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AMACR expression in Prostate cancer and its correlation with Gleason grading - A study at a tertiary care hospital in Himachal Pradesh

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Abstract

Introduction: Prostate carcinoma is currently the third most commonly diagnosed cancer with a worldwide prevalence of 1.4 million cases and an incidence of 7.3%. The main aim of our study was to ascertain the role of AMACR in the diagnosis of PCa and its correlation with Gleason grading.

Method: The study design was conducted in the departments of Pathology and Urology, Indira Gandhi Medical College, Shimla for a period of two years (w.e.f. June 1, 2019 to May 31, 2021). Routine H&E staining for histopathological diagnosis and IHC analysis for AMACR was carried out in all the cases.

Result: The most common Gleason Grade of PCa observed were 5(36.8%) followed by 4(29.4%) and 2 (16.2%) respectively. Majority (91.2%) of the cases of PCa showed AMACR positivity in the tumor cells. There was a positive correlation between AMACR staining intensity and the Gleason grade in our study (p value: 0.0001).

Keywords: Prostate Cancer, Gleason Grading, Immuno histochemistry, AMACR

Introduction

Prostate carcinoma is currently the third most commonly diagnosed cancer with a worldwide prevalence of 1.4 million cases and an incidence of 7.3%. The mortality attributed to it in the year 2020 was 3,75,000 making it the fifth leading cause of cancer related death in men.[1] In 2018, India had 25,696 new cases and 17,184 deaths related to PCa.[2]

The current gold standard for diagnosis of PCa is a prostatic biopsy, usually via a transrectal ultrasound (TRUS) approach. The diagnosis along with grading is usually established on hematoxylin and eosin (H&E)-stained sections.[3]

Prior to 1966, there was no standardized grading system for PCa which made its prognostic categorization difficult. To overcome this shortcoming, Donald F. Gleason developed a classification scheme from a randomized, which relies on low-power observation of glandular architecture.[4]

Markers detected by immuno histochemistry on tissue sections help to support a diagnosis of PCa especially in difficult cases and at metastatic sites. Many such molecular markers have been studied like PSA, PSMA, PSCA, EPCA, Chromogranin A, and AMACR.[5]

AMACR is a mitochondrial and peroxisomal enzyme involved in the metabolism of branched chain fatty acid and bile acid intermediates.[6] AMACR expression in PCa has been studied by various investigators and in these studies, the sensitivity of AMACR was more than 80%, making it a sensitive marker for Prostate carcinoma.[7,8,9] A few studies have been conducted to find out the correlation of AMACR with Gleason grading, some showed a positive correlation while others showed no corelation.[10,11,12]

The aim of this study was to ascertain the role of AMACR in the diagnosis of PCa and its correlation with Gleason grading.

Materials and methods

The study design was a hospital based cross-sectional observational study conducted in the departments of Pathology and Urology, Indira Gandhi Medical College, Shimla for a period of two years (w.e.f. June 1, 2019 to May 31, 2021). The study was approved by the institutional ethics committee. All the diagnosed cases of PCa during the mentioned time period (via TRUS guided biopsy or TURP chips) were included in the present study.

H&E-stained paraffin sections were examined by three observers for establishment of diagnosis of Carcinoma Prostate and Modified Gleason grading.

Gleason grading

(Based on the 2019 ISUP Consensus Conference on Grading of PCa).[4]

Gleason pattern 3

- Single, separate glands, may be either minute or large and cyst-like; glands having irregularly separated, ragged, poorly defined edge, looser than a nodule and being infiltrative.

Gleason pattern 4

- Small acinar structures, some with well-formed lumina, fusing into cords or chains.
- Cribriform glands with irregular edges.
- Hyper-nephroid pattern with nests of clear cells resembling renal cell carcinoma.
- Intraductal carcinoma, when admixed with invasive carcinoma.
- Glomeruloid pattern (a rare small cribriform variant containing a tuft of cells that is mostly detached from its surrounding duct space except for a single point of attachment)

Gleason pattern 5

- Comedo-necrosis: central necrosis with intra-luminal necrotic cells or karyorrhexis within papillary / cribriform spaces.
- Single cells, possibly forming cords, possibly with vacuoles (signet ring cells) but without glandular lumina.

The most prevalent pattern was scored as primary and the worst pattern was scored as secondary. The tumour was graded as the sum total of primary and secondary pattern as follows

Table 1:

Grade	Primary Pattern	Secondary Pattern	Score
1	3	3	6
2	3	4	7
3	4	3	7
4	4,3,5	4,5,3	8
5	4,5,5	5,4,5	9 and 10

*If tumor was minimal on biopsy (≤ 1 mm), Gleason grading was not done.

AMACR immuno stained sections were evaluated under the light microscope independently by three observers for expression of AMACR and staining intensity was assessed in carcinoma of the prostate. IHC staining intensity of 1+, 2+ or 3+ was considered as positive.

The cytoplasmic staining intensity & pattern were considered for scoring according to the following scheme:[11,12]

Table 2:

0 (negative)	No staining is observed
1+ (weak positive)	Weak scant faint granular staining, not seen on low-power magnification.
2+ (moderately positive)	Moderately continuous dark cytoplasmic or apical granular staining, significantly stronger than that of adjacent benign glands and visible on low-power magnification.
3+ (strong positive)	Strongly continuous dark cytoplasmic or apical granular staining, significantly stronger than that of adjacent benign glands and visible on low-power magnification.

Figure 1: prostatic adenocarcinoma, Gleason pattern 3 (h&e; 100x)

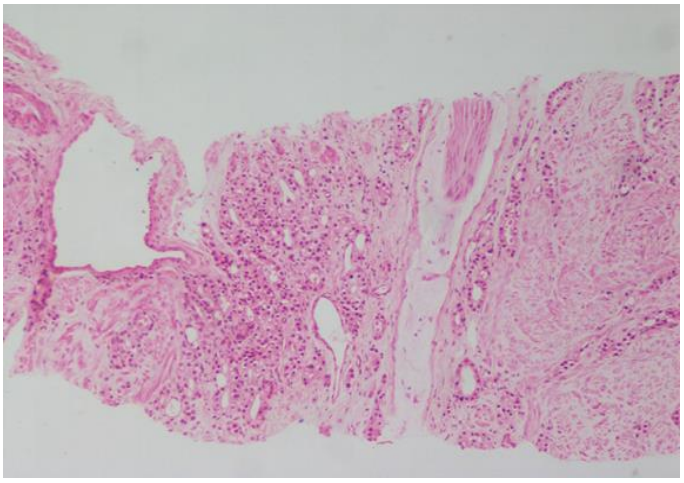


Figure 2: prostatic adenocarcinoma, cribriform like arrangement Gleason pattern 4 (h&e;400x)

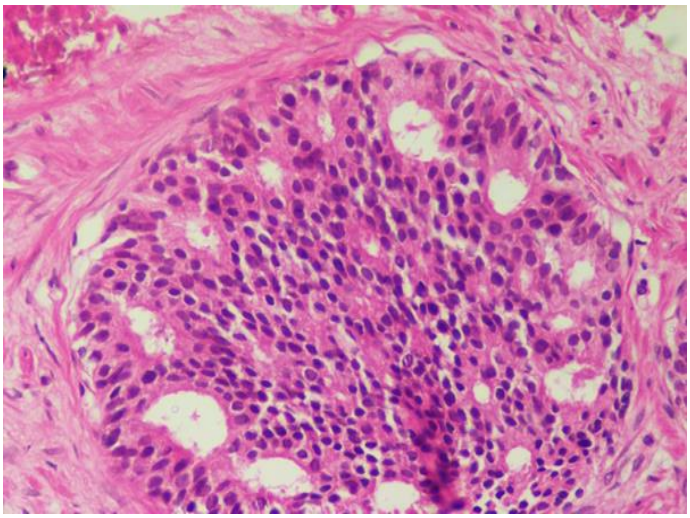


Figure 3: prostatic adenocarcinoma, arrangement in sheets; Gleason pattern 5 (H&E; 100X**)**

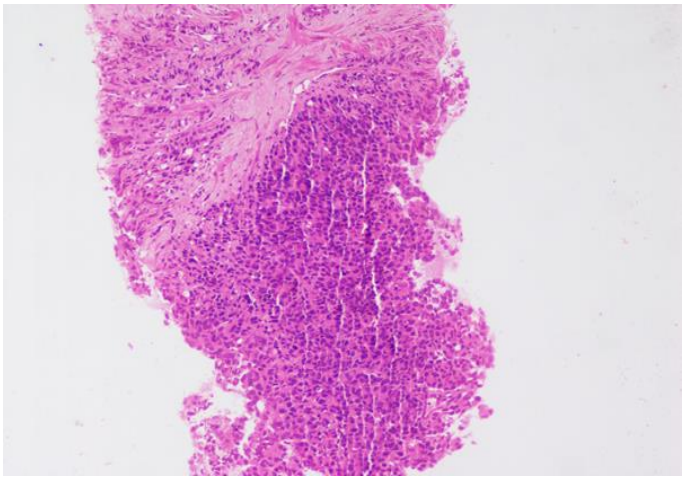


Figure 4: amacr negative staining in PCa (400x)

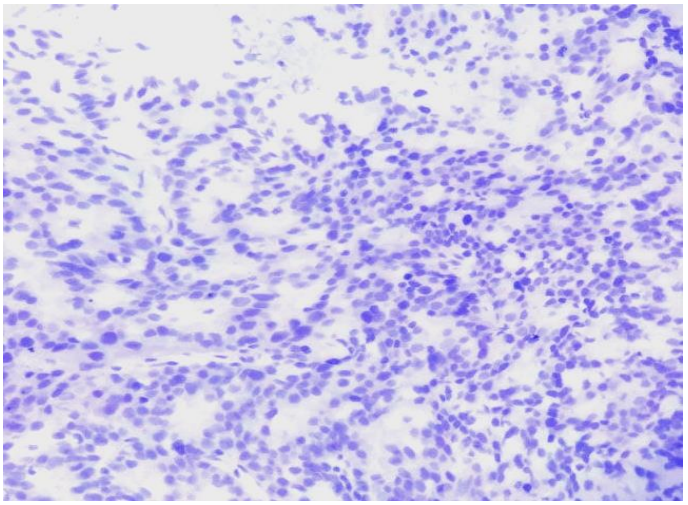


Figure 5: prostatic adencarcinoma, amacr 1+ staining (400x)

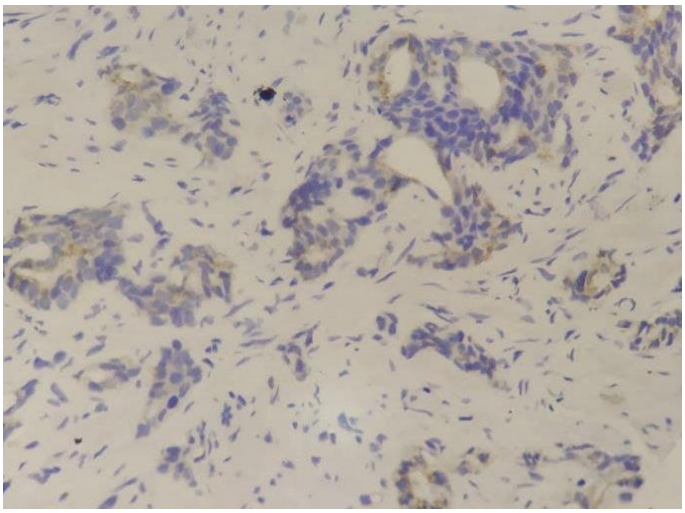


Figure 6: prostatic adencarcinoma, amacr 2+ staining (400x)

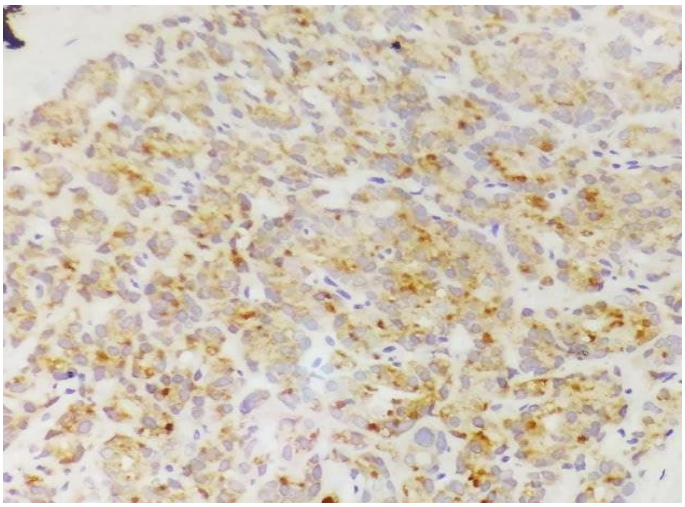
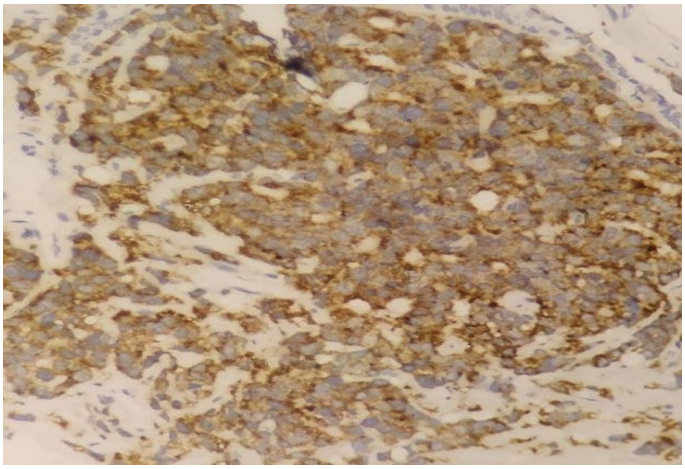


Figure 7: prostatic adenocarcinoma; amacr 3+ staining (400X)



Results

Sixty-eight patients diagnosed with PCa were included in the present study to evaluate their histopathological diagnosis, Gleason Grading and assessment of AMACR expression in relation to Gleason grade.

Table 3: Age Distribution of 68 patients of PCa

Age (years)	No. of cases	Percentage (%)
51-60	7	10.3
>60-70	26	38.2
>70-80	26	38.2
>80-90	9	13.2
Total	68	100.0

In our study, 52 (76.4%) patients were in the age group of >60 to 80 years. The youngest patient was 51 years old and the oldest patient was 88 years of age with a mean age of 71 years.

Table 4: Gleason score of the 68 cases of PCa in the present study

Gleason score Primary pattern+ Secondary pattern	No of cases	Percentage (%)
3+3=6	3	4.4
3+4=7	11	16.2
4+3=7	9	13.2
4+4=8	20	29.4
4+5=9 5+4=9	21	30.9
5+5=10	4	5.9
Total	68	100.0

As obvious from table no. 2, majority of the cases i. e 61(73.5%) had Gleason scores of 7 to 9. Gleason score of 6 and 10 were seen in 3 (4.4%) and 4 (5.9%) cases of PCa respectively.

Table 5: Gleason grade in 68 cases of PCa in the present study

Gleason grade	No of cases	Percentage (%)
1	3	4.4
2	11	16.2
3	9	13.2
4	20	29.4
5	25	36.8
Total	68	100.0

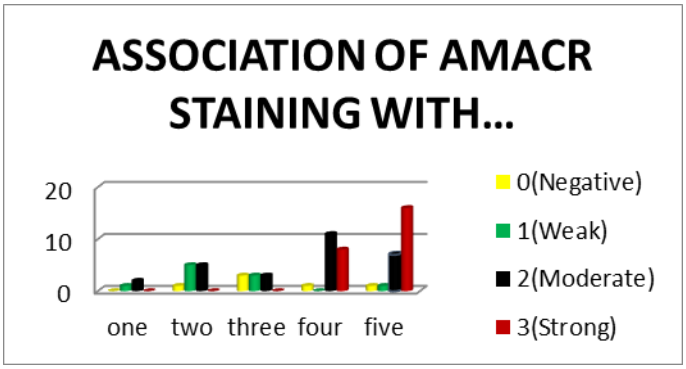
As seen in table no. 3, 45 (66.2%) cases of PCa had a Gleason grade of 4&5 followed by grade 2&3 in 20(29.4%) biopsies. However, 3(4.4%) patients had Gleason grade 1.

As obvious from table 8, moderate to strong (2+ and 3+) intensity was seen in 52 (76.5%) cases of PCa. Ten (14.7%) cases of PCa showed weak (1+) staining intensity. However, in our study, 6 (8.8%) cases did not show any AMACR expression.

Table 6: AMACR expression in 68 cases of PCa		
AMACR Staining intensity	No of cases	Percentage (%)
0 (Negative)	6	8.8
1+(Weak)	10	14.7
2+(Moderate)	28	41.2
3+(Strong)	24	35.3
Total	68	100.0

Table 7: AMACR expression with respect to Gleason Grade in 68 cases of PCa					
Gleason grade	AMACR staining intensity				
	0 (Negative)	1+ (Weak)	2+ (Moderate)	3+ (Strong)	TOTAL
1	0(0%)	1(33.3%)	2(66.7%)	0(0%)	3(100%)
2	1(9.1%)	5(45.5%)	5(45.5%)	0(0%)	11(100%)
3	3(33.3%)	3(33.3%)	3(33.3%)	0(0%)	9(100%)
4	1(5%)	0(0%)	11(55%)	8(40%)	20(100%)
5	1(4%)	1(4%)	7(28%)	16(64%)	25(100%)
Total	6(8.8%)	10(14.7%)	28(41.2%)	24(35.3%)	68(100%)
Chi square p value: 0.0001; Spearman r value: 0.578					

Graph 1:



As obvious from table 4, Grade 1,2 and 3 showed only weak to moderate staining intensity whereas majority of cases with grade 4 and 5 showed moderate to strong staining. Only 1 case of Gleason grade 5 was negative for AMACR and another 1 case showed weak positivity i. e 1+. Thus, A statistically significant association was found between AMACR expression and Gleason grade. (p=0.0001; r=0.578)

Discussion

PCa is usually seen as a disease of old age most of the patients being more than 60 years old. The mean age of the patients in our study was 70.8 years which was similar to Gaid et al[13] and Taheri et al.[14]

In our study, the most common PCa was found to be Moderately differentiated (Gleason grades 3&4) in concordance with the observations made by other investigators.[10,11,13] However, the proportion of Gleason grade 5 PCa i.e., poorly differentiated PCa in our study was also more compared to other studies in the literature.

Perineural invasion is a common finding seen in cases of PCa. In 2 recent studies, PNI was seen in 42.5% and 34.5% cases of PCa respectively.[14,16] However in the present study we observed PNI in relatively more cases i. e 58.8% of cases.

AMACR expression in PCa has been studied by various investigators and all of them have reported it as a sensitive maker for PCa.[13,6,7,17,18,9,11,12,19]

Table 8: Comparison of AMACR positivity in cases of Prostatic Adenocarcinoma in the present study with other studies in the literature.

Study and year	Total cases	AMACR staining	
		Positive	Negative
Jiang Z et al (2001)[6]	137	100%	0%
Ming Zhou et al (2002)[7]	72	83%	17%
Beach et al (2002)[9]	124	82%	18%
Murphy et al (2007)[17]	57	91%	9%
Ozgur et al (2013)[19]	68	90.6%	9.4%
Gaid et al (2017)[13]	49	100%	0%
Rathod et al(2019)[11]	40	90%	10%
Ayo Wole et al (2021)[12]	30	100%	0%
Present study	68	91.2%	8.8%

In the present study, AMACR expression was seen in 91.2% of the cases of PCa which was in concordance with other studies in the literature.

The studies conducted by Jain et al[10] and Rathod et al[11] showed no correlation between the AMACR staining intensity and Gleason Grading. However, Murphy et al[17] and Gaid et al[13] in their studies have found a correlation between the increasing AMACR staining intensity and the Gleason grade with a p value of <0.005 and 0.004 respectively. Similar to these 2 studies, we also observed a correlation between AMACR staining intensity and Gleason grade with a p value of 0.0001.

Conclusion

Our study included 68 patients of PCa. The diagnosis and Gleason grading of PCa was established on H & E-staining. The Gleason scores varied from 6 to 10. The most common score was 9 in 30.9% of the patients and the least common score was 6 in 4.4% of the patients. Also, the most common Gleason Grade of PCa observed were 5(36.8%) followed by 4(29.4%) and 2 (16.2%) respectively.

IHC for AMACR expression was performed in all the 68 cases of PCa. Majority (91.2%) of the cases of PCa showed cytoplasmic positivity in the tumor cells and staining intensity was moderate to strong in 76.5% of the cases. Weak staining intensity for AMACR was seen in 14.7% of cases and 8.8% did not show any AMACR expression. Thus, AMACR is a sensitive marker for PCa. There was a positive correlation between AMACR staining intensity and the Gleason grade in our study (p value: 0.0001). So, we infer that AMACR can be a sensitive diagnostic and prognostic marker for PCa.

Abbreviations

S.N	Abbreviation	Full form
1.	AMACR	Alpha-methyl acyl-CoA racemase
2.	H&E	Haematoxylin and Eosin
3.	HP	Himachal Pradesh
4.	IGMC	Indira Gandhi Medical College
5.	ISUP	International Society of Urological Pathology
6.	PCa	Prostate Cancer

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