



Immunoexpression of Cytokeratin 34 β E12 and Alpha-Methylacyl CoA Racemase (AMACR) in Prostatic Biopsy Specimens: A Cross-Sectional Study.

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Abstract

Background: Differentiating benign, atypical, and malignant prostatic lesions on routine H&E (Hematoxylin and Eosin) staining can be challenging, particularly in small biopsy specimens. Immunohistochemical markers such as CK34 β E12 (Cytokeratin 34 β E12), a basal cell marker, and AMACR (Alpha-Methylacyl CoA Racemase), a positive marker for prostatic carcinoma, can improve diagnostic accuracy by distinguishing benign mimickers from adenocarcinoma. This study evaluated the immunoexpression of CK34 β E12 and AMACR in prostatic biopsy specimens and assessed their association with histopathological diagnosis and tumor grade. **Methods:** This cross-sectional study was conducted in the Departments of Pathology and Urology, Regional Institute of Medical Sciences (RIMS), Imphal. Consecutive prostatic biopsy specimens, including core needle biopsies, transurethral resection of the prostate (TURP), and prostatectomy specimens from adults (>18 years), were included. Routine H&E examination was followed by immunohistochemistry using mouse anti-human AMACR (Clone 13H4) and CK34 β E12 (Clone MABT1530). Staining intensity was graded using predefined criteria, and associations with lesion type and histological parameters were analyzed statistically. **Results:** The study included 109 prostatic lesions, comprising 63 benign, 8 atypical, and 40 malignant cases. AMACR expression showed a statistically significant association with lesion type ($p < 0.001$). Among malignant lesions, 37 of 40 (92.5%) demonstrated moderate-to-strong AMACR positivity, whereas benign lesions were predominantly negative or mildly positive. CK34 β E12 exhibited preserved basal cell staining in benign lesions and loss of expression in malignant lesions, supporting its utility as a negative marker for carcinoma. The combined immunohistochemical profile effectively distinguished benign from malignant lesions and improved diagnostic confidence in atypical cases. **Conclusion:** Combined evaluation of CK34 β E12 and AMACR significantly enhances the diagnostic accuracy of prostatic biopsy interpretation. Their complementary staining patterns are particularly valuable in resolving morphologically equivocal lesions and reducing diagnostic uncertainty.

Keywords: Prostate Adenocarcinoma, CK34 β E12, AMACR, Immunohistochemistry, Prostatic Biopsy, Benign Prostatic Hyperplasia.



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INTRODUCTION

The prostate, derived from the Greek word “prostates” meaning “one who stands before” or “protector,” was first described by Niccolò Massa in 1536 and illustrated by Andreas Vesalius in 1538.[1] In adults, the prostate weighs approximately 20 g and is anatomically divided into peripheral, central, transition, and periurethral zones. Histologically, it comprises glandular acini embedded within abundant fibromuscular stroma and is lined by an outer basal cell layer and an inner columnar secretory cell layer.[2]

The histopathological spectrum of prostatic lesions includes inflammatory conditions, BPH (Benign Prostatic Hyperplasia), PIN (Prostatic Intraepithelial Neoplasia), and adenocarcinoma.[3] BPH is a common age-related disorder characterized by stromal and glandular proliferation within the periurethral region, leading to nodular enlargement.[4] Prostate cancer is the sixth most commonly diagnosed malignancy worldwide and the second leading cause of cancer-related mortality among men after lung cancer. Approximately 70% of prostatic adenocarcinomas arise in the peripheral zone, making digital rectal examination clinically valuable for detection.[5]

Screening modalities include PSA (Prostate-Specific Antigen) testing, digital rectal examination, and transrectal ultrasonography; however, histopathological examination of biopsy specimens remains the diagnostic gold standard.[6] Although PSA screening has facilitated earlier cancer detection, its limited specificity often results in unnecessary biopsies.[7] Standard systematic 12-core biopsy protocols and MRI-guided biopsies have improved the detection of clinically significant cancers while reducing overdiagnosis of insignificant lesions.[8]

Despite these advances, limited tissue in needle biopsies makes distinguishing small foci of carcinoma from benign mimickers-including adenosis, atrophy, basal cell hyperplasia, post-atrophic hyperplasia, mesonephric hyperplasia, and inflammatory atypia-a persistent diagnostic challenge.[9] The presence or absence of the basal cell layer is a critical histological criterion, with basal cells retained in benign glands but absent in adenocarcinoma, highlighting the importance of immunohistochemistry in difficult cases. Basal cell markers such as HMWCK (34βE12) and p63 serve as negative markers for carcinoma, although patchy basal cell staining may limit interpretation.[10] AMACR, a positive marker for malignant prostatic glands, demonstrates high sensitivity and specificity and, when used alongside basal cell markers, improves the detection of small foci of prostatic adenocarcinoma.[10]

Aims and Objectives

The study aimed to evaluate the diagnostic accuracy of Cytokeratin 34βE12 and AMACR in prostate lesions with the aim of reducing the chances of misdiagnosis. It assessed the immunoexpression of Cytokeratin 34βE12 and AMACR in benign, atypical prostatic lesions and prostatic carcinoma, and evaluated their association with different histopathological types and histological grades of prostate carcinoma.

MATERIALS AND METHODS

Study Design

This cross-sectional study was conducted at the Regional Institute of Medical Sciences (RIMS), Imphal, Manipur. The study was carried out in the Department of Pathology in collaboration with the Department of Urology. The study was conducted over a two-year period, from April 2024 to March 2026.

Inclusion and Exclusion Criteria

The study included prostatic specimens obtained through core needle biopsy, TURP (Transurethral Resection of the Prostate), and radical prostatectomy from patients aged 18 years and above. Patients who were already receiving treatment for carcinoma of the prostate, including systemic chemotherapy or radiotherapy, and those with metastatic prostate cancer were excluded from the study.

Sample Size Calculation

All the cases of prostatic lesion biopsy during the period of study will be included. It is calculated as:

$$n = \frac{4pq}{d^2}$$

Where n = sample size

P = 92% (Prevalence of Immunoexpression of AMACR in prostate lesion = 92%)³ q = 100-p = 8%

d=5, absolute allowable error n = 118

So, the sample size was 118.

Data Collection Tools

Data were collected using a predesigned proforma to record demographic and clinical details, including age, sex, occupation, smoking history, and relevant clinical information. Histopathological evaluation was performed using standard laboratory equipment, including an automated tissue processor, rotary microtome, binocular light microscope, oven, and pressure cooker. Immunohistochemical analysis was carried out using AMACR (Clone 13H4) and Cytokeratin 34βE12 (Clone MABT1530) monoclonal antibodies for the assessment of prostatic lesions.

Data Collection Procedure

Eligible prostatic specimens obtained through core needle biopsy, TURP, and radical prostatectomy were included after recording the patients' demographic and clinical details in a predesigned proforma. The specimens were fixed in 10% neutral buffered formalin, processed in an automated tissue processor through graded alcohol dehydration and xylene clearing, embedded in paraffin, and sectioned at 3–5 μm using a rotary microtome. The sections were mounted on slides and stained with H&E for routine histopathological examination. For immunohistochemistry, sections mounted on poly-L-lysine-coated slides underwent baking, deparaffinization, rehydration, antigen retrieval using IRIS buffer (pH 9) in a pressure cooker, blocking, and incubation with primary antibodies against AMACR and Cytokeratin 34βE12, followed by secondary antibody application, DAB chromogen development, and hematoxylin counterstaining. Immunostaining was interpreted according to predefined criteria for the intensity and extent of marker expression, while the final

histopathological diagnosis and reporting were performed in accordance with the College of American Pathologists (CAP) protocol, and the findings were correlated with the histopathological type and grade of the lesions.

Statistical Analysis

The collected data were entered into Microsoft Excel 2010 and analyzed using IBM SPSS Statistics Version 27 (IBM Inc., Armonk, New York, USA). Descriptive statistical analysis was performed to summarize the study variables. Age (in years) was expressed using appropriate descriptive measures, while categorical variables, including the nature of prostatic lesions (benign, atypical, and malignant), Gleason grade, perineural invasion, lymphovascular invasion, extraprostatic extension, tumor location, and pathological stage, were presented as frequencies and percentages. The Chi-square test was used to determine the association between the immunoexpression of Cytokeratin 34 β E12 and AMACR and the histopathological grades and stages of prostate carcinoma. A p-value of less than 0.05 was considered statistically significant at a 95% confidence interval.

RESULTS

Table 1 illustrates the baseline characteristics of the 111 prostatic specimens. The majority occurred in patients aged 61–70 years (39.62%), TURP was the commonest specimen (60.4%), and benign lesions predominated (56.8%).

Table 1. Clinicopathological Profile of the Study Population

Characteristic	Category	N	%
Age	≤50	8	7.20
Age	51–60	23	20.72
Age	61–70	44	39.62
Age	≥71	36	32.43
Specimen	TURP	67	60.4
Specimen	Biopsy	43	38.7
Specimen	Partial Prostatectomy	1	0.9
Nature	Benign	63	56.8
Nature	Malignant	40	36.0
Nature	Atypical	8	7.2

Table 2 shows that benign lesions were observed across all age groups, whereas malignant lesions became more frequent after 60 years of age. However, the association was not statistically significant (p=0.18).

Table 2. Age Group in Relation to Nature of Lesion

Age	Benign	Malignant	Atypical
≤50	6	2	0
51–60	15	6	2
61–70	25	16	3
≥71	17	16	3
Total	63	40	8

Table 3 demonstrates that benign diagnoses accounted for 66 cases after IHC resolution, with BPH being the commonest diagnosis (49.55%). Among eight atypical cases, three were reclassified as BPH and five as acinar adenocarcinoma.

Table 3. Final Histopathological Diagnosis after IHC

Diagnosis	n	%
Benign Prostatic Hyperplasia	55	49.55
Prostatitis	6	5.40
Basal Cell Hyperplasia	2	1.80
Atypical → BPH	3	2.70
Atypical → Acinar Adenocarcinoma	5	4.50
Acinar Adenocarcinoma	40	36.03
Total	111	100

Table 4 shows that high-grade tumours (Grade Groups 4–5) constituted 80% of malignant cases, and stronger AMACR staining was significantly associated with higher Gleason grade (p=0.01).

Table 4. Gleason Grade Distribution and AMACR Intensity

Gleason Group	Cases	Strong	Moderate	Mild
Grade 2	3	0	3	0
Grade 3	6	6	0	0

Grade 4	16	14	2	0
Grade 5	20	20	0	0
Total	45	40	5	0

Table 5 demonstrates preserved basal-cell staining in benign lesions, whereas loss of CK34βE12 expression predominated in malignant lesions ($p < 0.001$).

Table 5. CK34βE12 Staining Intensity According to Lesion Type

Lesion	Mild	Moderate	Strong	Negative
Benign	0	3	58	2
Atypical	0	2	1	5
Malignant	2	0	0	38
Total	2	5	59	45

Table 6 shows that malignant lesions predominantly exhibited moderate-to-strong AMACR positivity (37/40), whereas most benign lesions remained negative (59/63), with a highly significant association ($p < 0.001$).

Table 6. AMACR Staining Intensity According to Lesion Type

Lesion	Mild	Moderate	Strong	Negative
Benign	4	0	0	59
Atypical	0	3	2	3
Malignant	0	4	33	3
Total	4	7	35	65

Table 7 illustrates the complementary diagnostic role of CK34βE12 and AMACR. CK34βE12 positivity with AMACR negativity characterized benign lesions, while CK34βE12 negativity with AMACR positivity was strongly associated with malignant lesions ($p < 0.001$).

Table 7. Combined CK34βE12 and AMACR Immunoexpression

Marker	Benign	Malignant	Atypical
CK34βE12 Positive	61	2	3
CK34βE12 Negative	2	38	5
AMACR Positive	4	37	5
AMACR Negative	59	3	3
Dual Expression	2	2	0

DISCUSSION

The present study evaluated the immunoexpression of CK34βE12 and AMACR in benign prostatic lesions, atypical prostatic lesions, and prostatic adenocarcinoma, and correlated these findings with the histopathological spectrum and Gleason grade groups of prostatic carcinoma. A total of 111 prostatic lesions diagnosed on needle biopsy, TURP, and prostatectomy specimens were included.

The age of the patients ranged from 40 to 85 years, with the highest number of cases occurring in the 61–70-year-old age group. The mean age was 63.7 years for benign lesions (BPH, prostatitis, and basal cell hyperplasia), 66.6 years for atypical lesions, and 66.8 years for prostatic adenocarcinoma. These findings are comparable with those reported by Hassan et al.,[3] Bukhari et al.,[11] Azad et al.,[12] and Jain et al.,[13] indicating that both benign and malignant prostatic lesions predominantly affect older men.

Among the 111 prostatic lesions, benign lesions constituted the largest proportion with 63 cases (56.8%), followed by malignant lesions with 40 cases (36.0%) and atypical lesions with 8 cases (7.2%). This distribution is consistent with the observations of Hassan et al.,[3] Jain et al.,[13] Azad et al.,[12] and Malik et al.,[14] who similarly reported benign lesions as the predominant category.

Histopathologically, BPH was the most common lesion, accounting for 55 cases (49.55%), followed by acinar adenocarcinoma with 40 cases (36.03%), prostatitis with 6 cases (5.4%), and basal cell hyperplasia with 2 cases (1.8%). This pattern closely resembles the findings of Hassan et al.,[3] Garg et al.,[7] Kumaresan et al.,[15] Sadeghifar et al.,[16] Azad et al.,[12] Jain et al.,[13] and Malik et al.,[14] all of whom identified BPH as the predominant prostatic lesion.

Grading of malignant cases using the Gleason Grade Group system demonstrated that Grade Group 5 was the most frequent, comprising 20 cases (44.4%), followed by Grade Group 4 with 16 cases (35.6%), Grade Group 3 with 6 cases (13.3%),

and Grade Group 2 with 3 cases (6.7%). Similar predominance of higher-grade tumors (Grade Groups 4 and 5) has been reported by Jain et al.,[13] and Azad et al.,[12] suggesting that many patients present with advanced-grade disease at diagnosis.

Immunohistochemistry proved particularly valuable in resolving diagnostically challenging atypical lesions. Among the 8 atypical prostatic lesions, 3 cases (37.5%) demonstrated intact continuous basal cell staining with CK34 β E12 and absence of AMACR expression, supporting a benign diagnosis. In contrast, 5 cases (62.5%) lacked CK34 β E12 staining while exhibiting AMACR positivity, resulting in revision of the diagnosis to prostatic adenocarcinoma. The CK34 β E12 positivity observed in atypical lesions (37.5%) was lower than that reported by Hassan et al.,[3] (75%), Kumaresan et al.,[15] (69.2%), and Garg et al.,[7] (50%), but higher than Malik et al.,[14] (25%). Similarly, AMACR negativity in atypical lesions (62.5%) was comparable to Garg et al.[7] (64.3%), intermediate between Hassan et al.,[3] (75%) and Malik et al.,[14] (41.67%), and higher than Kumaresan et al.,[15] (50%). These findings reinforce the complementary diagnostic utility of basal cell markers and AMACR in evaluating suspicious prostatic foci.

Among the 45 cases of prostatic adenocarcinoma, 42 cases (93.33%) demonstrated AMACR positivity, with 35 cases showing strong positivity and 7 cases showing moderate positivity. Conversely, CK34 β E12 negativity was observed in 95.56% of malignant cases, reflecting the expected loss of the basal cell layer in invasive adenocarcinoma. The CK34 β E12 negativity in the present study closely corresponds with Hassan et al.,[3] (96%) while being slightly higher than Kumaresan et al.,[15] (84%); Garg et al.,[7] reported complete 100% negativity. Similarly, AMACR positivity of 93.33% closely aligns with Kumaresan et al.,[15] (92%) and Hassan et al.,[3] (92%), while remaining slightly lower than Garg et al.,[7] (100%).

The immunohistochemical profile observed in the present study supports the established diagnostic role of CK34 β E12 as a basal cell marker and AMACR as a positive marker for prostatic adenocarcinoma. The combined application of these markers significantly improves diagnostic accuracy, particularly in atypical or suspicious lesions where routine histopathological examination alone may not provide a definitive diagnosis.

Limitations

The present study included 111 cases, which was slightly lower than the calculated sample size of 118 due to the limited number of eligible cases available during the study period. This relatively smaller sample size may limit the generalizability of the findings.

Future studies with larger sample sizes and longer follow-up periods are warranted to further evaluate the diagnostic and prognostic significance of CK34 β E12 and AMACR expression in prostatic lesions and to validate their clinical utility across different histopathological subtypes and grades of prostatic carcinoma.

CONCLUSION

This study demonstrates that the combined use of CK34 β E12 and AMACR significantly improves the diagnostic accuracy of prostatic lesions, particularly in distinguishing benign, atypical, and malignant lesions. CK34 β E12 showed preserved basal cell staining in most benign lesions, whereas its loss of expression was strongly associated with prostatic adenocarcinoma. Conversely, AMACR was predominantly expressed in malignant glands and showed a significant association with higher Gleason grade groups, with staining intensity increasing with tumor grade. Importantly, the combined application of these markers enabled the reclassification of five atypical cases from benign to malignant, highlighting their value in resolving diagnostically challenging lesions. Overall, the complementary immunoexpression patterns of CK34 β E12 and AMACR support their routine use as an immunohistochemical panel to reduce misdiagnosis, enhance diagnostic confidence, and facilitate appropriate clinical management of patients with prostatic lesions.

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