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Original Article

Pathology Section

Role of Two Antibodies Panel High Molecular Weight Cytokeratin and Alpha-Methylacyl-CoA Racemase in Diagnosing Prostatic Lesions: A Cross-sectional Study

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ABSTRACT

Introduction: Prostatic diseases cause significant morbidity and mortality. Although histopathological examination is the gold standard for diagnosing prostatic lesions but diagnosis may be challenging in the presence of benign mimickers or a very small focus of malignancy. Immunohistochemical aid to morphology helps in making a timely and accurate diagnosis.

Aim: This study was done to evaluate the role of two antibodies panel High Molecular Weight Cytokeratin (HMWCK) and Alpha-Methylacyl-CoA Racemase (AMACR) in improving the diagnostic accuracy of prostatic lesions.

Materials and Methods: This was an observational cross-sectional study conducted in the Department of Pathology, Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIM and HS), Dehradun, Uttarakhand, India, from May 2019 to October 2020. Haemotoxylin and Eosin (H&E) stained sections of prostatic biopsies were classified into benign and malignant. Amongst malignant lesions, prostatic adenocarcinomas were graded according to Gleason's grading system and Gleason's scores were noted. One section from each was subjected to AMACR and HMWCK antibody tests. HMWCK was interpreted as negative/positive and continuous/discontinuous. For AMACR, both location and intensity of stain was observed. The parameters studied were Gleason's score, group grade, expression of HMWCK and AMACR. Categorical data was presented in form of frequency and percentage. Independent t-test, Yates Chi-square test were used. Data was entered in Microsoft (MS) excel sheet and analysis was done using CRAN R 2.1.

Results: Total of 80 prostatic biopsies were taken, 24 were malignant and 55 were benign and one was Benign Prostatic Hyperplasia (BPH) with a focus suspicious for malignancy showing atypical small acinar proliferation on histopathological examination. The mean age of non neoplastic cases was 67.68±8.56 years, while that of neoplastic lesions was 75.41±9.34 years. Amongst benign, 56.3% (31/55) cases were BPH, 43.6% (24/55) cases were BPH with associated lesions which included 62.5% (15/24) cases of BPH with non specific prostatitis; 29.2% (7/24) cases of BPH with adenosis and 8.3% (02/24) cases of BPH with basal cell hyperplasia. Of malignant cases, 24 cases were of adenocarcinoma with maximum cases having Gleason's score 9 (11/24;45.8%) and group grade V (18/24;75%). The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of HMWCK and AMACR were calculated using histopathology as the gold standard.

Conclusion: Although histopathology is the gold standard in prostatic biopsies but immunohistochemistry is additional diagnostic aid in confirmation of diagnosis. Immunohistochemistry not only confirms the histological diagnosis but is of great help in challenging cases. It has markedly increased the diagnostic accuracy.

Keywords: Benign prostatic hyperplasia, Immunohistochemistry, Prostate cancer

INTRODUCTION

Diseases of prostate gland are responsible for significant morbidity and mortality amongst adult males globally [1]. Benign Prostatic Hyperplasia (BPH) is the most common prostatic disease in males older than 50 years. The burden of prostatic carcinoma is expected to grow to 1.7 million new cases and 0.499 million new deaths worldwide by 2030, possibly due to population growth and aging of the global population [2]. This fact emphasises upon the need of increasing the prostatic needle biopsies and improving the skills of accurate diagnosis with minimal tissue. At times, a small focus of prostatic adenocarcinoma can be easily missed or benign mimickers of adenocarcinoma like atrophy, Basal Cell Hyperplasia (BCH), Atypical Adenomatous Hyperplasia (AAH)/adenosis, nephrogenic adenoma, clear cell cribriform hyperplasia, sclerosing adenosis and mesonephric hyperplasia are overdiagnosed [3].

It is unfortunate that, although small but there is a significant error in diagnosing prostatic biopsies due to limited biopsy specimens and presence of mimickers. Diagnostic difficulties in challenging cases comprise 1.5-9% of prostatic biopsies with a sole responsibility on the reporting pathologists [4].

The clinical evaluation of patient includes the presenting complaints, physical examination, Prostate Specific Antigen (PSA) levels, radiological and histopathological examination of the tissue obtained by transrectal prostatic biopsies with or without radiological guidance.

Although histopathological examination is the gold standard for diagnosing prostatic lesions but diagnosis may be challenging in the presence of benign mimickers, very small focus of malignancy or the presence of any unusual variant. Accurate diagnosis is important as overdiagnosis may lead to unnecessary treatment and underdiagnosis may be responsible for unnecessary delay in treatment and spread of the disease. Moreover, timely diagnosis of carcinoma improves the prognosis. In the present era, Immunohistochemistry (IHC) is a boon for the pathologists especially for the challenging cases. A very few studies in recent past have been done to signify the role of High Molecular Weight Cytokeratin (HMWCK) and Alpha-Methylacyl-CoA Racemase (AMACR) in diagnosing prostatic lesions [5-7].

High molecular weight cytokeratin (34βE12) is a cytoplasmic marker that highlights intermediate cytokeratin filaments in glandular basal cells and is most widely used marker to highlight the glandular basal cells. Alpha-methyl-CoA racemase (AMACR) is a mitochondrial and

peroxisomal enzyme that is involved in beta oxidation of branched chain fatty acids and fatty acid derivatives. It is a cytoplasmic marker with consistently significantly higher expression in carcinoma and prostatic intraepithelial neoplasia (PIN) than matched normal epithelium [8,9].

The aim of the present study was to evaluate the expression of HMWCK and AMACR in prostatic biopsies. The objectives were to find the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the expression of HMWCK and AMACR and to find the association of Gleason's score and group grade with AMACR. The current study not only emphasises upon the combined role of HMWCK and AMACR in diagnosing prostatic lesions but also signifies their role in the gray zone area.

MATERIALS AND METHODS

This was an observational cross-sectional study carried out in the Department of Pathology, Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIM and HS), Dehradun, Uttarakhand, India, from May 2019 to October 2020. The study was approved by the Institutional Ethical Committee (ECR/710/Inst/UK/2015/RR-18).

Inclusion criteria: Both transurethral resection of prostate specimen and needle biopsies were included in the study.

Exclusion criteria: Inadequate biopsies and cases with marked inflammation obscuring the epithelium were excluded from the study.

Sample size calculation: Assuming 3% of the subjects in population having factor of interest, for estimating the expected proportion with 4% absolute precision and 95% confidence, the sample size is calculated as 70. After adding 10% non respondents, the sample size is 77 and therefore included 80 participants.

Study Procedure

Prostatic tissue was fixed in 10% buffered formalin, paraffin embedded, sectioned and, Haematoxylin and Eosin (H&E) stained sections were studied under the light microscope. The lesions were classified into benign and malignant. The adenocarcinoma cases were graded and categorised using Gleason's scoring system [10]. Primary grade was assigned to the dominant pattern and secondary grade to subdominant pattern. The two numeric grades were added to obtain the combined Gleason's score. These were then grouped into different group grades according to Gleason's grading system [11].

One section each from a representative block was subjected to AMACR and HMWCK (34βE12) immunostain. Immunohistochemistry was performed on 4 µm thick sections using streptavidin-biotin immunoperoxidase technique (Dako-cytomation). Positive and negative controls were run simultaneously. The positive control used for AMACR was a known case of prostate adenocarcinoma and for HMWCK was normal prostate tissue.

High molecular weight cytokeratin (34βE12) was interpreted as negative/positive and continuous/discontinuous [4]. For the evaluation of immunostaining of AMACR both location and intensity of stain was observed i.e dark diffuse cytoplasmic or circumferential strong apical granular staining. The percentage positivity was graded from 0 to 3+ as 0% cells : 0+(Negative), 1-10% cells: 1+(Mild), 11-50% cells: 2+(Moderate), >51% cells: 3+(Strong). No staining or focal, weak non circumferential fine granular staining was considered as negative [1].

STATISTICAL ANALYSIS

The results were tabulated and the statistical analysis was performed using CRAN R 2.1. Data was expressed as a mean±Standard Deviation (SD) for quantitative variables, numbers and percentage. Sensitivity and specificity values were calculated for both the markers using histopathology as the gold standard. Comparison between multiple groups were made using Independent t-test and Yates Chi-square whichever was appropriate. A p-value of ≤0.05 was taken as significant whereas p-value of more than 0.05 was considered non significant.

RESULTS

The study included 80 subjects, of which 24 were malignant cases, 55 benign cases and one case was BPH with a focus suspicious of malignancy, showing atypical small acinar proliferation as per histopathology. Majority of the non neoplastic lesions were in the age group of 60-79 years and the maximum number of neoplastic cases were seen in age group of 70-89 years as shown in [Table/Fig-1]. The mean±SD age of non neoplastic cases was 67.68±8.56 years, while that of neoplastic lesions was 75.41±9.34 years. The difference was statistically significant (p-value=0.0006). Amongst the 55 benign lesions, 56.3% (31/55) cases were BPH and 43.6% (24/55) cases were BPH with associated lesions which included 62.5% (15/24) cases of BPH with non specific prostatitis; 29.2% (7/24) cases of BPH with adenosis and 8.3% (2/24) cases of BPH with basal cell hyperplasia and one case was diagnosed as BPH with a focus suspicious of malignancy on histopathology [Table/Fig-2]. The suspicious lesion turned out to be adenosis after immunohistochemical analysis. Out of 24 malignant cases, 22 cases were of prostatic adenocarcinoma and one case each of adenocarcinoma with mixed small cell and adenocarcinoma with focal squamous carcinoma. Additional focus of High-grade Prostatic Intraepithelial Neoplasia (HGPIN) was also seen in four cases of these 24 malignant cases.

Age group (years)	Non neoplastic cases n (%)	Neoplastic cases n (%)
40-49	2 (3.57)	0
50-59	7 (12.5)	1 (4.17)
60-69	23 (41.07)	6 (25)
70-79	22 (39.28)	9 (37.50)
80-89	2 (3.57)	7 (29.17)
90-99	0	1 (4.17)
Total	56	24

[Table/Fig-1]: Age-wise distribution of prostatic lesions.

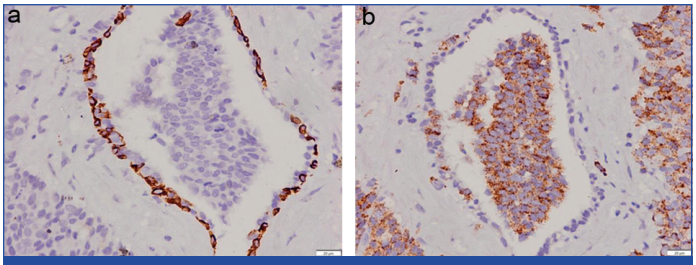
Type of prostatic lesions		Number of cases
Benign prostatic hyperplasias		31
BPH with associated lesions	BPH with non specific prostatis	15
	BPH with adenosis	7
	BPH with basal cell hyperplasia	2
	BPH with a focus suspicious of malignancy	1
Carcinoma		24
Total		80

[Table/Fig-2]: Distribution of cases according to histopathological diagnosis.
BPH: Benign prostatic hyperplasia

Maximum (11/24; 45.8%) cases had Gleason's score 9 and belonged to group grade V (18/24;75%). There was only one case (4.17%) in group grade I as well as group grade III [Table/Fig-3]. Four cases showed HGPIN along with carcinoma and one case in addition showed focus of intraductal carcinoma as shown in [Table/Fig-4], where AMACR positivity is seen in tumour cells present with in a duct showing positive HMWCK positivity in the glandular basal cells.

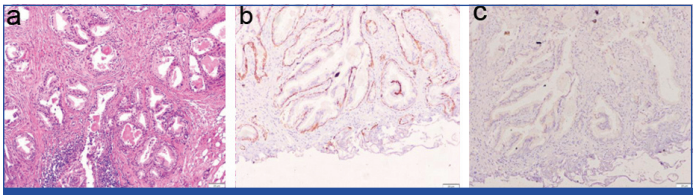
Group grade	Gleason's score	Number of cases (%)
I	<6	1 (4%)
II	3+4=7	2 (8.3%)
III	4+3=7	1 (4%)
IV	8	2 (8.3%)
V	9	11 (45.8%)
	10	7 (29.1%)
Total		24 (100%)

[Table/Fig-3]: Distribution of malignant cases in different group grade according to Gleason's score.

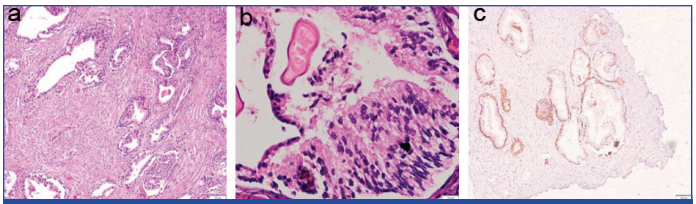


[Table/Fig-4]: (a) HMWCK positive expression in a focus of intraductal carcinoma (400X, IHC); (b) AMACR positive expression in a focus of intraductal carcinoma (400X, IHC).

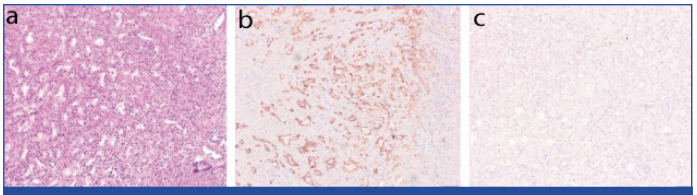
All non neoplastic lesions expressed HMWCK (34 βE12) positivity as shown in the case of BPH and a focus of basal cell hyperplasia [Table/Fig-5,6]. AMACR expression was seen in all the neoplastic lesions along with eight non neoplastic lesions [Table/Fig-7,8]. HMWCK positivity was also seen in four cases out of 24 neoplastic cases due to additional foci of HGPIN in these cases [Table/Fig-8]. AMACR positivity is seen in a case of prostatic adenocarcinoma along with negative expression of HMWCK as shown in [Tab/Fig-9]. Considering histopathology as the gold standard, the sensitivity, specificity, PPV and NPV of the expression of HMWCK and AMACR was calculated as shown in [Table/Fig-10,11]. The association of Gleason's score and group grade with AMACR was evaluated by Yates Chi-square test and p-value was found to be statistically insignificant in both the cases with value of 0.93 and 0.90, respectively [Table/Fig-12,13].



[Table/Fig-5]: (a) Glandular and stromal hyperplasia in benign prostatic hyperplasia (100X, H&E); (b) HMWCK positive immunostaining in benign prostatic hyperplasia (100X, IHC); (c) AMACR negative immunostaining in benign prostatic hyperplasia (100X, IHC).



[Table/Fig-6]: (a) A focus of basal cell hyperplasia(100X,H&E); (b) Basal cell hyperplasia (400X, H&E); (c) HMWCK positive expression at the focus of basal cell hyperplasia (100X, IHC).



[Table/Fig-9]: (a) Small fused glands in prostatic adenocarcinoma (100X, H&E); (b) Positive AMACR expression in prostatic adenocarcinoma (100X, IHC); (c) Negative immunostaining of HMWCK in prostatic adenocarcinoma (40X, IHC).

Variables	Value	95% CI	p-value
Sensitivity	100%	92.0-100.0	0.017*
Specificity	83.3%	61.8-94.5	0.013*
PPV	93.3%	83.0-97.0	0.02*
NPV	100%	80.0-100.0	0.03*

[Table/Fig-10]: Sensitivity and specificity of HMWCK (34βE12) CI-confidence interval.
*p-value ≤0.05 was considered statistically significant

Variables	Value	95% CI	p-value
Sensitivity	100%	82.8-100.0	0.04*
Specificity	87.2%	73.2-93.2	0.03*
PPV	75.0%	56.2-87.8	0.01*
NPV	100.0%	90.8-100.0	0.04*

[Table/Fig-11]: Sensitivity and specificity of AMACR, CI-confidence interval.
*p-value of ≤0.05 was considered statistically significant

Gleason's score	AMACR positivity			Yates Chi-square (p-value)
	1+	2+	3+	
<6	0	0	1	0.93
3+4=7	0	2	0	
4+3=7	0	0	1	
8	0	1	1	
9	3	2	6	
10	1	3	3	

[Table/Fig-12]: Association of Gleason score with AMACR.

Group grade	AMACR positivity			Yates Chi-square (p-value)
	1+	2+	3+	
Group grade I	0	0	1	0.90
Group grade II	0	2	0	
Group grade III	0	0	1	
Group grade IV	0	1	1	
Group grade V	4	5	9	

[Table/Fig-13]: Association of group grade with AMACR.

Type of lesions	HMWCK (34βE12) (Negative)	HMWCK (34βE12) (Positive)		AMACR (Negative)	AMACR (Positive)		
		Continous	Discontinous		1+	2+	3+
Non neoplastic lesions	0	45	11	48	6	1	1
Neoplastic lesion	20	1	3	0	4	8	12

[Table/Fig-7]: Comparison of HMCWK and AMACR immunostaining in non neoplastic and neoplastic lesion.

Diagnosis before IHC, based on histopathology	34βE12 negative	34βE12 positive		AMACR negative	AMACR positive	Final diagnosis after IHC
		Continous	Discontinous			
BPH (n=31)	0	24	7	31	0	31- BPH
BPH associated lesion (n=24)	0	12	3	15	0	15- BPH with prostatitis
	0	6	1	0	7	7- BPH with adenosis
	0	2	0	2	0	2-BPH with BCH
BPH with suspicious lesion (n=1)	0	1	0	0	1	BPH with adenosis
Adenocarcinoma (n=24)	20	0	0	0	20	20-Adenocarcinoma
	0	1	03	0	4	4-Adenocarcinoma with HGPIN

[Table/Fig-8]: Diagnosis before and after immunohistochemistry.
IHC: Immunohistochemistry; BCH: basal cell hyperplasia; HGPIN: High-grade prostatic intraepithelial neoplasia

DISCUSSION

Non neoplastic lesions of the prostate are more common than neoplastic lesions as seen in the present study and various previous studies [12,13]. Non specific prostatitis was the predominant subgroup in the category of BPH with associated lesions. This was in concordance with study by Mittal BV et al., [14]. The present study and study by Jain D et al., observed that the non neoplastic lesions occured at an average 10 years younger than the neoplastic lesions [1]. Most of the cases of prostate carcinoma were diagnosed above 50 years of age with maximum cases seen at more than 70 years of age.

Basal cell hyperplasia and atypical adenomatous hyperplasia were also observed in the present study. BCH is characterised by nodular and localised expansion of uniform, round to elongated glands with proliferating, small darkly staining basal cells with scanty cytoplasm and round spindly hyperchromatic nuclei. AAH is proliferation of small to medium sized glands lined by a single row of epithelial cells showing neither nuclear atypia nor prominent nucleoli. At times, it is difficult to differentiate adenosis from low-grade adenocarcinoma.

The age of presentation of BPH with basal cell hyperplasia was similar in the present study as well as study by Cleary RK et al., [15]. Atypical adenomatous hyperplasia (AAH) was observed in 11.6% of Transurethral Resection of the Prostate (TURP) specimens. This was in concordance with the reported incidence of AAH in literature which ranges from 2.2-19.6% in TURP specimens [16]. In the present study, group grade 5 and Gleason's score 9 was the most common grade and score observed, whereas, Jain D et al., Rathod SG et al., and Djavan B et al., have reported Gleason's score 6 and 7 to be more common pattern [1,6,17]. HGPIN was also seen in association with cases of adenocarcinoma and isolated cases of PIN were not observed in the present study. These results were in congruence with the results of Mc Neal JE et al., Horinger W et al., and Brawer KM [18-20].

High molecular weight cytokeratinc (34βE12) is a basal cell specific marker that was expressed in all the benign cases with 100% sensitivity and 83.3% specificity which was in congruence with the studies by Leong VW et al., and Kumaresan K et al., [21,22]. However, in a study by Malik N et al., the sensitivity of HMWCK was 92% and specificity was 100% [5]. It can express continuous and discontinuous pattern in benign, premalignant and sometimes malignant cases as in the present study. Manna AK et al., in their study also observed similar results with HMWCK showing continuous staining of basal cells in benign and premalignant lesions, whereas discontinuous staining was seen in malignant cases [23]. The variable discontinuous staining pattern in BPH could be because of patchy loss of basal cells or certain technical factors [24]. Fragmented pattern of basal cells in 5-23% of benign glands and 50% of adenosis has also been demonstrated in the study by Kumaresan K et al., [22].

Several studies have shown focal HMWCK (34βE12) expression in rare cases of prostatic adenocarcinoma. This could be explained by spread of prostatic adenocarcinoma intraductally, entrapment of benign glands that may also be mistaken as residual cells in prostate carcinoma or due to the presence of patchy cells with morphology of basal cells. As these cells give aberrant expression of the antigen in cancer, they give discontinuous immunostaining with HMWCK (34βE12) [25-28]. It was also observed that a focus of intraductal carcinoma was better appreciated with the help of HMWCK (34βE12) and AMACR rather than histopathology alone. Intraductal carcinoma has a prognostic value and is an independent predictor of the outcome.

Alpha-methylacyl-CoA racemase is a cancer specific marker that is strongly positive in malignant prostatic lesions but shows little or no immunostaining in benign lesions as observed in many studies. Luo J et al., observed that <4% of normal prostatic epithelium showed positive staining for AMACR, while in prostate carcinoma >95% stained positively [29]. The sensitivity ranged from 82-100% and specificity ranges from 79-100%. Other studies by Malik N et al., and Rather SG et al., found the sensitivity and specificity to be (89.96%, 76%)and (90%,100%) respectively [5,6]. In the present study, sensitivity and specificity was 100% and 85.7% respectively, which is analogous with the studies by Jiang Z et al., Luo J et al., and Beach R et al., [29-31].

In the present study, all the carcinoma cases showed positive cytoplasmic granular staining with AMACR with four cases in addition showing positive basal cell staining with HMWCK (34βE12). This corresponded to the focus of HGPIN and intraductal carcinoma.

There was one case of atypical foci with BPH. On histopathology, there were crowded glands and scattered poorly formed glands with nucleoli which resembled low-grade adenocarcinoma architecturally and morphologically. But on immunohistochemical analysis, HMWCK showed patchy discontinuous basal cell immunoreactivity along with AMACR immunoreactivity of 1+ intensity. The cytological characteristic of the cells lining these glands resembled the surrounding benign glands. Hence, correlating histopathology with IHC, a final diagnosis of adenosis was rendered. Both the cancer specific marker (AMACR) and basal cell marker (HMWCK) should be used to come to a diagnostic conclusion because single marker alone can be misleading due to their variable expression in benign, premalignant and malignant cases.

In the present study, three cases of HGPIN showed HMWCK (34βE12) discontinuous basal cell immunostaining and one case had continuous basal cell immunostaining. These three out of four cases of HGPIN showed strong granular cytoplasmic positivity with 3+ immunostaining with AMACR. Thus variable expression of the immunomarkers is seen in HGPIN. These findings were in accordance with the studies by Boran C et al., Zhou M et al., and Jiang Z et al., [30,32,33]. Rubin MA et al., and Luo J et al., reported that both invasive carcinoma and HGPIN had higher IHC staining scores than normal prostate epithelium [29,34].

Maximum cases of higher Gleason's score 9-10 and group V had 3+ intensity of AMACR. There was wide variation of AMACR intensity in different Gleason's scores. Statistically no association was observed between AMACR expression and Gleason's score (p-value=0.93) or AMACR expression with group grade (p-value=0.90), which was in congruence with the study by Rathod SG et al., Jain D et al., and Rubin MA et al., [1,6,34].

Limitation(s)

The lesions of the gray zone area could not be appropriately highlighted because of limited biopsy specimens due to pandemic era during the study period.

CONCLUSION(S)

Immunohistochemistry plays an important role as an adjunct to histopathology that remains the gold standard. It improves the accuracy of pathological diagnosis because of more objectivity. HMWCK (34βE12) and AMACR have good sensitivity for benign and malignant lesions, respectively. However, relying on any single marker is not recommended because of their variable expression in different prostatic lesions. Therefore, use of two antibodies panel can increase the level of confidence in establishing a definitive diagnosis especially in ambiguous lesions.

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