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Immunohistochemical Detection of Alpha-Methyl-Co-Racemase (AMACR) in Adenocarcinoma of Prostate

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Abstract: The aim of study was to evaluate the expression pattern of Alpha-methylacyl-CoA Racemase (AMACR) in prostate tumors. In order to examine the relationship between AMCAR expression and tumor, we analyzed a total of 10 cases of benign prostatic hyperplasia and 20 cases of prostate adenocarcinoma . Expressions pattern of AMCAR were analysed and found that only one case (10%) of benign prostatic hyperplasia showed expression whereas 19 cases (95%) of prostate adenocarcinoma showed expression at different levels of intensity. The difference in expression pattern among benign prostatic hyperplasia and prostate adenocarcinoma was statically significant ($p>0.05$). Expression profile were further categorised according to grade of the tumor and found that AMCAR was expressed in 4 (100%), 6 (100%) and 9 (90%) in well, moderate and poor differentiated prostate adenocarcinoma respectively. This difference of expression among the different types of grade was statistically insignificant ($p>0.05$). Our results concluded that AMCAR is a valuable diagnostic and prognostic marker of prostate cancer and one of the vital factors in pathogenesis of prostrate adenocarcinoma.

Key words: Prostate tumor, AMACR expression IHC, adenocarcinoma, vital factors, Saudi Arabia

INTRODUCTION

Cancer is a multi-factorial disease and various factors show different roles in the development and progression of tumors including alteration of gene functions (Rahmani *et al*.,2013). Prostate cancer is one of the most commonly diagnosed cancers and the sixth leading cause of cancer death in males worldwide (Baade *et al*., 2009). Additionally, it is consider one of the malignancies with highest frequency of genetic variations (Boland and Ricciardiello, 1999). Although, such type of cancer have been reported in all age groups but>80% of cases occur in age over 60 years (Jemal *et al*., 2009). The incidence of prostate cancer is increasing worldwide and it has been reported that prostate cancer is the second cause of death in males in Sudan as per Radiation and Isotope Center (Alam *et al*., 2012). Early detection and accurate diagnosis of prostate cancer is prime interest in medical sciences to control the death rate and management of prostate cancer. In this vista, prostate specific antigens are commonly used as screening tests of prostate cancer worldwide and since PSA screening test was introduced, the prostate cancer mortality has reduced. However, PSA has some limitations as it is not an ideal marker of aggressive cancers, because high-grade cancers generally produce less PSA than low-grade cancers, particularly when the levels of PSA are corrected with cancer volume (Partin *et al*., 1990). Therefore, there is an increasing demand for effective and affordable markers to diagnose and monitor the tumor development and progression via accurate screening for all type of prostate cancer. AMACR is an enzyme that shows a major role in the beta-oxidation of branched-chain fatty acids and highly expressed in prostate adenocarcinoma but not in benign prostatic glands (Jiang *et al*., 2001). In the present study, we examined the possible role of AMCAR in prostate cancer through immuno histochemistry.

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MARERIALS AND METHODS

Study design: This is a descriptive retrospective case/control study was conducted at Sudan University of Science and Technology and different histopathology laboratories in Khartoum State, Sudan.

Sample collection: A total of 30 patient, 10 cases of benign prostatic hyperplasia and 20 cases of prostate adenocarcinoma were collected and immunohistochemical staining was performed to evaluate the expression of AMCAR. Hematoxyline and Eosin stain was performed to confirm the tumor types. The study was approved by the local Ethics Committee of the Sudan University of Science and Technology.

Immunohistochemical staining: Formalin fixed paraffin-embedded tissue blocks were cut in 5 microns thick sections. Sections were dewaxed in hot plate oven and cleaned in two changes of xylene for five minutes each, then sections were hydrated through graded alcohol (100, 90, 70 and 50%) and finally to distilled water, 2 minutes for each change. An Immunohistochemical assay of AMCAR was performed on paraffin section using streptavidin-biotin method. Monoclonal mouse antihuman antibody (ChemMate ™ EnVision, +/ HRP /DAB, rabbit/ mouse two-step staining, Gene tech company limited, Shanghai, China) used as primary antibodies for AMCAR. Antigen retrieval was performed in citrate buffer with pH 6.0. After antigen retrieval slides were incubated for primary and secondary antibody with appropriate time and also sections were incubated with streptavidin peroxidase. Finally, chromogen Diaminobenzidine (DAB) was used and section were counter stain with hematoxylin.

Result interpretation: Positive results were interpreted as if>5 % cells per field showed brown cytoplasmic stain measured as positive and<5% cells per field were considered as negative. All quality control measurements were followed during staining procedure.

Statistical analysis: Chi square $(ë)^2$ test was performed to find out the association among AMCAR and other clinical parameters in benign prostatic hyperplasia and prostate adenocarcinoma. Statistical significance was defined as $p<0.05$.

RESULTS

A total of 30 cases of prostate tumors including 20 cases of prostate adenocarcinoma and 10 cases of benign prostatic hyperplasia were studied (Table 1). A total of 20 cases of prostate cancer/adenocarcinoma were graded and it was found that 4 (20%) are well differentiated adenocarcinoma, 6 (30.0%) are moderate differentiated adenocarcinoma and 10 (50%) are poorly differentiated adenocarcinoma (Fig. 1). The age of patients ranged between 53-85 years old with a mean of 69 years (±9.2 year). Out of 30, 6 patients were less than 61 years and 24 patients were>61.

Table 1: AMACR expression pattern in benign tumor and prostate cancer

	AMACR expression			
	Positive		Negative	
Histopathological diagnosis	N	%	N	%
Benign	1	10	9	90
Prostate cancer	19	90	1	5

p = 0.00

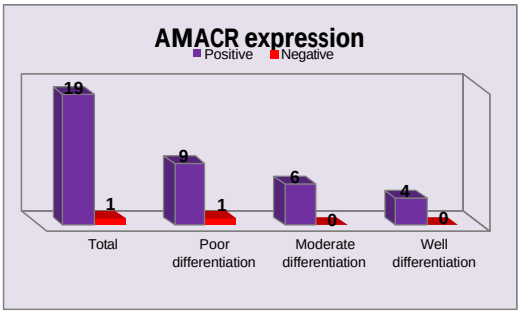

Fig. 1: Grade wise expression of AMACR in prostate carcinoma

The Cytoplasimc expression pattern via immunohistochemistry was analyzed in benign prostatic hyperplasia and prostate adenocarcinoma cases and results were interpreted (Fig. 2 and 3). In benign prostatic hyperplasia patients, AMACR was expressed only in 1 (10%) case, whereas 9 (90%) cases did not show any expression (Table 1).

On a sharp contrast, AMACR was highly expressed in all well and moderate differentiated prostate adenocarcinoma cases. All cases of well (4) and moderate (6) differentiated prostate adenocarcinoma. However, the poorly differentiated prostate adenocarcinoma showed 9 (90%) positive expression of AMACR (Fig. 3). The expression pattern among the different types of prostate adenocarcinoma was statically insignificant.

On the other hand, the expression profile was compared in benign prostatic hyperplasia and prostate adenocarcinoma cases and found that AMACR highly expressed in adenocarcinoma as compared to benign tumor and the differences in expression pattern were statically significant (P value <0.05).

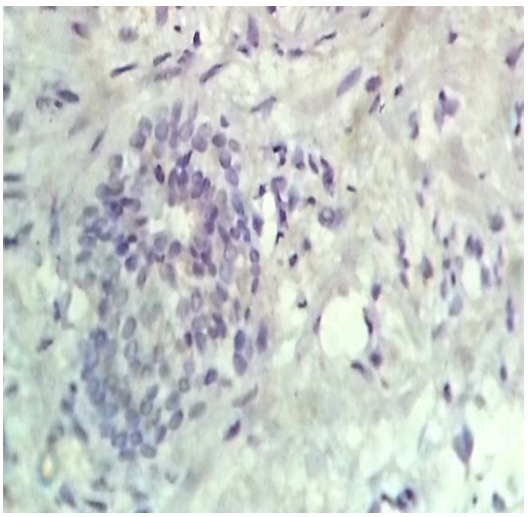

Fig. 2: AMACR was not expressed in benign prostatic hyperplasia (X40)

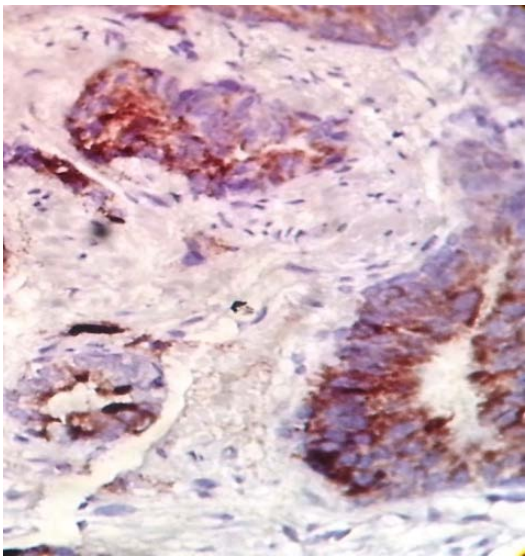

Fig. 3: Cytoplasmic expression of AMACR in prostate adenocarcinoma (X40)

DISCUSSION

Prostate cancer is one of the most common cancers among elderly men and is the second leading malignancy in the Western world. The incidence of prostate cancer is high in older age groups but exact reason behind the difference in incidence pattern among older age is not fully understood. It was proposed that it could be due to the long time exposures to carcinogens which may lead to failings of DNA repair mechanisms and aging (Rahmani *et al*., 2013;Horstmann *et al*., 2008). In this study, 30 patients with prostate tumor were studied and their age range between 53 to 85 years with the mean age of 69 years (±9.2) and predominant age was in more than 61 years (80.0%).

Our study showed that elderly men are at high risk of prostate cancer which is consistent with early findings that men over 60 years of age show histologic evidence of malignancy and higher incidence of tumor (Rahmani *et al*., 2013;Carter *et al*., 1990). Various types of screening test including PSA are used to diagnose prostate cancer, however PSA have some limitations. AMCAR has been shown to more sensitive and accurate marker in the diagnosis of prostate cancer. Earlier study showed that AMACR is considered to be very useful immunohistochemical marker for prostate cancer (Varma and Jasani, 2005). In this study, AMACR expression among prostate adenocarcinoma patients was found that 19 (95%) patients were positive for AMCAR and only one (5%) cases was negative. Among benign prostatic hyperplasia, out of 10 patients, only one patient (10%) was positive and 9 (90%) patients were negative for AMCAR expression. This study found that there is a relation between the expression of AMACR and the grade of prostate cancer. AMACR has been shown to be a highly sensitive and specific positive marker for prostate adenocarcinoma (Jiang *et al*., 2001). Additionally, it was found that AMACR is overexpressed in prostate cancer and it may be useful in the interpretation of prostate needle biopsy specimens that are diagnostically challenging (Rubin *et al*., 2002). Our results concluded that AMCAR is a valuable diagnostic and prognostic marker for prostate cancer.

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