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ORIGINAL ARTICLE

Immunohistochemical Evaluation of p63 and AMACR in Prostatic Lesions

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ABSTRACT

Background: Prostate cancer progression is relatively indolent and may be asymptomatic. The use of IHC markers may establish the diagnosis of carcinoma in a histo-morphologically suspicious focus of prostate gland.

Material & Method: Histologically diagnosed non neoplastic and neoplastic prostatic lesions were evaluated, and detailed histopathological parameters including Gleason's Grade Groups, the presence of cribriform architecture in Gleason's pattern 4, perineural invasion, and lymphovascular invasion were assessed. In addition, immunohistochemical expression of AMACR and p63 were recorded to correlate their diagnostic utility with the morphological features.

Results: Out of a total of 130 prostatic cases studied, 81 were benign and 35 were malignant. All benign cases demonstrated no AMACR expression in the epithelial cells, while showing 100% nuclear positivity for p63 in the basal cells. Among the 35 malignant cases, 30 (85.7%) exhibited positivity for AMACR immunostaining, with a statistically significant p-value of 0.00. A cribriform pattern corresponding to Gleason's pattern 4 was observed in 12 malignant cases, of which 11 (92%) showed AMACR positivity (p-value 0.003). A 87.5% (14/16) cases of Perineural expressed AMACR positivity (p-value 0.002). Out of 14 cases initially categorized as suspicious for carcinoma, 11 (79%) were proved prostatic adenocarcinoma following immunohistochemical evaluation, while 2 cases (14.2%) demonstrated positive immunostaining for both AMACR and p63.

Conclusion: The dual IHC staining approach allow a comprehensive analysis of both structural and molecular markers & improves diagnostic accuracy where histomorphology alone may be ambiguous & provides clarity where distinction of non-neoplastic from malignant lesions is challenging.

KEYWORDS: *AMACR, Benign Prostatic Hyperplasia, Prostatic Adenocarcinoma, p63.*

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INTRODUCTION

Prostate carcinoma is 2nd most frequent malignant neoplasm in males.[1] Prostate cancer progression is relatively indolent and can cause no health problem. Active survillence by Digital rectal examination or elevated serum PSA may primarily raise a suspicion of prostate cancer. Further advanced diagnostic techniques like ultrasound and imaging-guided biopsy with histopathological correlation may help to prove malignancy.[2] In prostate biopsy, cancer detection can be particularly challenging when the malignant focus is minute or histomorphological mimicker of malignancy make the interpretation difficult to resolve. Accurate recognition of architectural and cytological features of malignant glands requires a methodical and systematic approach. In such situations, histopathology in adjunct with immunohistochemistry can serve as a beneficial tool to confirm or exclude malignancy. By employing specific markers, pathologists are able to differentiate between benign mimickers of cancer, thereby improving diagnostic precision and reducing uncertainty in suspicious cases.[2]

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IHC markers may establish the diagnosis of carcinoma in a histo-morphologically atypical/ suspicious small focus of prostatic lesion. IHC expression for AMACR (α-Methylacyl-CoA racemase) enzyme implicated in beta oxidation of branched chain fatty acid, expressed in malignant prostate luminal cells but not in non-neoplastic prostatic epithelium. A transcription factor belonging to the p53 gene family is tumour protein 63 (p63). It is essential for normal stem cell function and has control over development and growth of prostate gland. In the prostate gland, the nuclear p63 IHC is expressed in basal cells and is absent in luminal cells. Using a cocktail of these IHC markers; a negative nuclear p63 in

basal cells and a positive cytoplasmic AMACR in luminal cells strongly suggest a diagnosis of prostatic carcinoma.[2]

Aim of this study is to evaluate and compare the expression of AMACR and p63 in prostatic lesions and to assess the utility of aforementioned markers in clarifying the suspicious cases.

Material and Method

This study was conducted in pathology department of L.N. Medical College Bhopal. Prostatic tissue specimen (TURP & Core Needle Biopsies) diagnosed with various prostatic lesions during the study period were included. Prostate cancer with prior Chemotherapy, Radiotherapy & all cases where core-needle biopsies will be inadequate (extensive necrosis, crushing or cautery artifacts) were excluded from study.

The sample size was 130 prostatic cases & for each case, relevant clinical data, laboratory investigations, serum PSA levels, and ultrasonography findings were documented in a structured proforma. In prospective cases, specimens were processed paraffin blocks were prepared, while in retrospective cases, paraffin blocks were retrieved from the archives. Sections were cut and stained with hematoxylin and eosin, following which a histopathological diagnosis was established. For the purpose of this study, cases were categoried as benign, suspicious & malignant.[3] All cases were subsequently subjected to IHC for p63 and AMACR.

Methodology of IHC

Immunohistochemical analysis was performed using p63 and AMACR antibodies with appropiriate positive and negative controls. BioGenex Super SensitiveTM Polymer HRP Detection System (a non – streptavidin – biotin proprietary micropolymer – Complex technology) was used.

Thin paraffin sections were placed on Poly-L-Lysine (Sigma chemical Co, USA) coated slides. After overnight incubation at 37 degrees Celsius dewaxing with xylene, 3 changes for 10 minutes each. Rehydrate the section through graded alcohol series and then rinsed with water for 15 minutes. For Antigen retrieval, slides were inserted in a slide holder containing antigen retrieval solution and place them into microwave for 2 cycles. An antigen retrieval system, BIOGENEX-EZ-Retriever System V3 (microwave), with Antigen Retrieval Solution, EZ-AR2 (HK522-XAK) QUARTETT was used at retrieval pH 9.0. Two cycles in microwave temperature at 80-850C for 5 minutes & 600C for 15 minutes were run. Slides were cooled for 20 minutes at room temperature & washed with buffer 3 times (pH 7.2-7.4). Peroxidase block (100 µl) done for 10 minutes at room temperature then Buffer wash for 3 times. Power block (100 µl) done 10 minutes at room temperature followed by Buffer wash, 3 times. Primary Antibody incubation (100 µl) done for 1-2 hr at room temperature. {Rabbit monoclonal AMACR (anti-P504S) ready to use. (Clone: 13H4, IgG class) from BioGenex. Fremont CA}followed by Buffer wash 3 times. Secondary Antibody (BioGenex) applied for 30 minutes. Followed by Buffer wash. DAB solution (100 µl) applied for 10-15 minutes. Wash in running tap water, Hematoxylin counter stain (100 µl) for 2-3 minutes, 1-2 dip in acid alcohol. Dehydration in 70 to 90% to absolute alcohol for 5 minutes each was done. For positive control use normal kidney tissue was used for AMACR where epithelial cells of proximal tubules showed strong, distinct granular staining (figure 4a).

Interpretation of IHC for p63: Visualization of brown color in Nucleus is considered immunostain positive. The p63 nuclear stain was recorded as positive if at least one basal cell will be identified in the acini.[4]

Interpretation of IHC for AMACR: A cytoplasmic or luminal brown staining is circumferential, dark, diffuse, or granular was considered positive. Absent to focal weak non-circumferential fine granular staining was interpreted as negative. The percentage positivity of 1-10%, 11-50% & >51% was graded 1+, 2+ & 3+ respectively.5 Statistical analysis was done & data was expressed as percentages and Chi square $\chi 2$ test was applied for comparing qualitative variables. p-value was obtained and was considered significant if ≤ 0.05.

RESULT

A total of 130 prostatic cases 81 cases were benign while 35 cases were malignant. All malignant cases were prostate adenocarcinomas with mean age of 66.0 years (ranging from 40 to 90).

Majority of the patient belongs to age group of 61-70 years (41.5%), followed by 71-80 years (26.2%). Most of the benign and malignant lesions were common in 7th decade of life comprising 36 (44.4%) and 12 (34.3%) of total cases respectively.

As with increasing age number of malignant cases increases in compare to benign cases. In the age group 81-90 yrs, 83.3% cases are malignant and only 16.6% cases are benign. There was no suspicious case detected in this age group.

All benign cases were of Bening Prostate Hyperplasia showed no AMACR positive p63 positive expression in the basal cells. Out of 35 malignant cases, 30 cases (85.7%) showed positivity for AMACR immunostaining while all were negativity for p63 immunostaining. AMACR was significantly expressed in malignant prostatic cases. (p-value 0.00). (Table 1, Figure 1)

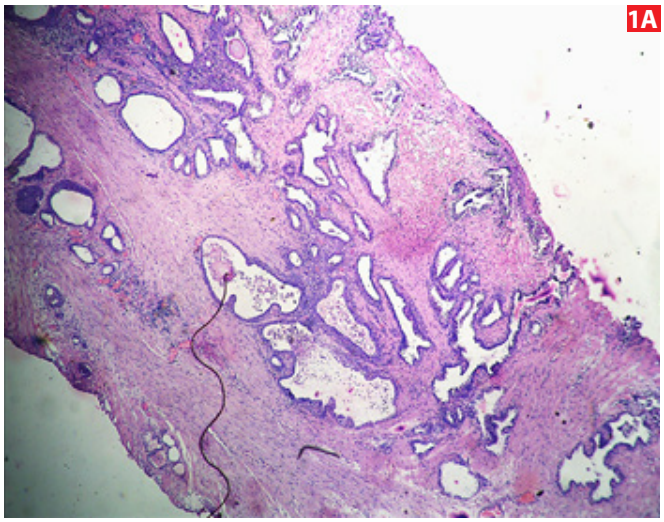
Among prostatic adenocarcinoma association between AMACR expression and Gleason's Grade Group was not found to be significant (p-value 0.65). (Table 2, Figure 2) Out of total 35 malignant prostatic cases, 34 cases showing Gleason's pattern 4, out of which 29 (85.3%) cases showed AMACR expression positivity and rest 5(14.7%) cases showed AMACR expression negativity. In this study 12 malignant cases showed cribriform pattern in Gleason pattern 4. In cribriform pattern 11/12 (92%) cases showed AMACR expression positivity. A correlation between

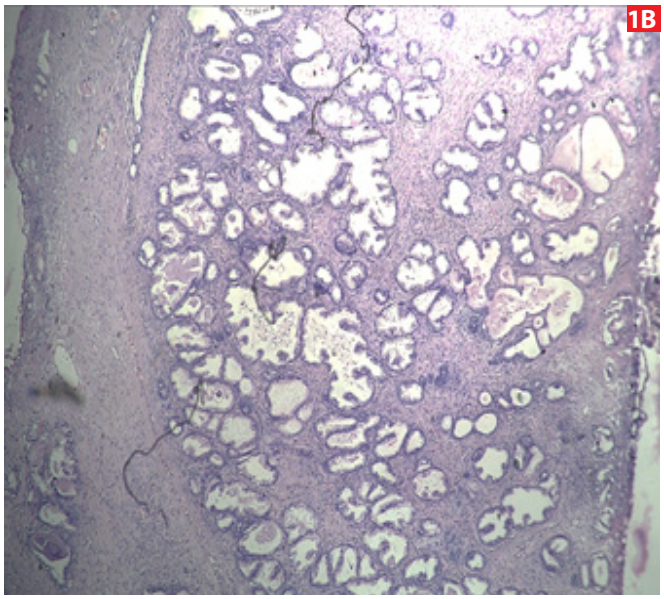
AMACR expression and cribriform in Gleason's pattern 4 was significant statistically (p-value 0.003). (Table 3, Figure 3,4)

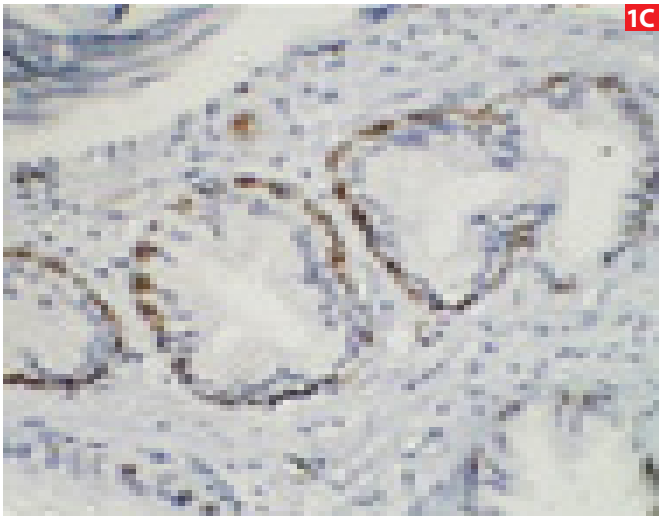
In the present study 16 cases showed perineural invasion. Out of which 14/16 (87.5%) cases showed AMACR expression positivity while 2/16 (12.5%) cases showed AMACR expression negativity. AMACR expression and perineural invasion showed a significant correlation (p-value 0.002). (Table 3, Figure 3d)

Out of total 35 malignant cases only 3 cases showed lympho-vascular invasion of which 2/3(66.6%) cases showed AMACR positivity. AMACR expression and lympho-vascular invasion showed no association (p-value is 0.56). (Table 3)

Of the 14 suspicious cases for malignancy, a change of diagnosis based on IHC, in 11/14 (79%) cases from suspicious for malignancy to prostatic adenocarcinoma. Out of 14 total suspicious for malignancy 2/14 (14.2%) expressed both AMACR & p63 positivity. A significant correlation was found between AMACR & p63 expression with suspicious for malignant cases. (Table 4, Figure 5)







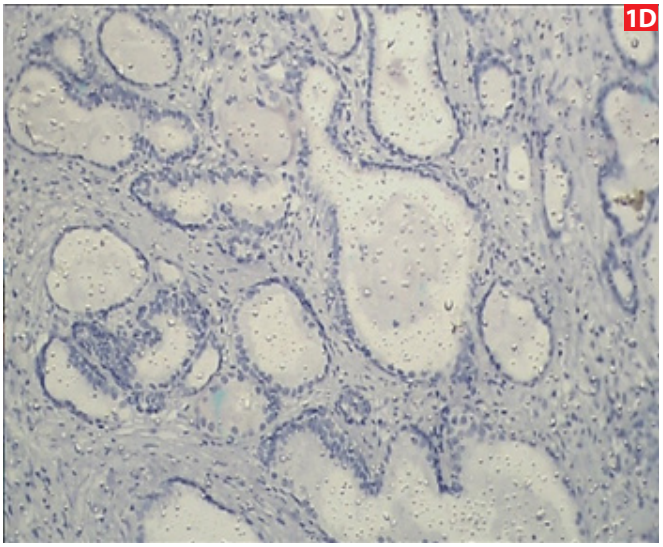
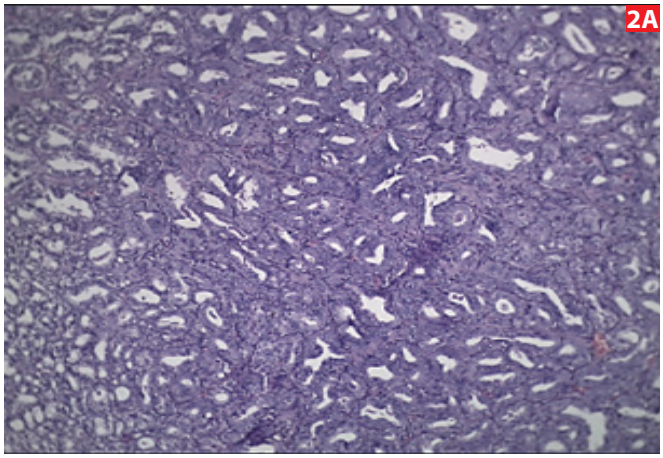
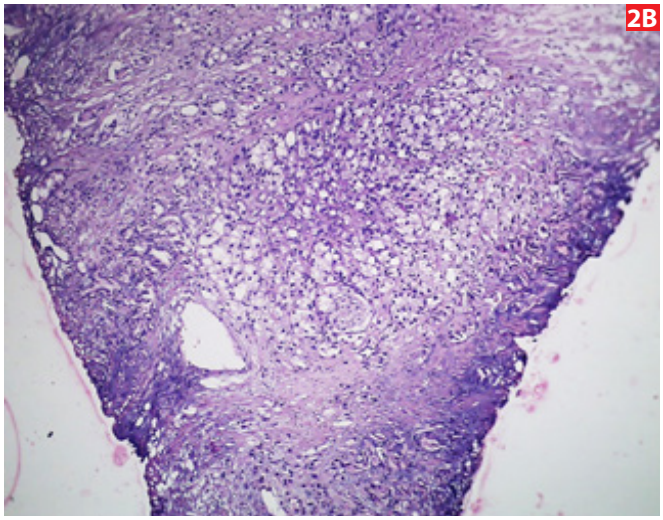
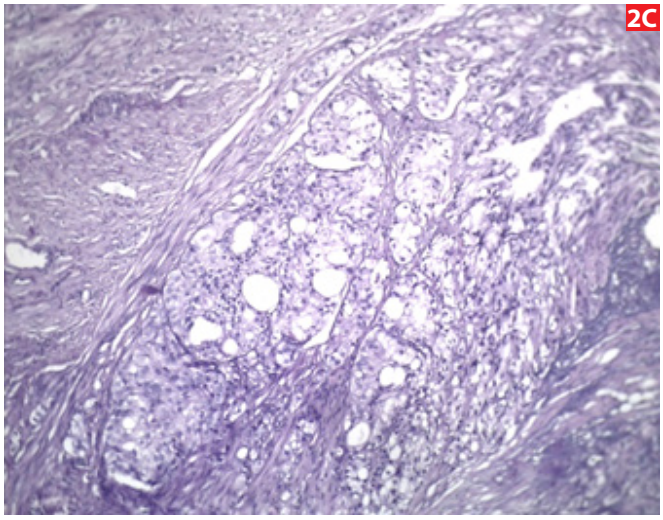
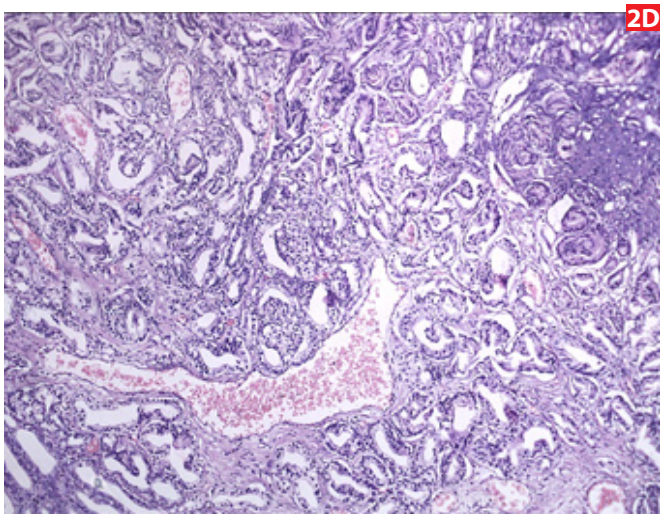


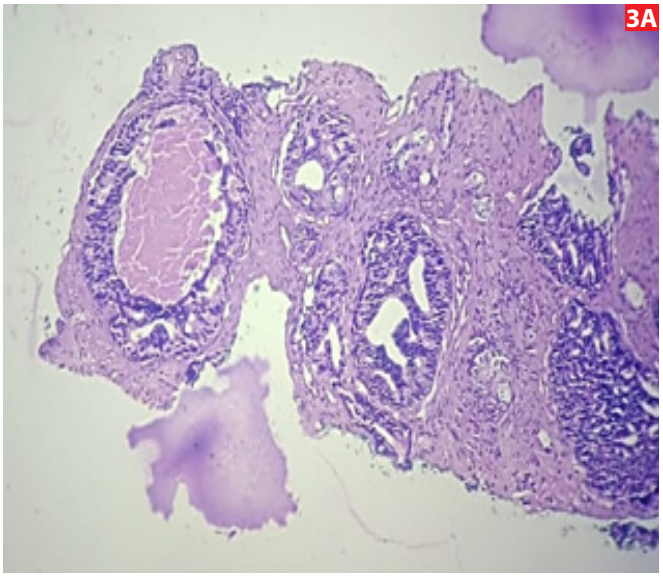
Figure 1: Histomorphology & Immunohistochemistry of Bening Prostatic Hyperplasia a) (H&E; x100). Inset H&E; x400 showing corpora amylacea. b) (H&E; x100). Inset H&E; x400 showing papillary infoldings c) Continuous strong nuclear positivity for basal cells (IHC for p63; x40). Inset IHC for p63; x400. d) Negative cytoplasmic staining for luminal cells (IHC for AMACR; x100).

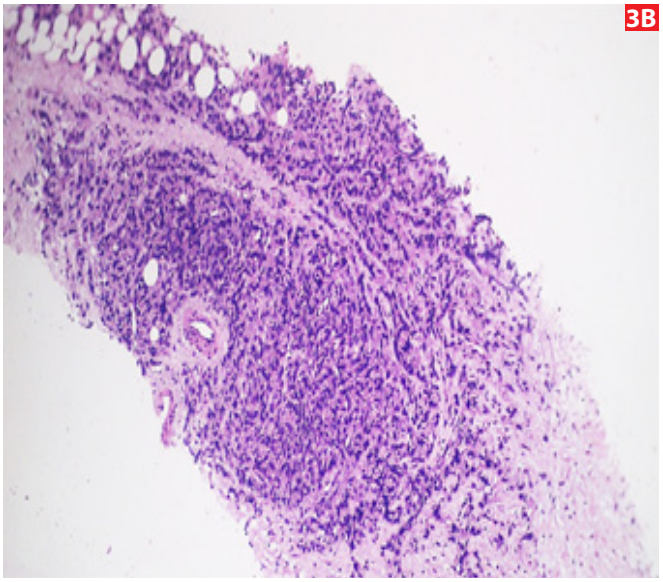












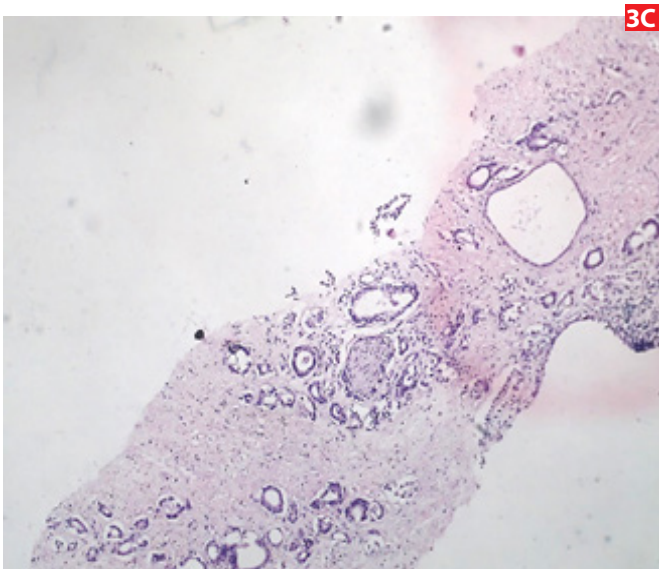


Figure 2: Histomorphology of Malignant Prostatic Lesions {Gleason's Pattern 4}: a) Fused Gland (H&E x100) b) Hypernephroid with PNI (H&E x100). Inset Hypernephroid (H&E x40) c) Cribriform Pattern (H&E x100) d) Glomeruloid pattern (H&E x 100). Inset Glomeruloid (H&E x400).

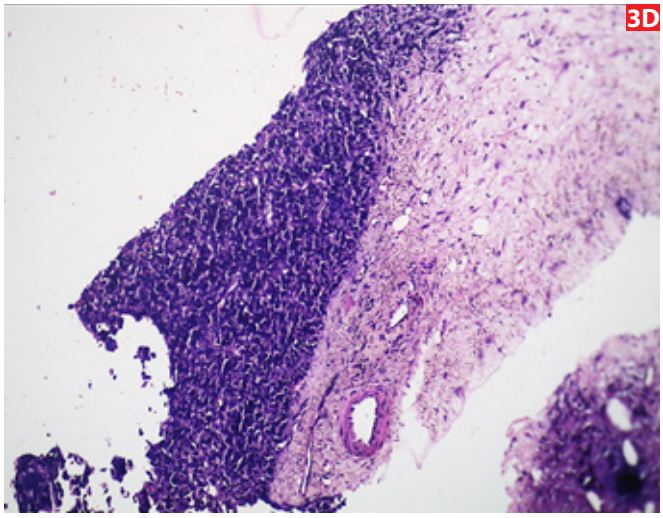
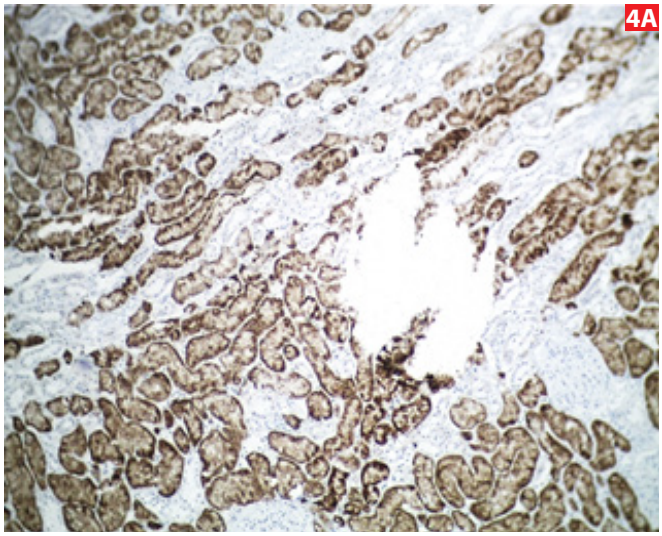
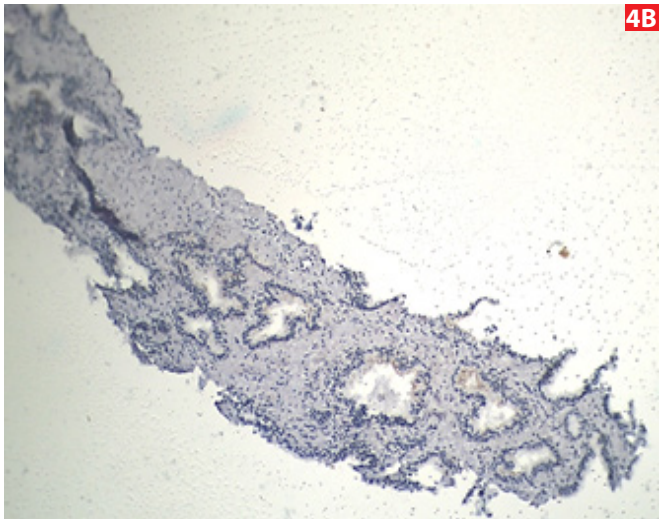
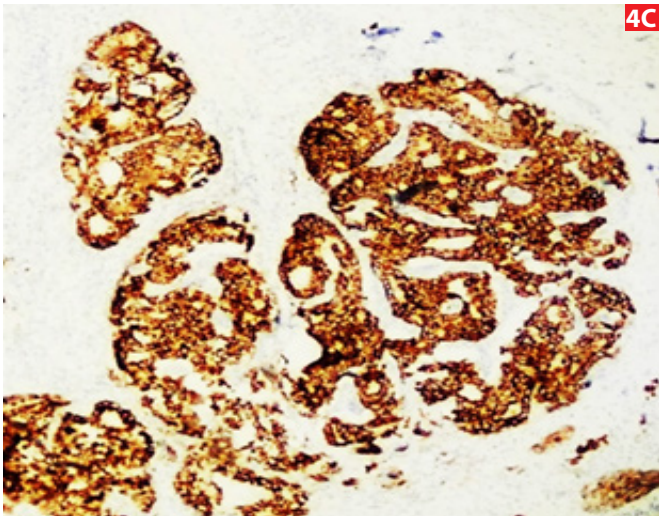


Figure 3: Histomorphology of Malignant Prostatic Lesions {Gleason's Pattern 5}: a) Comedo necrosis (H&E x100) b) Sheets of cells (H&E x100). Inset single cell infiltration showed prominent nucleoli (H&E x400) c) Solid pattern (H&E x100) d) Perineural invasion (H&E x 100).







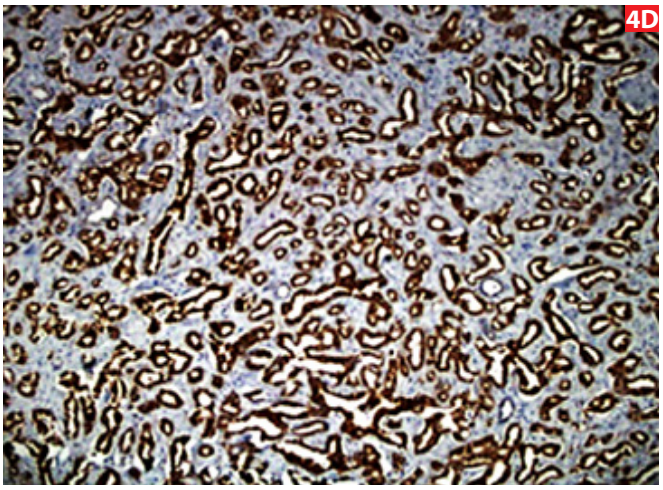
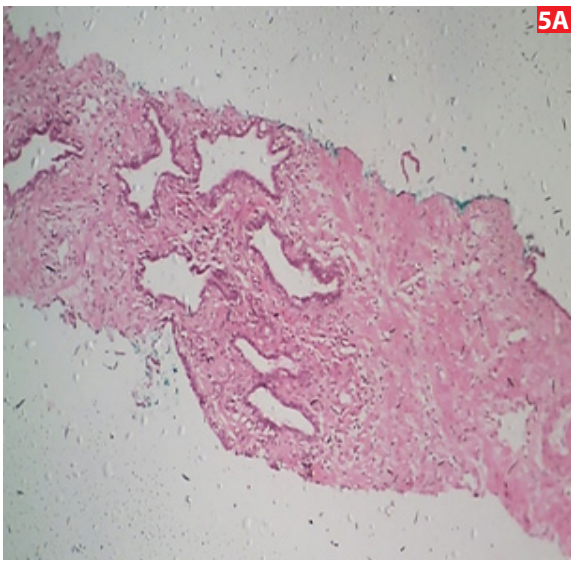
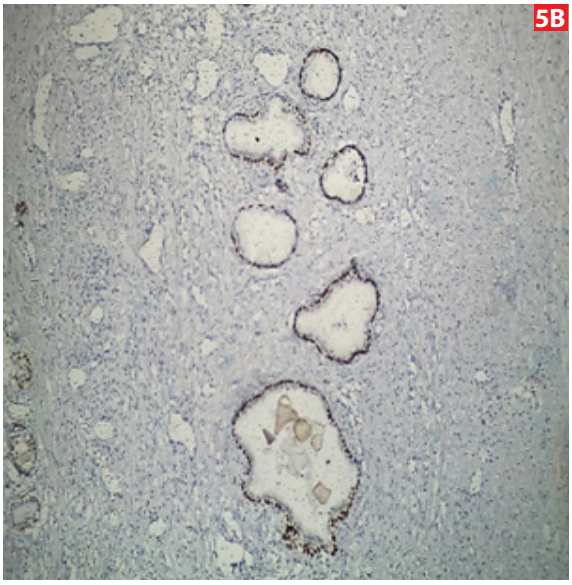
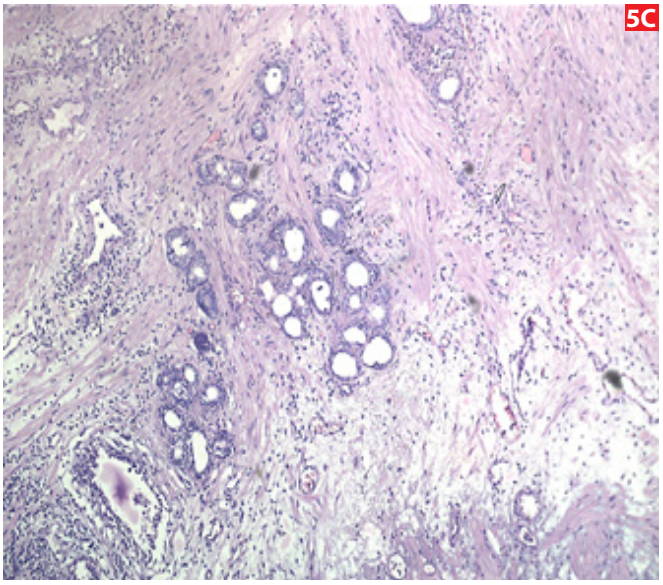


Figure 4: Control slides of IHC for AMACR: Strong & distinct granular Cytoplasmic staining in Epithelial cells of the Proximal Kidney Tubules a) IHC; x40 b) IHC; x100. Inset IHC; x400 c) Strong cytoplasmic staining of luminal cells (IHC for AMACR; x400) d) Diffuse cytoplasmic staining of luminal cells (IHC for AMACR; x100).







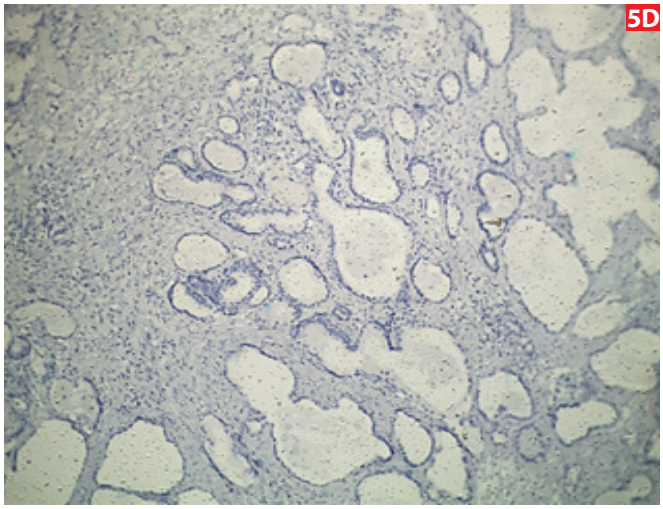


Figure 5: Histomorphology & Immunohistochemistry of Suspicious Prostatic Lesion a) Atrophy (H&E; x100); b) Atrophy: Continuous strong nuclear positivity for basal cells (IHC for p63; x100) c) Atypical Small Acinar Proliferation (H&E x100) d) Atypical small acinar proliferation: Negative cytoplasmic staining for luminal cells (IHC for AMACR; x100).

Table 1: AMACR & p63 expression in Benign and Malignant Prostatic Lesions.

Total no. of Prostatic cases 130	**P63**	**AMACR**	**Chi-square test value**	**p-value**
Benign (81)	81(100%)	00	105.99	0.000
Malignant (35)	00	30 (85.7%) *		
Total	81	30		

Table 2: Correlation of AMACR expression in Gleason's Grade Group in Malignant Lesions.

Gleason's Grade Group*	**Total cases**	**AMACR positive cases**	**AMACR negative cases**	**% of positive cases**	**Anova test value**	***p-value***
Grade Group 2	7	6	1	85%	1.45	*0.65*
Grade Group 3	3	2	1	66%		
Grade Group 4	4	3	1	75%		
Grade Group 5	21	19	2	90%		
Total	35	30	5			

** There was no case of Gleason Grade Group 1.*

Table 3: Distribution of Malignant lesions into Gleason's Grade Groups & other Histopathological Parameters & Correlation with AMACR expression.

Gleason's Grade Group#	Total number	Number of malignant cases showing Gleason pattern 4	Number of cases showing cribriform in pattern 4	Number of cases showing PNI	Number of cases showing LVI
Grade Group 2	07	07	01	02	00
Grade Group 3	03	03	02	02	00
Grade Group 4	04	03	02	01	01
Grade Group 5	21	21	07	11	02
TOTAL cases	35	34	12	16	03
IHC AMACR positive cases	30		11	14	02
p-value			*0.003*	*0.002*	*0.56*

No malignant case of Grade Group 1 was present

Table 4: Expression of AMACR & p63 in Suspicious Cases.

Total no. of suspicious cases n=14	AMACR	P63
Positive	11 (79%)	5(26%)
Negative	3(21%)	9(64%)

DISCUSSION

In this study, benign lesions were the most common, accounting for 62% (81/130) of cases, predominantly Benign Prostatic Hyperplasia, while all malignant cases were acinar adenocarcinoma. These results are similar to research by Singh V et al.[6] and Mahajan et al.,[7] who likewise found benign lesions to be the most common. However, Okonkwo et al.4,6 found a greater prevalence of malignancy, probably because they concentrated on core needle biopsies. Both benign & malignant cases were of average age 66-years, which is similar to Garg et al.9 The patients' ages ranged from 40 to 90 years, with the majority (41.5%) presenting in 7th decade, similar to Chauhan et al.,[8] Gleason Grade Group 5 accounted for 60% of malignant patients; Emiogun FE et al. similarly observed this pattern.[10] Overall, the study emphasizes the & the preponderance of benign prostatic hyperplasia with higher frequency of high-grade adenocarcinoma among malignant lesions.

The consistent role of p63 in benign prostatic lesions was confirmed in this investigation, as all benign patients (81/81, 100%) had p63 nuclear positivity with negative AMACR expression. This outcome is congruent with the findings of Gudeli V et al.,[12] who discovered AMACR negativity in all 139/139 (100%) benign cases, and Rathod et al.,[11] who showed p63 positivity in all 40/40 (100%) benign lesions. Together, our results support the diagnostic accuracy of p63 expression and the lack of AMACR as characteristics that set benign prostatic tissue apart.

Thirty (85.7%) of the 35 malignant patients in this investigation had cytoplasmic AMACR immunostaining positivity in luminal cells, whereas none of the malignant cases had p63 positive. AMACR's diagnostic utility was highlighted by its statistically substantial expression in malignant lesions. These results are in congruent with the study by Arthi and Dhanalakshmi et al.,[13] which found that just 2/19 (10.5%) had negative cytoplasmic immunostaining, while 17/ 19 malignant cases had AMACR positive. AMACR is a reliable marker for identifying malignant prostatic lesions, as demonstrated by this agreement.

Cribriform pattern of Gleason's pattern 4 are linked to the worst clinical course among other morphologic variants of in this study.[14] A significant correlation between AMACR & Gleason's pattern 4 was 29/34 (85.3%) of malignant cases that displayed this pattern. Additionally, 11/12 (92%) malignant cases with cribriform growth had positive AMACR expression, supporting the strong association between aggressive histologic features like of AMACR staining & Cribriform pattern of Gleason's pattern.[4]

Association of perineural invasion AMACR expression was found to be statistically significantly. This outcome is similar with the findings of Sahed RM et al.,[15] who similarly institute a strong link between perineural invasion and AMACR expression. Taheri et al.,16 on the other hand, noted that there

was no statistically significant correlation between AMACR expression and perineural invasion and that there was no difference in the frequency of perineural invasion between AMACR-positive and AMACR-negative patients. These conflicting studies demonstrate the diversity in the research about AMACR's function in relation to perineural invasion.

In the current study, immunohistochemical analysis employing p63 and AMACR changed the diagnosis to adenocarcinoma in 11 cases (78%) of the 14 cases that were first classified as suspicious for malignancy. It's interesting to note that two of these instances showed dual positivity for p63 and AMACR. A similar change in diagnosis from malignancy mimicker foci to confirm adenocarcinoma after IHC interpretation was observed by Fatima et al.17 3/6 doubtful cases in their series tested positive for AMACR alone, two for p63 alone, and one for both markers. Similarly, 38/50 morphologically doubtful foci were categorized as adenocarcinoma following IHC interpretation, according to Ahmed Ibn Edriss Mohamed et al.[18] Of them, two cases showed dual positivity for both markers, while 36 cases showed AMACR positivity & absence of p63. A positive AMACR positivity & absent p63 in basal cells, emerge as the most consistent pattern supporting adenocarcinoma. Hence, these results collectively demonstrate the diagnostic utility of AMACR and p63 immunostaining in resolving morphologically ambiguous cases, though isolated instances of dual positivity have been observed across different studies.

CONCLUSION

In prostate cancer detection, AMACR is a useful immunohistochemistry marker, especially when benign mimickers such adenosis, atrophy, adenosis, basal cell hyperplasia etc. pose diagnostic difficulties. Although AMACR is a useful tool due to its overexpression in malignant prostatic glands, it does not completely discern it because certain benign diseases may exhibit modest positivity. Because of this, it works well when coupled with basal cell markers like p63. The dual staining approach—AMACR positivity with basal cell marker negativity—provides strong confirmatory evidence of adenocarcinoma, whereas the inverse pattern suggests a benign diagnosis. This combination of AMACR and basal cell markers is especially essential in needle biopsy specimens, where morphology alone may be equivocal, and so serves as a dependable technique for increasing diagnostic accuracy in prostate cancer identification.

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