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Original Article

Diagnostic utility of immunohistochemical markers alpha methyl acyl coA racemase (AMACR) and Ets related gene (ERG) in prostate cancer

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Abstract: Objectives: To study the sensitivity and specificity of IHC markers AMACR and ERG in prostatic adenocarcinoma. Methods: The study was a prospective one and samples were collected from August 2014 to June 2016. A total of 186 samples were obtained from the Department of Urology, in which 112 of these were benign prostatic hyperplasia (BPH), and 71 were prostatic adenocarcinoma. The adenocarcinoma cases were evaluated by two histopathologists, and appropriate Gleason score was given according to the modified ISUP Gleason grading system (2016). IHC markers AMACR & ERG were performed on the adenocarcinoma cases and their sensitivity and specificity were calculated. Results: AMACR was a highly sensitive and specific marker for detecting prostatic carcinoma with a sensitivity and specificity of 95.8% and 96.5% respectively. ERG was a very specific marker with poor sensitivity in detecting prostate cancer. The sensitivity and specificity of ERG were 35.2% and 100% respectively. ERG expression decreased with increasing Gleason grade, PSA level, and tumour volume, which was statistically significant while the association of AMACR with Gleason grade or with tumor volume was not significant. Conclusion: ERG is a marker of early prostatic carcinogenesis and tumors may be positive or negative subtypes. Special histomorphologic features like perineural invasion, glomerulations, and intraluminal blue mucin were also studied. AMACR was a highly sensitive marker for detecting prostatic adenocarcinoma, while ERG was highly specific.

Keywords: AMACR, cancer, ERG, immunohistochemical, prostate

Introduction

The second most common cancer is prostate cancer and it ranks sixth among the leading causes of cancer-related deaths in men worldwide. Prostate needle biopsies provide diagnosis, Gleason grade, tumor volume, and presence or absence of perineural invasion. Even with multiple needle biopsy specimens, prostatic adenocarcinoma poses a diagnostic challenge. This diagnostic challenge can mainly be attributed to the presence of mimickers of prostate adenocarcinoma such as high-grade prostatic intraepithelial neoplasia (HGPIN), basal cell hyperplasia (BCH), adenosis, atrophy, and sclerosing adenosis [1]. Hence, there is a need to use appropriate immunohistochemical (IHC) markers to resolve the diagnosis.

This study was carried out to find the utility of IHC markers alpha methyl acyl-CoA racemase (AMACR) and the ETS (erythroblast transformation specific) related gene (ERG). AMACR, a peroxisomal enzyme, is overexpressed in prostatic adenocarcinoma glands with a sensitivity of 82-100%. ETS family of transcription factor ERG, a truncated protein, formed from the fusion of TMPRSS2 (an androgen-related gene) and ERG, involved in carcinogenesis of the prostate, is the second marker used in our study. ERG is reportedly positive in 50% of prostatic adenocarcinoma. In this study, we use anti-ERG as it is strongly expressed in fusion-positive prostatic adenocarcinoma [1].

Materials & methods

Ethical clearance number

The study was a prospective one and samples were collected from August 2014 to June 2016. A total of 186 samples were obtained from the

Table 1. The staining pattern of AMACR [1]

Sl. No.	Staining pattern	Score
1.	Non-circumferential staining	0
2.	Focal apical granular staining	1+
3.	Diffuse weak cytoplasmic staining	2+
4.	Strong cytoplasmic & luminal staining	3+

Table 2. The staining pattern of ERG [1]

Sl. No.	Staining pattern	Score
1.	Negative staining	0
2.	Weak staining	1+
3.	Strong but lighter than endothelial cells	2+
4.	Same as endothelial cells	3+

Proportion score.

Table 3. Proportion score used in the study [1]

No. of cells	Proportion score
< 5%	0
5-25%	1+
26-50%	2+
51-75%	3+
76-100%	4+

Overall comparison of Gleason grade with clinicopathologic characteristics.

Department of Urology. 112 of these were benign prostatic hyperplasia (BPH), and 74 were found to be prostatic adenocarcinomas. Out of the 74 adenocarcinoma cases, three were excluded because of exhausted blocks. TURP specimens, channel TURP specimens, and needle core biopsies were included in the study. The exclusion criteria included cases with evidence of prostatitis without evidence of hyperplasia or tumour, non-primary prostatic malignancies, prostatic adenocarcinoma diagnosed on histopathology but where IHC could not be performed because of exhausted blocks.

The diagnosis was based on the architectural pattern. Tumor volume was calculated by the percentage of tumor in each core and finally calculating the mean percentage of tumor of all cores. The tumor volume was divided as < 50% and > 50% tumor volume for comparison with Gleason grade. The adenocarcinoma cases were evaluated by two histopathologists, and appropriate Gleason score was given according to the modified ISUP Gleason grading system (2016) [2]. When there was a discrepancy in the scoring, a common consensus made by the two pathologists was considered.

Clinical parameters that were included in the study were the age at presentation, the pre-treatment PSA values, and bone scan. IHC was performed in 85 of the 112 BPH cases and 71 of the 74 adenocarcinoma cases. An intensity score and a proportion score were used to assess the staining characteristics of AMACR and ERG (**Tables 1**-**3**). The clones used for AMACR & ERG were P504S and EP111, both of which were rabbit monoclonal antibodies which were cytoplasmic and nuclear positive respectively. (**Figures 1**, **2**) Gleason grade compression was done as follows: 2-4 (grade 1), 5-6 (grade 2), 7 (grade 3) and 8-10 (grade 4) based on the study by Humphrey et al. [3]. PSA values were arbitrarily divided as < 10 ng/ml, 10-100 ng/ml, and > 100 ng/ml. Statistical tests were done using SPSS software version 20. The distribution of data on histopathology, the expression of AMACR and ERG were expressed as frequencies and percentages. The comparison of histopathology with AMACR and ERG expression and the association of categorical variables such as Gleason grade, and PSA levels with IHC expression was carried out by Chi-square test or Fisher exact test or Yates Chi-square Test, whichever is appropriate. The data on age, PSA levels, Gleason score, and tumor volume were expressed as mean with S.D or median with range. The diagnostic accuracy of AMACR and ERG were assessed by estimating sensitivity, specificity along with predictive values. All statistical analyses were carried out at 5% levels of significance and *P* value < 0.05 considered significant.

The Ethics clearance was taken from the Institute Ethics Committee. [IEC: ECR/342/Inst/PY/2013].

Results

A total of 183 specimens were studied, which included 112 benign prostatic hyperplasia (BPH) and 71 prostatic adenocarcinoma. The benign prostatic hyperplasia (BPH) specimens were taken as the comparison group for the 71 adenocarcinoma cases. The age group of prostate adenocarcinoma ranged from 50 to 89 years. The mean (SD) age for prostate adenocarcinoma was found to be 70 (8.2) years. Of

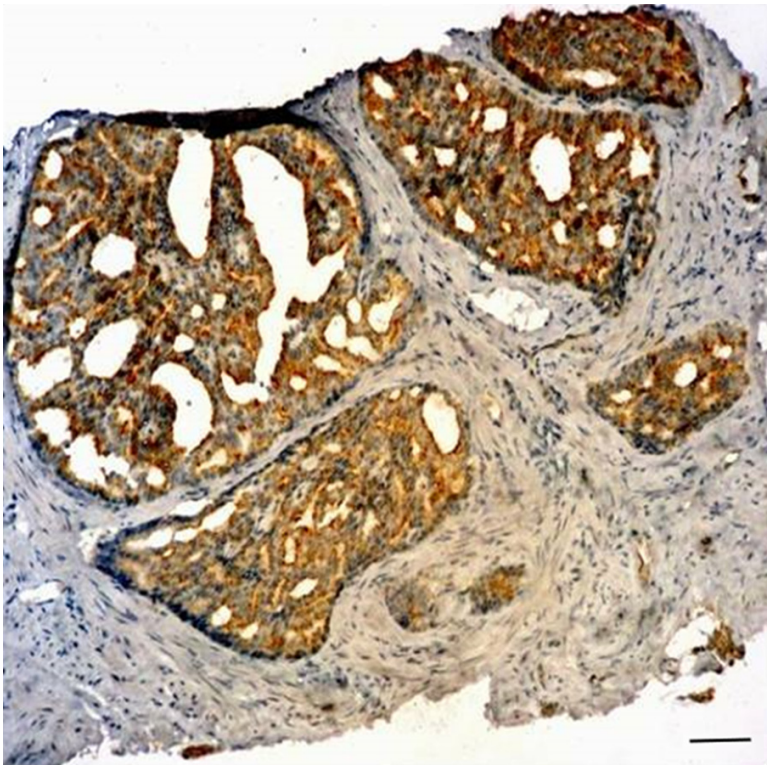

Figure 1. Strong cytoplasmic AMACR staining in a case of prostatic adenocarcinoma with Gleason score 4+4=8 (IHC, 100x), (Scale bar -100 μm).

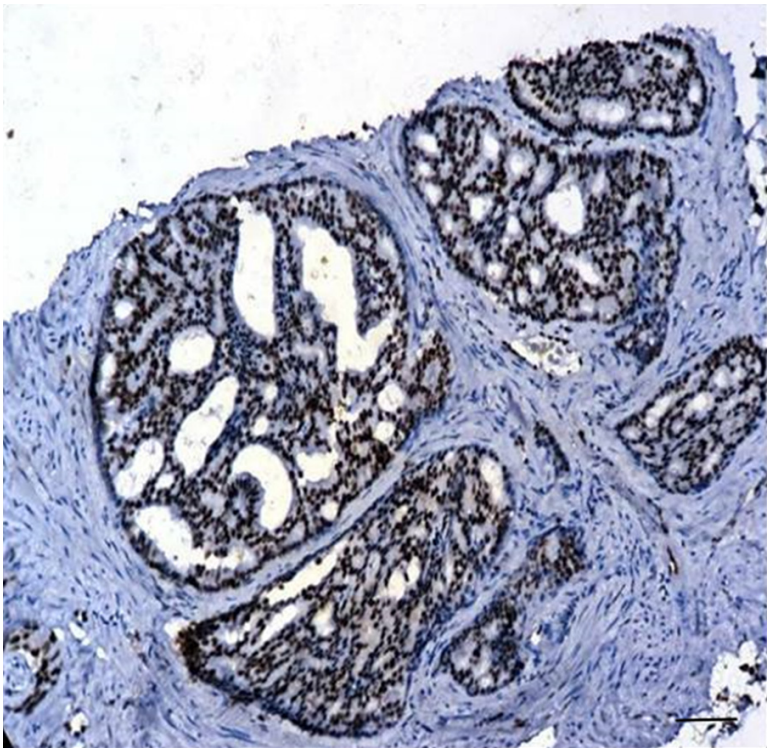

Figure 2. Strong nuclear ERG staining in a case of prostatic adenocarcinoma with Gleason score 4+4=8 (IHC, 100x) (Scale bar -100 μm).

the 71 adenocarcinoma cases, 67 were acinar adenocarcinoma, 2 were a mucinous variant of prostatic adenocarcinoma, and 2 were foamy gland variant of prostatic adenocarcinoma.

Nine different Gleason patterns were noted in the study, and it was found that the Gleason pattern (4+4) was the most common (22.5%). The least common patterns observed were (3+5) and (5+3), each constituting 4.2%. Based on the grade compression described earlier, it was found that 57.7% of cases were of higher grade (poorly differentiated), 29.6% of cases were intermediate grade, 12.7% were moderately differentiated grade (**Figures 3**-**5**).

Special histomorphological features in adenocarcinoma

The histomorphological features of prostatic adenocarcinoma such as the presence of blue mucin, glomeruloids, perineural invasion, associated HGPIN, and LGPIN were studied. Of the 71 adenocarcinoma cases, 19 (26.8%) cases showed associated high-grade prostatic intraepithelial neoplasia (HGPIN), 3 (4.2%) cases showed associated low grade prostatic intraepithelial neoplasm (LGPIN). 33 (46%) cases showed perineural invasion, lymphovascular invasion in 8 (11.3%) cases, glomeruloids in 10 (14.1%) cases, blue mucin in 42 (59.2%) cases. Of the 71 cases of adenocarcinoma studied, 19 cases showed associated HGPIN. The various patterns of HGPIN were studied. Among the HGPIN cases, cribriform pattern (42.1%) was the

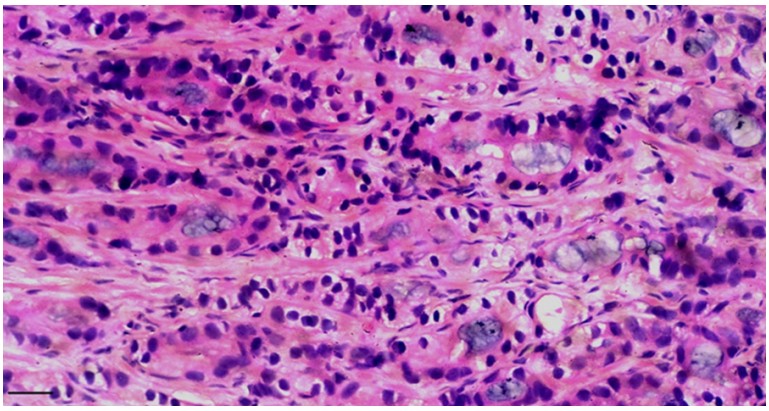

Figure 3. Prostatic adenocarcinoma with presence of blue-tinged mucinous secretions (H&E, 200x) (Scale bar -100 μm).

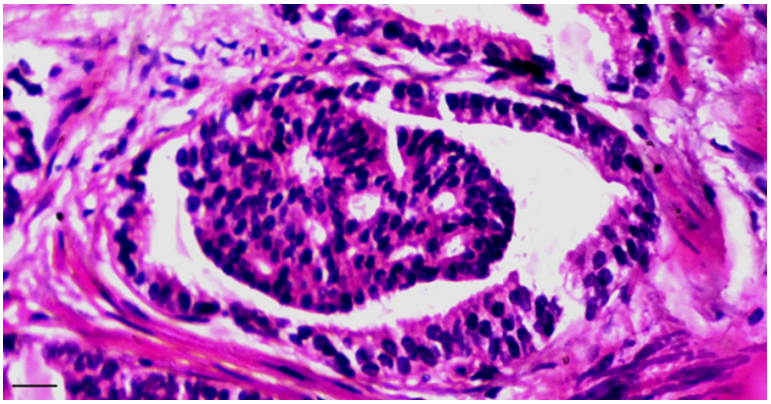

Figure 4. Prostatic adenocarcinoma with presence of glomerulations (H & E, 400x) (Scale bar -100 μm).

predominant pattern, followed by micropapillary (31.6%), tufted (15.8%), and flat type (10.5%). The clinicopathologic findings are compared in **Table 4**.

IHC expression

AMACR expression in the prostatic adenocarcinoma group and the comparison group: The sensitivity and specificity of AMACR were 95.8% and 96.5% respectively and the positive predictive and negative predictive values for AMACR were 95.8% and 96.5%. Among the 71 cases, 43 (60.6%) cases showed score 3 positivity and 25 (35.7%) cases showed score 2 positivity. Therefore, AMACR was considered positive or overexpressed in 68 cases. Two cases showed score 1 staining (weak intensity) and one case was negative. Cases were considered positive only if they showed score 2 and 3. The comparison group consisted of 85 cases (BPH cases) which were negative for adenocarcinoma. Two cases of foamy gland variant and the mucinous variant of prostatic adenocarcinoma were also included in the study. Both the cases of foamy gland variant and mucinous variant of prostatic adenocarcinoma were positive for AMACR (100%). Three cases showed an associated intraductal carcinoma prostate (IDC-P). Among the three cases, two were positive for AMACR and one case showed a weak 1+ staining intensity.

ERG expression in the prostatic adenocarcinoma groups with the comparison groups: The sensitivity and specificity of ERG were 35.2% and 100% respectively and the PPV and NPV were 100% and 64.9% respectively. Among the 71 cases, 12 cases showed score 3 positivity, and 13 cases showed score 2 positivity. Two cases showed weak staining (score 1). The remaining 44 cases were negative. Cases were considered positive only if they showed score 2 and 3. The comparison group consisted of 85 cases (BPH cases) which were negative for adenocarcinoma. Among the two cases of foamy gland variant of prostatic adenocarcinoma, both were negative for ERG, and one among the two cases of mucinous variant of prostatic adenocarcinoma was positive for ERG (50%). Two out of the three cases showing associated IDC-P were positive for ERG (66.7%). One case was negative.

Among the 71 cases of prostatic adenocarcinoma, 95.8% were positive and 4.2% cases were negative for AMACR. Among the BPH cases (comparison group), only 3.5% were positive for AMACR and 96.5% were negative for AMACR. Most cases of histopathologically diagnosed prostatic adenocarcinoma (95.8%) were positive for AMACR and the association was found to be statistically significant ($P < 0.0001$). Among the 71 cases of prostatic adenocarci-

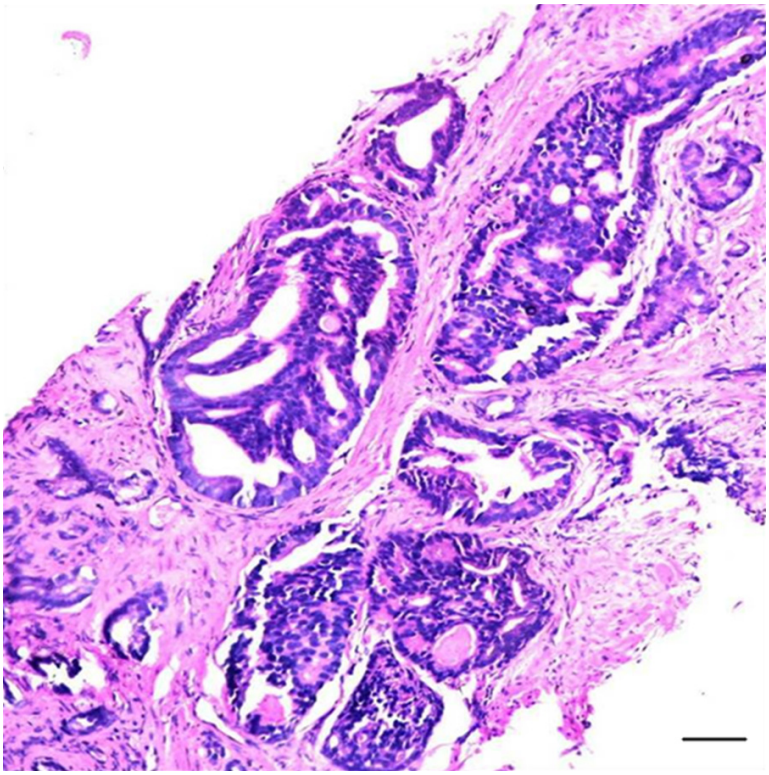

Figure 5. Gleason score 4+4=8 with presence of irregular cribriform glands (H&E, 100x) (Scale bar - 100 µm).

noma, 35.2% were positive and 64.8% cases were negative for ERG. Among the BPH cases (comparison group), none of the cases were positive for ERG and all cases (100%) were negative for ERG. The association was statistically significant (P < 0.0001). The association between Gleason grade and PSA levels with IHC expression was studied. It was found that AMACR expression with Gleason grades was not significant (P=0.94) but there was an increase in AMACR expression with increasing PSA levels and the association was significant (P=0.011).

The expression of ERG decreased with increasing Gleason grades (P=0.02) and PSA level (P=0.02) and this association was significant. Among the 19/71 cases of prostatic adenocarcinoma with HGPIN, 14 cases showed weak staining for AMACR (score 1). Four cases showed a score 2 staining. One case was negative. Among the 3/71 cases of LGPIN, all showed a weak score 1 staining intensity. Among the 19/71 cases of prostate adenocarcinoma showing an associated HGPIN, 8 cases showed weak staining for ERG (score 1). One case showed score 2 staining intensity. Ten cases were negative. Among the 3/71 cases of LGPIN, all showed a weak score 1 staining intensity.

Discussion

The demographic profile of the 71 patients with prostatic adenocarcinoma revealed the range of most of the cases of prostatic adenocarcinoma ranged between 70 to 79 years.

The age range in the present study was compared with the studies by *Garg et al*., *Barkzai et al*., and *George et al*. The number of prostatic adenocarcinoma cases in the present study was compared with other studies with similar results. The comparison of various Gleason grades was also studied. As compared to the study by *Varma et al.* and *Garg et al*., the present study had more cases of poorly differentiated tumors [4, 5] (**Table 5**). Special morphologic features like blue mucin, glomerulations, and perineural invasion were noted in adenocarcinoma which aids in the diagnosis of adenocarcinoma. Among the special histomorphologic features that were studied, the presence of blue mucin was the most common finding constituting 59.2%, followed by perineural invasion (46.0%), glomeruloids (14.1%), and lymphovascular invasion (11.3%). *Varma et al*. in their study of histomorphologic parameters of prostate carcinoma also obtained similar results except for perineural invasion.

Diagnostic utility of ihc markers in adenocarcinoma of prostate

AMACR which is overexpressed in prostatic adenocarcinoma is a peroxisomal and mitochondrial enzyme [6-9]. In various studies, it was found that AMACR had a sensitivity of 80-100% and a specificity of 79-100% [10-13].

The association between Gleason grade and AMACR expression was studied and was not

Table 4. Gleason score with age, bone metastasis, tumor volume, PIN, and perineural invasion

Gleason Grade	no. of patients (n=71)	Age (median)	Bone Metastasis (+)	Percent of core involved (%) (mean)	PIN	Perineural invasion
0-4 (I)	0	0	0	0	0	0
5-6 (II)	9 (4*)	70	2 (5.3%)	46.1%	4 (44.4%)	3 (33.3%)
7 (III)	21 (11*)	70	7 (18.4%)	50.7%	6 (28.6%)	10 (47.6%)
8-10 (IV)	41 (23*)	70	16 (42.1%)	63.6%	12 (52.2%)	20 (48.7%)

*-available cases with bone scan. **Table 4** shows the overall comparison of Gleason grade with age, bone scan, tumour volume, PIN and perineural invasion (PNI). It was observed that most of the patients belonged to the poorly differentiated category (57.7%) with an increasing tendency for bone metastasis, tumor volume and a higher percentage of perineural invasion as the grade increases. Though the presence of an associated PIN was found to drop in the intermediate category, the grade 4 tumours, however, showed a higher percentage of cases with PIN.

Table 5. Comparison of grades of tumour among various studies

Grade	Present Study (n=71)	*Varma et al.* (n=150)	*Garg et al.* (n=68)	*George et al.* (n=125)
Well differentiated	-	1 (0.7)	-	11 (8.8)
Moderately differentiated	9 (12.7)	74 (49.3)	19 (27.9)	31 (24.8)
Intermediate	21 (29.6)	49 (32.7)	35 (51.5)	-
Poorly Differentiated	41 (57.7)	26 (17.3)	14 (20.6)	82 (65.6%)

statistically significant (P=0.94). In the present study, we can infer that all the epithelial cells in adenocarcinoma express AMACR irrespective of the Gleason grade. Rubin et al. and Luo et al. also observed similar findings.

The correlation between PSA levels and AMACR expression was found to be significant with high expression of AMACR correlating with higher PSA levels (P=0.02). AMACR expression in PIN was associated with adenocarcinoma. Among the 19 cases showing an associated HGPIN, 14 cases showed a weak staining for AMACR (score 1). Four (5.6%) cases showed a score 2 positivity. ERG gene rearrangement is considered as the most widely known genetic change in prostatic carcinogenesis. This rearrangement juxtaposes the ETS family of genes (ERG) next to androgen promoter gene TMPRSS2. This leads to the invasiveness of the prostatic epithelial cells by elaboration of matrix metalloproteases. Studies have used anti-ERG antibodies and found the IHC results to correlate well with the results of ERG gene rearrangements by FISH [14, 15]. ERG gene rearrangement is noted in 50% of prostatic adenocarcinoma [16]. In the present study, ERG expression was studied in all the 71 adenocarcinoma cases by IHC and in 85 BPH cases which formed the control group. Immunostaining with p63 as a basal cell marker was performed in all cases for identifying PIN. ERG expression in adenocarcinoma and BPH of the prostate were studied and 35.2% of the adenocarcinoma cases were positive (with a score 3 and score 2 staining intensity), and none of the BPH cases were positive. The proportion of staining was found to be score 4 in all the adenocarcinoma cases. These were in concordance with other studies [1]. Various studies on ERG IHC in prostatic adenocarcinoma were studied with a sensitivity of 36% and 45% respectively and an almost 100% specificity in all these studies [1, 17-20].

In the present study, Gleason grades, PSA levels, tumor volume were compared with ERG expression and a decreasing tendency in ERG expression was noted with an increase in Gleason grades, PSA levels, and tumor volume respectively, and the association was found to be statistically significant. An association between ERG expression and Gleason grade was also demonstrated in other studies. Prior studies by *Wienmann et al.*, *Petterson et al.* found no significant association between ERG and Gleason grade [21, 22].

A study by *Darnel et al.* showed ERG rearrangement more associated with Gleason score 6 and 7 (82%) than Gleason score 8 (14%) and the association was found to be statistically significant. We can extrapolate the fact that ERG expression decreases with increasing tumour grade, PSA levels, and large volume

tumors. We can conclude that ERG expression may be an early event in prostatic carcinogenesis [23].

The ERG immunochemistry results were compared with that of the ERG rearrangement studied through FISH by various authors. The IHC expression of ERG was found to correlate with ERG rearrangement by FISH technique [24]. Among the 19 cases with HGPIN, one case showed a 2+(5.3%) positivity, 8 cases showed a weak 1+(42.1%) staining intensity and 10 cases were negative similar to a study by Perner et al. [24].

We can extrapolate from this finding that ERG expression is observed in early carcinogenesis and decreases as the grade, PSA levels, and tumor volume increase, similar to other studies. We can conclude that ERG expression may be an early event in prostatic carcinogenesis.

ERG expression can be used in molecular subtyping of prostatic adenocarcinoma. ERG status may define molecular subtypes that may provide context for other biomarkers. One example is that the presence of PTEN loss is associated with more adverse prognostic features in those with associated ERG positivity than those with ERG negativity [24, 25].

Similarly, tumors expressing CRISP3 with associated strong expression of PTEN and ERG are found to demonstrate a worse prognosis [26, 27]. ERG can be considered as a distinct molecular subtype with specific targeted therapies. In a study on radical prostatectomy specimens, the presence of ERG expression was not associated with biochemical recurrence instead were showing positivity for androgen receptors (AR). Thus, these patients can be candidates who would respond well to androgen therapy [28].

Negative expression

Among the three cases with associated intraductal carcinoma prostate [IDC-P], two showed 2+ positive staining for AMACR and ERG and the other one with 1+ staining for AMACR and ERG. *Robinson et al.* found the high expression of AMACR in IDC-P in his study. Han et al. found that 75% of cases with IDC-P showed ERG positivity. The morphologic criteria to detect IDC-P are the presence of dense/solid cribriform glands, the presence of nuclear pleomorphism and giant cells (6 times the size of adjacent nuclei), the presence of non-focal comedonecrosis, and the presence of large caliber glands. Reporting of IDC-P is vital in needle biopsies as well as in radical prostatectomy specimens since it is associated with a worse prognosis and is associated with a higher Gleason grade, a higher tumour volume, extraprostatic extension (EPE), pelvic lymph node metastasis, and the presence of extraprostatic extension [29, 30].

Conclusion

AMACR, as an immunohistochemical marker for prostatic adenocarcinoma, has high utility in the diagnosis of prostatic adenocarcinoma due to its high sensitivity and specificity in distinguishing benign from malignant lesions of the prostate. ERG is another marker with high specificity and low sensitivity in distinguishing benign from malignant lesions of the prostate. ERG due to its low sensitivity to detect adenocarcinoma cannot be utilized inadvertently for distinguishing benign from malignant lesions of the prostate. The association of AMACR with histopathologic diagnosis, Gleason score, tumor volume, PSA level, and bone metastasis was carried out. The association of AMACR score and PSA levels was statistically significant. However, there was no significant correlation between Gleason grade and AMACR expression. There was a relatively high ERG expression associated with low-grade tumor, low PSA level, and lesser tumour volume with a statistically significant association. This suggests ERG is expressed early in prostatic carcinogenesis. Studies have shown that ERG-positive subtypes express high levels of androgen receptors. Adenocarcinoma of the prostate can be further divided as ERG positive and ERG negative distinct tumor molecular subtypes for further enhancing the avenues for targeted therapies.

Disclosure of conflict of interest

None.

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