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Original Article

Pathology Section

Utility of Alpha-Methyl Acyl-CoA Racemase Marker in Prostatic Adenocarcinomas

VAHINI GUDELI[1], UMA RANI PALLIVILLA[2], SWATHI CHILKURI[3], HARSHINI REDDI[4], KRISHNA RAAVI[5], ASHA THOTA[6]

ABSTRACT

Introduction: Prostatic adenocarcinoma is the second leading cause of cancer related death in men in the western world and its incidence is increasing in Asian countries. Hence, it is of diagnostic challenge for pathologists to report in tissue biopsies especially in small focus of suspicious glands in radical prostatectomies and needle biopsies.

Aim: To evaluate the role of Alpha-Methyl Acyl CoA Racemase (AMACR) immunohistochemical marker in diagnosing prostatic adenocarcinomas and in benign conditions of prostate.

Materials and Methods: This was a cross-sectional study of 26 cases of prostatic adenocarcinomas, two cases of High Grade Prostatic Intraepithelial Neoplasias (HGPIN), 139 cases of Benign Prostatic Hyperplasia (BPH) and two cases of atypical adenomatous diagnosed on routine Haemotoxylin and Eosin (H&E) stained sections during a period of four years from January 2016 to December 2019 at ASRAM Medical College, Eluru. Immunohistochemistry with AMACR marker was done in all cases. Membranous staining pattern was accepted for prostatic adenocarcinomas. Semiquantitative scoring method was employed and tissue sections were examined at high power magnification (X400) by Olympus light microscope to evaluate AMACR expression.

Results: Out of the 26 cases of prostatic adenocarcinomas in the study, majority were Grade group IV according to Gleason scoring system. Majority of the prostatic carcinomas showed strong and diffuse AMACR positivity. All BPH and Atypical Adenomatous Hyperplasia (AAH) were negative to AMACR immunohistochemical marker.

Conclusion: The AMACR was found to be important diagnostic immune marker in prostatic adenocarcinomas especially in problematic situations where quantity and quality of tissue is limited.

Keywords: Diagnostic challenge, Immunohistochemical marker, Over expression, Radical prostatectomies

INTRODUCTION

Prostatic cancer is the second most leading cause of cancer related death in men in the western world. There is increasing incidence in Asia and diagnosing prostate cancer based on architecture or cytological clues is a challenge for pathologist and is difficult to diagnose in a small foci of suspicious gland in Transurethral Resection of the Prostate (TURP) chips and needle biopsies [1-3]. The tissue examination of a prostate needle biopsy or transurethral resection specimen of prostate, for presence of Prostrate Specific Antigen (PSA), is mandatory for the diagnosis of prostate cancer and permits patients to receive appropriate therapy.

The recent discovery of the P504S/AMACR, a newer immunohistochemical marker is found to be a useful aid in distinguishing prostatic cancer from its benign mimics and has high sensitivity and specificity. AMACR is over expressed in more than 90% of prostatic cancers. It is a peroxisomal and mitochondrial enzyme that is important for beta oxidation of dietary branched chain fatty acids and C27 bile acid intermediates [4,5].

The aim of the study was to analyse AMACR expression in prostatic adenocarcinomas, prostatic intraepithelial neoplasias and also in BPH, AAH in TURP chips. And, also to correlate prostatic adenocarcinomas with Gleason's grade.

MATERIALS AND METHODS

This was a cross-sectional study conducted from January 2016 to December 2019.The study was conducted at Asram Medical College, Eluru, Andhra Pradesh, India. Twenty-six cases of prostatic adenocarcinomas and two cases of prostatic intraepithelial neoplasia, along with 139 cases of BPH and two cases of AAH were included. All the diagnoses were based on H&E stain. The sample was time based and convenient sampling method was employed for sample size. The institutional ethical clearance was taken as no-IEC/ASR/APPROVAL/036/2019.

Inclusion criteria: The cases that were diagnosed as prostatic adenocarcinomas, prostatic intraepithelial neoplasia, BPH and AAH on H&E stained sections of TURP chips were included in the study. All prostatic samples included in the study were of TURP chips.

Exclusion criteria: Radical prostatectomy specimens were excluded from study.

Study Procedure

The paraffin blocks of these cases were retrieved and sections of 3-4 mm thickness were cut, de-paraffinised and rehydrated through graded series of alcohol. The standard indirect biotin- avidin immunohistochemical analysis with manual method was performed. Microwave antigen retrieval method with immunohistochemical marker-AMACR was applied. The AMACR clone used was 13H4 with dilution 1:100 and catalogue used was PRO78-6 mL RTU.

Membranous staining pattern was accepted, and the staining intensity was classified into four categories according to Rubin MA et al., [6]. The Staining pattern for AMACR was scored semi quantitatively as diffuse when all glands were positive, focal when some gland or gland portions were positive and rest negative. The intensity was scored as follows strong (3+), moderate (2+), weak (1+) and negative (0).

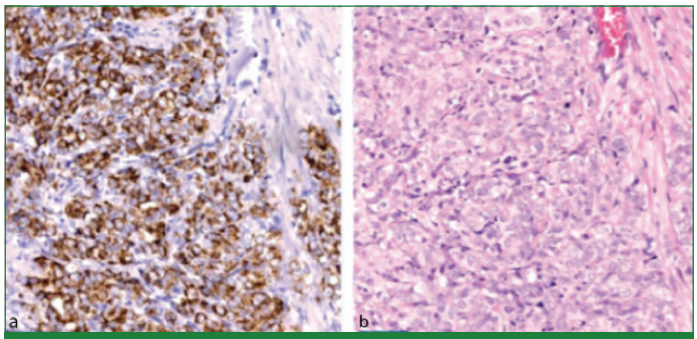
Normal kidney tissue was taken as positive control for AMACR where the epithelial cells of proximal tubules showed strong, distinct granular staining. Haematoxylin and eosin stained slides of these cases were reviewed.

STATISTICAL ANALYSIS

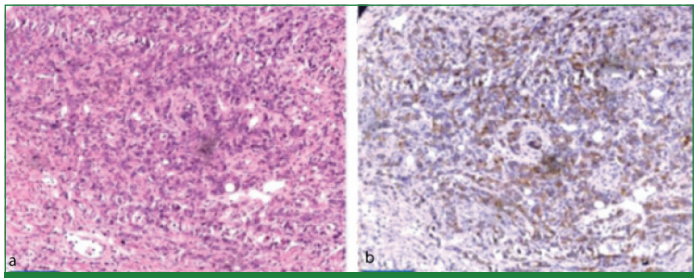
The data was entered and analysed with Excel, proportions were calculated and data presented in bar graph.

RESULTS

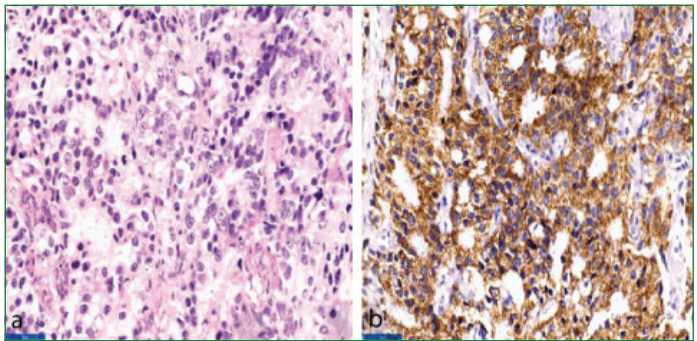
Out of 28 cases of prostatic malignancies included in the study, there were 26 cases of prostatic adenocarcinomas and two cases were of High-Grade Prostatic Intraepithelial Neoplasia (HGPIN). The histopathological patterns of prostatic adenocarcinomas included in the present study were hypernephroid [Table/Fig-1a,b], raggedly infiltrating [Table/Fig-2a,b], cribriform, sheets [Table/Fig-3a,b], and small glandular [Table/Fig-4a,b,5a,b]. Among these 26 cases of prostatic adenocarcinoma-14 cases (54%) were of Grade group IV, 6 cases (23%) of Grade group III and 5 cases (19%) of Grade group II and 1 case (4%) of Grade group I, according to the Gleason scoring system [Table/Fig-6,7] [7]. AMACR expression was found in all 26 cases (100%) of prostatic adenocarcinomas including a case of minimal cancer. AMACR was diffusely positive in 21 cases (81%) and focally positive in 5 cases (19%) [Table/Fig-7]. AMACR intensity was graded as Moderate (2+) in 10 cases (38%), Strong (3+) in 15 cases (58%), Weak (1+) in 1 case (4%) of prostatic adenocarcinomas [Table/Fig-7]. Both the cases (100%) of HGPIN showed moderate (2+) positivity. Atypical/suspicious focus adjacent to HGPIN in one case (microfocus of atypical glands) showed positivity. All one thirty nine cases (139) of BPH and two cases (2) of AAH were negative to AMACR stain though weak, focal positivity was seen in two BPH cases where the histopathology slides were reviewed and immunohistochemical procedure was repeated, subsequently were negative.



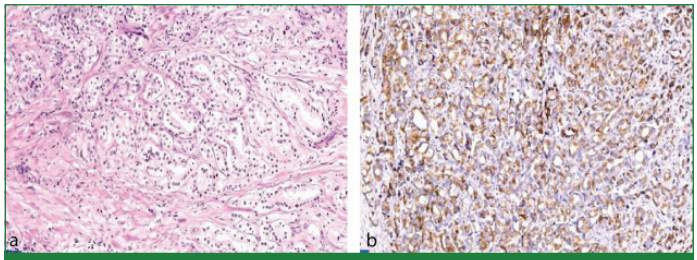
[Table/Fig-1]: a) Prostatic adenocarcinoma in small glandular and hypernephroid patterns with Immunohistochemistry of AMACR stain showing membranous positivity, magnification power X40; b) Prostatic adenocarcinoma in small glandular and Hypernephroid patterns on H&E Stain on Histopathological examination, magnification power X40.



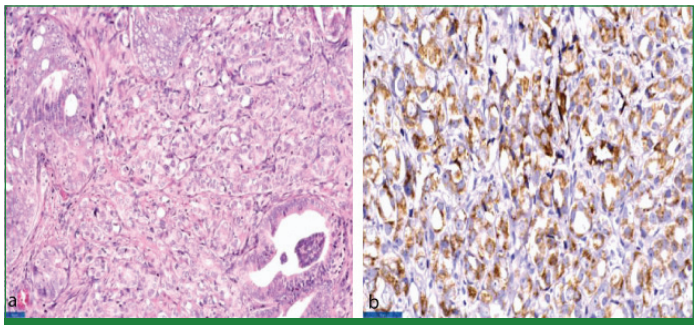
[Table/Fig-2]: a) Prostatic adenocarcinoma with variably sized raggedly infiltrating glands on histopathological examination, Haematoxylin & Eosin stain, magnification power X40, b) Same sections with Immunohistochemistry of AMCAR stain, showing membranous positivity, magnification power X40.



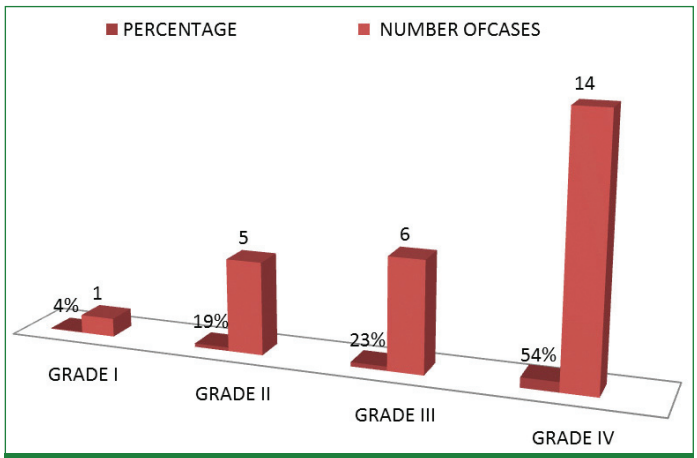
[Table/Fig-3]: a) Prostatic adenocarcinoma in Cribriform pattern, poorly differentiated glands and sheets on histopathological examination on H&E stain, power X40; b) Same sections with immunohistochemistry of AMACR stain, showing membranous positivity, magnification power X40.



[Table/Fig-4]: a) Prostatic adenocarcinoma in small glandular pattern on histopathological examination on H&E stain, magnification power X10; b) Immunohistochemistry AMCAR stain in the same sections showing membranous positivity power X40.



[Table/Fig-5]: a) Prostatic adenocarcinoma with small glandular pattern on histopathological examination on H&E stain, power X10; b) Same sections with immunohistochemistry of AMCAR stain showing membranous positivity, magnification power X40.



[Table/Fig-6]: Percentage/number of prostatic adenocarcinomas with Gleason grade.

Sl. No.	Age (years)	Diagnosis	Gleason's score	Grade group	AMCAR+/- intensity stain
1.	65	Adenocarcinoma	4+4=8	IV	3+strong
2.	72	Adenocarcinoma	4+4=8	IV	3+strong
3.	70	Adenocarcinoma	4+4=8	IV	3+strong
4.	65	Adenocarcinoma	3+5=8	IV	3+strong
5.	65	Adenocarcinoma	4+4=8	IV	3+strong
6.	67	Adenocarcinoma	4+4=8	IV	3+strong
7.	75	Adenocarcinoma	5+3=8	IV	3+strong
8.	72	Adenocarcinoma	3+5=8	IV	3+strong
9.	65	Adenocarcinoma	4+4=8	IV	3+strong
10.	72	Adenocarcinoma	3+5=8	IV	3+strong
11.	55	Adenocarcinoma	3+5=8	IV	3+strong
12.	67	Adenocarcinoma	4+4=8	IV	3+moderate
13.	57	Adenocarcinoma	4+4=8	IV	3+strong
14.	57	Adenocarcinoma	4+4=8	IV	3+strong
15.	70	Adenocarcinoma	4+3=7	III	3+strong
16.	69	Adenocarcinoma	4+3=7	III	3+strong
17.	63	Adenocarcinoma	4+3=7	III	2+moderate
18.	67	Adenocarcinoma	4+3=7	III	2+moderate

19.	65	Adenocarcinoma	3+4=7	III	2+moderate
20.	58	Adenocarcinoma	3+4=7	III	2+moderate
21.	67	Adenocarcinoma	3+4=7	II	2+moderate
22.	63	Adenocarcinoma	3+4=7	II	2+moderate
23.	68	Adenocarcinoma	3+4=7	II	2+moderate
24.	76	Adenocarcinoma	3+4=7	II	2+moderate
25.	65	Adenocarcinoma	3a+4b=7	II	2+moderate
26.	80	Adenocarcinoma	3a+2=5	I	2+moderate
27.	58	High grade PIN		IV	2+moderate
28.	60	High grade PIN			2+moderate

[Table/Fig-7]: List of prostatic adenocarcinomas with age, Gleason's score, grade group and AMCAR (Alpha-Methyl Acyl CoA racemase) stain intensity.

The correlation between prostatic adenocarcinoma score with Gleason's grade was calculated with excel. The R value was found to be 0.918 showing positive correlation.

DISCUSSION

Prostate cancer is the second leading cause of cancer-related deaths in men in the United States [8]. Even though the diagnosis can usually be made based on morphologic features, it is sometimes difficult to diagnose when the foci of cancer is small. In particular for small foci of cancer in needle biopsies and transurethral resection of prostatic chips [9]. Various benign conditions can mimic prostate cancer.

Hence, there is a need for immunohistochemical marker to differentiate prostatic adenocarcinoma from benign conditions of prostate and for diagnosing problematic cases.

The PSA, a prototypic cancer biomarker, highlights both normal and malignant prostatic epithelium and has limited specificity for detecting prostatic carcinomas. Thus, there has been an extensive study to find the positive and sensitive immune marker. AMACR a new potential prostatic adenocarcinoma specific marker has been reported to have sensitivity ranging from 82-100%, respectively [10-13]. In 2001, Jiang Z et al., investigated AMACR protein expression, using immunohistochemical methods, in 137 cases of prostate cancer and 70 cases of benign prostate specimens [14]. Recently, Magi-Galluzzi C et al., studied large numbers (209 cases) of prostate needle biopsy specimens with small foci (<5% of a core) of prostate carcinoma, including 34 cases from their institution and 175 cases from outside consultations [15]. Of small foci of prostate carcinoma, 88% were positive for AMACR. They found that the sensitivity varied among the different groups: 100% for the in-house cases and 80-87% for cases from outside institutions, which they suggested possibly related to differences in fixation processing in different pathology laboratories. Although it is extremely important to recognise negative staining of AMACR in some small cancers, they concluded that positive staining for AMACR could increase the level of confidence in establishing a definitive malignant diagnosis from the needle biopsy specimens and transurethral resection of prostate chips. All these studies have demonstrated that AMACR/P504S could be used successfully as part of the routine surgical pathology workup of difficult prostate biopsy specimens with "suspicious" small glands [15,16].

The AMACR immunostaining was strong in all prostate cancers with continuous dark diffuse cytoplasmic staining or circumferential apical granular staining pattern. The sensitivity of AMACR was 100% in the present study similar to 90-100% documented in other studies [17]. AMACR marker was found to be useful in diagnosing carcinomas especially in small foci in prostatic biopsies [18,19]. AMACR could contribute to prognosis, as it had a role in distinguishing ordinary from aggressive carcinoma [20]. The AMACR proved to be a useful tool which aided in diagnosis of minimal prostate cancer in the present study [Table/Fig-8] [14,15].

Studies and year	Sensitivity to AMACR Marker	Number of prostatic adenocarcinomas
Jiang Z et al., [14] (2001)	82-100%	137
Magi-Galluzzi C et al., [15] (2003)		
In-house cases	100%	34
Outside cases	80%-87%	175
Present study (2016-2019)	100%	26

[Table/Fig-8]: Percentage sensitivity of AMACR (Alpha-Methyl Acyl-CoA Racemase) in prostatic adenocarcinomas [14,15].

The AMACR staining pattern was coarse, strong, and granular in prostatic cancer cells and showed little or no expression in benign glands [21]. A diffuse staining pattern was not found in benign prostate glands.

Moreover, the small, benign glands, which can mimic cancer, including atrophy, basal cell hyperplasia, inflammatory glands, and urothelial epithelium/metaplasia and most cases of adenosis, did not show any expression of AMACR by immunohistochemical analysis with a monoclonal antibody (P504S). All one hundred thirty nine cases (139) of BPH were negative to AMACR stain in the present study [Table/Fig-9] [14,22].

Studies and year	Number of benign prostatic hyperplasia cases	AMACR staining result
Yang XJ et al., [22] (2002)	20	All negative
Jiang Z et al., [14] (2001)	70	All negative
Present study (2016-2019)	139	All negative

[Table/Fig-9]: AMACR (Alpha-Methyl Acyl-CoA Racemase) expression in Benign Prostatic Hyperplasia (BPH) [14,22].

Yang XJ et al., studied 40 cases of AAH by immunohistochemical analysis using the P504S monoclonal antibody and a basal cell-specific marker specific for 34 E12 [22]. AMACR was undetectable in 83% of AAH cases, focally expressed in 10%, and diffusely positive in 8%. Interestingly, two of seven AMACR-positive AAH cases were found adjacent to adenocarcinomas, which were strongly positive for AMACR. All the 20 BPH cases were negative, while all prostatic carcinoma cases showed diffuse AMACR staining pattern.

Gupta et al., recently found that 31% of cases of AAH expressed P504S/AMACR. [23]. The combination of AMACR/P504S and 34βE12 helps to distinguish AAH from prostatic adenocarcinoma, particularly in prostate needle biopsy specimens and transurethral resection of prostate chips. Two cases of AAH in the present study showed negativity to AMACR [Table/Fig-10].

Studies, year	Number/percentage of atypical adenomatous hyperplasias prostate	AMACR staining result
Yang XJ et al., [22] (2002)	33/40 (83%)	Negative
Jain D et al., [23] (2003)	69%	Negative
Present study (2016-2019)	2 (100%)	Negative

[Table/Fig-10]: AMACR (Alpha-Methyl Acyl-CoA Racemase) expression in Atypical Adenomatous Hyperplasia (AAH) [22,23].

Two possible premalignant lesions, HGPIN and AAH, might exhibit some or low reactivity for AMACR. Both PIN and AAH retain basal cells and positive immunostaining for 34βE12 or p63 can help in distinguishing PIN and AAH from prostate cancer [24-31]. However, small glands adjacent to HGPIN with AMACR staining and absence of basal cells might represent out-pouching of the PIN glands. The authors had single case of HGPIN showing focal positivity with AMACR in suspicious small glands indicating prostatic adenocarcinoma adjacent to prostatic intraepithelial neoplasia and after reviewing the histopathology slides, it was confirmed. Approximately, 20% of adenocarcinomas of prostrate are AMACR negative, so AMACR alone is not helpful for the diagnosis of adenocarcinomas [28].

Limtation(s)

The limitations of this study include focal, weak expression of AMACR observed in cases of HGPIN, AAH and also in some benign glands which could be misinterpreted as malignancy. Although, the authors repeated immunohistochemical staining and also reviewed the histopathology slides in those cases, a few technical problems like fixation timings contributed to such false positive results.

CONCLUSION(S)

The AMACR was found to be important diagnostic immune marker in prostatic adenocarcinomas especially in dilemmatic situations where quantity and quality of tissue is limited.

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PARTICULARS OF CONTRIBUTORS:

1. Professor, Department of Pathology, Asram Medical College, ELURU, Andhra Pradesh, India.
2. Associate Professor, Department of Pathology, Asram Medical College, ELURU, Andhra Pradesh, India.
3. Associate Professor, Department of Pathology, Asram Medical College, ELURU, Andhra Pradesh, India.
4. Postgraduate Student, Department of Pathology, Asram Medical College, ELURU, Andhra Pradesh, India.
5. Professor, Department of Pathology, Asram Medical College, ELURU, Andhra Pradesh, India.
6. Professor and Head, Department of Pathology, Asram Medical College, ELURU, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Uma Rani Pallivilla,
Flat No. 203, Sree Swetha Enclave Apartments, Sree Ramachandra Nagar Colony, Vijayawada-520008, Andhra Pradesh, India.
E-mail: gudelivahini@gmail.com

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