

# Diagnostic utility of $\alpha$ -methylacyl CoA racemase in prostate cancer of the Iranian population

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**Background:** Considering the great variations in the reported prevalence of prostate cancer across the world possibly due to different genetic and environmental backgrounds, we aimed to determine the expression pattern and the diagnostic utility of  $\alpha$ -methylacyl coenzyme A racemase (AMACR) among Iranian patients with prostate adenocarcinoma. **Materials and Methods:** In this cross-sectional study, formalin-fixed paraffin-embedded tissues of 58 patients with a definitive pathologic diagnosis of prostatic adenocarcinoma were evaluated. The expression of AMACR, intensity, and extensity of its staining was determined in selected samples by immunohistochemical technique. **Results:** AMACR expression was significantly higher in neoplastic compared to normal tissue ( $P < 0.05$ ). The expression of AMACR was significantly associated with the age of the patients ( $P = 0.04$ ). The intensity of the staining was associated with the grade of the prostate adenocarcinoma ( $P = 0.04$ ). There was no significant relationship between AMACR expression and perineural invasion. The sensitivity, specificity, positive predictive value, and negative predictive value of AMACR were 90%, 96%, 96%, and 90%, respectively. **Conclusion:** Findings from our study indicate that AMACR could be used as a diagnostic tool for the diagnosis of prostate adenocarcinoma. However, due to false-positive staining in the mimicker of prostatic adenocarcinoma, it is recommended to use it in combination with basal cell markers.

**Key words:** Adenocarcinoma, alpha-methylacyl-CoA racemase, biomarker, prostate

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## INTRODUCTION

Prostate cancer is one of the most common cancers in the male population worldwide.<sup>[1]</sup> It is considered to be one of the most important leading causes of death by cancer in men in western countries.<sup>[2]</sup> The incidence of prostate cancer in Asia has been reported to be lower than that in the western world.<sup>[3]</sup> Its incidence in Iran is even lower than that reported for Asian countries.<sup>[4]</sup>

Although prostate cancer is significantly less common in Iran than in developed countries, it is one of the

most frequently reported malignancies among Iranian males and is associated with high rates of morbidity and mortality.<sup>[5-7]</sup> As a result of the age-related nature of this cancer, it is believed that following the increase in the number of elderly in Iran, the prevalence of prostate cancer would also increase.<sup>[4]</sup>

The diagnosis of prostate cancer in patients with clinically localized lesions is typically determined by histopathological examination of prostate needle biopsy samples. In some atypical cases of prostate cancer or small foci of carcinoma in needle biopsy, diagnosis of

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the cancer is a challenging issue for pathologists. In such cases, immunohistochemistry (IHC) could be an essential diagnostic tool for the evaluation of the foci.<sup>[8,9]</sup>

Immunohistochemical stains of some basal cell markers could facilitate the diagnostic decision of the aforementioned difficult cases. Several markers including antikeratin 34BE12, p63, and  $\alpha$ -methylacyl coenzyme A racemase (AMACR) have been introduced and their utility has been evaluated in different studies.<sup>[10]</sup>

AMACR is a peroxisomal and mitochondrial enzyme that is found to be overexpressed in approximately 80%–100% of prostate adenocarcinoma glands.<sup>[11]</sup> Recently, the use of this marker has been on an increase in routine practice and the report of a systematic review study indicated that AMACR is potentially an important prostate tumor marker.<sup>[12]</sup>

Although the use of the marker has been reported in many studies, recent evidence showed that AMACR may also be expressed in benign prostate glands, atypical adenomatous hyperplasia, and high-grade prostate intraepithelial neoplasia.<sup>[13]</sup> In addition, different rates of sensitivity and specificity have been reported for the diagnostic utility of the marker, which is supposed to be due to the differences in diagnostic methods and ethnic and racial variations.<sup>[14-16]</sup>

There are shreds of evidence that Iranian men are racially and ethnically different from most of the other Asian men, and it is suggested that their biochemical parameters for the diagnosis of prostate adenocarcinoma could be different from others.<sup>[6]</sup> Therefore, considering the widely reported variations and poorly understood differences in the diagnostic validity of AMACR among different races, we determined the expression pattern of AMACR among Iranian male patients with prostate adenocarcinoma.

## MATERIALS AND METHODS

In this retrospective cross-sectional study, consecutive patients with a definite pathologic diagnosis of prostatic adenocarcinoma in the pathology archives of Azahra hospital, affiliated to Isfahan University of Medical Sciences, Isfahan, central Iran, in 2013–2014 were included. All sections were selected by the nonprobability convenience sampling method. The hematoxylin and eosin-stained slides of all cases were reviewed by an expert uropathologist for confirming the histopathologic diagnosis and selecting those that contain both neoplastic and normal tissues for IHC staining. Cases with inadequate biopsy or those who underwent chemotherapy or radiotherapy before the biopsy were excluded from the study.

The protocol of the study was confirmed by the Regional Ethics Committee of Isfahan University of Medical Sciences, Iran (research project number: 391449).

Demographic, clinical, and pathologic characteristics of included study participants were obtained from the patient's medical files. Tumor grade was determined according to the Gleason scoring system recommended by the International Society of Urological Pathology consensus on Gleason scoring of prostatic adenocarcinomas.<sup>[17]</sup>

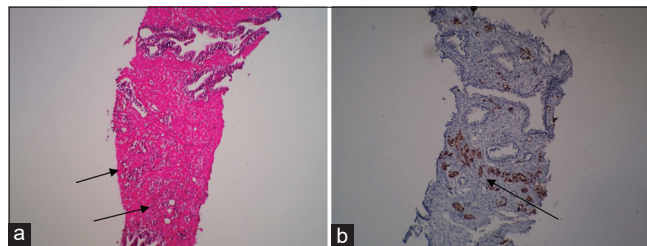
### Immunohistochemical staining

The expression of AMACR, its intensity, and the extensity of staining was determined using the IHC technique. Two 4- $\mu$ m-thick slides of formalin-fixed paraffin-embedded tissues were prepared for IHC staining. After preparation of the tissue, sections were placed on poly-L-lysine slides, deparaffinized, and dried in an oven at 60°C for 30–45 min. After rehydration, their antigens were retrieved by boiling in Tris-buffered saline by microwave heat-induced epitope retrieval method. A Polymer-Based (EnVision™) IHC method was used for the detection of P504s (monoclonal rabbit-Anti Human- Clone 13H4, Dako, Denmark) [Figure 1].

Normal prostate tissue in the same slide was used as negative control. IHC evaluation of AMACR expression in stained slides was performed by a single pathologist to exclude observer bias. The intensity of staining was scored on a scale of 0–3 as follows; 0: no cytoplasmic staining, 1: weak noncircumferential staining, 2: moderate circumferential staining, and 3: strong circumferential staining. The extensity of staining was scored as follows: absent (0%), trace (1%–5%), focal (6%–50%), and diffuse (>50%).<sup>[18,19]</sup>

### Statistical analysis

SPSS statistical software program version 20 (SPSS Inc., Chicago, IL, USA.) was used for statistical analysis. Data were analyzed using Student's *t*-test and the Chi-square test for comparing quantitative and qualitative variables, respectively. Mann-Whitney *U*-test was used for data that were not normally distributed.  $P < 0.05$  was considered as statistically significant. As normal tissues were obtained from the same set of patients, the McNemar test was used as a repeated measures version of a Chi-square test of independence in  $2 \times 2$



**Figure 1:** (a) The hematoxylin and eosin-stained slides of cases of prostate carcinoma (b) Immunohistochemical staining of prostate needle biopsy with  $\alpha$ -methylacyl coenzyme A racemase. \*Arrows indicate malignant glands

categorical analysis. Specificity, sensitivity, negative predictive value (NPV), and positive predictive value (PPV) were calculated for AMACR having the histopathologic diagnosis as the gold standard test. The receiver operating characteristic curve was generated and area under the curve (AUC) was calculated with its 95% confidence interval (CI) (When AUC equals 0.7–0.8 it has good discrimination, while an AUC of >0.81 is considered excellent<sup>(20)</sup>).

## RESULTS

In this study 58 cases of prostate adenocarcinoma with a mean age of 68.2 ± 2.8 years (ranging from 50 to 88), were evaluated for the presence of AMACR. The demographic and histopathologic characteristics of the studied population are presented in Table 1.

Histopathologic characteristics of positive and negative AMACR cases are presented in Table 2. The mean age of patients with positive expression of AMACR was significantly higher than those with negative expression of AMACR ( $P=0.04$ ). The mean of Gleason Score and frequency of perineural invasion was not statistically different in those with positive and negative AMACR expression.

In 31 (53.4%) cases, the grade of tumor according to the Gleason scoring system was ≤6 and in 27 (46.6%) cases, the grade was ≥7. AMACR expression was positive in 26 out of 31 (83.9%) cases with a Gleason score of ≤6 and 26 out of 27 (96.3%) cases with a Gleason score of ≥7 ( $P = 0.05$ ).

The frequency of different grades of staining intensity and extensity in cases with prostate adenocarcinoma grade ≤6 and ≥7 are presented in Figure 2. The intensity of staining was significantly higher in cases with higher grades of prostate adenocarcinoma ( $P=0.04$ ). The extensity of staining was not significantly associated with cancer grade.

AMACR was expressed in 2 out of 58 normal prostate tissues and 52 out of 58 prostate adenocarcinoma tissues ( $P < 0.05$ ). The sensitivity, specificity, PPV, and NPV of the marker were

90%, 96%, 96%, and 90%, respectively. AUC was 0.88 (95% CI: 0.80–0.97) which is considered excellent [Figure 3].

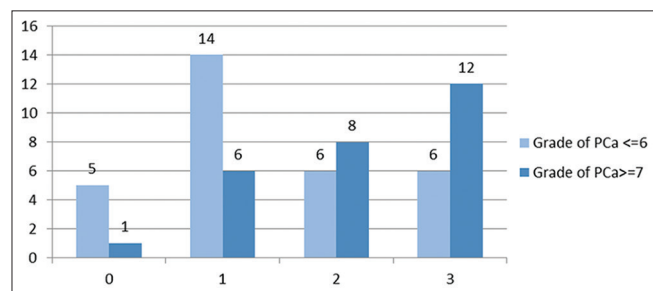
## DISCUSSION

Considering the limitations in basal cell markers in the diagnosis of prostate adenocarcinoma, such as aberrant

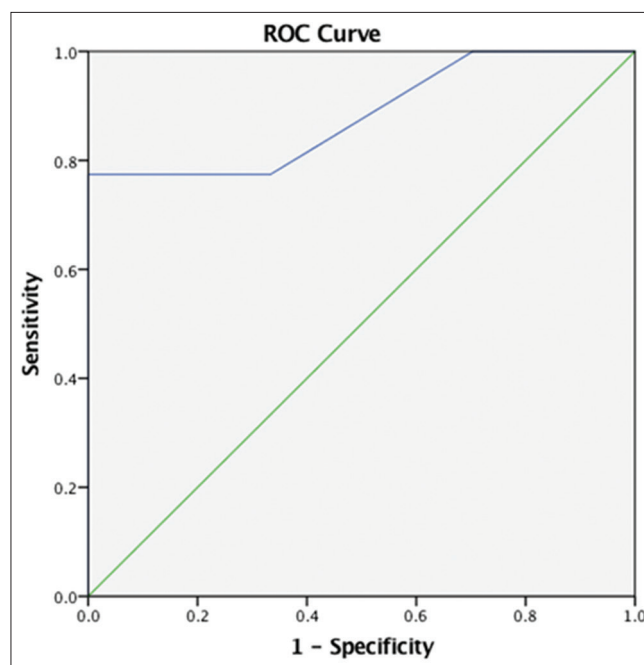
**Table 1: Demographic and histopathologic characteristics of the 58 patients with prostate adenocarcinoma**

Variables	n (%) or mean±SD
Age (years)	68.2±2.8
Gleason score	6.8±1.3
≤6	31 (53.5)
7	10 (17.2)
8	8 (13.8)
9	8 (13.8)
10	1 (1.7)
Perineural invasion positivity	20 (34.5)
AMACR positivity	52 (89.7)
Intensity of staining	
0 (AMACR negative)	6 (10.3)
1 (weak circumferential staining)	20 (34.4)
2 (moderate circumferential staining)	14 (24.1)
3 (strong circumferential staining)	18 (31.2)
Extensity of staining	
Absent (AMACR negative)	6 (10.3)
Trace (1%-5%)	0
Focal (5%-50%)	11 (19.0)
Diffuse (>50%)	41 (70.7)

AMACR=α-methylacyl coenzyme A racemase; SD=Standard deviation



**Figure 2:** Frequency of different grades of staining intensity in the two groups of subjects with grade ≤6 and ≥7 prostate adenocarcinoma ( $P = 0.04$  using Chi-square). (0: No cytoplasmic staining, 1: Weak noncircumferential staining, 2: Moderate circumferential staining, and 3: Strong circumferential staining)



**Figure 3:** Receiver operating characteristic curve and area under the curve for α-methylacyl coenzyme A racemase expression. Area under the curve: 0.88 (95% confidence interval: 0.80–0.97)

**Table 2: Histopathologic characteristics of positive and negative  $\alpha$ -methylacyl coenzyme A racemase cases**

	Positive AMACR cases (n=52)	Negative AMACR cases (n=6)	P*
Age (years)	69.1±9.86	60.7±4.6	0.04
Gleason score	6.8±1.3	6.3±0.8	0.40
Perineural invasion, n (%)	18 (34.6)	2 (33.3)	0.60

\*t-test or Mann-Whitney. AMACR= $\alpha$ -methylacyl coenzyme A racemase

diffuse expression of p63 in prostate adenocarcinoma or focal positivity of high molecular weight cytokeratin (HMWCK),<sup>[21]</sup> recent studies have recommended the use of another marker, AMACR.<sup>[22]</sup> In this study, it was shown that the expression of AMACR marker in prostate adenocarcinoma is significantly higher than normal tissue and was related to age. The intensity of the marker staining was also associated with the grade of the prostate adenocarcinoma.

The usefulness of AMACR immunostain in the diagnosis of prostate adenocarcinoma has been reported in several studies. In a meta-analysis study, reviewing 22 studies that included 4385 cases, Jiang *et al.* reported the diagnostic utility of this marker. However, most of the reviewed studies were from western or Asian countries that have no similar ethnicity with our country.<sup>[12]</sup> To the best of our knowledge, our study is the first study of the utility of AMACR in Iran, which is considered the strength of this study.

In previous studies, the reported expression rate of AMACR in prostate adenocarcinoma ranged from 62% to 100%.<sup>[23]</sup> Ozgur *et al.*,<sup>[24]</sup> in Turkey, evaluated the expression of AMACR in 64 prostate biopsies of prostate adenocarcinoma. AMACR expression was positive in 90.6% of the cases. They concluded that AMACR could be an important diagnostic marker in needle biopsies with limited quality and quantity. Singh *et al.*<sup>[25]</sup> in India, also reported a 95% and 92.5% sensitivity and specificity, respectively, for AMACR in diagnosing prostate adenocarcinoma. They recommended the use of a combination of AMACR and P63. In another study in India, 92% sensitivity and 100% specificity for AMACR in diagnosing morphologically difficult prostate adenocarcinoma was reported. They recommended the combination use of AMACR and HMWCK.<sup>[26]</sup>

In this study, AMACR expression was associated with age. To our knowledge, there are no studies demonstrating the age-associated changes of AMACR in prostate adenocarcinoma. Gologan *et al.*<sup>[27]</sup> investigated age-related changes of the marker in normal patients and those with benign prostatic lesions and demonstrated that it is age-related with a decreasing trend in younger males specifically those aged <45 years. This higher expression of AMACR in patients with prostate adenocarcinoma might be due to the background increase of AMACR in normal males, however, to confirm this hypothesis, it is recommended to perform further studies with larger sample size to confirm this association.

In this study, the mean of Gleason Score was not different in cases with positive and negative expression of AMACR, but the staining was more intense in higher grades of prostate adenocarcinoma. Our results in this regard were in line with the study of Murphy *et al.*<sup>[19]</sup> The lower intensity of AMACR is an important factor to consider in interpreting the AMACR positivity in prostate specimens with lower Gleason Scores, which are more difficult to categorize as neoplastic or benign tissue.

We observed that the rate of perineural invasion was not significantly different in cases with positive and negative AMACR expression. Although there are shreds of evidence that the presence of perineural invasion in needle biopsies is associated with an increased risk of extraprostatic involvement of cancer, recent studies did not indicate such a relationship.<sup>[28]</sup>

The limitations of the current study were small sample size and lack of samples of other prostatic lesions for comparing AMACR expression.

## CONCLUSION

It could be concluded from this study that AMACR could be used as a diagnostic tool for the diagnosis of prostate adenocarcinoma. However, due to false-positive staining in mimickers of prostatic adenocarcinoma, it is recommended to use it in combination with other basal cell markers.

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## Conflicts of interest

There are no conflicts of interest.

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