

**EFFICACY OF IMMUNOHISTOCHEMISTRY IN PROSTATE NEEDLE BIOPSIES**Tameem Afroz<sup>1</sup>, Sistla Radha<sup>2</sup><sup>1</sup>Senior Consultant, Department of Anatomical Pathology and Cytology, Global Hospitals, L. B. Nagar, Hyderabad, Telangana.<sup>2</sup>Consultant and HOD, Department of Anatomical Pathology and Cytology, Global Hospitals, L. B. Nagar, Hyderabad, Telangana.**ABSTRACT****BACKGROUND**

Prostate needle biopsies can pose a major diagnostic challenge when it comes to differentiating adenocarcinoma and its variants from its benign mimics. In needle biopsies, when the suspicious focus is small, morphological features may not suffice to differentiate it from its morphologic mimics like atrophy, basal cell hyperplasia, reactive inflammatory changes, seminal vesicles and adenosis. Immunohistochemical marker for basal cells, p63 and prostate cancer specific marker, Alpha-Methylacyl-CoA Racemase (AMACR) help in overcoming such diagnostic dilemmas.

**MATERIALS AND METHODS**

We analysed 157 prostate core needle biopsies over a period of 2 years. Routine Hematoxylin and Eosin (H and E) sections and immunohistochemical markers for basal cells (p63) and prostate cancer specific marker (AMACR) were used. Prospective study was done on prostate needle core biopsies. Biopsy was done under ultrasound guidance with an 18-gauge needle. Biopsy was done in patients with raised serum PSA levels for exclusion of prostate carcinoma.

**RESULTS**

Over a period of two years, 157 prostate core needle biopsies were studied. 83 were benign lesions comprising 69 benign prostatic hyperplasias, five basal cell hyperplasias, four granulomatous lesions and three showed atrophic changes. Two biopsies morphologically resembled seminal vesicles. Prostate cancer specific marker, AMACR was negative in all, but two lesions. In these two lesions, it showed weak nonspecific staining. Basal cell marker p63 showed a continuous staining pattern highlighting the basal cells in all the 69 cases of benign prostatic hyperplasia, 5 cases of basal hyperplasia showed positivity in all the hyperplastic basal cells. In the two cases of seminal vesicles, it showed intense basal cell positivity. It showed a discontinuous pattern in two of the four granulomatous lesions and showed a weak, but a continuous staining pattern in the atrophic lesions. 74 were adenocarcinomas; the predominant Gleasons grade was (3+3). AMACR showed a sensitivity of 93% and a specificity of 97%. It had a positive predictive value of 0.97 and a negative predictive value of 0.94. Basal cell marker, p63 showed absent staining in all the 74 cases.

**CONCLUSIONS**

With the advent of prostate specific antigen serum screening and routine use of transrectal ultrasonography, there is a manifold increase in early detection of prostate adenocarcinomas. 18-gauge needle prostate biopsy under transrectal ultrasound guidance is a preferred method for detection of adenocarcinoma because it is associated with low morbidity and it provides information regarding the grade and extent of carcinoma. However, prostate adenocarcinoma has a number of morphological mimics with various architectural patterns. Immunohistochemistry plays a major role in overcoming diagnostic dilemmas encountered due to the presence of morphological and cytological equivocal features in small volume biopsies. To conclude, in morphologically equivocal glandular architectural patterns and cytological features, combination of immunostains highlighting the basal cells and prostate cancer associated marker can help the pathologist to arrive to a diagnosis on the limited amount of tissue available at his disposal. However, applications of both the immunostains have their inherent limitations.

**KEYWORDS**

Prostate Adenocarcinoma, p63, AMACR.

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**BACKGROUND**

Worldwide, prostate cancer is the second most common malignancy in men after lung cancer.<sup>1</sup> With advent of Prostate Specific Antigen (PSA) screening; there is a manifold increase in the number of prostate biopsies being performed. Prostate needle biopsy has a low morbidity, but can present a diagnostic challenge in view of the small volume of tissue submitted for histologic examination. Prostate adenocarcinoma has a great number of mimics ranging from its variants, benign lesions as well as normal

histoanatomic structures. Absence of basal cells is an important criterion for diagnosis of prostate adenocarcinoma. Basal cell markers like p63 and High Molecular Weight Cytokeratin (34 $\beta$ E12) are the commonly used to highlight the basal cells. p63 is a homologue of the p53 tumour suppressor gene.<sup>2</sup> It has a selective expression in the basal cell compartment of various epithelial tissues and is sensitive in identifying the nuclei of basal cells in benign prostatic lesions.<sup>3,4</sup> 34 $\beta$ E12 is a high molecular weight cytokeratin expressed in the cytoplasm of basal cells.<sup>5</sup> p63 being a nuclear stain is a more reliable tool in highlighting basal cells compared to 34 $\beta$ E12, which is a cytoplasmic stain.<sup>3</sup> Prostate cancer specific marker (AMACR) is a protein whose expression is increased in prostatic adenocarcinoma.<sup>6</sup>

Its gene is located on 5p13 and the gene product resides in peroxisomes and mitochondria.<sup>7</sup>

The objective of this study was to assess the utility of basal cell marker (p63) and prostate cancer specific marker (AMACR) in distinguishing prostate adenocarcinoma and its variants from its benign mimics on prostate needle core biopsies. This study is one of the very few studies available in Indian literature evaluating usefulness of both p63 and AMACR on prostate biopsies.

### Aims and Objectives

To analyse the efficacy of immunohistochemistry using a basal cell marker and a cancer specific marker in limited volumes prostate carcinomas.

To differentiate the benign mimics from prostate adenocarcinoma.

### MATERIALS AND METHODS

Prospective study was carried out on prostate needle core biopsies received over a period of two years from 2008-2010. Formalin fixed, paraffin embedded sections were stained by routine Haematoxylin and Eosin (H and E) stain and immunohistochemistry was done using commercially available primary antibodies of p63 and AMACR using modified labelled streptavidin biotin technique with 3-diaminobenzidine tetrahydrochloride as chromogen. Counter staining was done with haematoxylin. Evaluation of immunohistochemical stains was done in conjunction with the morphology. For basal cell stains, benign glands served as inbuilt internal controls by highlighting the circumferential nuclear staining of the basal cells. Positive immunohistochemical staining was defined as nuclear reactivity for p63. AMACR was interpreted as positive for carcinoma when it showed a circumferential, strong and cytoplasmic granular quality.<sup>8</sup> Biopsies with diffuse background staining throughout were excluded from the study. Staining for basal cells as well as AMACR was interpreted following recommended guidelines.<sup>9</sup>

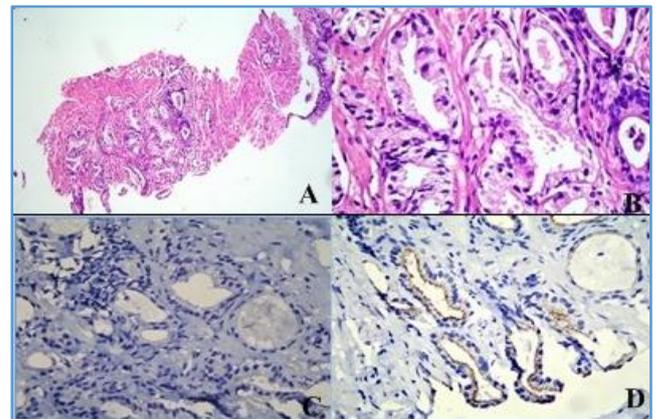
### RESULTS

A total of 157 prostate core needle biopsies were analysed over a period of 2 years. Biopsies were stained using routine H and E stains and immunohistochemistry was done using a

basal cell marker, p63 and a prostate cancer specific marker, AMACR.

Out of the 157 biopsies, 83 were benign lesions comprising 69 benign prostatic hyperplasias, five basal cell hyperplasias, four granulomatous lesions and three showed atrophic changes. Two biopsies morphologically resembled seminal vesicles. AMACR was negative in all, but two lesions. In two lesions, it showed weak nonspecific staining. This staining pattern was interpreted as negative as it did not conform to the strict criteria used for interpretation of positive staining. P63 showed a continuous nuclear staining pattern in all benign prostatic hyperplasias, it showed positivity of all the basal cells on basal cell hyperplasias in two cases of granulomatous lesions, it showed a discontinuous staining pattern. In all the three cases of atrophy, it showed a continuous, but weak staining pattern.

74 were adenocarcinomas; the commonest Gleasons grade was (3+3). AMACR showed strong cytoplasmic positivity in 69 cases, five cases were negative. P63 showed loss of basal staining pattern in all the 74 cases (Figure 1). Six cases of adenocarcinoma were associated with high-grade prostatic intraepithelial neoplasia (HG-PIN). AMACR was positive in four such foci (Figure 2). There was no difference in the staining pattern or intensity of AMACR in such foci. P63 showed a continuous staining pattern demonstrating the intactness of the basal layer in all these 6 cases.



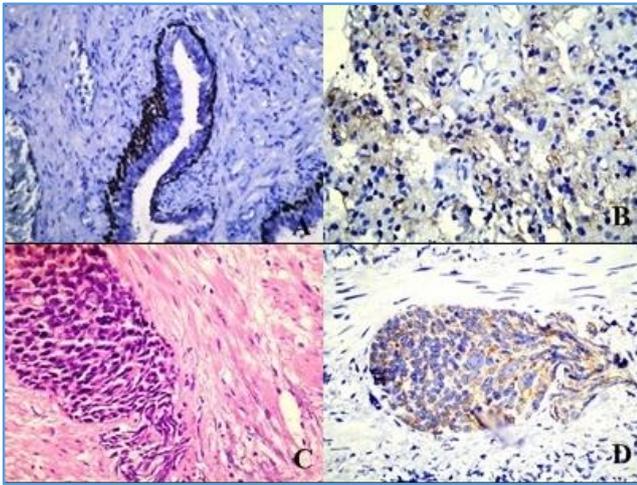
**Figure 1A, B, C, D**

**Figure 1A:** Needle core biopsy showing a focus of adenocarcinoma (H and E x40).

**Figure 1B:** Same focus of adenocarcinoma showing nuclear details (H and E x400).

**Figure 1C:** Absence of p63 staining of basal cells in the same focus (x400).

**Figure 1D:** Cytoplasmic granular positivity for AMACR in tumour cells (x400).



**Figure 2A, B, C, D**

**Figure 2A:** Strong nuclear positivity in basal cells of p63 in a benign gland (x400).

**Figure 2B:** AMACR staining pattern in a focus of adenocarcinoma (x400).

**Figure 2C:** Focus of High-Grade Prostatic Intraepithelial Neoplasia (H and E x400).

**Figure 2D:** High-Grade Prostatic Intraepithelial Neoplasia with AMACR staining (x400).

## DISCUSSION

Diagnosis of small foci of prostate cancer (<5% of the biopsy core) and distinguishing these foci from benign mimics is one of the greatest challenges in surgical pathology practice.

Although, diagnosis can be made based on histomorphological features such as architectural growth patterns, nuclear atypia and absence of basal cells morphology when used alone is not entirely sensitive or specific to establish a definitive diagnosis of prostate cancer.

Widespread use of serum PSA screening and manifold increase in use of 18-gauge needle biopsy has led to a large increase in prostate core biopsies in routine surgical pathology practice. In such biopsies, interpretation of small foci of atypical glands can be extremely challenging. Immunohistochemistry with basal cell marker and prostate cancer specific markers are potent tools in the hands of surgical pathologist to overcome such diagnostic dilemmas.

Basal cell marker p63 is a homologue of the tumour suppressor gene, p53.<sup>10</sup> It is expressed in the basal cells of epithelium in a variety of human tissue.<sup>3</sup> A study by Parsons et al, which included needle biopsies as well as prostatectomy specimens has shown that immunohistochemical staining for p63 selectively labels the basal cells in prostate. In our study, p63 was used as a basal cell marker. P63 staining was negative in morphologically suspicious foci. In the eighty three lesions classified as benign, p63 showed a continuous staining pattern outlining the basal cells. It showed a weak, but continuous staining pattern in three cases of atrophy and a discontinuous staining pattern in 4 cases of granulomatous lesions. This demonstrated that p63 has a high sensitivity in outlining the basal cells in benign lesions effectively preventing an erroneous diagnosis of malignancy (false positives) in needle

core biopsies of prostate. P63 alone may not clear all the suspicion, which was raised as demonstrated by the discontinuous staining pattern in the granulomatous lesions as well as the weak staining pattern in atrophy, which might be difficult to interpret. Atrophy might itself present with architectural distortion. In addition, atrophy post radiation might present with cytological atypia also.

Prostate cancer associated marker AMACR (P504S) gene was identified by combination of complementary DNA subtraction and high through output microarray overexpressed by malignant glands. It is not expressed by benign glands.<sup>9</sup> The gene product is a protein whose activity is increased in prostatic adenocarcinoma.<sup>11</sup> Its overexpression is linked to prostate cancer irrespective of the grade or the stage of the cancer.<sup>12</sup> In our study, AMACR staining was interpreted according to the recommended guidelines.<sup>9</sup> AMACR showed a strong circumferential granular quality of staining in 69 of the 74 cases of adenocarcinoma. Five cases were negative. In all the 74 cases of carcinoma, p63 was negative.

Six of the seventy four cases of carcinoma, in addition had high-grade PIN changes. AMACR was positive in four such cases. P63 showed continuous staining pattern highlighting the intactness of the basal layer in all the six cases.

Out of the benign lesions, all but two cases were negative for AMACR staining.

In our study, AMACR showed a sensitivity of 93% and a specificity of 97%. Negative predictive value was 0.94 and positive predictive value was 0.97. This is in comparison with a sensitivity of 97% and a specificity of 92% in literature.<sup>13</sup>

A note of caution in application of AMACR is in the differential diagnosis of tumours, which clinically present with bladder outlet obstruction. 31% of bladder carcinomas per se express AMACR reactivity.<sup>14</sup>

Similarly, prostate carcinomas, which are subjected to hormonal manipulation may show a loss of reactivity. Up to 29% of such tumours may lose their AMACR reactivity.<sup>15</sup>

Using AMACR as a positive marker for identification of prostate adenocarcinoma alone or p63 alone to identify the presence of basal cells may not suffice because AMACR and p63 stains both have their inherent advantages and disadvantages. Hence, a combination of these markers is recommended in morphologically and cytologically equivocal lesions.

## CONCLUSIONS

Prostate core biopsies with suspicious foci on morphology are a diagnostic dilemma. Morphology with cytological features and architectural pattern along with a combination of basal stain (p63) highlighting the intactness of the basal layer and prostate cancer associated marker (AMACR) can help the surgical pathologist in overcoming such diagnostic dilemmas. To conclude, in morphologically equivocal glandular architectural patterns and cytological features, combination of immunostains highlighting the basal cells and prostate cancer associated marker can help the pathologist to arrive to a diagnosis on the limited amount of tissue

available at his disposal rather than submitting the patient to a new series of biopsies.

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