Original Article

Sensitivity of Alpha-Methylacyl-COA Racemase (AMACR) Staining in Prostatic Adenocarcinoma

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ABSTRACT

OBJECTIVES: To determine the sensitivity of Immunohistochemistry for confirmation of diagnosis of prostate adenocarcinoma 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 September 2012 to March 2013, taking a sample size of 80 biopsy proven cases of prostatic Adenocarcinoma with Non-probability purposive sampling technique. 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.

RESULTS: The mean ± SD age of patients was 67.81±7.25 years with a range from 51 to 85 years. The mean ± 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 Adenocarcinoma, PSA, Gleason Scoring, Immunohistochemistry.

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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 adenocarcinoma[8]. 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 deceptive[8]. 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 from September 2012 to March 2013, taking a sample size of 80 biopsy proven cases of prostatic adenocarcinoma with Non-probability purposive sampling technique. This study was approved by College of Physicians and Surgeons Pakistan. 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 µm 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.

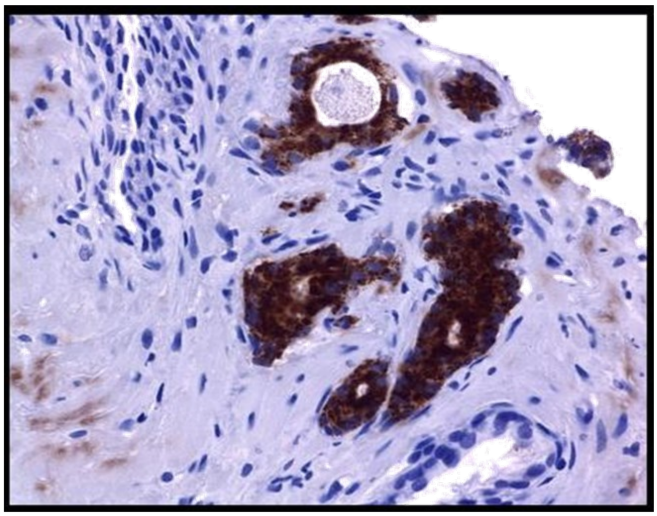
RESULTS

In current study, 80 biopsy proven cases of prostatic adenocarcinoma were included. The mean ± SD age of patients was 67.81±7.25 years with a range from 51 to 85 years. The mean ± 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 (Figure I), while remaining 8.8% (n=7) were AMACR negative.

FIGURE I: FOCUS OF NEOPLASTIC PROSTATE GLANDS SHOWING POSITIVE STAINING OF AMACR



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 I).

Effect of Level of differentiation on frequency of AMACR expression is given in Table II, 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 III).

TABLE I: EFFECT OF AGE ON FREQUENCY OF AMACR EXPRESSION

Age category (Years)	AMACR expression		Total
	Positive	Negative	
51-60	8 (80%)	2 (20%)	10 (100%)
61-70	37 (90.2%)	4 (9.8%)	41 (100%)
71-80	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%)**

Pearson Chi-Square = 2.690, P value = 0.442, df= 3

TABLE II: EFFECT OF LEVEL OF DIFFERENTIATION ON FREQUENCY OF AMACR EXPRESSION

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%)**

Pearson Chi-Square = 32.944, P value <0.001, df= 2

TABLE III: EFFECT OF GLEASON SCORE ON FREQUENCY OF AMACR EXPRESSION

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%)

Pearson Chi-Square = 33.254, P value <0.001, df= 5

DISCUSSION

In this study patient's age ranged from 51 to 85 years with a mean ± 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 reflects the fact that prostatic cancer is 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 mortality associated with prostatic cancer and other reason is high. Therefore, in higher ages the number of patients living with prostatic adenocarcinoma is least. These findings match those of Ahmad Z 2009[10] who reported that mean age of patients of prostatic adenocarcinoma was 72.1 years.

Contrary to findings of this study, Sreekumar A 2004[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 broad range some of 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 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 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 been reported the expression of this enzyme in adenocarcinoma of prostate in combination with morphological findinds[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 al[9] reported 85% positivity of AMACR staining in prostatic adenocarcinoma. Accordingly, Magi-Gulluzi C 2003[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 XJ 2002[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 ± 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.

Ethical permission: College of Physicians & Surgeons Pakistan dissertation approval letter (Duplicate) No. CPSP/REU/HSP-2009-074-308, dated 7-3-2020.

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AUTHOR CONTRIBUTIONS

Kumar S: Histological Examinations, Manuscript writing
Bukhari U: Histological Examinations, Manuscript writing.
George A: Data Analysis & interpretation
Memon Y: Data Analysis & interpretation
Sikandar B: Data Analysis & interpretation
Khan N: Data Analysis & interpretation
Bukhari A: Sample collection.

REFERENCES

1. Hasan IA , Gaidan HA, Al-kaabi MM. Diagnostic Value of Cytokeratin 34 beta E12 (Ck34βE12) and α-Methylacyl-CoA racemase (AMACR) Immunohistochemical Expression in Prostatic Lesions. Iran J Pathol. 2020; 15(3): 232-38.
2. Alam MS , Ali A, Mehdi SJ, Alyasiri NS, Kazim Z, Batra S, et al. HPV Typing and Its Relation With Apoptosis in Cervical Carcinoma From Indian Population. Tumour Biol. 2012; 33(1): 17-22.
3. Hanif M, Zaidi P, Kamal S, Idrees S, Rasool S. Significance of prostate specific antigen in prostate cancer patients and in non-cancerous prostatic disease patients. J Pak Med Assoc. 2007; 57: 248.
4. Humphrey PA. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. J Clin Pathol. 2007; 60: 35-42.
5. Gladell P, Daniel JL, Mahul BA. Best Practice in Diagnostic Immunohistochemistry Prostate Carcinoma and Its Mimics in Needle Core Biopsies. Arch Pathol Lab Med. 2008; 132: 1388–96.
6. Rathod SG, Jaiswal DG, Bindu RS. Diagnostic utility of triple antibody (AMACR, HMWCK and P63) stain in prostate neoplasm. Family Med Prim Care. 2019; 8(8): 2651-5.
7. Abdelaziz MS, Mohagir HE, Babiker AY. Immunohistochemical detection of Alpha- Methyl-Co-Racemase (AMACR) in adenocarcinoma of Prostate. Res J Med Sci. 2016; 10 (6): 707-10.
8. Kumarvesan K, Kakkar N, Verma A, Mandal AK, Sing SK, Joshi K. Diagnostic utility of Alpha-Methyl Acyl COA Racemase (P504S) and HMWCK in morphologically difficult prostate cancer. Diag Pathol. 2010; 5: 83.
9. Tariq H, Ahmed R, Muhammad I, Afzal S, Hashmi SN, Hamdani S, et al, Immunohistochemical expression of Alpha Methylacyl-COA Racemase (amacr) in carcinoma Prostate in Pakistani population. Pak Armed Forces Med J. 2017; 67(6): 1054-57.
10. Ahmad Z, Qureshi A, Idrees R, Aftab K. Prostatic carcinoma: a Pakistani perspective. Asian Pac J Cancer Prev. 2009; 10(2): 323-4.
11. Sreekumar A, Laxman B, Rhodes DR. Humoral immune response to alpha-methylacyl - CoA racemase and prostate cancer. JNCI. 2004; 96: 834-43.
12. Bhurgri Y, Bhurgri A, Hasan SH. Cancer incidence in Karachi, Pakistan: First results from Karachi Cancer Registry. Int J Cancer. 2000; 85: 325-9. doi: 10.1002/(sici)1097-0215(20000201)85:3<325: aid-ijc5>3.0.co;2-j.

13. Rosai J. Rosai and Ackerman's Surgical Pathology. 9th ed. New York, NY: Mosby;2004: 1361-85.
14. Epstein JI, Herawi M. Prostate needle biopsies containing prostatic intraepithelial neoplasia or atypical foci suspicious for carcinoma: implications for patient care. J Urol. 2006: 175-820.
15. Yano M, Imamoto T, Suzuki H. The clinical potential of pretreatment serum testosterone level to improve the efficiency of prostate cancer screening. Eur Urol. 2007; 51: 375-80.
16. Schmitz W, Fingerhut R, Conzelmann E. Purification and properties of an alpha-methylacyl-CoA racemase from rat liver. Eur J Biochem. 1994; 222(2): 313-23.
17. Yokomizo Y, Miyoshi Y, Nakaigawa N. Free PSA/ total PSA ratio increases the detection rate of prostate cancer in twelve-core biopsy. Urol Int. 2009; 82: 280–5.
18. Polascik TJ, Oesterling JE, Partin AW. Prostate specific antigen: a decade of discovery–what we have learned and where we are going. J Urol. 1999; 162: 293-306.
19. Yamada H, Tsuzuki T, Maeda N, Yamauchi Y, Yoshida S, Ishida R, et al Alpha methylacyl-CoA racemase (AMACR) in prostate adenocarcinomas from Japanese patients: is AMACR a "race" dependent marker? Prostate. 2013; 73: 54-9.
20. Nadler RB, Humphrey PA, Smith DS. Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. J Urol. 1995; 154: 407-13.
21. Kuefer R, Varambally S, Zhou M. Alpha-methylacyl-CoA racemase: expression levels of this novel cancer biomarker depend on tumor differentiation. Am J Pathol. 2002; 161: 841-8.
22. Zha S, Ferdinandusse S, Denis S. Alpha-methylacyl-CoA racemase as an androgen-independent growth modifier in prostate cancer. Cancer Res. 2003; 63: 7365-76.
23. Varma M, Jasani B. Diagnostic utility of immunohistochemistry in morphologically difficult prostate cancer: review of current literature. Histopathol. 2005; 47: 1-16.
24. Brustmann H. p40 as a basal cell marker in the diagnosis of prostate glandular proliferations: a comparative immunohistochemical study with 34betaE12. Pathol Res Int. 2015; 2015.
25. Jain D, Gupta S, Marwah N, Kalra R, Gupta V, Gill M, et al. Evaluation of role of alpha-methyl acyl-coenzyme A racemase/P504S and high molecular weight cytokeratin in diagnosing prostatic lesions. J Cancer Res Therapeut. 2017; 13(1):21-25.
26. Rashed HE, Kateb I, Ragab E, Shaker E. Evaluation of minimal prostate cancer in needle biopsy specimens using AMACR (P504S), P63 and KI67. Life Sci J. 2012; 9: 12-21.
27. Molinie V, Fromont G, Sibony M, Vieillefond A, Vassiliu V, Cochand-Priollet B, et al. Diagnostic utility of a p63/alpha-methyl-CoAracemase (p504s) cocktail in atypical foci in the prostate. Mod Pathol. 2004; 17(10): 1180-90.
28. Magi-Galluzzi C, Luo J, Isaacs WB. Alpha-methylacyl-CoA race-mase: a variably sensitive immunohistochemical marker for the diagnosis of small prostate cancer foci on needle biopsy. Am J Surg Pathol. 2003; 27: 1128–33.
29. Yang XJ, Wu CL, Woda BA. Expression of alpha-methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. Am J Surg Pathol. 2002; 26: 921-5.

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