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Anti-PSA immunoreactivity in primary prostatic tissues from Black African men

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Summary

Prostatic specific antigen (PSA) immunoreactivity is the most commonly used histological marker to identify epithelial cells of prostate origin. Unlike tissues from white men in which grade-related variability has been reported, the pattern of PSA immunoreactivity in prostatic tissues from black African men is presently unknown. This study was done to evaluate the pattern of PSA staining in sections of normal, benign hyperplastic and malignant prostatic glands from men from this sub-population. PSA immunostaining was done on 4-μm serial sections from archival specimens of benign prostatic hyperplasia (BPH) and carcinoma of the prostate (CaP) obtained from black African men using standard immunoperoxidase techniques. The intensity of PSA immunoreactivity of the glands was scored using a semi-quantitative method. PSA expression decreased with increasing de-differentiation of the tissue histotype with poorly differentiated tumours staining least. PSA immunoreactivity was strong in 100% of normal glands and 84% of BPH glands and moderate in the rest. In contrast, PSA immunopositivity was strong in 32% of CaP glands, moderate in 26%, weak in 34% and absent in 8%. Statistical comparison revealed that PSA expression was significantly higher in benign tissues (normal/atrophic and BPH) than in CaP glands [p = <0.0001]. Our findings show that PSA immunoreactivity is grade-related in prostatic tissues from black men and this has implications for clinical diagnosis and research. It also confirms the limitations of PSA-testing in diagnosing CaP, and indicates that newer immunohistochemical tests for malignant prostatic cells should be acquired by Sub-Saharan laboratories.

Keywords: *PSA immunoreactivity, prostate, BPH, prostate cancer, black African, immunohistochemistry*

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Résumé

L'immunoreactivité à l'antigène prostatique spécifique (APS) est le plus utilisé marqueur histologique pour identifier l'origine des cellules prostatiques épithéliales. Contrairement aux tissus des hommes européens ayant différent degré et fréquence d'antigène prostatique spécifique. L'immunoreactivité des tissus prostatique chez les hommes noir Africain reste inconnu. Cette étude avait pour objectif d'évaluer la fréquence des APS tenté des sections normales, hyperlastique et maligne des glandes prostatiques des hommes dans le sud du Sahara. APS était faite sur 4um sections des échantillons obtenu des Africain de l'hyperplasie bénigne prostatique et du cancer de la prostate obtenu des Africains utilisant les techniques standard d'immunoperoxidase. L'intensité de l'APS immunoreactive des glandes était évaluée utilisant une méthode semi quantitative. L'expression de l'APS augmentant avec la différentiation tissues mais était faiblement différentiée par les teintures. L'APS immunochimie était de 100% pour les glandes normales et 84% pour les cas bénignes et modérés. Au contraire l'APS immunochimie était de 32% pour les glandes cancéreuses, 26% pour les cas modérés, faible chez 34 cas et absent chez 8 cas. La comparaison statistique révélait que l'expression de l'APS était significativement plus élevée aux tissues bénignes que les glandes cancéreuses P=0.0001. Ces résultats démontrent que l'APS immunochimie varie avec le degré de tissus prostatiques chez les noirs et a des implications de diagnostic cliniques et de recherche. Ceci confirme les limitations du dépistage dans le diagnostic de l'APS ou cancer prostatique et indique des nouvelles méthodes immunohistologiques doivent être développés pour les tests des cellules du cancer prostatiques dans les laboratoires au Sud du Sahara.

Introduction

Although newer and more specific tests have been developed for the identification of prostatic epithelial cells [1-3] anti-prostate specific antigen .(PSA) immunoreactivity remains the most commonly used

marker for these cells [4]. The sensitivity and specificity of anti-PSA antibodies has therefore remained of importance in clinical studies aimed at accurately diagnosing and staging cases of carcinoma of the prostate (CaP). To this end, monoclonal antibodies have been found to be more specific but less sensitive than polyclonal antibodies [5]. Furthermore, the heat-induced epitope retrieval (HIER) technique is considered superior to the older antigen retrieval methods in exposing PSA epitopes and increasing the sensitivity and specificity of the antibodies [5]. Despite these advances, immunoreactivity to anti-PSA monoclonal antibodies has been found to decrease with increasing tumour grade in prostatic tissues from white men [5, 6]. In addition, PSA is an androgen response element and its expression is regulated by androgen receptors (AR) in prostatic epithelial cells [7, 8]. We and others have previously detected sub-population differences in the expression of AR in the epithelium and stroma of benign and malignant prostatic tissues from black men [9, 10]. Racial differences have also been reported in the expression of other antigens whose activity is regulated by AR [11]. These studies suggest that PSA expression in prostatic tissues from black men may be different from that reported in white men.

Despite the above reports however, we could find no studies specifically directed at assessing PSA expression in prostatic tissues from black men. We now report on a study in which we evaluated the level of PSA immunostaining in normal/atrophic, benign hyperplastic and malignant prostatic tissues from native black African men.

Materials and methods

Materials

Sections from archival specimens of transurethral and retropubic prostatectomies for 19 cases of benign prostatic hyperplasia (BPH) and 39 cases of carcinoma of the prostate (CaP) were obtained from the Pathology Department of the University College Hospital (UCH), Ibadan Nigeria and the African Institute of Urology, Chilongwe, Zimbabwe. All the tissues had been fixed using similar protocols in 10% buffered formalin and embedded in paraffin. Routine haematoxylin and eosin staining of representative sections from each block was undertaken for histological evaluation by experienced pathologists (JOO and CAM). 4-μm serial sections were prepared from selected blocks, and stored in slide racks at room temperature until stained.

The primary malignant prostatic tumours were graded using the Gleason score [12], and classified as well differentiated (G1, Gleason score 2-4), moderately differentiated (G2, Gleason score 5-7) or poorly differentiated (G3, Gleason score 8-10). There were 11, G1 tumours; 10, G2 tumours; and 18, G3 cancers. The monoclonal anti-PSA antibody used in this study was obtained commercially (NCL-PSA Novocastra, Northumbria, UK). The sensitivity and specificity of this antibody had been confirmed in our earlier report [13].

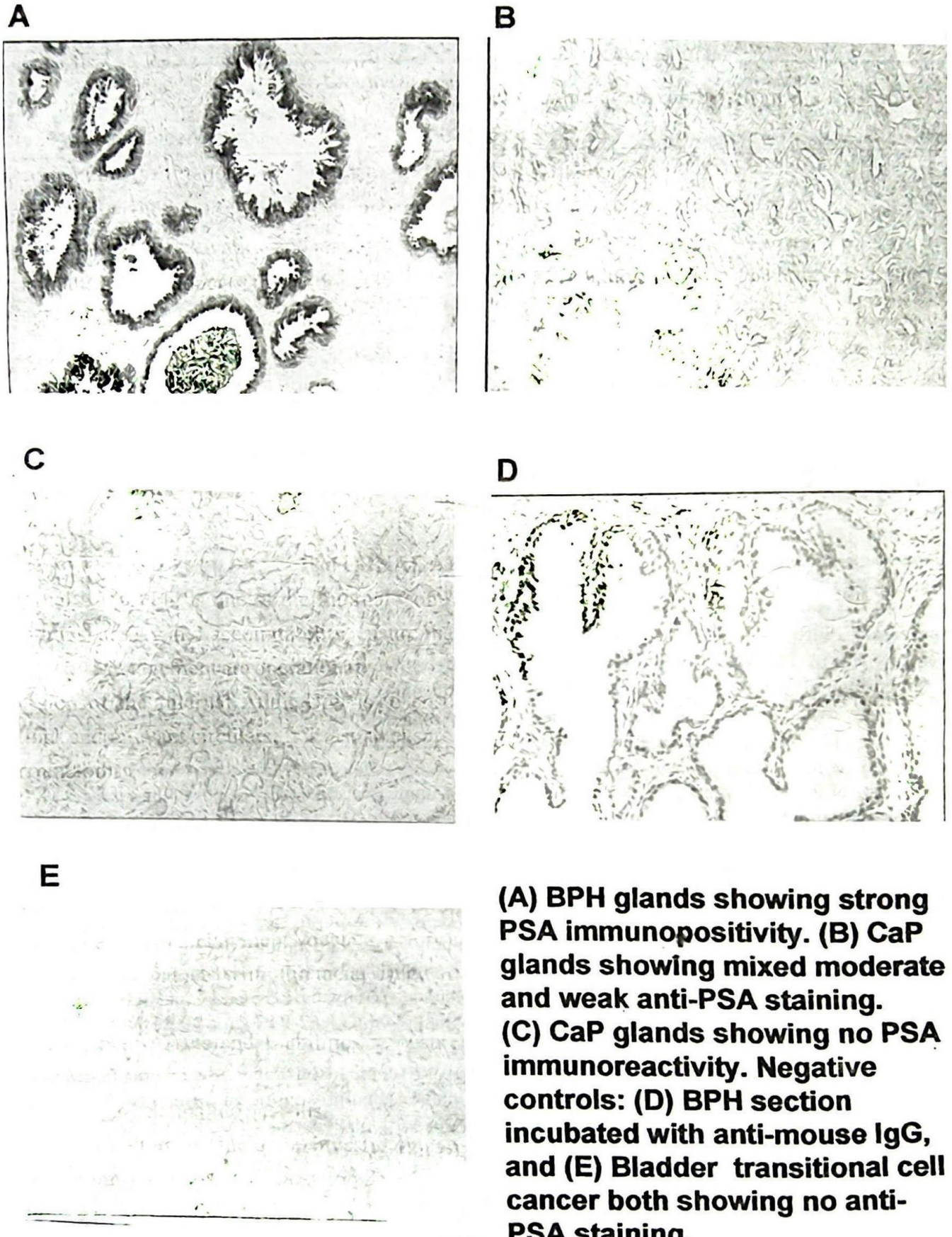
PSA Immunostaining

Anti-PSA immunostaining was done as previously described [13] by one of the authors (EOO). All sections were stained within 3 weeks of preparation from archival blocks to avoid antigen degradation [13]. Briefly, archival sections were deparaffinized and equilibrated (Tris-buffered saline [TBS]), and peroxidase activity was quenched with hydrogen peroxide solution. Heat-induced epitope retrieval (HIER)of the PSA epitopes was achieved by microwaving the sections in 10mM citrate buffer pH 6.0. The sections were then blocked with 20% normal goat serum. A pre-diluted solution of mouse monoclonal anti-human PSA was added and the sections incubated for 2hrs at room temperature or overnight at 4°C. The sections were rinsed with TBS and incubated with goat secondary antibody. The avidin-biotin complex was then applied to the sections followed by a freshly prepared chromogen mix of buffered 3',3' diaminobenzidine tetrahydrochloride. The sections were counterstained with Mayer's haematoxylin, then dehydrated, cleared and mounted with XAM neutral mounting medium (BDH Poole, Dorset, UK).

Positive and negative controls were included in all staining runs. The positive control was BPH sections known to be PSA positive. Negative controls were: (1) substitution of monoclonal anti-PSA with mouse IgG at the same dilution as the primary antibody; (2) sections of transitional bladder carcinoma incubated with anti-PSA.

Evaluation of Immunochemistry

The staining patterns of the sections were assessed independently, and without prior knowledge of histological grading, by an assessor (EOO) and scored visually. Areas of inflammation, infarction and tissue autolysis within the sections were excluded



(A) BPH glands showing strong PSA immunopositivity. (B) CaP glands showing mixed moderate and weak anti-PSA staining. (C) CaP glands showing no PSA immunoreactivity. Negative controls: (D) BPH section incubated with anti-mouse IgG, and (E) Bladder transitional cell cancer both showing no anti-PSA staining.

Fig. 1:

Table 1: PSA Expression in Prostatic Glands in Black African Men

Histology (Total no)	Mean Score (SD)	Intensity of immunoreaction Strong (%, CI)	Moderate (%, CI)	Weak (%, CI)	Absent (%, CI)
Benign Tissues (Cum)	74 (13)	16 (84, 62-94)	3 (16, 6-38)	0 (0, 0-17)	0 (0, 0-17)
Normal/ Atrophic (19)	77 (7)	19 (100, 83-100)	0 (0, 0-17)	0 (0, 0-17)	0 (0, 0-17)
BPH (19)	71 (22)	16 (84, 62-94)	2 (11, 1-32)	1 (5, 0-25)	0 (0, 0-17)
CaP Cum (39)	43 (35)	12 (32, 18-50)	10 (27, 14-44)	13 (35, 20-53)	3 (8, 2-22)
G1 (11)	57 (27)	5 (46, 17-77)	3 (28, 6-61)	3 (28, 6-61)	0 (0, 0-29)
G2 (10)	50 (30)	4 (40, 12-77)	3 (30, 7-65)	3 (30, 7-65)	0 (0, 0-27)
G3 (18)	29 (34)	3 (18, 4-43)	4 (24, 7-50)	7 (41, 18-67)	3 (18, 4-43)

Key
SD = Standard Deviation *HGPIN = High grade prostatic intra-epithelial neoplasia*
CI = Confidence Interval *CaP = Primary prostatic cancer*
BPH = Benign prostatic hyperplasia *Cum = Cummulative*

Table showing that anti-PSA expression decreased in prostatic tissues from native African men with increasing de-differentiation of the tissue histotype with poorly differentiated tumours staining least (normal/atrophic and BPH glands v CaP glands, p = <0.0001)

from analysis. False positivity due to formalin pigments was excluded by viewing the slides with polarized light. The PSA score was determined as has been previously described using the modified H-Score that takes into account the number of cells with a positive membranous reaction weighted with the intensity of staining in pre-selected homogeneous areas of normal and benign hyperplastic glands in BPH sections and malignant glands in CaP sections [10, 14]. The final scores were classified as; 0 = negative, 1- 33% = low/weak expression, 34- 66% = moderate expression, >66% = high/strong expression.

Statistical methods
The results were reported as proportions within histological groups together with the corresponding 95% confidence intervals. Statistical comparison of PSA scores in the main prostatic tissue histotypes (normal, benign hyperplastic, and malignant cell types) was done using the paired and unpaired Student t-test as appropriate. All tests were two-sided, and were performed at the 0.05 level of significance.

Results

Pattern of PSA immunostaining
PSA was expressed by prostatic epithelial cells only and not by the surrounding stromal elements. Furthermore, expression decreased with increasing de-differentiation of the tissue histotype with poorly differentiated tumours staining least (figs. 1a-d). Furthermore, whilst PSA immunostaining was uniform in normal/atrophic and BPH glands, a mixed intensity of immunopositivity was observed in CaP glands. As such CaP sections were scored using the areas of highest PSA immunoreactivity. For details of immunostaining score see table 1.

Sections from BPH glands
Normal/atrophic and BPH glands in sections from BPH glands were all positive for PSA. Anti-PSA staining was strong in normal/atrophic glands in all 19 sections (100%, C.I 83-100%). In contrast, PSA immunopositivity in BPH glands was strong in 16/19 sections (84%, C.I. 62-94%), moderate in 2/19 (11%, C.I. 1-32%), and weak in 1/19 (5%, C.I. 0-25%). Statistical comparison of individual immunostaining scores revealed no significant differences between PSA expression in normal/atrophic and BPH glands [$P = 0.08$].

Sections from CaP glands
Anti-PSA reaction was strong in malignant glands in 12/39 sections (32%, C.I. 19-48%), moderate in 10/39 (26% C.I.15 to 42%) and weak in 13/39 (34% C.I. 21 to 50%). Malignant glands in 3/39 CaP sections were negative for the marker (8%, C.I. 3-

21%). Overall, the intensity of PSA staining appeared to decrease with increasing de-differentiation of the epithelium but there was no significant difference between adjacent histological grades [p = 0.08]. PSA expression was however significantly lower in poorly differentiated tumours when compared to well/moderately differentiated cancers [p = 0.008]. Of note in this regard was the fact that the 3 PSA - negative sections were from anaplastic (G3, Gleason score 10) tumours. Furthermore, PSA immunoreactivity was significantly higher than observed in benign prostatic glands tissues (normal/atrophic and BPH) in BPH sections when compared to CaP glands ($P = <0.0001$).

Discussion

The description of the pattern of anti-PSA immunoreactivity in native black African men is of clinical and research importance. This is because the newer immunohistochemical or genetic tests with which prostatic cells may be identified are largely unavailable in Africa, and international collaborative research projects on prostatic diseases often involve comparative studies on tissues from pathology departments from the continent [10, 13, 15].

Our finding that PSA immunostaining decreases with increasing malignancy of prostatic tissues in our cohort of black African men is therefore of significance. This is because it confirms earlier reports from studies on tissues from white men [5, 6] and shows that the changes in PSA expression associated with grade are similar in tumours from black and white men. In addition, the fact that all the PSA-negative tissues were cases of anaplastic (Gleason Score 10) tumours is also similar to previous reports [5] and provides further evidence that the lack of PSA immunoreactivity does not exclude prostatic origin of the suspected malignant epithelium. Indeed this finding may explain, at least in part, the relatively low serum levels of PSA detected in some cases of high grade CaP [16].

The results of this present study were also significant for another reason. Prostate cancers have a more aggressive biology in blacks [17-19] and PSA production is a marker of the aggressiveness of the disease [20]. PSA expression is regulated by AR [7, 8] and it has been previously shown [9, 10] that black men with CaP have higher epithelial AR expression than Caucasians. We therefore expected that PSA immunopositivity would be higher in prostatic epithelium from black men. As such the similarity of PSA immunoreactivity in our samples and those of previous studies [5] was surprising and we are presently unable to explain this similarity. However, as the three PSA-negative cases were AR positive tumours we postulate that, similar to observations in the serum levels of the marker in some patients with advanced cancers [16], tissue expression PSA may become dissociated from androgen regulation in high grade tumours.

A limitation of this study is the relatively small number of samples evaluated from the two participating centres. However the similarity of the distribution of results in the samples from the centres lends credence to the validity of the results. However, a larger study may be required to confirm our findings.

Conclusion

Although this study incorporates a relatively small number of specimens, the findings of this study show that, similar to tissues from white men, PSA immunoreactivity shows grade-related variability in prostatic tumours from native black African men and this has implications for the clinical diagnosis of CaP and research (especially collaborative projects). It also confirms the limitations of PSA-testing as the sole diagnostic tool for CaP, and indicates that pathology laboratories in Sub-Saharan Africa should acquire newer immunohistochemical tests for malignant prostatic cells to maintain the diagnostic and research standards. These findings may need to be confirmed by a larger study.

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