

Sensitivity of Alpha-Methylacyl-Coa Racemase (Amacr) Staining in Prostatic Adenocarcinoma

Uzma Bukhari^{1*}, Suresh Kumar¹, Aneeta George¹, Yusra Memon¹, Asma Bukhari²

¹Dow University of Medical & Health Sciences, Karachi, Pakistan

²Riphah International University, Islamabad, Pakistan

*Corresponding author: Uzma Bukhari, Dow University of Medical & Health Sciences, Karachi, Pakistan, Tel: +92-3343319897; E-mail: Uzmabukhari@gmail.com

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ABSTRACT

Objectives

Prostate Adenocarcinoma is a major health problem worldwide. Karachi Cancer Registry of Pakistan has reported that prostatic adenocarcinoma ranks at number six among cancers in males in Pakistan. Immunohistochemical marker Alpha-methylacyl-CoA Racemase has valuable role in diagnosis. This study was conducted to determine the sensitivity of this marker in our setup.

Methodology

This descriptive cross sectional study was conducted at Pathology Department of Shaukat Khanum Memorial Cancer Hospital and Research Center, in a period of six months from 13 September 2012 to 13 March 2013, taking a sample size of 80 biopsy proven cases of prostatic Adenocarcinoma. SPSS version 16 was used for data analysis. The qualitative variables like expression of AMACR were presented as frequencies and percentages. Chi square was used with a p value <0.05 taken as significant.

Sampling technique: Non-probability purposive sampling.

Results

The mean \pm SD age of patients was 67.81 ± 7.25 years with a range from 51 to 85 years. The mean \pm SD Gleason score of these patients was 6.99 ± 1.52 . About 51.2% (n=41) patients were of age group 61-70 years, 33.8% (n=27) were between 71-80 years, only 2.5% (n=2) were of age 81 years and above. Well-differentiated cases of prostatic adenocarcinoma were 3.8% (n=3), 52.5% (n=42) were moderately while 43.8% (n=35) were poorly differentiated. Out of all 100% (n=80) cases 91.2% (n=73) were expressed positive with AMACR staining while remaining 8.8% (n=7) were AMACR negative.

Conclusion

AMACR staining is highly sensitive diagnostic tool and should be carried out in all patients who present with doubtful picture of prostatic cancer.

Keywords: AMACR Staining, Prostatic PSA, Gleason Scoring, Adenocarcinoma, Immunohistochemistry.

INTRODUCTION

Carcinoma of Prostate represents commonest cancer among males. In the United States, this is the second cause of cancer related deaths.¹ This malignant tumor rank 3rd in carcinoma incidence and sixth in carcinoma mortality worldwide with incidence is increasing worldwide.² Prostatic adenocarcinoma is third common tumor in our population.² The reported incidence of prostate cancer in Pakistan is 3.8%. This under reporting is primarily due to lower life expectancy and lack of screening.³

The initial evaluation for prostate cancer by the gold standard triad comprising of digital rectal examination DRE, PSA level and transrectal ultrasonography is followed by surgical pathological evaluation of prostate specimens by needle biopsy.³ Morphological diagnosis of the malignant neoplasm is critical for maximum patient survival. Although histological features of adenocarcinoma are sufficient for morphological diagnosis, immunohistochemistry is necessary for confirmation of diagnosis of prostate adenocarcinoma particularly due to benign mimickers of prostatic carcinoma.⁴ Thus immunohistochemical analysis not only helps to distinguish these benign conditions from malignant ones but also mandatory for confirmation of diagnosis in difficult cases.^{5,6}

Alpha-methylacyl coenzyme A Racemase (AMCAR) has an important value in oxidation of fatty acid. AMCAR positive cases revealed cytoplasmic or granular staining with no immunostaining in non-neoplastic glands.⁷ AMCAR staining alone or in combination not only confirms prostate adenocarcinoma but it also identifies those malignancies which lack qualitative or quantitative features, have unusual morphological features and also in the setting of prior

treatment. AMCAR has 92% sensitivity and 100% specificity in detecting adenocarcinoma⁸. The use of AMCAR as a regular marker for detecting prostatic adenocarcinoma is not only time and cost effective but also raises the diagnostic accuracy in detecting cases of prostatic adenocarcinoma especially where histology is deceptive⁸. Therefore, the study aimed to determine the sensitivity of Alpha-methylacyl-CoA racemase (AMACR) stain in prostatic adenocarcinoma in our setup.

METHODOLOGY

This descriptive cross sectional study was conducted at Pathology Department of Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore, Pakistan in a period of six months from 13 September 2012 to 13 March 2013, taking a sample size of 80 biopsy proven cases of prostatic adenocarcinoma. College of physicians and Surgeons has approved the study. Sampling technique was Non-probability purposive sampling. Consent of patient along with history was taken on designed proforma. All poorly preserved and poorly fixed specimens were excluded from the study.

A total of 80 cases of prostatic adenocarcinoma found suitable by inclusion criteria were included in this study. Each case was given a medical record number. Socio-demographic information (name, age, full address, history of frequency and urgency of micturation, family history of any cancer and prostate cancer) were obtained along with data of PSA level. All the information was recorded on specially designed proforma. Gross examination was performed for large specimen i-e radical prostatectomy and TURP specimens. In case of radical prostatectomy specimens, representative areas from intra-tumoral, marginal para-tumoral and distant normal prostate tissues were sectioned. The small tissues i.e., U/S- guided needle core biopsies were processed as such. All tissues were subjected to automated histology tissue processor for dehydration, clearing, impregnation and embedding steps of tissue processing. Paraffin embedded blocks were prepared and two sections from each block 4-6 μ m for haematoxylin and eosin staining and one section, 3-5 run for immunohistochemical expression of AMACR were cut with the help of rotary microtome. The sections for immunohistochemical staining were collected on positive charged slides.

Immunohistochemical stain AMACR was performed according to the specification given by the manufacturer. The morphology was reviewed by histopathologist to establish the diagnosis. The result of AMACR immunostaining was based on cytoplasmic staining. The lesion was designated positive when it was strong and circumferential or significantly stronger than that of background benign glands. The immunostaining with AMACR was graded as weak, moderate or strong. The lesion was designated negative for AMACR when no cell expresses at detectable level or show very weak, focal staining with AMACR. SPSS version 16 was used for data analysis.

RESULTS

In current study, 80 biopsy proven cases of prostatic adenocarcinoma were included. The mean \pm SD age of patients

was 67.81 ± 7.25 years with a range from 51 to 85 years. The mean \pm SD Gleason score of these patients was 6.99 ± 1.52 which ranged from minimum of 4 to maximum score 9.

It was noted that more than half of patients i-e 51.2% (n=41) were of age group 61-70 years, about one third i-e 33.8% (n=27) were between 71-80 years of age while in 51-60 years age group there were 12.5% (n=10) patients and 2.5% (n=2) were of age 81 years and above. It was seen that 3.8% (n=3) were well differentiated, about 52.5% (n=42) were moderately differentiated while other 43.8% (n=35) were poorly differentiated cases of prostatic adenocarcinoma.

Regarding the sensitivity of AMACR expression it was noted that out of all 100% (n=80) cases of prostatic adenocarcinoma, about 91.2% (n=73) were expressed positive with AMACR staining (Fig-01), while remaining 8.8% (n=7) were AMACR negative. Similarly true Positive results of AMACR staining were 91.2% while false negative results of AMACR staining were 8.8%. When stratified analysis was performed with p value of <0.05 taken as significant, it was seen that age of patients was an effect modifier for the frequency of AMACR expression patients of prostatic Adenocarcinoma. Accordingly, positivity of AMACR expression increased from 80% in 51-60 age groups to 100% in 81 years and above age group patients. (p-value 0.442) (Table-1)

Effect of Level of differentiation on frequency of AMACR expression is given in Table 02, according to which it was noted with high significance (p value <0.001) that among cases of well differentiated prostatic adenocarcinoma (n=3), the AMACR expression was negative in all i-e; 100% while it was 97.1% among those who had poorly differentiated picture of Prostatic adenocarcinoma. The study also evaluated the effect of Gleason score on frequency of AMACR expression. It was seen that in Gleason score 4 (n=3) it was negative in all i.e.; 100% which increased gradually with increasing Gleason score till 8 & 9 where the sensitivity of AMACR expression was 94.7% & 100% respectively. The results were highly significant. (p value <0.001) (Table 3)

Age category	AMACR expression		Total
	Positive	Negative	
51-60 years	8 80%	2 20%	10 100%
61-70 years	37 90.2%	4 9.8%	41 100%
71-80 years	26 96.3%	1 3.7%	27 100%
81 and above	2 100%	0 0%	2 100%
Total	73 91.2%	7 8.8%	80 100%

Table 1: Effect of age on frequency of AMACR expression.

Pearson Chi-Square = 2.690

P value = 0.442

df= 3

Level of differentiation	AMACR expression		Total
	Positive	Negative	
Well differentiated	0 0%	3 100%	3 100%
Moderately differentiated	39 92.9%	3 7.1%	42 100%
Poorly differentiated	34 97.1%	1 2.9%	35 100%
Total	73 91.2%	7 8.8%	80 100%

Table 2: Effect of Level of differentiation on frequency of AMACR expression.

Pearson Chi-Square = 32.944

P value <0.001

df=

Gleason score	AMACR expression		Total
	Positive	Negative	
4	0 0%	3 100%	3 100%
5	14 93.3%	1 6.7%	15 100%
6	12 92.3%	1 7.7%	13 100%
7	13 92.9%	1 7.1%	14 100%
8	18 94.7%	1 5.3%	19 100%
9	16 100%	0 0%	16 100%
Total	73 91.2%	7 8.8%	80 100%

Table 3: Effect of Gleason score on frequency of AMACR expression.

Pearson Chi-Square = 33.254

P value <0.001

df=

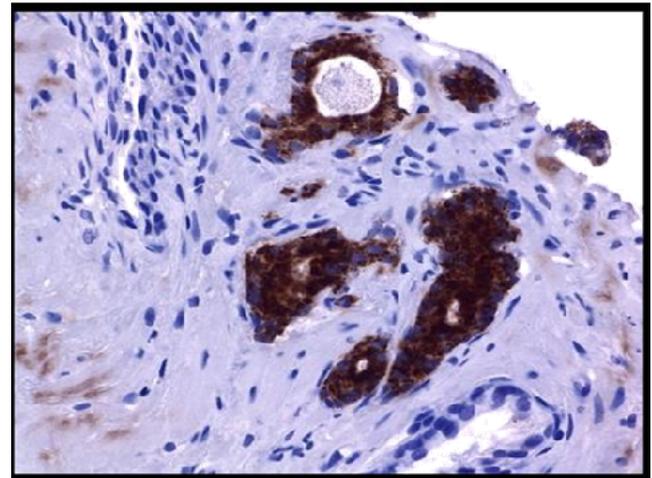


Figure 1: Focus Of neoplastic Prostate glands showing positive staining of AMACR.

DISCUSSION

In this study patient's age ranged from 51 to 85 years with a mean \pm SD age of 67.81 ± 7.25 years. Nearly two-thirds (63.7%) patients were between the age of 51 to 70 years (i.e. 51.2%+12.5%). These statistics reflect the fact that prostatic cancer is a disease of old age with highest prevalence in 6th and 7th decades of life. These results are in agreement with Tariq H et al, 9 who also reported similar findings. After this age both mortality associated with prostatic cancer and other reasons is high. Therefore, in higher ages the number of patients living with prostatic adenocarcinoma is least. These findings match those of Ahmad et al, (2002)10 who reported that mean age of patients of prostatic adenocarcinoma was 72.1 years.

Contrary to findings of this study, Sreekumar A et al.11 in their study from India, found a younger mean age of presentation of prostatic adenocarcinoma i.e.; 59.8 years. The similar authors described that their patients ranged from 41 to 83 years which is a broad range; some of the patients were quite younger than others.11

Prostate cancer diagnosis at any age has a major impact on a man's quality of life.12,13 Morphological diagnosis of the malignant neoplasm is critical for maximum patient survival.14-16. Sometimes on the basis of morphology, it is difficult to give a final diagnosis, especially in trucut biopsies having tiny foci of carcinoma.17,18. Therefore, to solve this issue, immunohistochemistry has an important role to differentiate prostate adenocarcinoma from benign mimickers and to establish the correct morphological diagnosis.19-22.

On the basis of immunohistochemistry, panels of markers are required. There is still no single specific marker that has yet been discovered for the confirmation of adenocarcinoma. 17,18,23 These panels include at least one basal cell-specific marker and the prostate cancer-specific marker, alpha-methyl-CoA-Racemase. This enzyme plays an important role in the diagnosis. Multiple authors have reported the expression of this enzyme in adenocarcinoma of prostate in combination with morphological findings.24-26

Clinicians are found that using this enzyme marker in conjunction with other prostatic markers gives more accurate results than AMACR alone.^{23,27} All the 80 patients were purposively selected cases of prostatic cancer. Only few cases were well-differentiated prostatic adenocarcinoma while more than half (52.5%) were moderately differentiated or poorly differentiated were almost half (43.8%). When these specimen from these cases were analyzed with AMACR staining it was noted that 91.2% cases were positively detected as prostatic adenocarcinoma; thus proving the sensitivity of AMACR staining at the rate of 91.5%. About 8.5% specimen results turned out to be false negative. These finding are in matching with other studies. Tariq H et al⁹ reported 85% positivity of AMACR staining in prostatic adenocarcinoma. Accordingly, Magi-Gulluzi C, et al.²⁸ found that out of 209 cases of prostatic cancer, 87% were picked right by the AMACR staining. In some other samples of other institution, the similar study found sensitivity of AMACR staining up to 100%. Another study by Yang et al.²⁹ the sensitivity of AMACR staining was noted to be nearly 100%.

The ages of patients of prostatic cancer affected the frequency of AMACR staining results viz the frequency of positive results increased with increasing age of patients (from 80% in 51-60 age group to 100% in 81 years and above age group) but the results were not statistically significant. None of other authors took account of such findings in their studies.

The current study also graded the prostatic adenocarcinoma as per Gleason scoring system. Because we had taken the diagnosed cases of prostatic adenocarcinoma therefore none of patients had a score of <3 out of 10. Minimum score was 4 and maximum was 9 with a mean \pm SD score of 6.99 ± 1.52 . It was highly significantly noted that with the increasing the Gleason score the sensitivity of AMACR staining increased from Zero percent (in those who had minimum score) to 100% (in those who had maximum score). Level of differentiation of prostatic adenocarcinoma also affected the sensitivity of AMACR staining in similar pattern.

Though some studies have worked upon the identifying the biomarkers of prostatic disease and their efficiency but in our knowledge this study was first of its kind in Pakistan to assess the sensitivity of AMACR staining in histopathologically diagnosed cases of prostatic adenocarcinoma. Overall, the study has given good insight into the availability & reliability of a good diagnostic tool at such a time when the value of prostatic specific antigen is losing focus of clinicians and pathologists.

The current study has some limitations as well. It was a study with a limited scope due to short time duration and resources. It did not take account of prostatic specific antigen so as to compare or associate it with the sensitivity of AMACR staining which if done would have better cleared the perspectives of the study. Secondly, the study did not evaluate the strength of AMACR staining.

CONCLUSION

Prostatic adenocarcinoma is a very common morbid condition and leading cause of death in elderly age men therefore, it should be diagnosed correctly and well early before its

metastasis in order to decrease the morbidity and mortality. AMACR staining is highly sensitive diagnostic tool for this and should be carried out in all the patients who present with doubtful picture of prostatic adenocarcinoma.

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