CAN THE INTRAPROSTATIC CONCENTRATION OF EPIDERMAL GROWTH FACTOR INFLUENCE THE VARIANCE OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS IN PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA?

F. DI SILVERIO, A. SCIARRA, S. MONTI, P. CASALE AND F. SECCARECCIA

From the Department of Urology U. Bracci and II Endocrinology, University La Sapienza, and Laboratory of Epidemiology and Biostatistics, Istituto Superiore di Sanita, Rome, Italy

ABSTRACT

Purpose: Except for prostate volume, little is known about the factors influencing serum prostate specific antigen (PSA) levels. Considering that dihydrotestosterone and epidermal growth factor are regulators of the proliferation and differentiation in the epithelial component of human prostate tissue and that PSA is produced only by the epithelial cells of the gland, studies were performed on patients with a histological diagnosis of benign prostatic hyperplasia (BPH) to establish whether a significant association exists between the intraprostatic concentration of dihydrotestosterone or epidermal growth factor and serum PSA levels.

Materials and Methods: A total of 20 patients with BPH who had not been previously treated were part of a larger study on the correlation among PSA, prostate volume and age, and were evaluated according to the algorithm in the guidelines of the international consultation on BPH. All men underwent open suprapubic prostatectomy to enucleate the entire adenoma and in each case sections were made in the periurethral, subcapsular and intermediate zones of the BPH tissue. Dihydrotestosterone and epidermal growth factor concentrations were evaluated by radioimmunoassay in the periurethral zone and in total BPH tissue.

Results: In these 20 patients with BPH serum PSA levels were significantly associated with epidermal growth factor but not with dihydrotestosterone concentrations in total BPH tissue ($r = 0.7762$, $p = 0.00002836$ and $r = 0.3923$, $p = 0.0956307$, respectively). A stronger association was found between PSA levels and the periurethral concentration of epidermal growth factor and dihydrotestosterone ($r = 0.8117$, $p = 0.000005$ and $r = 0.5656$, $p = 0.0098326$, respectively). On the contrary, epidermal growth factor and dihydrotestosterone were not significantly associated with prostate volume ($p = 0.957415$ and $p = 0.531439$, respectively).

Conclusions: To our knowledge this study is the first report in the literature to demonstrate an association between serum PSA, and dihydrotestosterone and epidermal growth factor levels, particularly in the periurethral zone of human BPH tissue. These data suggest the importance of epidermal growth factor and dihydrotestosterone in influencing serum PSA levels.

KEY WORDS: prostatic hyperplasia, prostate-specific antigen, epidermal growth factor–urogastrone

Prostatic tissue is a target of androgen action, and adequate hormonal support is required for the differentiation and development of the normal gland, and for its structural and functional integrity.[1] However, investigations of primary cultures of normal rat prostate or cell lines derived from normal canine prostate indicate that androgens directly promote prostatic epithelial differentiation but do not seem to affect substantially cell proliferation of the adult gland.[2] This finding suggests that the growth promoting effects of sex steroids may be, in part, indirect and mediated by other substances, such as peptide growth factors acting as local regulators of cellular replication and differentiation through paracrine and autocrine processes.[3] A major growth factor in prostatic tissue is the epidermal growth factor. Immunohistochemical analyses reveal a higher epidermal growth factor content in the prostatic epithelium than in stroma.[4] In fact, epidermal growth factor is mitogenic mainly for epithelial and mesodermal tissue, as demonstrated in experiments on various cell lines derived from normal prostate and androgen dependent lymph node (LNCaP) metastasis of human prostate cancer.[5,6]

Prostatic specific antigen (PSA) is a serine protease pro-

duced by the benign and malignant prostatic epithelium that can be measured in serum samples by immunoassay.[7] In patients with benign prostatic hyperplasia (BPH) a factor most likely responsible for the age related increase in serum PSA may be the concomitant increase in prostatic volume as men age. However, the differences observed in the rate of changes in PSA levels in these patients may reflect the histological heterogeneity of BPH. Weber et al demonstrated that PSA is produced only by the epithelial component of BPH.[8] Thus, men with a predominance of epithelial hyperplasia would be expected to have more marked increases in PSA during periods of epithelial proliferation than those with primarily stromal hyperplasia. Considering that dihydrotestosterone and epidermal growth factor indirectly and directly regulate the proliferation and differentiation of the epithelial component of prostate tissue and that PSA is produced only from the epithelial cells of the gland, we verified in patients with histologically proved BPH whether a significant association exists between the intraprostatic concentration of dihydrotestosterone or epidermal growth factor and serum PSA levels and, if so, whether this association may indicate the importance of epidermal growth factor and dihydrotestosterone in influencing serum PSA levels.

MATERIALS AND METHODS

The study population comprised 20 patients with a histological diagnosis of BPH who had chosen to undergo surgical treatment. These patients were part of a larger study on the relationship among age, PSA and prostate volume in men with lower urinary tract symptoms.[9] Details concerning the study design and criteria used in the selection and evaluation of patients taking part in that investigation have been published previously.[10] The 20 men, 54 to 77 years old (mean age ± standard deviation [SD] 67 ± 6.5), had no history of outlet surgery, histologically proved prostate cancer, neurogenic disorders, urethral stricture, urinary retention, prostatitis or other conditions known to interfere with normal voiding (except BPH) and had not received any treatment with agents known to influence vesicourethral function, prostate growth and serum PSA levels. Moreover, they had not received any medical treatment for at least 1 year. Mean International Prostate Symptom Score was 16.3 ± 3.5 and none of the patients had imperative indications for surgical treatment as defined by the international consultation guidelines.[11] There was no evidence of prostate cancer on digital rectal examination or transrectal ultrasonography.

Serum PSA concentration was determined with the Tandem-R* PSA assay. Subjects with a PSA greater than 4 ng./ml. underwent a sextant biopsy (3 cores for each side) of the prostate. Of the 20 patients 8 had a PSA of greater than 4 but less than 10 ng./ml. Following sextant biopsy none of the patients had prostate cancer on histological examination. Prostate volume was uniformly determined in all men by transrectal ultrasonographic (7.5 MHz. biplanar endorectal transducer) estimates of the anteroposterior, transverse and sagittal prostate dimensions assuming a prolate ellipsoid shape.[12]

All 20 untreated patients agreed to undergo open suprapubic prostatectomy. According to the international consultation on BPH,[11] patients chose the treatment in consultation with the urologist who discussed all options in the same order and in a neutral nonjudgemental manner. Patients

* Hybritech Inc., San Diego, California.

were fully informed of the risks and benefits of watchful waiting, and medical and surgical therapies. The potential benefits of various options were described relative to the severity of the condition. All descriptions and success/failure probabilities supplied to the patient were based on information in the literature.

In each case sections of the adenoma enucleated by prostatectomy were obtained in the periurethral, subcapsular and intermediate zones, as described previously (see figure).[13] Each section parallel to the longitudinal axis of the urethra was approximately 0.6 cm. thick and weighed 1 gm. Histological confirmation of BPH was obtained in all cases. Tissue was pulverized in liquid nitrogen and homogenized in 5 volumes of TEGM buffer as previously described.[14] Total tissue represented the sum of aliquots of the different pulverized tissue sections proportionally mixed according to the initial weight.

The intraprostatic content of dihydrotestosterone was determined in duplicate by radioimmunoassay in tissue homogenate after extraction with ice-cold acetone and ether, and purification on celite microcolumns eluted with a isoctane-benzene ratio of 85:15 volume-to-volume.[15] Specific antibody for dihydrotestosterone (2,000 counts per minute tritium dihydrotestosterone) was added to the homogenate for recovery calculation (mean recovery 66 ± 16% SD). Accuracy, evaluated by adding known amounts of dihydrotestosterone to tissue samples of different weight, revealed coefficients of variation of less than 10%. The interassay coefficient of variation was less than 11% and the intra-assay coefficient of variation was less than 10%. The blank value evaluated on stripped tissue sample was 1 pg. per tube.

Immunoreactive epidermal growth factor was evaluated in duplicate by radioimmunoassay in tissue homogenate using a homologous kit after extraction with ice-cold acetone, stirred at 4C and centrifuged at 1,400 g. The resulting pellets were dried under nitrogen and the epidermal growth factor was extracted from acetone powder by homogenization in 10 volume (weight per vol.) of ice-cold extraction solution (1% trifluoroacetic acid, 1% sodium chloride and 5% formic acid in
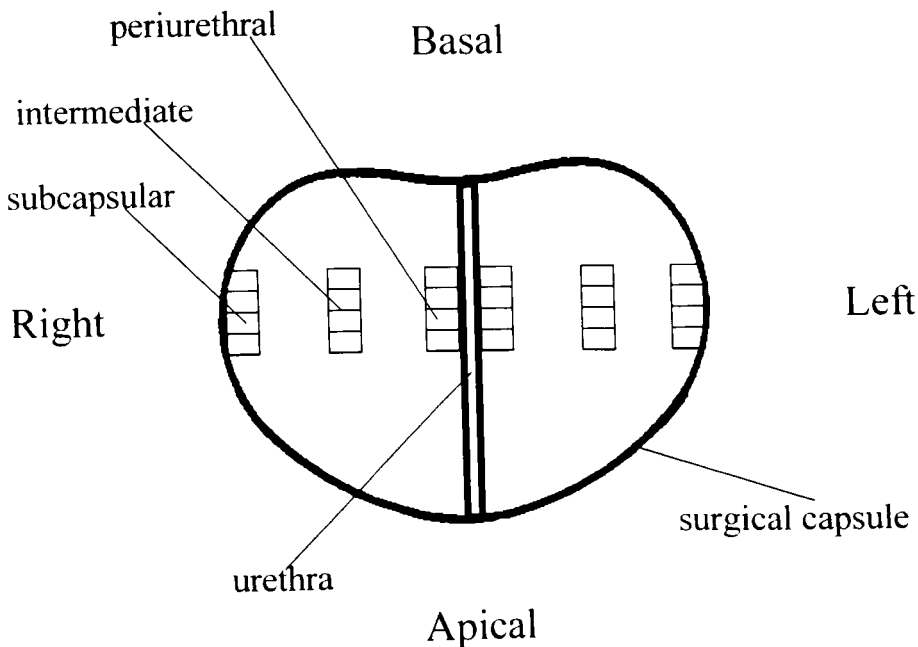
BPH tissue map of frontal (coronal) middle plane of adenoma enucleated by prostatectomy shows method used to evaluate dihydrotestosterone and epidermal growth factor concentrations in different zones. On this plane sections parallel to longitudinal axis of urethra were obtained in periurethral, subcapsular and intermediate zones.

1N hydrochloric acid) and stirred at 4C. After centrifugation at 1,400 and 100,000 g., the epidermal growth factor containing supernatants was purified on Sep-pak C18 Cartridges* and eluted with 4 ml. 80% acetonitrile (20% water containing 0.1% trifluoroacetic acid).[14] [125]Epidermal growth factor (3,000 counts per minute) was added to each homogenate for recovery calculation (mean recovery 66 ± 15% SD). The interassay and intra-assay coefficients of variation were 5.1 and 4.7%, respectively. We considered the concentrations of dihydrotestosterone (pg./gm. wet weight tissue) and epidermal growth factor (ng./gm. wet weight tissue) in total tissue and in the periurethral area of BPH.

STATISTICAL ANALYSIS

Descriptive statistics were used to characterize prostate volume, serum PSA, and total and periurethral concentrations of dihydrotestosterone and epidermal growth factor in BPH tissue of the 20 patients. Because of the log-normal distribution, PSA levels, prostate volume, and total and periurethral concentrations of dihydrotestosterone and epidermal growth factor were log-transformed for analysis. For the same reason these variables have been presented as median with 25th and 75th percentiles. Pearson's product-moment correlation coefficients were calculated to assess the association between PSA and epidermal growth factor (total and periurethral epidermal growth factor values), PSA and dihydrotestosterone (total and periurethral dihydrotestosterone values), prostate volume and epidermal growth factor (total and periurethral), prostate volume and dihydrotestosterone (total and periurethral), prostate volume and PSA, and epidermal growth factor and dihydrotestosterone (periurethral and total values).

Univariate linear regression models solved with PSA as dependent variable and epidermal growth factor or dihydrotestosterone, respectively, as independent variables, and with prostate volume as dependent and epidermal growth factor or dihydrotestosterone, respectively, as independent variables were performed. To establish whether changes in PSA levels may be directly influenced by changes in epidermal growth factor concentration independent of modifications in dihydrotestosterone concentration and in prostate volume, multiple linear regression models were performed with PSA as dependent, and epidermal growth factor and dihydrotestosterone or epidermal growth factor and prostate volume, respectively, as independent variables.

RESULTS

Descriptive analysis. Mean age plus or minus SD for the 20 men with histologically proved BPH was 67.2 ± 6.5 years (range 54 to 77). With regard to the outcome variables the median (25th, 75th percentiles) serum PSA concentration was 4.50 ng./ml. (3.20, 6.30). Overall serum PSA was less than 4 ng./ml. in 12 men (60%), and between 4 and 10 ng./ml. in 8 (40%). Median prostate volume was 79.5 ml. (62.0, 96.0). Median concentration of total and periurethral epidermal growth factor was 14.699 (9.724, 18.250) and 18.399 (14.499, 23.250) ng./gm. wet weight tissue, respectively. Median concentration of total and periurethral dihydrotestosterone was 5,081 (4,569, 6,435) and 7,200 (5,270, 8,059) pg./gm. wet weight tissue, respectively.

Pearson's product-moment correlation coefficients and multiple linear regression models. The associations among serum PSA, prostate volume, epidermal growth factor and dihydrotestosterone are presented in table 1. As in a previous study,[13] a positive correlation between total epidermal growth factor and total dihydrotestosterone concentration was observed (r = 0.4888, p = 0.0315013). A stronger correlation was found between the periurethral concentration of

* Water Associates, Milford, Massachusetts.

TABLE 1. *Association among serum PSA levels, prostate volume, and total and periurethral concentration of dihydrotestosterone and epidermal growth factor in BPH tissue*

	Pearson's Product-Moment Correlation Coefficient	p Value
PSA + periurethral epidermal growth factor	0.8117	0.000005
PSA + total epidermal growth factor	0.7762	0.00002836
PSA + periurethral dihydrotestosterone	0.5656	0.0.0098326
PSA + total dihydrotestosterone	0.3923	0.0956307
Prostate vol. + periurethral epidermal growth factor	0.0535	0.829773
Prostate vol. + total epidermal growth factor	0.0133	0.957415
Prostate vol. + periurethral dihydrotestosterone	0.2546	0.295687
Prostate vol. + total dihydrotestosterone	0.1546	0.531439
Periurethral epidermal growth factor + periurethral dihydrotestosterone	0.7239	0.000217856
Total epidermal growth factor + total dihydrotestosterone	0.4888	0.0315013
Prostate vol. + PSA	0.1111	0.65421

epidermal growth factor and dihydrotestosterone (r = 0.7239, p = 0.000217856). In particular, a statistically significant association was noted between PSA and total epidermal growth factor (p = 0.00002836), whereas association between PSA and total dihydrotestosterone did not reach statistical significance (p = 0.0956307). Also in this case a stronger association was found between the periurethral concentration of dihydrotestosterone and epidermal growth factor (table 1).

No significant association was observed between prostate volume and epidermal growth factor or dihydrotestosterone (table 1). The association between PSA and epidermal growth factor, and PSA and dihydrotestosterone may suggest the importance of epidermal growth factor and dihydrotestosterone concentrations in influencing PSA serum levels. Considering the total values of epidermal growth factor and dihydrotestosterone, the regression analysis suggested that 60% of the serum PSA level variance could be accounted for by the intraprostatic concentration of epidermal growth factor (R^2 = 0.6026, p = 0.00002836) and only 15% by the intraprostatic concentration of dihydrotestosterone (R^2 = 0.1539, p = 0.0956307). Considering the periurethral values of the 2 variables, the results of the regression analysis suggested that 66% of the serum PSA level variance could be accounted for by the concentration of epidermal growth factor (R^2 = 0.6589, p = 0.000005), whereas 32% could be accounted for by the concentration of dihydrotestosterone (R^2 = 0.3200, p = 0.0098326). Moreover, the multiple linear regression model showed that changes in serum PSA level that could be accounted for by the intraprostatic concentration of epidermal growth factor were independent of changes in dihydrotestosterone concentration and prostate volume (table 2).

DISCUSSION

Functionally, PSA is a kallikrein-like serine protease that is produced by the epithelial cells lining the acini and ducts of the prostate gland.[16] A complete understanding of the regulation of PSA expression is important because of its potential usefulness as a model and its clinical relevance. Except for prostate volume, little is known about the factors that influence serum PSA levels. Regulators of PSA production at the cellular and molecular level, and routes of metabolism and excretion have been poorly defined. The expression of PSA protein has been shown to be correlated with fluctuating androgen levels during male development.[17] Furthermore, the evaluation of patients with BPH and treated with

TABLE 2. Multiple linear regression model (y = PSA)

			(y = log PSA)		
	Coefficient	p Value		Coefficient	p Value
PSA as dependent variable, and epidermal growth factor and dihydrotestosterone as independent variables					
α	−0.23		α	−0.49	
Log-transformed periurethral dihydrotestosterone	−0.08	Not significant	Log-transformed total dihydrotestosterone	0.03	Not significant
Log-transformed periurethral epidermal growth factor	0.93	<0.01	Log-transformed total epidermal growth factor	0.91	<0.01
R^2 = 0.6599			R^2 = 0.6028		
PSA as dependent variable, and epidermal growth factor and prostate volume as independent variables					
α	−0.32		α	−0.16	
Log-transformed vol.	−0.08	Not significant	Log-transformed vol.	−0.12	Not significant
Log-transformed periurethral epidermal growth factor	0.89	<0.001	Log-transformed total epidermal growth factor	0.92	<0.001
R^2 = 0.6635			R^2 = 0.6127		

antiandrogens has shown a direct correlation between serum testosterone and serum PSA.[8]

Studies suggest that in vitro PSA production by the human prostate cancer cell line LNCaP may be regulated by androgens and various growth factors.[18] Gleave et al demonstrated that castration of male mice bearing LNCaP tumors produces a rapid decrease in serum PSA independent of changes in tumor volume.[19] Administration of 3 mg./kg. testosterone daily in castrate male mice resulted in a marked and rapid increase in serum PSA levels. Within 24 hours of the first injection PSA levels increased independent of tumor volume. Northern blot analysis was also performed to assess the effect of androgens and growth factors on PSA messenger ribonucleic acid (mRNA) levels.[19] The authors observed that changes in PSA mRNA expression were not always accompanied by corresponding changes in growth rate of LNCaP cells. Androgens increased LNCaP cell growth as much as 180% and increased PSA mRNA expression 4-fold, while basic fibroblast growth factor stimulated LNCaP cell growth 180% but decreased PSA mRNA expression by 50%. Transforming growth factor-β inhibited LNCaP cell growth by 70% and yet increased PSA mRNA expression 180%.

Considering that dihydrotestosterone and epidermal growth factor are regulators of proliferation and differentiation in the epithelial component of human prostate tissue and that PSA is produced only from the epithelial cells of the gland, we attempted to establish in patients with a histological diagnosis of BPH whether a significant association exists between the intraprostatic concentration of dihydrotestosterone or epidermal growth factor and serum PSA. All patients underwent open suprapubic prostatectomy to enucleate the entire adenoma and to obtain in each case sections in the periurethral, subcapsular and intermediate zones of BPH tissue. Dihydrotestosterone and epidermal growth factor concentrations were evaluated in the periurethral area and in the total tissue of BPH by mixing equal aliquots of the 3 zones of the prostate. Each sample was approximately the same weight to avoid interferences in results. All values were expressed per gm. wet tissue to compare our results with those reported in the literature.[20]

In a previous study we attempted to establish whether androgens and epidermal growth factor are uniformly distributed from the periurethral to more peripheral zones of BPH tissue or whether they show regional differences.[13] Lawson reported that for basic fibroblast growth factor in the periurethral zone dihydrotestosterone, testosterone and epidermal growth factor showed significantly higher values than those in the subcapsular zone (p <0.001).[21] Moreover, a positive linear correlation among epidermal growth factor, testosterone and dihydrotestosterone was observed (epidermal growth factor and testosterone r = 0.562, p <0.002; epidermal growth factor and dihydrotestosterone r = 0.652,

p <0.001). The question is whether the periurethral epidermal growth factor levels, which correspond to the highest values of testosterone and dihydrotestosterone, reflect a local hyperproduction and express the biological effects of this peptide.[20] The highest androgen content in the periurethral zone may also reflect a transfer of testosterone and/or dihydrotestosterone from the vas deferens or the deferential veins where the concentrations of these compounds are particularly high.[22] In fact, man and dog are the only species that may be affected by BPH, as the vas deferens reaches the urethra by passing through the prostate.[23] Moreover, the high concentrations of dihydrotestosterone in the periurethral zone could enhance the expression of type 2, 5-α-reductase,[24] contributing to the increase in the hormonal differences between periurethral and peripheral areas of this gland. In the present study we confirmed a positive correlation between epidermal growth factor and dihydrotestosterone concentration in BPH tissue (r = 0.4888, p = 0.0315013 for total values and r = 0.7239, p = 0.000217856 for periurethral values).

To our knowledge our study is the first report in the literature to demonstrate a statistically significant association between serum PSA, and dihydrotestosterone and epidermal growth factor levels, particularly in the periurethral zone of human BPH tissue (PSA and periurethral dihydrotestosterone p = 0.0098326, PSA and periurethral epidermal growth factor p = 0.000005). In our sample the regression analysis indicates that 66% of serum PSA level variance could be accounted for by the periurethral concentration of epidermal growth factor, whereas 32% could be accounted for by periurethral dihydrotestosterone levels. The stronger association between PSA and epidermal growth factor than between PSA and dihydrotestosterone, and the multiple linear regression model suggest that epidermal growth factor can directly influence serum PSA levels independent of dihydrotestosterone (table 2). Moreover, the absence of a significant association between epidermal growth factor or dihydrotestosterone and prostate volume (p = 0.957415 and p = 0.531439, respectively, for total values, and p = 0.829773 and p = 0.295687, respectively, for periurethral values), between prostate volume and serum PSA (p = 0.65421) and the multiple linear regression model (table 2) suggest that changes in serum PSA levels are not always accompanied by corresponding changes in prostate volume, and that PSA expression may be directly influenced by androgens and growth factors independent of modifications in prostate size. However, a limit of the analysis could be that the high values of prostate volume in our patients with BPH do not represent the entire range of variation of this parameter.

Several authors reported the effect of prostate size on PSA level in large series of patients.[25] However, serum PSA is variably elevated by the glandular component of BPH. In a

previous report on the relationship among age, PSA and prostate volume in men with lower urinary tract symptoms and in those with the histological diagnosis of BPH, the results of the regression analysis suggested that only 23% of the variance in PSA with age could be accounted for by prostate volume.[9] Since the number of subjects in our study is limited, large prospective studies are needed to confirm these findings. Moreover, serum PSA levels should represent a steady state between total PSA production, and its rate of metabolism and excretion. In patients with BPH physiological barriers that maintain PSA in the prostatic duct system may become more permeable and, thus, allow PSA to enter the general circulation more easily via the capillaries and lymphatics.[26] Routes of excretion of PSA are not yet established, and the effects of liver and renal dysfunction on serum PSA levels remain to be defined. Consequently, it is difficult to estimate the influence of leak factors involved in PSA production and PSA serum concentration. Measurement of intraprostatic levels or Northern blot analysis to assess the direct effect of epidermal growth factor on PSA mRNA would be more helpful in establishing the relationship of epidermal growth factor to PSA in human BPH tissue.

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