

## Evaluation of the Diagnostic Value of Immunohistochemistry Staining for P63, Alpha-methyl Acyl-CoA Racemase, CK5/6, and 34βE12 in Prostate Carcinoma

### Abstract

**Background:** Prostate cancer is the most common male malignant tumor. Misdiagnosis in benign and malignant lesions of the prostate may lead to unnecessary or delayed treatment. **Aim:** The goal of this study was to determine the association between P63, 34βE12, CK5/6, and alpha-methyl acyl-CoA racemase (AMACR) biomarkers in a wide range of benign and malignant prostate tumors. **Settings and Design:** A total of 98 blocks of prostate tissue during the years 2006–2010 (in a single clinical center) were selected. Of these, 70 cases were malignant and 28 were benign. **Subject and Methods:** Immunohistochemistry (IHC) was done for P63, AMACR, CK5/6, and 34βE12 biomarkers. Chi-square and *t*-test were used for analyzing statically. **Results:** Of the 70 malignant specimens (prostate adenocarcinoma [PA]) stained by AMACR, 66 cases (94%) were positive. Of the 28 benign prostate samples, 27 cases (96%) were negative for AMACR. The sensitivity of the AMACR staining was 94% and its specificity was 96% in the diagnosis of adenocarcinoma of the prostate. The sensitivity of the biomarkers 34βE12 was 97% and its specificity was 100%. In the case of P63 and CK5/6, sensitivity and specificity were 98%, 96%, 98%, and 82%, respectively. Among basal cell biomarkers, 34βE12 had the highest specificity values. **Conclusion:** Due to the high sensitivity and specificity values of P63, AMACR, CK5/6, and 34βE12 biomarkers in the prostate lesions, the IHC method can be used for more reliable diagnosis and differentiation of benign and malignant types. By this approach, the possibility of pathologic diagnostic error between benign lesions and PA will be reduced.

**Keywords:** 34βE12, alpha-methyl acyl-CoA racemase, CK5/6, P63, prostate cancer

### Introduction

Prostate adenocarcinoma (PA) is the most common cancer in males. It affects 1 in 9 men older than 65 years old and after lung cancer is the most common cause of cancer deaths in men.<sup>[1,2]</sup>

Prostate cancer is the third most common cancer among old (>50 years old) Iranian men, and it has been reported that 7%–9% of men are involved. Previous studies have shown that the incidence of this cancer has increased 3.7 times (9.6/100,000). The mortality rate in men is higher than in other malignancies. In general, the prevalence of this cancer in Iran is similar to Eastern Mediterranean countries. Among the risk factors for this cancer in Iran is lifestyle, nutritional, and environmental factors.<sup>[3]</sup>

Histologically, the prostate consists of glands which covered by two types of cells, the basal cells, and the luminal secretory cells. The natural secretion of the prostate is a neutral mucinous substance. This natural secretory system is significantly altered in neoplastic conditions so that the most adenocarcinomas secrete a combination of acidic and neutral mucins. Some deal, this property has diagnostic value and can be detected by immunohistochemistry (IHC) and histochemical techniques.

In the PA, malignant glands are covered by a layer of columnar epithelial cells, and unlike benign cases, the outer basal layer is not present.<sup>[1,4]</sup> It is difficult to distinguish benign glands from malignant forms solely based on morphological manifestations, particularly if the area of interest is not widespread. Therefore, basal cell IHC detection is an important criterion for

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distinguishing malignant and invasive forms from benign types.<sup>[5]</sup> Several basal cell biomarkers such as 34 $\beta$ E12, keratin 5.6, and p63 have been used to diagnose prostate cancer. The trouble is that not all benign cases are stained with basal cell biomarkers uniformly and in some cases, such as atypical adenomatous hyperplasia or partial atrophy, mimic PA. Furthermore, some morphological variants of the PA are focally stained with basal cell biomarkers.

Alpha-methyl acyl-CoA racemase (AMACR) plays an important role in the  $\beta$ -oxidation of branched fatty acids. It suggested that the expression of this biomarker increases in PA. Recently, it has been shown to increase the expression of this biomarker in prostate cancer and unlike the basal cell biomarker, it is known to be a reliable biomarker of PA. In some cases, however, adenocarcinoma has negative staining for this biomarker.<sup>[6,7]</sup> Concurrent uses of AMACR and basal cell biomarkers in the diagnosis of prostate cancer can be very beneficial.

Given the high prevalence of prostate cancer and the need for proper diagnosis in malignant cases, this study was done to evaluate the diagnostic value of basal cell biomarkers and AMACR in prostate cancer.

## Subject and Methods

The procedures followed were by the ethical standards of the responsible committee on human experimentation Kermanshah University of Medical Science and with the Helsinki Declaration of 1975, as revised in 2000.

### Patient samples

Prostate samples consisting of two classes of PA and nonadenocarcinoma cases were selected by evaluation of the electronic pathology documents in a single clinical center from 2006 to 2010. Slides were reviewed by a pathologist and the previous diagnosis was confirmed. After confirmation of diagnosis, the best samples (paraffin-embedded blocks) were selected and used for IHC staining.

Each of these biomarkers stains a special part of the cells. For example, P63 is a nuclear biomarker, 34 $\beta$ E12 and CK 5/6 are membranous, and finally, AMACR is a cytoplasmic biomarker. Stained slides were observed and recorded by two pathologists separately. In the case of discrepancy between pathologists, slides checked again and the results obtained by consensus. The final results were entered into the SPSS program and statistical analysis was carried out.

### Immunohistochemistry staining method

Immunohistochemical staining of prepared slides was performed using antibodies against P63, CK5/6, 34 $\beta$ E12, and AMACR. Table 1 shows a list of the sources of these antibodies. The method of IHC staining was done as follows: de-paraffinization was conducted on 4  $\mu$ m tissue sections in a hot air oven (60°C–65°C) for 24 h. The

**Table 1: Sources of antibodies to P63, CK5/6, 34 $\beta$ E12, and AMACR**

Antibody	Source	Company
P63	Mouse anti-human monoclonal	Master diagnóstica
CK5/6	Rabbit anti-human monoclonal	Master diagnóstica
34 $\beta$ E12	Mouse anti-human monoclonal	Master diagnóstica
AMACR	Rabbit anti-human monoclonal	Master diagnóstica

slides were rehydrated in xylene and a graded sequence of ethanol solutions (45 min). Then, immersed in *Tris* buffer jar (pH = 9) and warmed (20 min) in the autoclave at 121°C, followed by washing in phosphate-buffered saline (PBS) solution to retrieve antigens. After that for decreasing intracellular peroxidase activity, slides were soaked in a solution of 3% hydrogen peroxide in methanol (15 min), washed with PBS (10 min). The slides were incubated with primary and secondary antibodies for 60 and 45 min, respectively, in a humid and dark place at room temperature. Then, washed them in PBS and coated with a chromogenic surface solution tetrahydrochloride and 3,3'-diaminobenzidine for 5 min. The counterstaining was performed for 30–60 s with hematoxylin then lithium carbonate 1% (5 min) and washed in water. Stained slides were immersed in graded ethanol series and then xylene for transparency and dehydration of tissues. Then, mounted slides were examined under a microscope.<sup>[8]</sup>

### Scoring of sections

The percentage of stained myoepithelial cells with IHC markers (extensiveness) classified by the semi-quantitative approach as negative, 1%–10%, 11%–50%, 51%–90%, and more than 90%. In each sample, if the percentage of stained myoepithelial cells was at least 10%, it was considered positive and negative if it was <10%.

The intensity of staining with basal biomarkers (34 $\beta$ E12, CK5/6, and P63) was graded as negative, weak, moderate, and strong.

AMACR staining intensity was classified as follows: negative, weak (weak nongranular cytoplasmic staining), moderate (weak or moderate granular cytoplasmic staining), and strong (highly granular cytoplasmic staining).

### Statistical analysis

The results were statistically analyzed by the Chi-square and *t*-test using the SPSS software (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc).

## Results

The average age of patients (benign and malignant) was 70  $\pm$  8.6 years. The maximum age was 93 years and the minimum age was 53 years.

### Immunohistochemistry staining result of alpha-methyl acyl-CoA racemase

Of the 70 malignant specimens (PA) which stained with AMACR, 66 (94%) cases were positive and 4 (6%) cases were negative. Of the total 28 cases of benign samples, 27 (96%) cases were negative for AMACR and only one case (4%) was positive. Results revealed that there was a significant statistical relationship between the positive IHC staining of AMACR and prostate malignancy ( $P = 0.001$ ) [Figure 1 and Tables 2, 3]. In the diagnosis of PA, the sensitivity of the AMACR was 94% and its specificity was 96%. The positive predictive value of this biomarker was 98% and its negative predictive value was 87%.

### Immunohistochemistry staining result of 34 $\beta$ E12

Of the total 70 PA cases, only 2 (3%) were positive and 68 (97%) were negative for this biomarker. In the benign group, all samples (28 cases) were positive. There was a statistically significant relationship between the positive IHC staining of 34 $\beta$ E12 and the benign group ( $P = 0.001$ ) [Figures 2a, b and Tables 2, 3]. The sensitivity of this biomarker was 97% and its specificity was 100%. The positive and negative predictive values were 93% and 100%, respectively.

### Immunohistochemistry staining result of P63

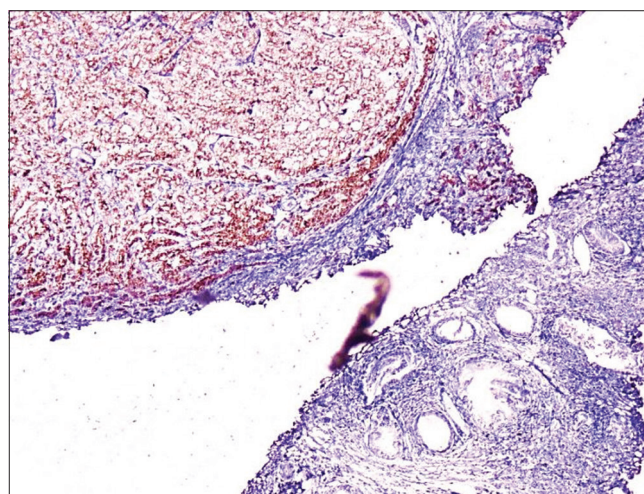
Of the total 70 cases of malignant prostate lesions, 69 (98.6%) were negative for the P63 biomarker and only one (1.4%) was positive. Of the total 28 benign specimens, 27 cases (96%) were positive and one (4%) was negative. There was a statistically significant relationship between P63 IHC staining and the differentiation of benign cases from malignant prostate lesions ( $P = 0.001$ ) [Figure 3a, b and Tables 2, 3]. The sensitivity and specificity of the biomarker were 98% and 96%, respectively, and the positive and negative predictive values were 96% and 98%, respectively.

**Table 2: The results of frequency of P63, CK5/6, 34 $\beta$ E12, and AMACR biomarkers in benign and malignant cases**

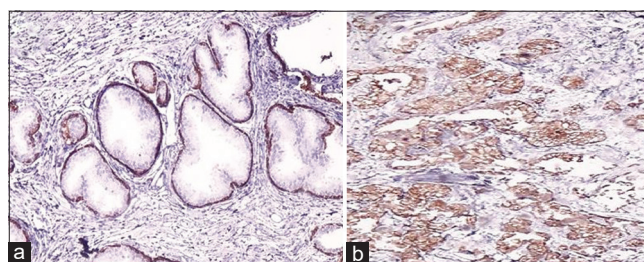
Biomarker	IHC staining result	Malignant	Benign	total
AMACR	Positive	66	1	67
	Negative	4	27	31
	Total	70	28	98
34 $\beta$ E12	Positive	2	28	30
	Negative	68	0	68
	Total	70	28	98
P63	Positive	1	27	28
	Negative	69	1	70
	Total	70	28	98
Ck 5/6	Positive	1	23	24
	Negative	69	5	74
	Total	70	28	98

### Immunohistochemistry staining result of CK5/6

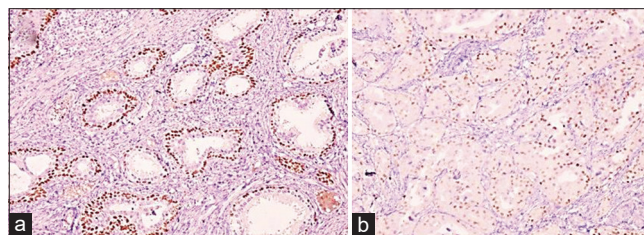
Of the total 70 malignant specimens that stained for this basal cell biomarker, 69 (98.6%) cases were negative and one (1.4%) was positive. Of the total 28 benign cases, 23 (82%) cases were positive and 5 (18%) cases were negative for CK5/6. There was a statistically significant relationship between the incidence of CK5/6 and benign prostate samples ( $P = 0.001$ ) [Figures 4a, b and Tables 2, 3]. The sensitivity of the CK5/6 biomarker in distinguishing benign and malignant cases was 98% and its specificity was 82%. Positive and negative predictive values were 95% and 93%, respectively.



**Figure 1: Light microscopy, immunohistochemistry staining of alpha-methyl acyl-CoA racemase in malignant (above), and benign (below) prostate specimen. Brown cytoplasmic staining indicates a positive reaction of the malignant glands to this marker ( $\times 100$ )**



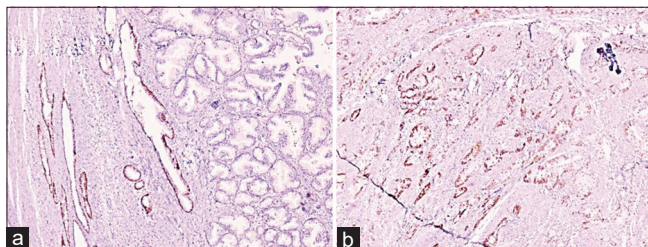
**Figure 2: Light microscopy, immunohistochemistry staining of 34 $\beta$ E12 in benign (a), and malignant (b) prostate specimen. Brown membranous staining indicates a positive reaction of the malignant glands to this marker ( $\times 400$ )**



**Figure 3: Light microscopy, positive nuclear staining for P63 in benign (a), and prostate adenocarcinoma (b). Brown nuclear indicates a positive reaction of the malignant glands to this marker ( $\times 400$ )**

**Table 3: The percentage results of P63, CK5/6, 34βE12, and AMACR biomarkers in benign and malignant cases**

Biomarker	Tumor types	>90%	51%-90%	10%-50%	<10%	0%	Total
AMACR	Benign	0	0	1	9	18	28
	Malignant	31	29	6	1	3	70
34βE12	Benign	15	12	1	0	0	28
	Malignant	0	2	0	7	61	70
P63	Benign	4	19	4	1	0	28
	Malignant	0	0	1	1	68	70
Ck 5/6	Benign	0	8	15	5	0	28
	Malignant	0	1	0	1	68	70

**Figure 4: Light microscopy, immunohistochemistry staining of CK5/6 in benign (a), and malignant (b) prostate specimen. Brown cytoplasmic indicates a positive reaction of the malignant glands to this marker (×400)**

## Discussion

PA is the most common cancer in the male that affects one in nine men over 65 years old. This type of cancer after lung cancer is the most common cause of death in men.<sup>[2]</sup> Occasionally, it is difficult to differentiate malignant glands from benign ones solely based on morphological manifestations, especially when the area involved is small. Basal cell IHC is one of the most important criteria for distinguishing malignant and invasive forms from benign ones. Various basal cell biomarkers such as 34βE12, keratin5/6, and P63 have been used to diagnose PA. The problem is that not all benign cases are stained with basal cell biomarkers and, in some cases such as atypical adenomatous hyperplasia or partial atrophy, they mimic PA. On the other hand, some morphological variants of the PA are focally stained with basal cell biomarkers.

Another biomarker for PA is AMACR, which, unlike basal cell biomarker, is a positive biomarker for prostate cancer, although some adenocarcinomas react negatively to this biomarker. Concurrent use of AMACR and basal cell biomarkers in the diagnosis of prostate cancer can be useful, and it is, therefore, important to know the predictive value of each of these biomarkers.<sup>[9]</sup>

In this study, which included 98 prostate specimens (70 malignant and 28 benign), the sensitivity and specificity of the AMACR biomarker for prostate cancer diagnosis were 94% and 96%, respectively. Furthermore, in the case of basal cell biomarkers (34βE12, CK5/6, and P63), there was a statistically significant relationship between these biomarkers and benign samples. In this study, the sensitivity of basal cell biomarkers, as a negative marker for prostate cancer, was higher than 95%.

Among three biomarkers, 34βE12, CK5/6, and P63, the best specificity was found in 34βE12 (100%) and the least specificity is shown for CK5/6 (82%).

In a study conducted by Boran *et al.* in Turkey, 98 prostate biopsy specimens (from 2003 to 2007) included 65 adenocarcinoma samples and 33 nonadenocarcinoma cases were stained with basal cell biomarkers (34βE12, CK5/6, P63, and bcl2) and AMACR. The study found that 34βE12 had the highest sensitivity and specificity among basal cell biomarkers (95% sensitivity and 98% specificity) and 34βE12 was identified as the best negative marker for cancer which should, in concurrent with AMACR, be used as a positive marker.<sup>[10]</sup>

Shah *et al.* conducted a study for comparing specific basal cell biomarkers P63 and 34βE12 to diagnose prostate cancer. They reported that none of the identified prostate cancer specimens (100% specificity) had responded to these biomarkers. They concluded that 34βE12 and p63 are highly specific for the basal cell.<sup>[11]</sup>

Basal epithelial cells are heavily stained with P63 in normal, benign hyperplasia, and intraepithelial neoplasm cases. On the other hand, a very high percentage of adenocarcinomas (90%) react negatively with p63. Parsons *et al.* studied the expression of P63 as a basal cell biomarker in prostate lesions, and of 233 PA samples, 212 cases were found negative for P63. In <1% of the PA, the P63 biomarker is positive, although the staining is very weak.<sup>[12]</sup>

Browne *et al.* examined 171 IHC-stained prostate specimens for AMACR and basal cell biomarkers and suggested that concurrent use of basal cell biomarkers (34βE<sub>12</sub> and P63) and AMACR may be helpful in the diagnosis of prostate cancer.<sup>[13]</sup>

In the report of Puebla-Mora *et al.*, 37 of the 41 cases of adenocarcinoma (90%) had a positive cytoplasmic AMACR response.<sup>[14]</sup>

This result was also reported in 6 of the 22 benign prostatic lesions (27%). The AMACR sensitivity for the detection of prostate carcinoma was 90% and the specificity was 72%.<sup>[14]</sup> Rubin *et al.* conducted a study of 94 cases of needle biopsy in the prostate, found 97% sensitivity and 100% specificity for the diagnosis of PA when using AMACR biomarker.<sup>[15]</sup>

## Conclusion

The findings of this study reveal that using IHC might help differentiate controversial lesions of the prostate. Concurrent use of AMACR and basal cell biomarkers may be helpful in the proper diagnosis of prostate cancer and IHC is recommended to reduce diagnostic error in suspected cases. In our study, we found that among all types of basal cell biomarkers suggested, aiding in diagnosis 34 $\beta$ E12 biomarkers appears to be more appropriate than others, and the use of this basal cell biomarker is recommended.

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## Conflicts of interest

There are no conflicts of interest.

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