Original Article

Diagnostic Utility of Immunochemical Technique Using p63 and Alpha Methylacyl Coenzyme A Racemase (AMACR) in the diagnosis of Core-Needle Biopsy of the Prostate: Experience in a Tertiary Academic Institution in Nigeria

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Abstract

Background: Prostate adenocarcinoma is one of the most common malignancies and a leading cause of cancer-related mortality among males worldwide. There are challenges associated with confident/equivocal diagnosis of prostate carcinoma on small prostate samples from core-needle biopsies diagnosed histologically because of certain mimickers of prostatic carcinoma. Hence, there is a need to improve the diagnostic accuracy of the histological diagnosis of core-needle biopsies by utilizing immunohistochemical profiling to overcome these challenges. The aim of this study was to use p63 and alpha-methylacyl coenzyme A racemase (AMACR) immunostains to confirm hematoxylin and eosin (H and E) diagnosed adenocarcinomas and clarify equivocal diagnoses as well as correlate the H and E diagnoses with immunohistochemical diagnoses. **Materials and Methods:** This was a 3-year retrospective study of core-needle prostatic biopsies processed at the Anatomic and Molecular Pathology Department of Lagos University Teaching Hospital, Lagos, Nigeria. The formalin-fixed paraffin-embedded tissue blocks were retrieved, and new slides were prepared in cases where old slides were faded. The routinely processed slides were reviewed and classified into the following categories: benign, malignant (adenocarcinoma), and equivocal lesions (i.e., lesions considered suspicious for adenocarcinoma). The cases diagnosed as adenocarcinoma and equivocal lesions were then subjected to immunohistochemistry (IHC) using p63 and AMACR monoclonal antibodies to confirm the diagnoses of prostate adenocarcinoma and clarify the equivocal diagnoses. Based on the findings on IHC, the cases were reclassified as either adenocarcinoma, benign or indeterminate lesions (i.e., lesions that could not be classified as either benign or adenocarcinoma due to poor staining quality). **Results:** A total of 221 prostatic core biopsies met the inclusion criteria for this study. Out of these, histological diagnoses of prostatic adenocarcinoma were made in 113 cases (51.1%), 86 cases (38.9%) were benign, while equivocal cases accounted for 22 cases (10%). The result showed that out of 113 H and E diagnosed prostatic carcinoma that were subjected to p63 and AMACR stains, 101 (89.4%) of them were found to be truly adenocarcinoma, while 7 (6.2%) were benign and 5 cases (4.4%) were indeterminate lesions. The results of p63 and AMACR on the 22 histologically diagnosed equivocal prostatic lesions showed that 13 (59.1%) of the cases were adenocarcinoma, 7 cases (31.8%) were benign while 2 cases (9.1%) were indeterminate lesions. These p63 and AMACR immunostain results on routinely diagnosed prostatic carcinoma and equivocal diagnoses showed a statistically significant difference in the diagnostic potential of p63 and AMACR IHC when compared to the H and E as a diagnostic tool ($P \leq 0.001$). **Conclusion:** We conclude that although histopathological examination of H and E sections remains the gold standard in the diagnosis of prostatic adenocarcinoma, the adjunctive use of p63 and AMACR immunostains is of great value in confirming small foci of adenocarcinoma, resolving morphologically equivocal cases and excluding benign mimickers as confounder in the diagnosis prostatic adenocarcinoma in small prostate samples obtained by core-needle biopsy.

Keywords: AMACR, benign lesion, immunostains, P63, prostate adenocarcinoma

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Introduction

Prostate adenocarcinoma is the most frequent malignant neoplasm in males.[1] It accounted for 6.1% to 19.5% of all cancer cases in Nigeria and the incidence is increasing.[2] In the United States of America, prostate cancer remains the most common malignancy affecting men and the second leading cause of cancer-related death.[3] When compared to African-American men, Nigerian men are ten times more likely to have prostate cancer and 3.5 times more likely to die from it.[2] Environmental and most importantly, genetic factors have been incriminated as the reasons for the geographic differences in incidence.[3] Advancing age (≥50 years), race, genetic/familial, hormonal, and dietary factors have been demonstrated as important risk factors in the development of prostate cancer.[4-7] In Nigeria,[8-15] the reported mean age of patients with prostate adenocarcinoma varies between 60.5 and 71.4 years while it is most frequently diagnosed in patients within the aged group 65–74 years in the USA and Canada.[16]

The 2016 World Health Organization (WHO) classification of prostate cancer recognizes many different histological types, grouped under six categories: epithelial, mesenchymal, neuroendocrine, hematolymphoid, miscellaneous, and metastatic tumors.[17]The epithelial cancers are the most common types encountered, and acinar adenocarcinoma is the most common variant, accounting for >95% of all prostate cancers, and hence, prostate cancer is almost synonymous with acinar adenocarcinoma.[4,17] The stage and grade of prostate cancer are the most important predictors of its behavior and outcome and are key parameters guiding patients' treatment.[4,18]

The Gleason scoring system is a widely accepted grading scheme use for prostate carcinoma.[18]

In this system, cancers are divided into five grades (grade 1–5) based on the glandular architectural patterns of differentiation of the tumor, best assessed at low power magnification. Grade 1 cancers are the most well-differentiated cancers, while grade 5 cancers are the least differentiated, exhibiting no glandular features. Because most tumors exhibit more than one pattern, a primary grade is allocated to the most prominent pattern and a secondary grade to the next frequent pattern. The final Gleason score (GS) for prostate cancer is the sum of the numeric values of its primary and secondary grades.[18] A minimum GS 2 (grades 1 + 1) is allocated for well-differentiated tumors while a maximum GS 10 (grade 5 + 5) is allocated for poorly differentiated tumors. The progression-free survival is about 90% for patients with GS ≤6 but this decreases significantly with GS ≥7.[19] In 2016, the International Society for Urologic Pathology (ISUP) and WHO adopted a simple patient-centric grading system where GSs are combined into 5 prognostic grade groups, grade groups 1–5.[20,21] This new grade group system addresses the deficiencies of the Gleason scoring system and correlates well with the risk of biochemical recurrence and death from prostate cancer.[20,21] In this system, tumors with GS ≤6 are in grade group 1, grade group 2 consist of tumors with GS 7 (3 + 4), grade group 3 are tumors with GS 7 (4 + 3), while grade group 4 are those with GS 8 and grade group 5 are those tumors with GS 9 and 10.[21]

The diagnosis of specific prostate disease is crucial to guiding clinical decisions and planning patients' management. Many approaches help to identify pathological processes within the prostate gland. These include digital rectal examination, transrectal prostate ultrasound, serum prostate-specific antigen, and prostate biopsy for histological analysis.[4,22] Histological analysis of prostate samples obtained by guided core-needle biopsy (transrectal/transperineal approach) is the gold standard for diagnosis of prostate diseases in general and prostate adenocarcinoma in particular.[4,22] Perineural invasion, mucinous fibroplasia, and glomeruloid formation are pathognomonic histomorphologic features of prostate cancer.[4,22,23] These features are uncommonly encountered; hence, a diagnosis of prostate adenocarcinoma is based on an array of atypical architectural, histological, and cytological characteristics seen at histology.[4,18,23]

Making this diagnosis on routine hematoxylin and eosin (H and E)-stained slides could be challenging because several benign or nonprostatic lesions and normal structures can mimic prostate carcinoma, especially in a small piece of tissue obtained by guided needle biopsy, even when it is adequate (contains ≥1 prostatic gland).[4,24-26] Furthermore, repeat core-needle biopsies initially reported as atypical/suspicious for the malignant prostate disease could be reported as negative, even though the patient has the extensive disease.[4,26-28] Some benign mimickers of prostate cancer from tissue obtained by core-needle biopsy include prostate atrophy, atypical adenomatous hyperplasia, postatrophy hyperplasia, metaplasia of prostate gland, and seminal vesicles.[1,29] Other pitfalls of limited prostate H and E-stained core-needle biopsy include missing the small focus of adenocarcinoma and misdiagnosing of secondary tumors involving the prostate as primary prostate cancer. These secondary tumors include malignant spreads from the lungs, skin (melanoma), gastrointestinal, kidneys, testes, and endocrine glands.[1]

Underdiagnosis or overdiagnosis of prostate adenocarcinoma have ramifications for record-keeping, the patients, and are potential bases of litigation against pathologists.[23,30] Overdiagnosis of prostate carcinoma implies misleading cancer registration and statistics as well as mislabeling and overtreatment of patients with the attendance medical, psychosocial, and fiscal implications.[23,30] These challenges of erroneous diagnoses of prostate carcinoma associated with small H and E-stained core-needle biopsies can be unraveled using the appropriate interpretation of immunohistochemical stains. P63 monoclonal antibody is a sensitive nuclear marker of prostatic basal cells while AMACR is a cytoplasmic immunostain which stains the same antigen overexpressed by malignant prostate luminal cells.[4,31,32] A negative p63 immunostain on prostatic cord-needle is strongly suggestive of routinely diagnosed prostate carcinoma while a positive AMACR stain of luminal cells does the same.

Used alone, each of these antibodies has limited sensitivity and specificity, however, when used together, either as preformed p63/AMACR immunohistochemical cocktail, double sequential stains or stained individually but interpreted together has been found to be a more useful adjunct to confirm histological diagnoses of all adenocarcinomas and clarify equivocal cases.[31-38] Sanderson *et al*.[33] showed that p63/AMACR immunohistochemical cocktail achieves an excellent diagnostic utility of 97.2% sensitivity and 99.7% specificity when applied on core-needle biopsies for the diagnosis of prostatic adenocarcinoma. Hameed *et al*.[37] demonstrated similar sensitivity and specificity when these immunostains are used on core-needle biopsies separately but interpreted together. Therefore, combine use of these immunostains (p63 and AMACR) is very sensitive for prostatic adenocarcinoma, high-grade prostatic intraepithelial neoplasm (HGPIN) and helpful in identifying benign mimickers of prostatic adenocarcinoma on limited prostate specimens, thus, improving diagnostic accuracy and reducing misdiagnoses.

The aim of this study was to determine the diagnostic value of the use of p63 and AMACR immunostains to confirm H and E diagnosed adenocarcinomas and clarify equivocal diagnoses as well as correlate the H and E diagnoses with immunohistochemical diagnoses.

Materials and Methods

Study design

This is a 3-year retrospective study of all the cases of prostate gland core-needle biopsy received at the Anatomic and Molecular Pathology Department of Lagos University Teaching Hospital (LUTH), Lagos, Nigeria, between January 1, 2014, and December 31, 2016. The demography and biodata of the patients were retrieved from the departmental database. The H and E-stained slides and paraffin-embedded tissue blocks were retrieved from the archives. New slides were prepared in cases where the old slides have become faded. The H and E sections were reviewed, and the diagnoses classified into three categories for the purpose of this study: benign (no atypical acini/cells seen in routine section), malignant (adenocarcinoma), and equivocal cases (lesions that on routine histology could not be confidently defined as malignant or benign but considered suspicious for malignancy because of small foci of atypical cells/acini).

All prostate core-needle biopsies received at the department during the study period were included in the study. All cases where the core-needle biopsies were inadequate and the tissue blocks could not be retrieved or with insufficient clinical data (age of patient) were excluded from the study. Ethical clearance for this study was granted by LUTH's Health Research Ethics Committee.

Setting

The LUTH is a 250-bedded capacity tertiary hospital located in the densely populated (estimated population of 20 million people) and highly cosmopolitan state of Lagos, the commercial capital of Nigeria. Clienteles utilizing the LUTH facilities include inhabitants of Lagos state and inhabitants of surrounding states of the federation as well as neighboring West African countries. The Anatomic and Molecular Pathology Department of LUTH has a histopathology unit and mortuary unit. The histopathology unit is manned by 12 consultant pathologists and 14 pathology residents, a couple of medical laboratory scientists and support staff. It provides primary autopsy pathology, surgical pathology, cytopathology, immunohistochemistry (IHC) services, as well as secondary consultation of cases from other pathology centers.

Tissue preparation, immunochemical staining technique and interpretation

H and E diagnosed cases of adenocarcinomas and suspicious lesions were subjected to IHC using p63 and AMACR monoclonal antibodies to confirm the diagnosis of adenocarcinoma as well as clearly characterized the equivocal diagnoses. Immunohistochemical staining for p63 and AMACR antibodies was performed individually on two sets of slides prepared from the same tissue block. This was done because p63/AMACR antibody cocktail could not be adequately sourced during the study.

Three sets of slides per block of freshly cut (4 μm) formalin-fixed paraffin-embedded prostate core-needle biopsies tissue were mounted on super frost slides, dewaxed with graded alcohols and xylene, and gradually hydrated. One set of these slides was stained routinely with H and E for confirmation of diagnosis and determination of GS if it malignant while the remaining two sets were stained for p63 and AMACR "Biocare Medical, Walnut Creek, CA" containing p63 (clone BC4A4) and AMACR (clone AMACR) monoclonal antibodies, respectively, using standard immunohistochemical techniques. Antigen retrieval was performed by treating the slides with a 1-mmol/L concentration of EDTA (PH 8.0) for 30 min in a vegetable/rice steamer. The antibodies received from Biocare Medical were incubated for 30 min at room temperature. A biotin-free detection system was used within 15 min of incubation: DAKO (Carpentaria, CA) Cytomation–Envision + Dual link Polymer HRP. DAKO Cytomation DAB+, HRP was used as the chromogen.

The cellular localization of the monoclonal antibodies was distinct on the slides with p63 exhibiting nuclear staining while AMACR showing granular and apical cytoplasmic reactivity. The p63 and AMACR antibodies panel on H and E diagnosed prostatic carcinoma, and equivocal cases were scored by semiquantitative methods.[39] The positivity for prostatic adenocarcinoma was defined as zero nuclear stains of p63 antibody for basal cells and a strong intensity of AMACR antibody stain for luminal cells under ×10 objective. The staining patterns (scored as absent or present) of AMACR and p63 immunoreactivity were recorded for adenocarcinoma and benign acini (mimickers of prostatic adenocarcinoma). The p63 nuclear stain was recorded as positive if at least one

basal cell is identified in the acini, and the AMACR stain is also considered positive if cytoplasmic reactivity is clearly distinct and more intense than background staining and the negative control from benign prostatic acini. The intensity of AMACR staining at ×10 objective was assessed in relation to negative control and scored from 0 to 4. We classified the diagnoses into the following categories: malignant (prostate adenocarcinoma), benign prostate lesions, and indeterminate lesions where the staining was of poor quality and a definite diagnosis of adenocarcinoma or benign prostate lesion could not be given.

All statistical analyses were carried out using the Statistical Package for the Social Science (SPSS) version 16 (IBM Corp, Armonk, NY, USA). Descriptive statistics of selected categorical and numerical variables using percentages, range, mean, and standard deviation (SD) were presented in prose and tables. Chi-square tests were employed to determine whether there is any association between discrete variables. Difference between H and E diagnoses and IHC diagnoses with $P < 0.05$ was considered as statistically significant.

Results

In total, 310 prostate biopsies were received at the department during the study period. 286 (92.3%) out of the total sample received were core-needle prostate biopsies and 221 (77.3%) biopsies met the inclusion criteria for this study. The H and E diagnoses of the 221 core biopsies consisted of 113 (51.1%) cases of prostatic adenocarcinoma, 86 (38.9%) cases of benign prostate lesions, and 22 (10%) cases of equivocal lesions. The age range of the patients with prostatic adenocarcinoma was 45–93 years, with mean (±SD) age of 69.5 ± 10.2 at diagnosis with peak age incidence seen within the age range of 60–69 years (36.3%). The ages of patients with benign lesions ranged from 49 to 86 years with mean (±SD) age of 67.4 ± 8.6 and the highest incidence seen in patients with the age range of 60–69 years (37.2%). For the equivocal lesions, the ages ranged from 46 to 82 years with the mean (±SD) age of 65.2 ± 9.3 with age group 60–69 years (40.9%) as the peak age incidence. For the adenocarcinoma cohort, the ISUP/WHO 2016[20,21] grade group 5 (GS 9 and 10) was more commonly encountered (35.4%), followed by grade group 4 (GS 8) accounting for 32.7%, while the least common grade group was 2 and 3, which constituted 10 (8.8%) and 11 (9.7%) cases, respectively.

The result showed that out of 113 H and E diagnosed prostatic adenocarcinomas that were subjected to p63 and AMACR stains, 101 of them were found to be truly adenocarcinoma (true-positive rate of 89.4%) while 7 were benign prostatic lesion (false-positive rate of 6.2%) and 5 cases (4.4%) could not be categorized (indeterminate lesion) because of poor staining quality [Table 1]. The result of p63 and AMACR on the 22 cases routinely diagnosed as equivocal prostatic lesions showed that 13 (59.1%) of the cases were adenocarcinomas [Figures 1-6], 7 cases (31.8%) were benign prostate lesions [Figures 4-6], while 2 cases (9.1%) were indeterminate lesions [Table 2].

These findings of p63/AMACR immunostain results on H and E diagnosed prostate carcinoma and equivocal prostatic lesions showed a statistically significant difference on the diagnostic potential of p63/AMACR antibodies IHC when compared to H and E as a diagnostic tool ($P \leq 0.001$) [Table 3].

Discussion

Given the prevalence and lethality of prostate adenocarcinoma among males locally and globally, it is imperative that its pathological diagnosis is rendered accurately and in a timely fashion utilizing the histological and immunohistochemical techniques available at a facility. The mean age of patients diagnosed with prostatic adenocarcinoma in this study was 69.5 ± 10.2 years which is in agreement with other published studies from Nigeria that varies from 60.5 to 71.4 years.[8-15] The modal age group of 60–69 years recorded in this study

Table 1: Results of P63 and alpha-methylacyl coenzyme A racemase immunohistochemistry on 113 cases diagnosed on hematoxylin and eosin as prostatic adenocarcinoma

Immunohistochemical diagnosis (P63/AMACR)	Frequency (%)
Carcinoma	101 (89.4)
Benign	7 (6.2)
Indeterminate	5 (4.4)
Total	113 (100)

AMACR: Alpha-Methylacyl Coenzyme A Racemase

Table 2: Results of P63 and alpha-methylacyl coenzyme A racemase immunohistochemistry on cases diagnosed on hematoxylin and eosin as suspicious for adenocarcinoma/equivocal prostatic lesions

P63/AMACR	Frequency (%)
Carcinoma	13 (59.1)
Benign	7 (31.8)
Indeterminate	2 (9.1)
Total	22 (100)

Table 3: Comparison between hematoxylin and eosin diagnoses and P63/methylacyl coenzyme A racemase immunohistochemical diagnoses

H&E	P63/AMACR			Total (%)
	Carcinoma	Benign	Indeterminate	
Carcinoma	101 (89.4)	7 (6.2)	5 (4.4)	113 (100)
Suspicious Cases	13 (59.1)	7 (31.8)	2 (9.1)	22 (100)
Total	114 (84.4)	14 (10.4)	7 (5.2)	135 (100)
χ^2, P	14.4, 0.001*			

AMACR: Alpha-Methylacyl Coenzyme A Racemase, H&E: Hematoxylin and Eosin

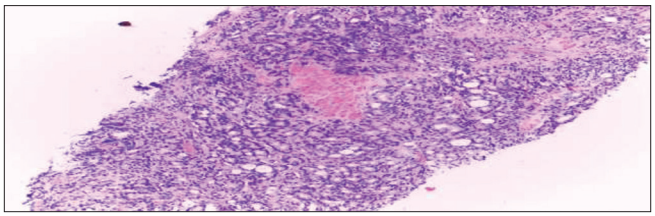

Figure 1: Photomicrograph of core-needle biopsy (H and E) showing prostatic carcinoma, Gleason score 4 + 4 (ISUP/WHO Grade Group 4) (H and E, ×40)

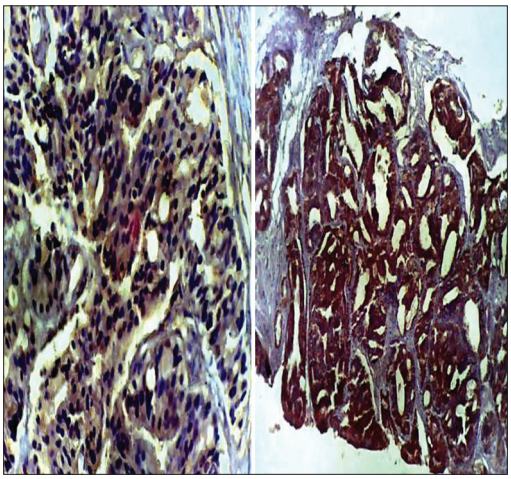

Figure 3: Photomicrograph of p63 negativity and AMACR positivity of core-needle biopsies placed side by side showing prostatic carcinoma. Sections taken from the same block showing same field views, ×40

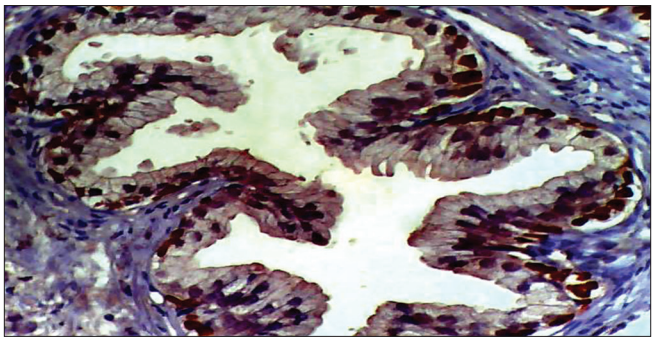

Figure 5: Photomicrograph of core-needle biopsy showing basal cells p63 immunostain (nuclear) positivity of benign prostatic lesion, ×100

is similar to the documented report in Ibadan by Ogunbiyi and Shittu.[10] However, this is slightly lower than that reported by the Surveillance, Epidemiology and End Result Program (SEER) of National Cancer Institute, USA, wherein a modal age group of 65–74 was found.[16] This slight difference could be attributed to the fact that SEER's study population had a mixture of different races while the index study population was entirely Africans.

High-grade prostate adenocarcinoma, Gleason grade group 5 (GS 9 and 10), was the most common grade recorded in this study (68.1%). This confirms the findings of earlier study in this center where high-grade prostate adenocarcinoma was

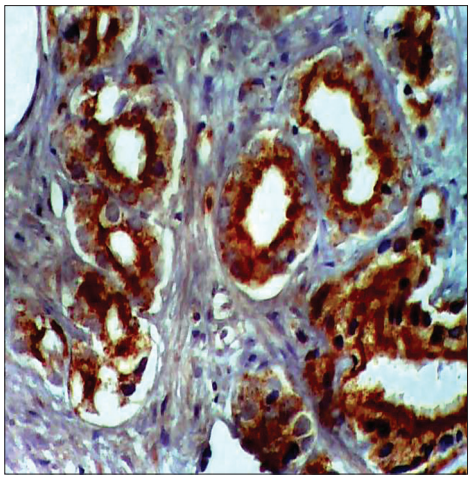

Figure 2: Photomicrograph of core-needle biopsy of prostatic adenocarcinoma showing luminal cells AMACR immunostain (luminal) positivity, ×100

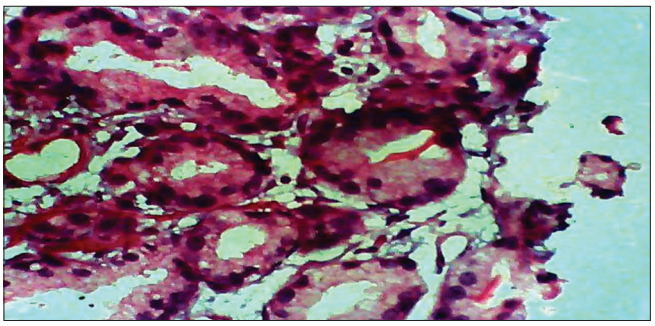

Figure 4: Suspicious case of prostatic core-needle biopsy showing atypical adenomatous hyperplasia (adenosis) (H and E, ×40)

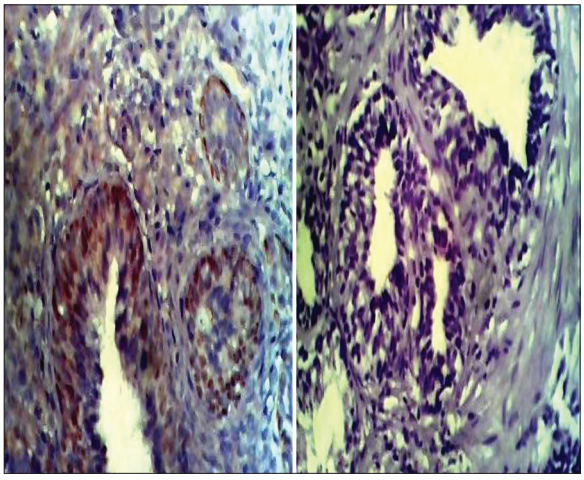

Figure 6: H and E equivocal case of prostatic core-needle biopsy demonstrated by P63/AMACR as showing basal cell hyperplasia. The photomicrograph on the left shows strong p63 positive immunostain for the basal cells and on the right shows AMACR negative immunostain for the luminal cells, ×40

in the majority (40%)[13] and is in consonance with a report from Zaria where they represented 49.5%.[14] In contrast, the least common low-grade cancers (13.3%) in our study were

the most common grade reported by Mohammed *et al*.[9] in Kano (64.2%), Akang *et al*.[15] in Benin (64%), Nigeria and Magoha[40] in Nairobi and Kenya (49.2%). We are unsure if these variations in the GS/grades are representative of the intrinsic biologic features of the cancers in these regions across the country. In a review article, Humphrey *et al*.[17] stated that undergrading which occurs in as much as 42% of cases is the most common error in assessing GS, especially in core-needle biopsies, whereas overgrading occurs in a mean of about 15% of cases. He surmised that errors and discrepancies in the grading of needle core biopsies could arise from the difficulty in appreciating an infiltrative growth pattern, tissue distortion, pathologist experience, and intra- and interobserver variability.

The thrust of this study was to assess the diagnostic value of adjunctive use of immunostains in confirming H and E diagnosed prostate adenocarcinoma and clarify suspicious cases. We demonstrated a statistically significant difference in our ability to confidently diagnose an overt prostate adenocarcinoma or a suspicious one when double immunostains of p63 and AMACR are used together with H and E sections versus only H and E sections ($P \leq 0.001$) [Table 3]. With the input of p63 and AMACR immunostains, >6% of the originally routinely diagnosed prostatic carcinomas were found to be benign, and this gives credence to the objective of using p63 and AMACR immunostains to confirm H and E diagnosis of adenocarcinoma on core-needle biopsies. Our finding is in tandem with that of Vikram *et al*.[34] who showed a statistically significant change when H and E diagnosed prostatic carcinoma, and suspicious cases were exposed to p63 and AMACR IHC ($P = 0.013$). Although the high true-positive rate of H and E diagnosis of prostatic adenocarcinoma (89.4%) in our center is testament to the morphological diagnostic acumen of our pathologists, we can only rue the many negative ways overtreated patients whose appropriate diagnoses fell through the cracks as a result of absent use of these stains have been affected.

The size of the value of these immunostains was more profound when 22 H and E diagnosed equivocal prostatic lesions were subjected for confirmatory diagnosis. The exact pathology was delineated in 90.9% of the cases with 31.8% (7 cases) of them confirmed as benign [Figures 4-6] and 59.1% (13 cases) confirmed as prostatic adenocarcinoma [Figures 2 and 3]. These findings authenticate those of Vikram *et al*.,[34] Jiang *et al*.,[31] and Naoto[36] who proved the usefulness of p63 and AMACR immunostains in deciding the final diagnosis of atypical gland foci in small prostatic needle biopsy specimens. Vikram *et al*.[34] showed that when 40 named H and E equivocal cases of prostate core-needle biopsy were subjected to p63 and AMACR immunostains, the diagnoses changed in 32.5% (13) of the cases. In 11 cases, the diagnoses changed from benign to malignant, 1 case from benign to HGPIN, while in another 1 case, it changed from malignant to benign.

Although urologic surgery practices vary within and outside the country in terms of clinical approaches to pathology reports of equivocal prostate needle biopsies deemed suspicious for malignancy, given our results, it is obvious that first time use of these stains will reduce incidences of multiple clinical follow-up visits/surveillance or rebiopsies and enable the early institution of specific management strategy.

With major efforts in the push for mass screening of men for the detection of early prostate cancer characterized by small foci of cancer on prostate needle biopsy specimens, it is pertinent not to miss these.[23,28] We contend that although this is challenging to a surgical pathologist, the combine use of negative and positive immunostains with high sensitivity and specificity for the detection of small foci of prostate carcinoma together with H and E slides will help clarify diagnoses on limited prostate specimens in this preclinical setting.

The limitation of this study includes that initial poor fixation of prostate tissue could cause distortion of nuclear and cytoplasmic details of prostatic acini cells. Furthermore, poor storage of tissue blocks or damage may affect the staining pattern of AMACR and p63 monoclonal antibodies. These could result in false-negative and false-positive antigen-antibody reactions.

Conclusion

We conclude that although histopathological examination of H and E sections remains the gold standard in the diagnosis of prostatic adenocarcinoma, the adjunctive use of p63 and AMACR immunostains are of great value in confirming small foci of adenocarcinoma, resolving morphologically equivocal cases and excluding benign mimickers as confounder in the diagnosis of prostatic adenocarcinoma in small prostate samples obtained by core-needle biopsy. Thus, we recommend their use to ascertain the exact nature of pathology on difficult small prostate samples obtained by core-needle biopsy to minimize the impacts of under and overtreatment of patients.

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Conflicts of interest

There are no conflicts of interest.

References

1. Miller DC, Hafez KS, Stewart A, Montie JE, Wei JT. Prostate carcinoma presentation, diagnosis, and staging: An update form the National Cancer Data Base. Cancer 2003;98:1169-71.
2. Abdulkareem F. Epidemiology and incidence of common cancers in Nigeria. Cancer Reg Epidemiol Workshop 2009;1:33-6.
3. Villers A, McNeal J, Freiha F, Stamey T. Multiple cancers in the prostate. Morphologic features of clinically recognised versus incidental tumors. Cancer 1992;70:2313-8.
4. Epstein JI. Prostate. In: Kumar V, Abbas AK, Aster JC, editors. Robbins and Cotran Pathologic Basis of Diseases. 9th ed. Philadephia: Elsevier Saunder; 2015. p. 1860-934.
5. Bostwick DG, Burke HB, Djakiew D, Euling S, Ho SM, Landolph J, *et al*. Human prostate cancer risk factors. Cancer 2004;101:2371-490.
6. Fleshner N, Bagnell PS, Klotz L, Venkateswaran V. Dietary fat and

prostate cancer. J Urol 2004;171:S19-24.
7. Gao X, LaValley MP, Tucker KL. Prospective studies of dairy product and calcium intakes and prostate cancer risk: A meta-analysis. J Natl Cancer Inst 2005;97:1768-77.
8. Ekwere PD, Egbe SN. The changing pattern of prostate cancer in Nigerians: Current status in the southeastern states. J Natl Med Assoc 2002;94:619-27.
9. Mohammed AZ, Alhassan SU, Edino ST, Ochicha O. Histopathological review of prostatic diseases in Kano, Nigeria. Niger Postgrad Med J 2003;10:1-5.
10. Ogunbiyi JO, Shittu OB. Increased incidence of prostate cancer in Nigerians. J Natl Med Assoc 1999;91:159-64.
11. Obiorah CC, Nwosu SO. A histopathological study of carcinoma of the prostate in Port Harcourt, Nigeria. Niger J Clin Pract 2011;14:363-8.
12. Yawe KT, Tahir MB, Nggada HA. Prostate cancer in Maiduguri. West Afr J Med 2006;25:298-300.
13. Anunobi CC, Akinde OR, Elesha SO, Daramola AO, Tijani KH, Ojewola RW. Prostate diseases in Lagos, Nigeria: A histologic study with tPSA correlation. Niger Postgrad Med J 2011;18:98-104.
14. Dawam D, Rafindadi AH, Kalayi GD. Benign prostatic hyperplasia and prostate carcinoma in native Africans. BJU Int 2000;85:1074-7.
15. Akang EE, Aligbe JU, Olise EG. Prostate tumours in Benin City, Nigeria. West Afr J Med 1996;15:56-60.
16. Ineke Neutel C, Gao RN, Blood PA, Gaudette LA. The changing age distribution of prostate cancer in Canada. Canada J Public Health 2007;98:60-4.
17. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO classification of tumours of the urinary system and male genital organs-part B: Prostate and bladder tumours. Eur Urol 2016;70:106-19.
18. Humphrey PA. Gleason grading and prognostic factors in carcinoma of the prostate. Mod Pathol 2004;17:292-306.
19. Sweat SD, Bergstralh EJ, Slezak J, Blute ML, Zincke H. Competing risk analysis after radical prostatectomy for clinically nonmetastatic prostate adenocarcinoma according to clinical Gleason score and patient age. J Urol 2002;168:525-9.
20. Epstein JI. New prostate cancer grade group system correlates with prostate cancer death in addition to biochemical recurrence. Br J Cancer 2016;114:1069-70.
21. Epstein JI, Zelefsky MJ, Sjoberg DD, Nelson JB, Egevad L, Magi-Galluzzi C, *et al*. A contemporary prostate cancer grading system: A validated alternative to the Gleason score. Eur Urol 2016;69:428-35.
22. Berney DM, Montironi R, Egevad L. Pathology in prostate research: Optimizing the pathological data. Acta Oncol 2011;50 Suppl 1:49-52.
23. Epstein JI, Potter SR. The pathological interpretation and significance of prostate needle biopsy findings: Implications and current controversies. J Urol 2001;166:402-10.
24. Lane RB Jr., Lane CG, Mangold KA, Johnson MH, Allsbrook Jr. Needle biopsies of the prostate: What constitutes adequate histologic samples? Arch Pathol Lab Med 1998;51:749-57.
25. Dogan HS, Aytac B, Kordan Y, Gasanov F, Yavascaoglu İ. What is the adequacy of biopsies for prostate sampling? Urol Oncol 2011;29:280-3.
26. Iczkowski KA, Casella G, Sappala RJ, Jones GL, Mishler BA, Qian J, *et al*. Needle core length in sextant biopsies influences prostate cancer detection rate. Urology 2002;59:698-703.
27. Epstein JI, Walsh PC, Sauvageot J, Carter HB. Utility of repeat sextant and transition biopsies for assessing extent of prostate cancer. J Urol 2009;158:1886-90.
28. Epstein JI, Steinberg GD. The significance of low-grade prostate cancer on needle biopsy. A radical prostatectomy study of tumor grade, volume, and stage of the biopsied and multifocal tumor. Cancer 1990;66:1927-32.
29. Xu Y, Wang Y, Zhou R, Li H, Cheng H, Wang Z, *et al*. The benign mimickers of prostatic acinar adenocarcinoma. Chin J Cancer Res 2016;28:72-9.
30. Esserman LJ, Thompson IM, Reid B, Nelson P, Ransohoff DF, Welch HG, *et al*. Addressing over diagnosis and overtreatment in cancer; A prescription for change. Lancet Oncol 2014;15:234-42.
31. Jiang Z, Wu CL, Wada BA, Dresser K, Xu J, Fanger GR, *et al*. P504S/alpha-methylacyl-CoA racemase: A useful marker for diagnosis of small foci of prostate carcinoma on needle biopsy. Am J Surg. Pathol 2002;26:1169-74.
32. Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, *et al*. α-Methlacyl CoA racemase; A new molecular marker for prostate cancer. Cancer Res 2002;62:2220-6.
33. Sanderson SO, Sebo TJ, Murphy LM, Neumann R, Slezak J, Cheville JC. An analysis of the p63/α-Methylacyl coenzyme a racemase immunohistochemical cocktail stain in prostate needle biopsy specimens and tissue microarrays. Am J Clin Pathol 2004;121:220-5.
34. Vikram S, Manu V, Ajay M, Dutta V, Mani NS, Patrikar S. Diagnostic utility of p63 and AMACR in resolving suspicious foci in prostatic needle biopsy and transurethral resection of prostate specimen. J Can Res Ther 2014;10:686-92.
35. Dabir PD, Ottosen P, Høyer S, Hamilton-Dutoit S. Comparative analysis of three- and two-antibody cocktails to AMACR and basal cell markers for the immunohistochemical diagnosis of prostate carcinoma. Diagn Pathol 2012;7:81.
36. Naoto K. Application of combined immunohistochemical panel of AMACR/p63 cocktail, cytokeratin 5 and D2-40 to atypical glands in prostate needle biopsy. Malaysian J Pathol 2014;36:169-73.
37. Hameed O, Sublett J, Humphrey PA. Immunohistochemical stains for p63 and alpha methylacyl-Coa racemase, versus a cocktail comprising both, in the diagnosis of protatic carcinoma in core needle biopsy tissues. Am J Surg Pathol 2005;29:579-87.
38. Trpkov K, Bartczak-McKay J, Yilmaz A. Usefulness of cytokeratin 5/6 and AMACR applied as double sequential immunostains for diagnostic assessment of problematic prostate specimens. Am J Clin Pathol 2009;132:211-20.
39. Xu J, Stolk JA, Zhang X, Silva SJ, Houghton RL, Matsumura M, *et al*. Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. Cancer Res 2000;60:1677-82.
40. Magoha GA. Management and survival in advance prostate cancer in Nairobi. East Afr Med J 2000;77:260-3.