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EVALUATION OF DIAGNOSTIC UTILITY OF A-METHYL ACYL CO A RACEMASE (AMACR) AND 34BETAE12 IMMUNOHISTOCHEMICAL MARKER IN PRORATION OF IN PROSTATE CANCER

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ABSTRACT

Objective: Prostate cancer is the most common cause of death in men after lung cancer. Achieve an accurate diagnosis with histological study common problems. Because of early detection of prostate cancer is causing a delay in treatment methods immunohistochemistry staining especially in uncertain a case is useful and necessary. 34BetaE12 marker in the cytoplasm of basal cells in the prostate gland is seen as continuous stained around the glands. Positive immunoreactivity for 34BetaE12 in the suspicious or abnormal glands rules out the diagnosis. **Aim:** So, the current study assayed the utility of α-methyl acyl Co A Racemase (AMACR) and 34BetaE12 immunohistochemical marker in proration of in prostate cancer. Methods: Cross-sectional and case-control study was done on 31 cases of prostate adenocarcinoma (target group) and 31 benign prostatic hyperplasia (control group) who needs needle biopsy, transurethral resection of the prostate (TURP) or open prostatectomy patients. After preparation, the samples immunohistochemicaly stained for AMACR and 34BetaE12 marker and examined using microscope. **Results**: According to the results, the mean age of

patients with prostate adenocarcinoma and benign prostatic hyperplasia were 71.64±9.21 and 68.16±12.04 years, respectively. In 31 cases, AMACR marker expression was negative in benign prostatic hyperplasia. In prostate adenocarcinoma cases, negative, medium and strong expression were 6(19.4%), 1(3.2%) and 24 (77.4%), respectively. 34BetaE12 marker in all benign prostatic hyperplasia and prostate adenocarcinoma cases had positive and negative expression, respectively. **Conclusion**: these results suggest positive expression of AMACR marker and negative expression of 34BetaE12 marker in adenocarcinoma cases and it is better to use both on diagnosis the adenocarcinoma from benign samples.

Keywords: Prostate adenocarcinoma, Benign prostatic hyperplasia, AMACR, 34BetaE12

INTRODUCTION

Prostate cancer is the most frequently diagnosed noncutaneous malignancy in men, and the second leading cause of male cancer-related mortality after lung cancer (Jemal et al. 2010). The incidence of prostate cancer in Asia has been increasing, although it is lower than that in the western world. Diagnosis of prostate cancer glands can sometimes present a diagnostic challenge for pathologists, since prostate carcinoma can mimic benign prostate glands (Jiang et al. 2013) and the architectural or cytologic clues for the diagnosis of carcinoma may not always be seen in small foci of suspicious glands (Pe´rtega-Gomes et al. 2012). Prostatic needle biopsy, the preferred method for diagnosing early prostate cancer, provides specific information with low morbidity (Molinié et al. 2006). Also, immunohistochemistry can be a useful adjuvant for diagnosing limited adenocarcinoma of the prostate but, as with any immunohistochemical technique, there are problems of sensitivity and specificity (Molinié et al. 2006).

The degradation of branched-chain fatty acids is predominantly due to β-oxidation. A well-characterized enzyme, α-methylacyl CoA racemase (AMACR) plays an important role in β-oxidation of branched-chain fatty acids because it catalyzes the conversion of several (2R)-methyl-branched-chain fatty acyl-CoAs to their (S)-stereoisomers in mitochondria and peroxisome (Lou et al. 2002). α-Methylacyl-coenzyme A racemase (AMACR,1 EC 5.1.99.4, also known as P504S) catalyzes the 1,1-proton transfer reaction (Ouazia et al. 2010) that effects the reversible epimerization of the coenzyme A (CoA) thioesters derived from a variety of (2R)- and (2S)-methyl branched fatty acids

including pristanic acid and its chain-shortened derivatives, a number of C_{27} bile acid intermediates and 2-arylpropionic acids (e.g., ibuprofen) (Bhaumik et al. 2007). Only stereoisomers with the 2-methyl group in the (S)-configuration can be degraded via β-oxidation (Wanders et al. 2001). Recently, AMACR, also known as P504S, was identified by microarray screening of prostate carcinoma. High immunoreactivity for AMACR was reported in prostate cancer, but not in benign prostatic tissue (Rubin et al. 2002). AMACR is a mitochondrial and peroxisomal enzyme that plays an important role in beta-oxidation of branched-chained fatty acids through the inter conversion between the (2R)- and (2S)- methyl branched-chain fatty acyl-CoAs (Li et al. 2014).

Elevated levels of AMACR have been associated with various cancers and the enzyme serves as a biomarker for prostate cancer (Allen et al. 2008). It is reported AMACR is abundantly expressed in prostate cancer, the concentration of which is even increased in premalignant lesions (Zeng et al. 2015). Some other studies have reported that the overexpression of AMACR can also occurs in hepatocellular carcinoma, breast cancer, renal cancer and other cancers (Yang et al. 2014). Since it has been proven that AMACR can serve as a novel diagnostic biomarker of cancer, the sensitive and selective determination of AMACR is of particular importance in cancer diagnosis (Li et al. 2014). Findings suggest AMACR has the potential to be a useful diagnostic marker for prostate cancer in clinical practice. However, little is known whether this enzyme is up-regulated in colon cancer (Jiang et al. 2002). In a study Jiang et al. (2003) reported AMACR may also serve as a molecular marker for colon cancers and its precursor lesions.

34βE12 is a high-molecular-weight cytokeratin that is expressed in the cytoplasm of basal cells rather than in luminal or secretory cells. P63 is a homologue of the p53 tumor suppressor gene. It has selective expression in the basal cell compartment of various epithelial tissues and is sensitive in identifying the nuclei of basal cells in benign prostatic lesions. It has been shown to be a reliable adjunctive tool for identifying basal cells, comparing favorably with high molecular- weight cytokeratin staining. Combining antibodies to these 2 markers in a cocktail has led to an increased detection rate of basal cells over either marker used alone owing to the improved sensitivity of nuclear and cytoplasmic staining for p63 and 34βE12, respectively, and a decrease in

staining variability (Leong et al. 2007). Negative 34βE12 immunohistochemical staining in small foci of atypical glands in prostatic needle biopsy specimens does not necessarily mean that the lesion is malignant because some benign lesions may have discontinuous basal cells and the entire lesion may not be represented on the needle biopsy (Jiang et al. 2005). This has increased the diagnostic accuracy of prostate cancer worldwide. Basal cell markers such as HMWCK (34βE12) and CK 5/6 and P63 are very useful for demonstration of basal cells as their presence argues against a diagnosis of invasive prostatic carcinoma (Kumaresan et al. 2010). It is reported combination of 34βE12and AMACR is of great value in combating the morphologically suspicious cases and significantly increasing the diagnostic accuracy in prostate cancer.

Recently, lower levels of prostate specific antigen (PSA) have been used to indicate the need for a prostate biopsy, and there have been an increasing number of cores taken in each biopsy session (Leite et al. 2010). Based on the literature there is no previous report on examine accuracy of two techniques with each other on detection of prostate adenocarcinoma and benign prostatic hyperplasia. So, the hypothesis of the current study was to determine utility of AMACR and 34BetaE12 immunohistochemical marker in proration of in prostate cancer.

MATERIAL AND METHODS

This cross-sectional and case-control study on patients referred for prostate adenocarcinoma and benign prostatic hyperplasia to the Ahwaz University of Medical Sciences, Ahwaz, Iran in a one year period from 2015- 2016. In this study, patients divided into 2 experimental groups, 31 cases of prostate adenocarcinoma (target group) and 31 benign prostatic hyperplasia (control group). The inclusion criteria were benign prostatic hyperplasia or prostate adenocarcinoma. After preparation, samples were collected and stained using immunohistochemical technique with AMACR and 34BetaE12 marker. The degree of staining was compared with H&E lab collection tissue slide samples. Then the sating graded as follows:

0: no cell stained

+1: weak stained

+2: moderate stained

+3: strong stained (Kumaresan et al. 2010).

Statistical analysis

Data was processed in excel and analyzed using SPSS 21.0 for Windows (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered as significant differences between treatments.

RESULTS

The results of diagnostic utility of α-methyl acyl Co A Racemase (AMACR) immunohistochemical marker in proration of 34BetaE12 in prostate cancer is presented in tables 1-4 and figure 1-2. According to the results, average age of the patinas in benign prostatic hyperplasia and prostate adenocarcinoma groups were 68.16 ± 12.04 and 71.64 ± 9.21, respectively.

The results of the staining with AMACR in benign prostatic hyperplasia and prostate adenocarcinoma patients are shown in table 2. As seen, there was no staining with AMACR using needle biopsy in benign prostatic hyperplasia patients. Also, all benign prostatic hyperplasia negative was detected using TURP. In prostate adenocarcinoma cases, 16 (51.6%) strongly detected with needle while 3 (9.6%) and 5 (16.1%) cases detected using TURP and prostatectomy.

Based on the results, the strong staining was detected in needle biopsy using 34BetaE12 marker in benign prostatic hyperplasia group. Also, in prostate adenocarcinoma cases, the negative 34BetaE12 staining detected via needle, TURP and prostatectomy were 22 (70.9%), 4 (12.9%) and 2 (16.2%), respectively (table 3).

The results for degree of the staining using AMACR and 34BetaE12 markers are presented in table 4. According to the results, using AMACR staining, 31 (100%) negative detected in benign prostatic hyperplasia while 24 (77.4%) cases found strong standing in benign prostatic hyperplasia group. Furthermore, in staining with 34BetaE12 marker, 31 (100%) cases with strong staining were detected in benign prostatic hyperplasia cases. Also, in prostate adenocarcinoma patients 31 (100%) negative detected found using 34BetaE12 marker (table 4).

Based on the compression results for staining with AMACR and 34BetaE12 on benign prostatic hyperplasia the highest negative staining was found using AMACR while the higher was detected using 34BetaE12 on benign prostatic hyperplasia cases (figure 1).

Additionally, the AMACR and 34BetaE12 markers significantly had higher staining detection in prostate adenocarcinoma patients (figure 2).

Table 1: The average age of the patinas included into study (n=31 for each group).			
	Mean age ± Sd	Minimum	maximum
benign prostatic hyperplasia	68.16 ± 12.04	32	88
prostate adenocarcinoma	71.64 ± 9.21	57	86

Table 2. the results of the staining with AMACR in benign prostatic hyperplasia and prostate adenocarcinoma patients					
		staining with AMACR			
		Negative	Weak	Moderate	Strong
benign prostatic hyperplasia	Needle	-	-	-	-
	TURP	31 (100%)	-	-	-
	Prostatectomy	-	-	-	-
prostate adenocarcinoma	Needle	5 31 (16.1%)	-	1 (3.2%)	16 (51.6%)
	TURP	1 (3.4%)	-	-	3 (9.6%)
	Prostatectomy	-	-	-	5 (16.1%)
Total	Needle	5 (8%)	-	1 (1.8%)	16 (25.8%)
	TURP	32 (51.6%)	-	-	3 (4.8%)
	Prostatectomy	-	-	-	5 (8%)
AMACR: methyl acyl Co A Racemase, TURP: transurethral resection of the prostate					

Table 3: The results of the staining with 34BetaE12 in benign prostatic hyperplasia and prostate adenocarcinoma patients					
		staining with 34BetaE12			
		Negative	Weak	Moderate	Strong
benign prostatic hyperplasia	Needle	-	-	-	-
	TURP	-	-	-	31 (100%)
	Prostatectomy	-	-	-	-
prostate adenocarcinoma	Needle	22 (70.9%)	-	-	-
	TURP	4 (12.9%)	-	-	-
	Prostatectomy	2 (16.2%)	-	-	-
Total	Needle	22 (35.4%)	-	-	-
	TURP	4 (6.6%)	-	-	31 (50%)
	Prostatectomy	5 (8%)	-	-	-
AMACR: methyl acyl Co A Racemase, TURP: transurethral resection of the prostate					

Table 4. the degree of the staining using AMACR and 34BetaE12 markers			
	staining with AMACR		
	Negative (<10%)	Moderate (11-50%)	Strong (>50%)
benign prostatic hyperplasia	31 (100%)	-	-
prostate adenocarcinoma	6 (19.4%)	1 (8.2%)	24 (77.4%)
Total	37 (59.7%)	1 (1.6%)	24 (38.7%)
		staining with 34BetaE12	
benign prostatic hyperplasia	-	-	31 (100%)
prostate adenocarcinoma	31 (100%)	-	-
Total	31 (50%)	-	31 (50%)

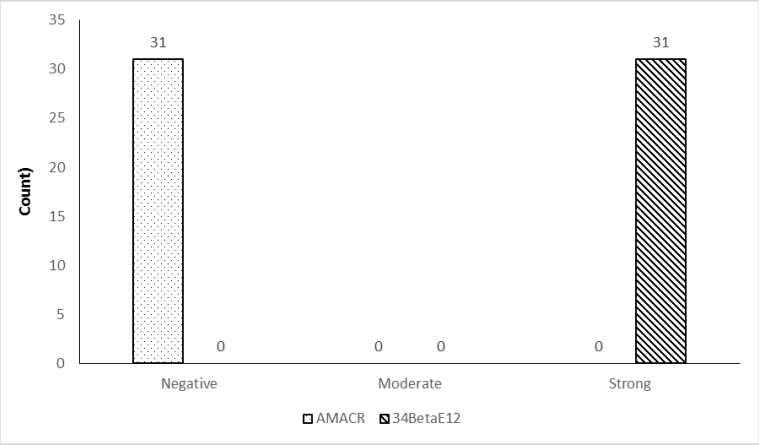

Figure 1. the compression results for staining with AMACR and 34BetaE12 marker on benign prostatic hyperplasia

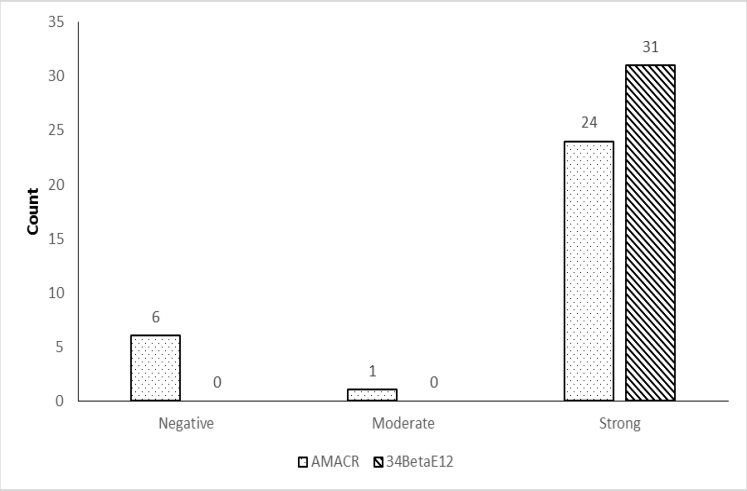

Figure 2. the compression results for staining with AMACR and 34BetaE12 marker on prostate adenocarcinoma

DISCUSSION

Prostate carcinoma is the most common form of cancer in men and the second leading cause of death. The advent of prostate-specific antigen screening has led to a significant increase both in the number of prostate needle biopsies performed and in the number of difficult biopsies with a small foci of adenocarcinoma and atypical glands suggestive but not diagnostic of adenocarcinoma (Kumaresan et al. 2010). In this study, prostate adenocarcinoma cases, negative, medium and strong expression were 6(19.4%), 1(3.2%) and 24 (77.4%), respectively. 34BetaE12 marker in all benign prostatic hyperplasia and prostate adenocarcinoma cases had positive and negative expression, respectively. Results suggest positive expression of AMACR marker and negative expression of 34BetaE12 marker in adenocarcinoma cases and it is better to use both on diagnosis the adenocarcinoma from benign samples.

The diagnosis of prostate cancer is made by use of traditional histological parameters, including architecture, nuclear features and ancillary features (if necessary) rather than any single diagnostic feature. Tissue diagnosis of prostate cancer can be difficult due to the presence of either a small focus of cancer or due to the many benign mimickers of malignancy like adenosis, atrophy, partial atrophy, basal cell hyperplasia, clear cell hyperplasia, post atrophic hyperplasia, nephrogenic adenoma, mesonephric hyperplasia, radiation atypia, seminal vesicle and Cowpers glands (Epstein and Yang, 2002).

In recent years basal cell markers and prostate biomarker AMACR have been

used as adjuvant to morphology in diagnostically challenging cases with a very high sensitivity and specificity. This has increased the diagnostic accuracy of prostate cancer worldwide. Basal cell markers such as 34bE12 and CK 5/6 and P63 are very useful for demonstration of basal cells as their presence argues against a diagnosis of invasive prostatic carcinoma (Kumaresan et al. 2010). A notable advantage to an AMACR immunostain is that a diagnosis of malignancy is substantiated by a positive signal rather than loss of a signal. Multiple studies have now evaluated the utility of AMACR immunostain in the diagnosis of prostate cancer. But there are varied reports regarding the expression of AMACR in prostate cancer which ranges from 62% to 100% (Skinnider et al. 2004).

Zhou et al (2004) found AMACR expression in 70-77% of pseudohyperpalstic carcinomas and 62-68% of foamy gland cancers. Although AMACR is a useful immunohistochemical marker for prostate cancer, it has significant limitations. It is so emphasized that AMACR should be interpreted in the appropriate morphological context and in conjunction with basal cell markers (Kumaresan et al. 2010). So, because of that in the current study we used combination of two biomarkers for diagnosis accuracy in benign prostatic hyperplasia and prostate adenocarcinoma patients. Jiang et al (2004) found that the AMACR and 34bE12 immunohistochemistry in the workup of 41 foci of so-called atypical small acinar proliferation led to a 76% agreement rate between the 3 pathologists participating in the study.

The staining appearance of 34βE12 was also different from that of AMACR such as homogeneous cytoplasmic reactivity for 34βE12 and granular positivity for AMACR. From a practical perspective, it is not often that 34βE12 immunostaining of the basal cells would be found in the same gland for which AMACR reactivity is expected because the latter would highlight malignant prostatic glands for which 34βE12 would not be positive because basal cells are absent in malignant glands (Leong et al. 2007). To the best of our knowledge, our study is the first report on evaluating the usefulness of AMACR and 34βE12 in benign prostatic hyperplasia and prostate adenocarcinoma. In conclusion these results suggest positive expression of AMACR marker and negative expression of 34BetaE12 marker in adenocarcinoma cases

and it is better to use both on diagnosis the adenocarcinoma from benign samples.

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