Immunohistochemical expression of Alpha-Methylacyl-CoA Racemase in Benign Prostate Hyperplasia and Adenocarcinoma Prostate.

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ABSTRACT

Objective: To determine the association of Alpha- Methylaceyl-CoA racemase expression in adenocarcinoma prostate and benign prostate hyperplasia on immunohistochemistry.

Study Design: Comparative Cross Sectional

Place and Duration: This study was carried out at the Department of Pathology, Pakistan Navy Station Shifa Hospital, Karachi from January 01, 2018 till February 28th, 2019.

Methodology: A total of 74 prostatic specimens were recruited in the study. Out of which 37 specimens were that of prostatic adenocarcinoma and the remaining 37 were of benign prostate hyperplasia. All specimens were subjected to immunohistochemical staining with Alpha- Methylacyl-CoA racemase. Statistical analysis was done by using SPSS version 23.0. The association of extent of Alpha- Methylacyl-CoA racemase staining between adenocarcinoma and hyperplasia group was assessed by using Chi square test x^2.

Results: Out of the 37 cases of adenocarcinoma stained for Alpha- Methylacyl-CoA racemase, 9 (24.3%) cases showed reactivity in >90% of tumor cells, 16(43.2%) cases showed reaction in almost 51-90% of cells with strong intensity of staining ,the remaining 12 (32.4%) cases exhibited reactivity in 10-50% of tumor cells with moderate intensity (+2) of staining. Among the 37 cases of benign hyperplasia prostate which were subjected to Alpha- Methylacyl-CoA racemase immune staining, all cases showed negative immuno-expression for Alpha- Methylacyl-CoA racemase. There was a statistically significant association of expression of Alpha-Methylacyl-CoA Racemase in prostatic adenocarcinoma group as compared to benign prostatic hyperplasia with a p-value of 0.001.

Conclusion: Alpha- Methylacyl-CoA Racemase expression is significantly associated with prostatic adenocarcinomas as compared to benign prostatic hyperplasia and should be used as a diagnostic tool for differentiating between the two.

Keywords: Adenocarcinoma prostate, AMACR (Alpha- Methylacyl-CoA racemase), Benign prostate hyperplasia, Immunohistochemistry.

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INTRODUCTION

Prostate carcinoma is one of the most commonly occurring malignancies affecting elderly men and a prominent cause of mortality around the world[1]. According to the recent cancer registry report 2016; prostate cancer is the second most commonly occurring malignancy among Pakistani men[2]. A total of eight studies were analyzed for evaluating the prevalence of prostate cancer. The overall prevalence of prostate cancer in these studies was approximately in the range of 2 to 8 % and the overall prevalence was found out to be 5%[3]. Several risk factors might be involved in the carcinogenesis of this lesion, these comprise of men above the age of 50 years, androgens, genetic factors, environmental factors, family history, high fat diet, alcohol consumption, cigarette smoking and certain acquired somatic mutations[4].

Benign prostatic hyperplasia (BPH) is a nonmalignant enlargement of the prostate caused by cellular hyperplasia. It is a common age-related pathology affecting 70% of men aged 70 years or over[5]. It is one of the leading diagnoses affecting men of elderly age group. By age of 50 years, about 50% of men are recognized as BPH. Up to 80 years, 90% of men are suffering from this pathology, and the highest occurrence is among men aged 70 to 79 years[6,7].

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The histological diagnosis of prostate cancer on biopsy specimens is extremely challenging for pathologists. The difficulty arises due to the scanty amount of tissue accessible for histological evaluation and quite often in biopsy samples only a few glands bearing suspicion of malignancy are present amidst the benign glands[8].

Despite the fact that there are a few characteristic histological findings which are conclusive for diagnosis, principally it is determined on the basis of a variety of architectural, cytological and additional findings. Morphologically, prostate cancer is problematic to diagnose in that the cues leading to the diagnosis of malignancy may be subtle, rising more chances of under diagnosis. There are also many benign lesions resembling malignant lesions that can incite the histopathologist to an inaccurate diagnosis of malignancy[9]. Immunohistochemistry plays a vital role in confirming the diagnosis of various malignant lesions including prostate adenocarcinoma. A variety of immunohistochemical markers have been used for the accurate diagnosis of adenocarcinoma prostate[10].

AMACR (Alpha-methyl-aceyl-coA-racemase) plays a key role in the beta oxidation of branched chain fatty acids and the bile acid intermediates dihydroxycholestanoic acid and trihydroxycholestanoic acid. The gene for AMACR is located on chromosome 5p13 and encodes a 382 amino acid long protein. It has been reported as potential immune marker for the diagnosis of prostate cancer[11].

In our study, we aimed to determine the association of Alpha-methylacyl-CoA racemase expression in adenocarcinoma prostate and benign prostate hyperplasia on immunohistochemistry at a tertiary care hospital in Karachi. The study was done to obtain more data on the immunohistochemical markers used for the diagnosis of prostate adenocarcinoma in our part of the world. So, we conducted this study with an objective to determine the association of Alpha- Methylaceyl-CoA racemase expression in adenocarcinoma prostate and benign prostate hyperplasia on immunohistochemistry.

METHODOLOGY

This is a comparative cross sectional study based on the analysis of prostatic samples, comprising of both prostatectomies and transurethrally resected prostatic biopsies received at the Department of Pathology, PNS Shifa Karachi during one year period from January 2017 till February 2018.The samples were collected by using non probability convenience sampling technique. Histologically, confirmed cases of adenocarcinoma prostate and benign prostatic hyperplasia were subjected to immunohistochemistry evaluation for AMACR. Ethical approval was taken from the ERC (Ethical Review Committee) of the Bahria University of Medical & Dental College (BUMDC), reference no ERC 44/2018 dated Oct 02, 2018.

Sample size was calculated by using the method of single proportion on www.openepi.com software. Male patients who were willing to participate between 50 years to 85 years were included in the study. Poorly fixed tissue samples and samples having inadequate material having atrophy were excluded. H&E stained slides were reviewed to confirm the diagnosis. The most representative section was used for immunohistochemical analysis. In order to perform immunohistochemistry sections of 3 to 5 µm thickness were taken from Formalin Fixed Paraffin Embedded blocks and picked up on poly-L- lysine coated slides. Antigen retrieval was done by using retrieval solution (pH 6.0 citrate buffer 10X) in water bath at 98-99ºC for 40 minutes. Container was removed from water bath and cooled at room temperature (15 to 20 minutes).Retrieval solution was discarded and section was rinsed two to three times. Endogenous peroxide was blocked using hydrogen peroxide blocking solution. Primary antibodies were applied to cover the section. AMACR dilution was done as per the company specified protocol and was incubated for 1 hour at room temperature. Slides were then incubated with HRP polymer for 10 minutes. Chromogen was applied for 20 minutes and all the slides were counterstained with Hematoxylin, dehydrated and mounted. Between each step, the slides were washed with phosphate buffer solution (PBS).

Anti AMACR (clone 13H4) ready to use monoclonal rabbit antibody against AMACR and was procured from DAKO Denmark. EnVision FLEX, Mini Kit (ready to use) was procured from Meximp Technologies. Normal kidney tissue was used as positive control for AMACR. AMACR showed continuous diffuse or granular cytoplasmic staining of glandular epithelium. The extent of staining was estimated in percentage by counting at least 50 nuclei, calculating the ratio of reactive nuclei to total number of nuclei and multiplying it by 100. The percentage positivity was graded from 0 to 4+as follows: 0 when negative staining was observed, 1+when 1-10% cells were stained,2(+) when 11-50% of cells were stained, 3(+) when 51–90% and 4(+) when the majority of cells (>90%) showed positive staining.

Relevant data was collected on self-designed proformas. Statistical analysis was performed using SPSS version 23. Mean and standard deviation were calculated for quantitative variables while percentages and frequencies were calculated for qualitative variables. Chi square was applied and p-value of less than 0.05 was considered significant at 95% confidence interval.

RESULTS

Among total of 74 patients studied, the mean age of patients with benign prostate hyperplasia was found to be 64.11±8.75 years, while those of adenocarcinoma prostate was70.0±7.4 years.

Table-I: Extent of AMACR immunohistochemical staining in benign prostate hyperplasia and adenocarcinoma prostate (N=74)

% of stained cells	AMACR (Benign prostate hyperplasia)		AMACR (Adenocarcinoma prostate)	
	n	%	n	%
>90%	0	0.0	9	24.3
51 -90%	0	0.0	16	43.2
11-50%	0	0.0	12	32.5
1-10%	2	5.41	0	0.0
0%	35	94.59	0	0.0
TOTAL	**37**	**100.0**	**37**	**100.0**

Table-I shows the extent of AMACR staining in cases of benign prostate hyperplasia and adenocarcinoma prostate. Table-II shows the intensity of AMACR staining in cases of benign prostate hyperplasia and adenocarcinoma prostate.

Table-II: Immunohistochemical staining intensity of AMACR in benign prostate hyperplasia and adenocarcinoma prostate

Intensity of staining	AMACR (Benign prostate hyperplasia)		AMACR(Adenocarcinoma prostate)	
	No.	%	No.	%
Negative(0)	35	94.59	0	0.0
Weak(+1)	2	5.41	0	0.0
Moderate(+2)	0	0.0	12	32.44
Strong(+3)	0	0.0	25	67.56
TOTAL	**37**	**100.0**	**37**	**100.0**

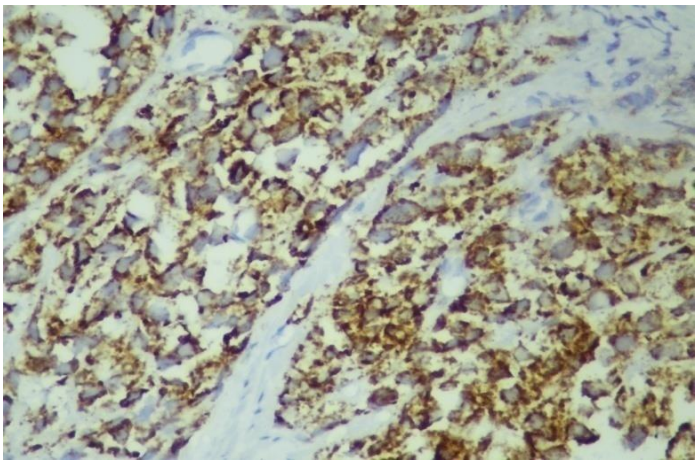

Figure-1: Adenocarcinoma prostate having Gleason score 5+4, showing strong AMACR expression. IHC x20

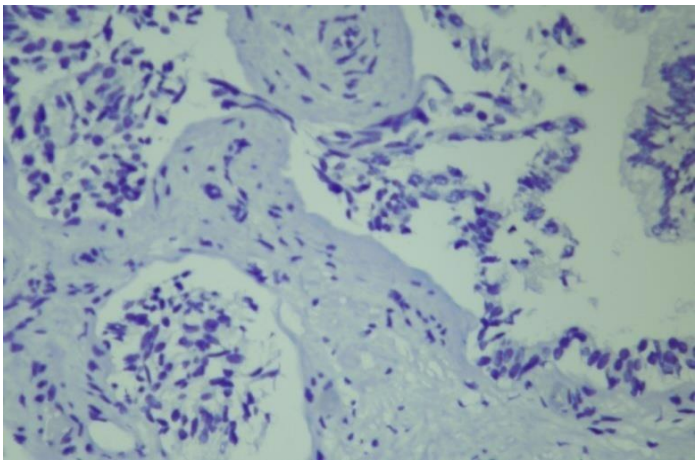

Figure-2: Absence of staining of AMACR in the benign glands of prostate on IHCX20

Out of the 37 cases of adenocarcinoma prostate subjected to AMACR staining, 25 cases showed strong intensity of staining (+3). Out of these 25 cases, 9 (24.3%) cases showed reactivity in >90% of tumor cells, (as seen in Fig-1), 16(43.2%) cases showed reaction in almost 51-90% of cells, 12(32.4%) cases exhibited reactivity in 10-50% of tumor cells with moderate intensity (+2) of staining(Table-I and Table-II). Among the 37 cases of benign hyperplasia prostate which were subjected to AMACR immune staining, 35cases (94.59%) showed negative immuno-expression for AMACR, as (seen in Fig-2) whereas, the remaining 2 cases (5.41%) stained <10% for AMACR (Table-I and Table-II)

Chi square analysis was done to compare the expression between two groups. The prostatic adenocarcinoma showed a statistically significant association of AMACR positivity as compared to benign prostatic hyperplasia cases. The p value was 0.001.

DISCUSSION

Immunohistochemistry (IHC) is an important adjuvant technique for pathologists as it precisely visualizes the dissemination and measure of a specific molecule in tissue specimen utilizing the specific antigen antibody reaction[12]. It has been an efficient tool in the diagnosis of prostate cancer, however the accurate diagnosis of prostate cancer can be challenging due to the availability of limited tissue and the presence of many benign mimickers of malignancy. In this study we determine the association of Alpha- Methylacyl-CoA racemase expression in adenocarcinoma prostate and benign prostate hyperplasia on immunohistochemistry.

In the present study, the mean age of patients having adenocarcinoma prostate was 70.0+7.4 years. This is concordant with study carried out by Bhatta and Hirachan, who reported a mean age of patients suffering from prostate cancer was found out to be 72.9 ± 5.2 years[13]. In the current study, patients suffering from benign prostate hyperplasia had the mean age of64.11±7.5 years. Our research findings correlate with the study carried out in the Chandka Medical College, Hospital Larkana. This study analyzed 103 patients suffering from BPH and the study revealed that the mean age of the patients was 62.29 ± 7.67 years[14].

Jiang et al proclaimed P504S (AMACR) as a novel immune marker for the diagnosis of prostate carcinoma. Immunohistochemistry was performed by using a rabbit monoclonal antibody on a total number of 207 cases which included 137 diagnosed cases of prostate cancer and 70 that of benign prostate hyperplasia. Results of this study revealed strong positive expression of AMACR in all malignant lesions[15].

Our study findings correspond with the study carried out at the Adelaide and Meath hospital, Ireland in which 101 prostatic cases were included. Out of these 101 cases, 57 cases were that of carcinoma while, the remaining 44 cases were diagnosed as benign prostate hyperplasia. Among the 57 cancer cases, 91% showed at least focal positive staining for AMACR strong or moderate staining in at least 1% of tumor glands while, 53% showed diffuse tumor. In the benign cases, the majority of glands were negative for AMACR[16].

Similar results were reported in the study conducted at Postgraduate Medical institute Lahore, in which 50 specimens of benign prostate hyperplasia and 50 cases of malignant lesions were subjected to immunohistochemistry with AMACR. It was observed that each hyperplastic lesion was negative whereas, malignant cases showed positive AMACR expression[17].

Tariq et al., emphasized on the timely diagnosis of prostatic carcinoma in routine practice, the study analyzed a total of 80

cases out of which, 85% were positive for AMACR while 15% were found out to be negative[18].

Our study results are in accordance with the study carried out at the Zagazig University, Egypt in which a total of 60 prostatic specimens were immune stained for AMACR, including 30 cases of adenocarcinoma prostate and 30 specimens of benign prostate hyperplasia. Results of the study revealed that 90% cases of prostate adenocarcinoma showed AMACR expression whereas no expression was noted in the benign glands. There were significant differences in AMACR expression between benign and malignant lesions ($p<0.001$)[19].

Our study findings correspond to the study carried out in Department of Pathology, Government Medical College, Kottayam evaluated a total number of 120 cases and immunohistochemistry was performed. 93 cases were diagnosed as prostate carcinoma and graded using the Gleason scoring, 85 cases showed positivity for AMACR, 5 cases were found to have a suspicion of malignancy, 4 cases displayed positivity for AMACR and 3 cases were confirmed as PIN of which 2 cases showed over expression of AMACR. Benign prostatic hyperplasia was observed in 19 cases all of which showed negative staining for AMACR[20].

Similar results were seen in a prospective study carried out at the Department of Pathology Rothak University in which a total of 50 cases were recruited in the study. Out of the 50 cases, 37 cases were malignant lesions and 13 cases comprised of benign prostate hyperplasia. AMACR was determined by immunohistochemical staining. The obtained results were analyzed and evaluated using Chi-square statistical test. The results of this study revealed that AMACR was not expressed in any of the 13 cases of benign lesions of the prostate while in malignant lesions of prostate it was expressed in 89.18% cases. There was statistically significant difference in expression of AMACR between benign and malignant lesions of the prostate, indicated by a significant p-Value of 0.001[21].

CONCLUSION

Alpha- Methylacyl-CoA Racemase expression is significantly associated with prostatic adenocarcinomas as compared to benign prostatic hyperplasia and should be used as a diagnostic tool for differentiating between the two.

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AUTHOR'S CONTRIBUTION

Nomani BH: Conceived idea, Data collection, Immunohiosto chemical analysis of slides, Data interpretation, Statistical analysis, Manuscript writing.

Alamgir M: Data interpretation, Proof reading of manuscript

Wasti H: Data collection, Statistical analysis, Proof reading of manuscript

Nadeem S: Data collection, Proof reading of manuscript

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