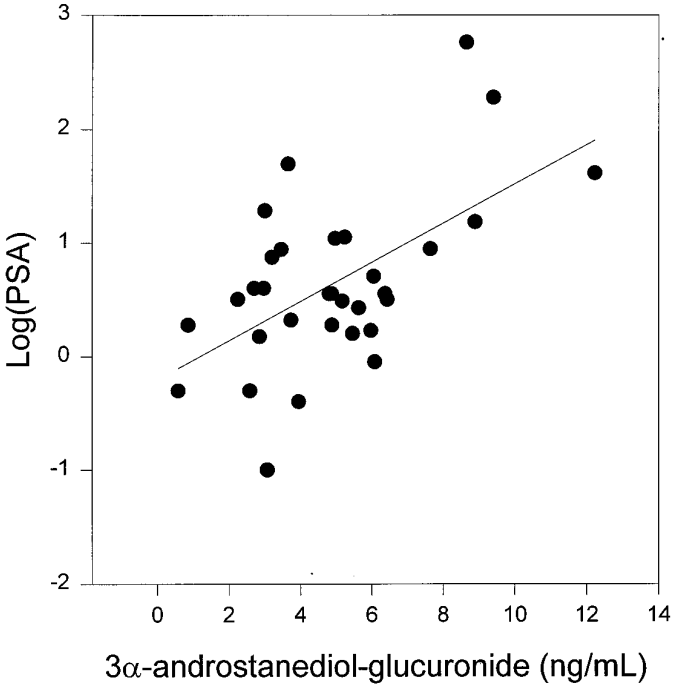

FIG. 1 Correlation between 3α-AG and log (PSA) for 32 patients' sera. The Pearson r = 0.58 ($P < 0.001$). The regression equation is $y = 0.17x - 0.20$. The values of PSA before logarithmic transformation are in pg/mL.

TABLE 2. Correlations among PSA, 3α-AG, and patient

Correlation	Spearman	Number of patients	*P* value
PSA and 3α-AG	0.42	32	0.2
PSA and age	−0.40	72	<0.001
3α-AG and age	−0.52	32	0.002
Correlation	**Pearson**	**Number of patients**	***P* value**
Log (PSA) and 3α-AG	0.58	32	<0.001
Log (PSA) and age	−0.37	72	0.001
3α-AG and age	−0.58	32	<0.001

patients and controls are shown in Figs. 3 and 4. Although patients tended to have higher PSA and 3α-AG than controls, there were still many patients who had values overlapping with those of controls. The ROC curve was slightly better for 3α-AG compared with that for PSA, but none of the markers could provide both more than 80% sensitivity and specificity at any single cutoff point (Fig. 5).

Based on the limited number of patients who had testosterone data, we found that serum testosterone levels tended to be positively correlated with 3α-AG (Pearson r of 0.62, $P = 0.02$) and PSA (Spearman r of 0.47, $P = 0.09$) and were negatively correlated with age (Pearson r of −0.53, $P = 0.05$).

Obese women tended to have a higher hirsutism score than nonobese women (median scores 16 *vs.* 10, respectively, $P = 0.01$). However, using both Wilcoxon rank sum testing and one-way ANOVA, we could not find any significant associations between either obesity or ovulation and age, 3α-AG, PSA, or testosterone (data not shown).

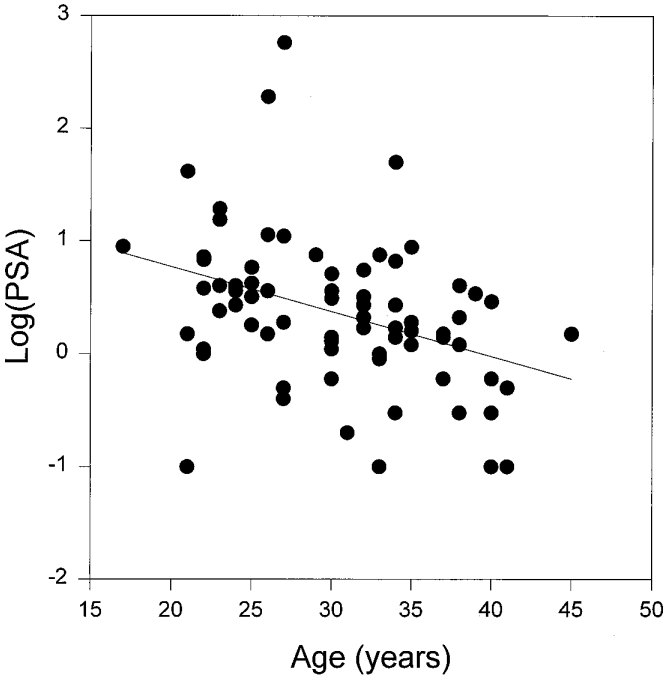

FIG. 2. Correlation between log (PSA) and age for 72 patients' sera. The Pearson r = −0.37 (P = 0.001). The regression equation is y = −0.040x + 1.57. The values of PSA before logarithmic transformation are in pg/mL.

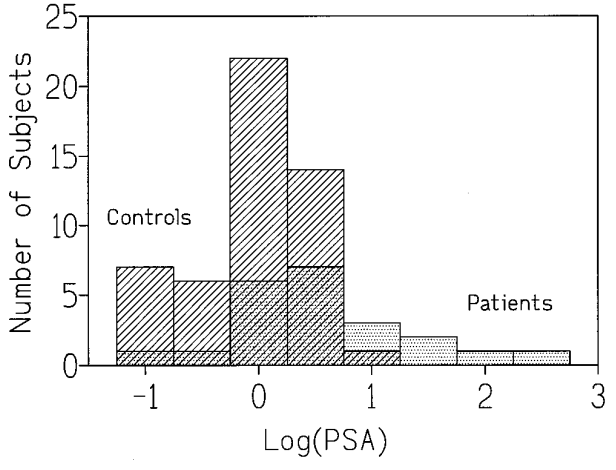

FIG. 3. Distribution of log (PSA) values between patients (*dotted bars*) and control subjects (*hatched bars*). For discussion, see text.

Discussion

PSA was thought to be absent from female tissues until our recent finding of its presence in female breast tissue. Our studies have demonstrated that the female breast is one of the female tissues that is capable of producing PSA (9, 11). Tissue culture experiments indicate that the production of PSA by steroid hormone receptor-positive breast cancer cells is under the control of steroid hormones, including androgens and progestins (10). Oral contraceptives increase PSA production in breast tissue (11). Amniotic fluid and milk contain significant amounts of PSA, and the levels of PSA in these fluids change with gestational age or postdelivery time (18, 19). Serum PSA levels are significantly higher in pregnant

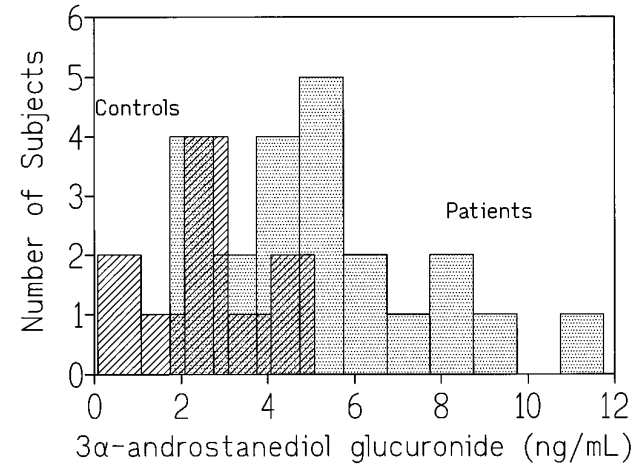

FIG. 4. Distribution of 3α-AG values between patients (*dotted bars*) and control subjects (*hatched bars*). For discussion, see text.

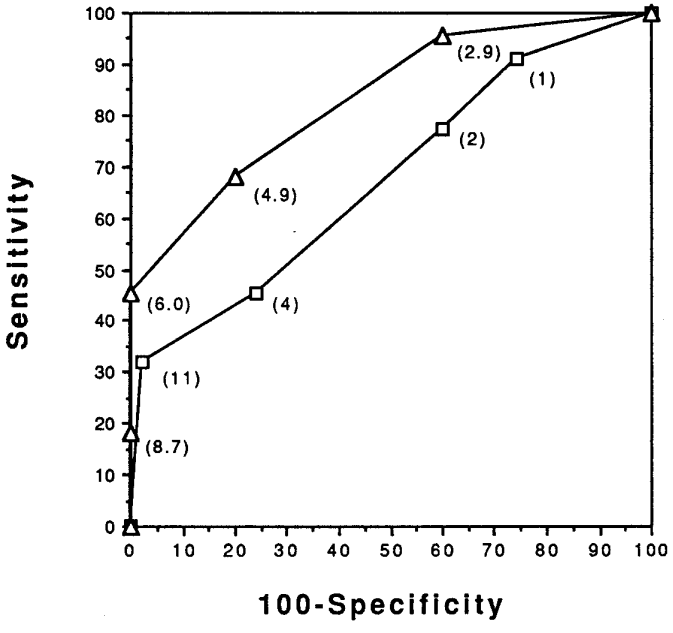

FIG. 5. Receiver operating characteristic curves for PSA (□) and 3α-AG (Δ)at various cutoff points shown in *brackets*. Units are in pg/mL for PSA and ng/mL for 3α-AG.

women in comparison with nonpregnant women (18). Taken together, these data suggest that PSA is expressed in breast and possibly other female tissues and that PSA expression is under the regulation of steroid hormones, especially androgens and progestins. The major difference in PSA expression between men and women is that the breasts express relatively low levels compared with the levels of male prostate.

Because of the relationship between PSA production and androgen regulation, we hypothesized that PSA may be a marker of androgen action in women. Women with higher levels of androgen may have higher levels of PSA compared with women with normal levels of androgen. Hirsutism represents a state of androgen excess in women. In this study, we report significantly elevated serum PSA levels in hirsute women compared with normal women.

The source of androgen excess in patients with idiopathic

hirsutism is considered to be increased peripheral conversion of androstanediol and testosterone to DHT via the pivotal enzyme, 5α-reductase. Although DHT is the most potent endogenous androgen, it is considered to be a poor circulating marker of androgenicity. Instead, the conjugated metabolite of DHT, namely 3α-AG, is considered to be an excellent serum marker of 5α-reductase activity and peripheral androgen action and of the clinical manifestations of hirsutism (20). For these reasons, we chose 3α-AG as a serum marker for determining the relationship of peripheral hyperandrogenism to circulating PSA levels.

Comparison of serum PSA with 3α-AG for the diagnosis of hirsutism showed that PSA did not provide better sensitivity and specificity than did 3α-AG. The two markers correlate significantly with each other. An inverse correlation between age and PSA or 3α-AG was observed in these women. We do not as yet know which female tissue produces and releases PSA into the circulation in hirsute women. The most likely candidate is the female breast because this tissue has steroid hormone receptors and is capable of producing high levels of PSA, especially after steroid hormone stimulation (11). Nevertheless, our data show that only a relatively small proportion of patients with hirsutism produces more than 10 pg/mL PSA (Fig. 3).

In summary, we found that serum PSA levels were increased significantly in women with hirsutism. PSA levels in female serum were positively correlated with serum 3α-AG and were inversely correlated with patient age. Therefore, PSA in serum can now be regarded as another biochemical marker of androgen action in female peripheral tissues.

References

1. **McCormack RT, Rittenhouse HG, Finlay JA, et al.** 1995 Molecular forms of prostate specific antigen and the human kallikrein gene family: a new era. Urology. 45:729–744.
2. **Wang MC, Valenzuela LA, Murphy GP, Chu TM.** 1979 Purification of human prostate-specific antigen. Invest Urol. 17:159–163.
3. **Papsidero LD, Wang MC, Valenzuela LA, Murphy GP, Chue TM.** 1980 A prostate antigen in sera of prostatic cancer patients. Cancer Res. 40:2428–2432.
4. **Iwakiri J, Granbois K, Wehner N, Graves HC, Stamey T.** 1993 An analysis of urinary prostate specific antigen before and after radical prostatectomy: evidence for secretion of prostate specific antigen by the periurethral glands. J Urol. 149:783–786.
5. **Catalona WJ, Smith DS, Ratliff TL, et al.** 1991 Measurement of prostate specific antigen in serum as a screening test for prostate cancer. N Engl J Med. 324;1156–1161.
6. **Luke MC, Coffey DS.** 1994 Human androgen receptor binding to the androgen response element of prostate specific antigen. J Androl. 15:41–51.
7. **Young CY-F, Montgomery BT, Andrews PE, Qiu S, Bilhartz DL, Tindall DJ.** 1991 Hormonal regulation of prostate specific antigen messenger RNA in human prostatic adenocarcinoma cell line LNCap. Cancer Res. 51:3748–3752.
8. **Diamandis EP, Yu H.** 1995 New biological functions of prostate specific antigen? J Clin Endocrinol Metab. 80:1515–1517.
9. **Yu H, Diamandis EP, Sutherland DJA.** 1994 Immunoreactive prostate specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. Clin Biochem. 27:75–79.
10. **Yu H, Diamandis EP, Zarghami N, Grass L.** 1994 Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. Breast Cancer Res Treat. 32:291–300.
11. **Yu H, Diamandis EP, Monne M, Croce CM.** 1995 Oral contraceptive-induced expression of prostate specific antigen in the female breast. J Biol Chem. 270:6615–6618.
12. **Yu H, Diamandis EP, Levesque M, Asa S, Monne M, Croce CM.** 1995 Expression of the prostate-specific antigen gene by a primary ovarian carcinoma. Cancer Res. 55:1603–1606.
13. **Yu H, Diamandis EP.** 1995 Measurement of serum prostate specific antigen levels in females and in prostatectomized males with an ultrasensitive immunoassay technique. J Urol. 153:1004–1008.
14. **Diamandis EP, Yu H, Melegos DN.** 1996 Ultrasensitive prostate specific antigen assays and their clinical application. Clin Chem. 42:853–857.
15. **Hatch R, Rosenfield RL, Kiru MH, Tredway D.** 1981 Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 140:815–830.
16. **Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP.** 1996 Ultrasensitive detection of prostate specific antigen by a new time resolved immunofluorometric assay and the Immulite immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. Clin Chem. 42:675–684.
17. **Norang R, Tran T, Savjani G.** 1994 Solid phase radioimmunoassay kit for the quantitative measurement of androstanediol glucuronide in unextracted serum or plasma. Clin Chem. 40:1132 (Abstract).
18. **Melegos DN, Yu H, Allen LC, Diamandis EP.** 1996 Prostate specific antigen in amniotic fluid of normal and abnormal pregnancies. Clin Biochem. 29:555–562.
19. **Yu H, Diamandis EP.** 1995 Prostate specific antigen in milk of lactating women. Clin Chem. 41:54–58.
20. **Horton R, Lobo R.** 1986 Peripheral androgen metabolism and the role of androstanediol glucuronide. In: Horton R, Lobo RA, eds. Clinics in endocrinology and metabolism, Vol 15. Philadelphia: WB Saunders; 293–306.