

**Original Article****Correlation between Serum PSA Level & AMACR Expression  
in Prostatic Cancer**

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**Abstract**

**Introduction:** Prostate cancer is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide. To diagnose prostate cancer, no specific single histological feature is sufficiently available. A positive diagnostic marker specific for prostatic adenocarcinoma may enhance the ability to detect small foci of cancer and reduce diagnostic difficulties. This study focuses on correlation between serum PSA level and AMACR (a proven biomarker) expression in prostate cancer. **Methodology:** Twenty one prostatic needle biopsies and 16 TURP specimens as cases and 37 TURP specimens as controls were included in this study. Serum PSA levels of patients were collected preoperatively. Specimens were processed routinely for H and E and IHC using FLEX monoclonal Rabbit anti-human AMACR. Histopathological and IHC results were analyzed. **Results:** Among the 37 cases 35 were diagnosed as prostatic adenocarcinoma and 2 were diagnosed as suspicious for malignancy histopathologically. AMACR was not expressed in any of the 37 benign controls. Among the 35 histopathologically diagnosed prostatic adenocarcinoma cases, 34 showed positive AMACR expression in various intensity and 01 showed negative AMACR expression. Among the 02 histopathologically diagnosed suspicious for malignancy cases, both showed strong AMACR expression. There was statistically significant difference in expression of AMACR between cases and controls, indicated by  $p < 0.05$ . Correlation between serum PSA level and AMACR expression were observed. Among the 4 patients containing serum PSA level 4 to 10 ng/ml 2 cases showed expression of AMACR strongly, 1 case showed expression of AMACR moderately and 1 case showed negative expression of AMACR. Among the 33 patients containing serum PSA level  $> 10$  ng/ml, 15 cases showed expression of AMACR strongly, 13 cases showed expression of AMACR moderately and 5 cases showed expression of AMACR mildly. **Conclusion:** AMACR can be used as a biomarker for prostatic carcinoma in addition to histopathological study and PSA level.

**Keywords:** Alpha Methyl Acyl Coenzyme A Racemase (AMACR), Prostate Specific Antigen (PSA), Prostatic cancer.

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## Introduction

Prostate cancer is the most frequently diagnosed non-cutaneous malignancy in men, and the second leading cause of male cancer-related mortality in the United States [1]. It is responsible for 6.6% of all deaths in men over age of 55 years [2]. In South-East Asian region, the incidence and mortality due to prostatic cancer are 4.7% and 4% respectively [2]. In Bangladesh, the prevalence of prostate cancer is 2.3% in the last 5 years [3].

Prostate-specific antigen (PSA) blood test in addition to digital rectal examination (DRE), have traditionally been the preferred modalities to screen for prostate cancer. PSA has significant limitations because serum PSA is not specific to prostate cancer. One of the major limitations of PSA screening is that serum PSA can be elevated in patients with other common benign conditions, such as benign prostatic hyperplasia (BPH), prostatitis or other minor clinical procedures such as trans-rectal ultrasound [4]. PSA is organ specific but not cancer specific [5].

The diagnosis of prostate cancer is based on a combination of architectural, cytological and ancillary features rather than any single diagnostic feature. Accurate tissue diagnosis can be very challenging due to the presence of either a small focus of cancer or due to the presence of many benign mimickers of malignancy [6].

Now a days, there is widespread use of serum PSA as a mass screening test for prostate cancer. So, there has been an ever increasing number of prostate needle biopsies and the need to give an accurate diagnosis despite the limitations. Under diagnosis of a small focus of prostatic adenocarcinoma might delay early treatment and cause severe adverse consequences for patients [6]. Therefore, a prostate carcinoma specific marker could be of great importance and usefulness as an adjunct to

facilitate critical diagnostic decisions with high sensitivity and specificity [1]. Recently, a positive marker for carcinoma prostate, alpha-methyl acyl-coenzyme A racemase (AMACR) has been reported to have sensitivity ranging from 82-100% [6].

AMACR is an enzyme that is consistently over expressed in prostate cancer epithelium; hence it becomes an ideal specific biomarker for cancer cells within the prostate gland [4]. More recently, a study conducted by Jain et al 2017, took total 50 cases including 37 cases of malignant lesions and 13 cases of benign lesions of prostate [5]. They found that AMACR was not expressed in any of the 13 cases of benign lesions and it was expressed in 33 of 37 malignant lesions [5]. Hence, evaluation of AMACR as new biomarker of prostatic adenocarcinoma is needed.

So, the study is designed to use AMACR as an immuno-histochemical biomarker and its contribution in diagnosis of prostate cancer especially in needle biopsy specimens. In addition, the sensitivity and specificity of AMACR for the detection of prostate cancer were also evaluated.

## Methodology

This cross-sectional study was carried out in the department of pathology Rajshahi Medical College, Rajshahi, Bangladesh from March 2017 to February 2019. Approval for the research protocol was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi prior to the commencement of the study. Routine haematoxylin and eosin stains were done in department of Pathology, Rajshahi Medical College. The immuno-staining was done in department of Pathology, Bangabondu Sheikh Mujib Medical University (BSMMU). Transurethral resection of the prostate (TURP) and needle biopsy specimens of prostate that were histopathologically diag-

nosed as carcinoma or suspicious for malignancy were taken as case. Histopathologically diagnosed BPH patients were taken as control. Poorly fixed samples, inadequate biopsies and samples with marked inflammation were excluded.

In this study, 35 histopathologically diagnosed prostate cancer and 2 histopathologically diagnosed suspicious for malignancy were taken as cases. Subsequently age matched 37 patients with histopathologically diagnosed BPH were selected as control. Serum PSA levels of all patients were collected preoperatively. Tissue samples were obtained from TURP and needle biopsies. A pre-tested questionnaire used to collect data from cases and controls including complete history, physical examination, information on hematological and biochemical investigations.

The specimens were fixed in 10% formalin. Tissue processing and staining were done according to standard protocol followed at department of Pathology, Rajshahi Medical College. Sections were studied under light microscope and classified into benign, malignant and suspicious for malignancy. Carcinoma cases were histologically graded according to the Gleason scoring system and 2014 WHO/I-SUP consensus conference criteria for grading of prostate cancer. Associated prostatic tissue changes such as tumor invasion, prostatitis and others were also analyzed.

Immunohistochemical staining was carried out using Dako Envision detection technique. Four micrometer thick sections of formalin fixed, paraffin-embedded tissues were used. The sections were deparaffized in hot air oven, dewaxed in Xylene and rehydrated in descending graded alcohol. Antigen was retrieved by placing the slides in preheated antigen retrieval solution. Blocking endogenous peroxidase

and incubated with a rabbit monoclonal antibody AMACR ( p504 S, clone no 13H4) in appropriate dilutions were done. Standard immunohistochemical method was applied for subsequent staining. Tumor cells were scored positive if there was golden brown cytoplasmic or membrane staining in the neoplastic cells. Negative diagnosis was made when no golden brown staining was noted.

### Interpretation of Immunohistochemistry

Positive staining for AMACR pertained to dark diffuse or granular, cytoplasmic or luminal, but circumferential. The percentage of positivity were graded from 0+ to 3+ as follows-

0% cells (0+, negative)

1-10% cells (1+, mild)

11-50% cells (2+, moderate)

>51% cells (3+, strong)

The adjacent benign glands did not show any staining for AMACR.

Negative staining pertained to no staining or focal, weak non-circumferential fine granular staining.

### Results

Thirty five histopathologically diagnosed prostate cancer and 2 histopathologically diagnosed suspicious for malignancy were taken as cases. Subsequently age matched 37 patients with BPH were selected as control. Patients (case) with age ranged from 55 to 87 years (mean age was  $67.92 \pm 8.5$  years) and control group with age ranged from 51 to 85 years (mean age was  $64.76 \pm 9.67$  years). The subjects were divided into 4 different age groups, up to 60 years, 61- 70 years, 71-80 years and 81- 90 years. The number and frequency of cases found in different age groups were 9 (24.3%), 19 (51.4%), 7 (18.9%) and 2 (5.4%) respectively (Table 1). 21 (56.8%) needle biopsies and 16 (43.2%) TURP specimens were included as case

and 37 (100%) TURP specimens were included as control (Table 2). Among the total 37 cases 35 (94.6%) were diagnosed as prostatic adenocarcinoma and 2 (5.4%) suspicious for malignancy histopathologically (Figure-I). The histopathologically diagnosed prostatic adenocarcinoma cases were graded according to the Gleason scoring system and 2014 WHO/ISUP consensus conference criteria for grading of prostate cancer. Out of 35 adenocarcinoma cases combined Gleason score 6, 7, 8, 9 and 10 were 2 (5.71%), 12 (34.29%), 9 (25.71%), 11 (31.43%) and 1 (2.86%) respectively (Table 2). According to 2014 WHO/ISUP consensus conference criteria for grading of prostatic cancer, grade group 1, grade group 2, grade group 3, grade group 4 and grade group 5 were 2 (5.7%), 3 (8.6%), 9 (25.7%), 9 (25.7%) and 12 (34.3%) respectively (Figure-II). Immunohistochemical staining were done in all the 37 cases and 37 controls of prostatic lesions and results were analysed. In histopathologically diagnosed prostatic adenocarcinoma cases it was expressed in 34 (97.14%) out of 35 cases in various intensity. Among the 2 histopathologically diagnosed suspicious for malignancy cases, all (100%) patients showed strongly positive AMACR expression. On the other hand, among the 37 benign controls, all (100%) patients showed negative AMACR expression (Table-4). There was statistically significant difference in expression of AMACR between cases and controls, indicated by  $p < 0.05$ . Using standard formula for diagnostic accuracy calculation, the sensitivity, specificity and diagnostic accuracy of AMACR for prostatic carcinoma were 97.14%, 100% and 98.61% respectively.

**Table 1:** Distribution of the patients according to age group (n=74).

Age group (years)	Case Frequency n (%)	Control Frequency n (%)
≤60	9 (24.3)	17 (45.9)
61-70	19 (51.4)	11 (29.7)
71-80	7 (18.9)	6 (16.2)
81-90	2 (5.4)	3 (8.1)
<b>Total</b>	<b>37 (100)</b>	<b>37 (100)</b>

Case: Min = 55 Y, Max = 87 Y; Mean = 67.92 (±8.5)

Control: Min = 51 Y, Max = 85 Y; Mean = 64.76 (±9.67)

**Table 2:** Distribution of prostatic specimens in cases & controls (n =74).

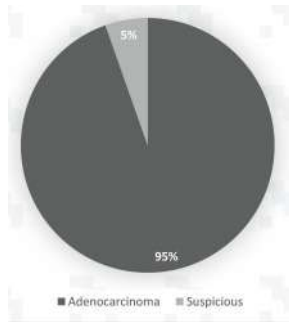
Type of specimen	Case Frequency n (%)	Control Frequency n (%)	Total
Needle biopsy	21 (56.8)	0 (0)	21 (28.4%)
TURP	16 (43.2)	37 (100)	53 (71.6%)
<b>Total</b>	<b>37 (100)</b>	<b>37 (100)</b>	<b>74 (100%)</b>

**Table 3:** Frequency of combined Gleason score of histopathologically diagnosed prostatic adenocarcinoma cases (n=35).

Combined Gleason score	Frequency n (%)
6	2 (5.71)
7	12 (34.29)
8	9 (25.71)
9	11 (31.43)
10	1 (2.86)
<b>Total</b>	<b>35 (100)</b>

**Table 4:** Correlation of AMACR expression with cases and controls (n=74).

	Positive Frequency n (%)	Negative Frequency n (%)	Total	p value
Case	36 (48.6)	1 (1.4)	37 (50%)	
Control	0 (0)	37 (50)	37 (50%)	<0.001
<b>Total</b>	<b>36 (48.6)</b>	<b>38 (51.4)</b>	<b>74 (100%)</b>	

**Figure i:** Pie chart showing histopathological diagnosis of the cases (n=37).**Table 5:** Frequency of serum PSA levels of cases and controls (n=74).

Serum PSA Level (ng/ml)	Case Frequency n (%)	Control Frequency n (%)	p value
<4	0 (0)	21 (56.76)	<0.05
4 to 10	4 (10.8)	15 (40.54)	
>10	33 (89.2)	1 (2.7)	
<b>Total</b>	<b>37 (100)</b>	<b>37 (100)</b>	

Case: Min = 7.80 ng/ml, Max= 383 ng/ml; Mean = 140.30±81.75

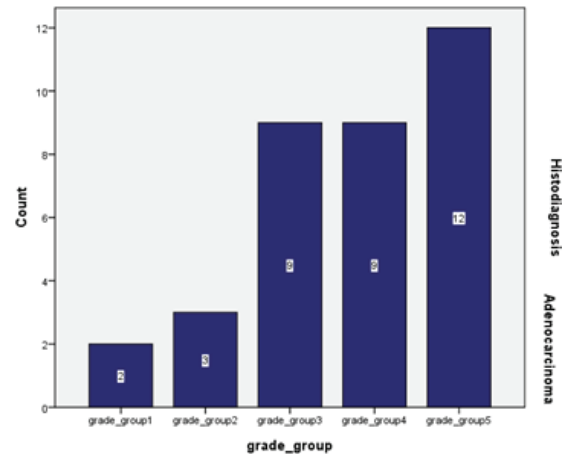
Control: Min = 2.25 ng/ml, Max = 10.2 ng/dl; Mean = 4.56±2.05

**Table 6:** Correlation between serum PSA levels and AMACR expression among cases (n=37).

Serum PSA level (ng/ml)	AMACR expression				Total	p value
	Strongly positive (>50% cells)	Moderately positive (11-50% cells)	Mildly positive (1-10 % cells)	Negative (0% cells)		
4 - 10	2 (5.4%)	1 (2.7%)	0 (0%)	1 (2.7%)	4 (10.8%)	<0.05
>10	15 (40.5%)	13 (35.1%)	5 (13.5%)	0 (0%)	33 (89.2%)	
<b>Total</b>	<b>17 (45.9%)</b>	<b>14 (37.8%)</b>	<b>5 (13.5%)</b>	<b>1 (2.7%)</b>	<b>37 (100%)</b>	

## Discussion

Prostate carcinoma is the 2nd 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. The diagnosis of prostate cancer is made by use of tradi-

**Figure ii:** Distribution of the patients of histopathologically diagnosed prostatic adenocarcinoma according to 2014 WHO/ ISUP modified Gleason grade group (n = 35).

tional 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



recent years basal cell markers and prostate biomarker Alpha-Methylacyl- CoA- Racemase (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 [6].

In this study, serum PSA levels of patients were collected preoperatively and immunohistochemical staining were done in all the 37 cases and 37 controls of prostatic lesions and results were analyzed. In histopathologically diagnosed prostatic adenocarcinoma cases it was expressed in 34 (97.14%) out of 35 cases in various intensity. Among the 2 histopathologically diagnosed suspicious for malignancy cases, all (100%) patients showed strongly positive AMACR expression. On the other hand, among the 37 benign controls, all (100%) patients showed negative AMACR expression. For accuracy of test to be calculated histopathologically confirmed carcinoma cases and histopathologically confirmed BPH controls were considered as gold standard. Confirmed prostatic carcinoma as evidenced by histopathological findings were 35 (94.6%) out of 37 patients (Figure ii). Among the 35 histopathologically confirmed carcinoma cases 34 cases showed positive AMACR expression at different intensity. On the other hand, all the 37 benign controls showed negative AMACR expression. AMACR expression of cases in relation to serum PSA level were observed. It is seen that among the 4 (10.8%) patients containing serum PSA level 4 to 10 ng/ml 2 (5.4%) cases showed expression of AMACR strongly, 1 (2.7%) case showed expression of AMACR moderately and 1 (2.7%) case showed negative expression of AMACR. Among the 33 (89.2%) patients containing serum PSA level >10 ng/ml, 15 (40.5%) cases showed expression of AMACR strongly, 13 (35.1%) cases

showed expression of AMACR moderately and 5 (13.5%) cases showed expression of AMACR mildly. A statistically significant correlation was observed between serum PSA level and AMACR expression (Table 6). Using standard formula for diagnostic accuracy calculation, the sensitivity, specificity and diagnostic accuracy of AMACR for prostatic carcinoma were 97.14%, 100% and 98.61% respectively. These results are almost similar to Rubin, et al. (2002) [8] who demonstrated 97% sensitivity of AMACR in the detection of prostate cancer. Shrivastava, et al. (2019) [7] reported 100% sensitivity of AMACR in the detection of prostate cancer in their study. Difference in sensitivity of AMACR in different studies including absent staining in prostatic adenocarcinoma can be a result of using different antibodies as polyclonal anti-AMACR is 100% sensitive while the sensitivity of monoclonal anti-AMACR in detecting prostate cancer is only 94% [6].

This study found that AMACR is over expressed in prostatic adenocarcinoma in both needle biopsy and TURP specimens. A statistically significant correlation between serum PSA level and AMACR expression was also observed. However a diagnosis of benignancy should not be made based only on a negative AMACR staining as AMACR can sometimes be negative in adenocarcinoma [7]. Results of AMACR staining should be interpreted only in the context of strict morphologic correlation. Also it is better to combine AMACR with a negative marker of prostatic adenocarcinoma like a basal cell marker as the contrasting staining results for adenocarcinoma (positive staining with AMACR and lack of staining with basal cell marker) will not only complement each other but will also increase the diagnostic confidence.

**Conclusion**

From the above study we can conclude that, AMACR can be used as a biomarker for prostatic carcinoma in addition to histopathological study and PSA level.

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