

Androgen receptor (AR) expression in different Gleason scores of prostatic adenocarcinoma by immunohistochemistry

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ABSTRACT

Background: Prostatic cancer is highly ominous in men and survives hormonal insufficiency. We studied Androgen Receptor (AR) expression in various Gleason scores (GS) of prostatic adenocarcinoma by using immunohistochemistry.

Materials and methods: This descriptive analytical study was carried out at King Edward Medical University/Mayo Hospital, Lahore in 2016. Slides were prepared from 60 paraffin blocks of prostatic adenocarcinoma and stained for evaluation of different parameters like Gleason score (GS), histopathological grades (well differentiated, intermediate or high grade) and AR immunohistochemistry score. Data entry and analysis was carried out using SPSS version 22. The relationship between different parameters was determined by Pearson chi-square test. A p-value of < 0.05 was considered statistically significant.

Results: Out of 60 cases, 15 (25%) had intermediate and 45 (75%) had high grade tumors. AR was positive in 59 (98.33%) cases. Among the positive cases, 5 (8.33%) had +1 score, 25 (41.67%) has +2 and 29 (48.33%) had +3 score. A significant association of AR expression with GS and histological grades was found.

Conclusion: AR positivity is significantly associated with Gleason Score and histological grading of prostate adenocarcinoma, hence it may prove a useful diagnostic and prognostic marker for carcinoma prostate.

Keywords:

Androgen receptors, Gleason's scoring, Prostatic adenocarcinoma

INTRODUCTION

The worldwide ranking of prostatic adenocarcinoma shows that it is the second most frequent cause of malignancy related deaths in males.¹ It shows an aggressive behaviour and poor prognosis in developing countries like Pakistan.² Many risk factors have been discussed but race, advancing age and a positive family history are important ones.³ Histological diagnosis is made on biopsy by using Gleason score, formulated initially by Donald Gleason, determined by adding the two numbers assigned for various morphological patterns starting from 1 to 5. Now it has been categorized in Gleason grade groups by WHO.⁴ Growth of prostatic gland is dependent on nuclear androgen receptor either normal or malignant. Newly diagnosed and untreated prostate cancer cases have shown correlation of low serum testosterone levels with higher Gleason score and increased AR expression.⁵

Cancers that do not respond to surgical treatment finally become androgen independent, resulting in failure of anti-androgen therapy.⁶ This biomarker will

also help the oncologists to assess prognosis and prediction to targeted therapy for prostate carcinoma. The aim of this study was to find the expression of androgen receptors in patients with prostatic adenocarcinoma.

MATERIALS AND METHODS

This retrospective and descriptive cross-sectional study was carried out at the Department of Pathology, King Edward Medical University (KEMU), Lahore. Sixty formalin fixed, processed and paraffin embedded tissue blocks of diagnosed cases of prostate adenocarcinoma were collected from December 2015 to December 2016, after the approval by Ethical Board/Institutional Review Board (IRB) and Advanced Study & Research Board (AS&RB) of KEMU.

The obtained formalin-fixed, paraffin-embedded tissue blocks were cut by microtome and the slides were prepared and stained with hematoxylin and eosin (H&E) as well as with androgen receptor protein by using immunohistochemistry technique. The hematoxylin and eosin stained slides of all sixty cases were evaluated for Gleason scoring and histological grading of prostate adenocarcinoma. Prostate adenocarcinoma is graded and categorized according to Modified Gleason scoring system. This system is based

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on various morphological patterns. Gleason grade 1 consists of closely packed glands which are uniform and medium sized but separate forming round to oval well circumscribed nodule. Gleason grade 2 consists of more loosely packed and less uniform glands showing minimal infiltration of tumor nodule. Gleason Grade 3 consists of well formed, smoothly circumscribed glands smaller than Gleason Grade 1 or 2 with marked variation in size and shape showing infiltration of non-neoplastic prostatic glands. Gleason grade 4 consists of poorly formed, raggedly infiltrating glands with ill-defined lumina as well as fused glands, chains or solid masses along with complex papillary and irregularly bordered cribriform glands. Hypernephroid and mucinous variants show this pattern. Gleason grade 5 consists of singly scattered cells or cords and solid sheets without any glandular differentiation. This include signet ring cells, adenocarcinoma and sarcomatoid variant of Adenocarcinoma prostate.

Gleason scores of similar behaviors are categorized in the form of groups. Well differentiated/low grade tumors have Gleason score of 6, intermediate/moderately differentiated tumors have Gleason score of 7 and high grade/poorly to undifferentiated tumors have Gleason score of 8-10.

Prostate tumors usually show more than one patterns (Gleason grades) in the same biopsy. The commonest pattern is called primary or dominant grade whereas the second most frequent pattern is called secondary grade. Each pattern is individually graded from 1 to 5, followed by the two numbers being added together in order to get a combined Gleason grade or Gleason score ranging from 2-10 where Gleason score of 2 represents tumors uniformly composed of Gleason pattern 1, and Gleason score of 10=5+5 signifies undifferentiated tumors e.g.; Sarcomatoid variant of Adenocarcinoma prostate.

WHO modified Gleason scoring system into 5 following Grade groups to help clinicians:

- Group 1: Gleason score (3+3=6)
- Group 2: Gleason score (3+4=7)
- Group 3: Gleason score (4+3=7)
- Group 4: Gleason score (3+5, 4+4, 5+3=8)
- Group 5: Gleason score (5+4, 4+5 and 5+5=10)

Formalin fixed, paraffin wax embedded tissue blocks of histopathologically diagnosed cases with representative areas of tumour were cut into 4µm thick section, placed on positively charged glass slides, fixed in oven (hot plate) at 58°C to 65°C for 50-60 minutes, dewaxed in xylene solution for 5 minutes followed by rehydration done in (descending order) in 100%, 80%,

50% concentration of alcohol, each for 3 minutes. Slides were then placed into Target Retrieval Solution, washed with Tris-buffered Saline (TBS), blocked with Blocking Reagent (Peroxidase), washed with Washing Buffer two times, each for 10 minutes. Then applied 50ul Anti Androgen Receptor (SP107) Rabbit Monoclonal Primary Antibody [200R-18 Cell Marque] with dilution of 1:30 and incubated followed by Secondary Antibody (HRP) application. Antibody was visualized by using Diaminobenzidine (DAB) Chromogen and counterstained with Hematoxylin.

For androgen receptor status, nuclear labeling in more than 10% neoplastic cells was considered as the cut-off point for positivity. Positive controls included prostate benign hyperplasia for AR and sections incubated without primary antibodies served as negative controls. In order to assess the intensity of AR immunostaining from 0-3+ a visual scoring technique was devised, for each of 100 randomly selected nuclei.

The intensity of androgen receptor antibody staining in tumor cells was categorized into scores as: No staining = 0, weak staining = 1+ (Heterogeneous, faint granular nuclear staining), moderate staining = 2+ (more than weak staining and less than strong staining), and strong staining = 3+ (homogeneous, granular dark brown nuclear staining)

Data entry and analysis was done by using SPSS version 22. Quantitative variables were presented as mean ± SD. Qualitative variables were presented as frequency and percentages. The relationships between Gleason scores, histological grade and AR were determined by Pearson Chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

This study included a total of 60 cases of prostate adenocarcinoma of prostate cancer. According to these histological grades, 7 (11.67%) cases had low grade or well differentiated tumor, 5 (8.33%) cases had intermediate grade tumor and 48 (80%) cases had high grade tumor. There were 7 (11.67%) patients who had a Gleason score of 6, 5 (8.33%) cases had Gleason score of 7, 19 (31.67%) cases had Gleason score of 8, whereas 26 (43.33%) cases had Gleason score of 9 and further 3 (5%) cases had Gleason score of 10. Among 7 cases of prostate adenocarcinoma with Gleason score of 6, Androgen receptor (AR) immunostaining score was 3+, 2+ and 1+ in 3 cases, 2 cases and 2 cases respectively. Two out of 5 cases labeled with Gleason score of 7 showed AR staining score of 3+ while the remaining three of them showed AR staining score of 1+.

Table 1: Intensity of androgen receptor immunostaining in different Gleason scores

Gleason scores	Androgen receptor immunostaining score				Total	p-value
	0 (no staining)	1+ (weak staining)	2+ (moderate staining)	3+ (strong staining)		
6	0	2	2	3	07	0.000
7	0	3	0	2	05	
8	0	0	11	8	19	
9	0	1	11	14	26	
10	1	0	0	2	03	
Total	01	06	24	29	60	

A p-value was determined by Pearson Chi-square test (<0.05 was taken as significant)

Table 2: Association of histological grade and androgen receptor intensity score

Histological grades	Androgen receptor score				Total	p-value
	0 (no staining)	1+ (weak staining)	2+ (moderate staining)	3+ (strong staining)		
Low grade	0	2	2	3	7	0.002
Intermediate grade	0	3	0	2	5	
High grade	1	1	22	24	48	
Total	1	6	24	29	60	

A p-value was determined by Pearson Chi-square test (<0.05 was taken as significant)

No case had a score of zero or 2+. Nineteen cases were identified as Gleason score of 8. Eleven of them showed AR intensity score of 2+ and eight cases showed AR intensity score of 3+. Twenty-six cases were diagnosed as Gleason score of 9, of which fourteen cases showed AR intensity score of 3+, eleven cases were identified with AR intensity score of 2+ and only one of them was labeled as AR intensity score of 1+. Only three cases with Gleason score 10 were identified. Two of them showed AR intensity score of 3+ and a single case had zero AR intensity score. This was histologically classified as sarcomatoid variant of prostate adenocarcinoma. Chi-square test was used to find out the p-value which was 0.000 showing strong association between Gleason score and androgen receptor intensity score (Table 1).

The androgen receptor positivity was detected in 59 (98.33%) cases and not detected in only 1(1.67%) case. In cases where androgen receptor was detected, 6 cases (10%) had 1+ score, 24 cases (40%) had 2+ score and 29 cases (48.33%) had score of 3+. As in 1(1.67%) case Androgen was not detected so its score was 0. Out of total 60 cases 6 cases (10%) showed weak staining, 24 cases (40%) showed moderate staining and 29 cases (48.33%) showed strong androgen receptor immunostaining. Only one case showed no staining at all.

DISCUSSION

Prostatic cancer, the second commonest malignancy of males, shows high incidence rate in United States of America.⁷ Its incidence in our country is low due to deficient screening methods and low life expectancy.⁸ Drift to cities as well as variable socioeconomic status are factors responsible for its developing risk.⁹ Gleason

scoring system is good prognostic tool used for grading of prostate adenocarcinoma.¹⁰ Sixty diagnosed cases of prostatic adenocarcinoma with different Gleason scores were included in this study. There were 3 (5%) patients who were diagnosed with Gleason score of 10, 26 (43.33%) patients with Gleason score of 9, 19 (31.67%) patients had Gleason score of 8, 5(8.33%) with Gleason score 7 and 7 (11.67%) patients with Gleason score 6. Another study at Karachi described 18 (34%) patients with Gleason score 6, 22 (41.5%) having Gleason score 7, 7 (13.2%) with Gleason score 8 and 6 (11.3%) patients with Gleason score 9.⁵ In a recent study conducted at RMDC, Lahore, 5 (41.66%) patients had Gleason score 9, 1 (8.33%) patient had Gleason score 7, 1 (8.33%) patient had Gleason score 6, 4 (33%) patients reported as Gleason score 8.¹¹

Androgens and androgen receptors have important roles both in the development of normal as well malignant prostate gland.¹² The intensity of AR staining on IHC is determined by assigning certain scores ranging from 0-3, where 0 means no staining, 1 means weak equivocal staining, 2 means unequivocal moderate staining and 3 represents strong staining.⁵ In the current study the androgen receptor was detected in 59 cases (98.33%) and was not detected in only 1 case (1.67%) so its score was graded as 0. In cases with AR positivity, 6 cases (10%) have 1+ score, 24 cases (40%) had 2+ score and 29 cases (48.33%) had a score of 3+. In another study high AR expression was observed in 56.2% cases of prostatic adenocarcinoma of a total of 121. The expression of AR in their study was correlated significantly with Gleason score.⁵ According to results of an earlier study by Osman et al¹³ a significant inverse correlation was observed between AR expression in

prostate cancer and tumor grade. But no such correlation was found in the current study. According to Lisa Das et al, AR expression did not show significant correlation with Gleason score.¹⁴ In present study, seven out of sixty cases were of low grade (Gleason score 6), of which 2 cases, 2 cases and 3 cases showed weak=1+, moderate=2+ and strong staining intensity=3+ respectively. Five cases represented intermediate grades (Gleason score 7) of which 3 cases showed weak=1+, 2 cases showed strong staining intensity=3+ while none of the cases showed moderate staining intensity of 2+. Both low and intermediate grades have not shown any case of negative or zero AR score. Forty-eight cases out of a total of sixty cases were of high grade (Gleason score 8-10), of which only one case was labeled as AR negative or zero staining. These high grade cases showed staining scores as 0, 1+, 2+ and 3+ in 1 case, 1 case, 22 cases and 24 cases respectively. The single case of AR negative was histologically classified as sarcomatoid variant of prostatic adenocarcinoma. The p -value was 0.001 showing significant association between AR scoring, Gleason score and histological grade. In the present study only one case was detected as AR- negative or zero staining. This AR negative case was graded as high grade prostatic adenocarcinoma (with the Gleason score of 10) and with sarcomatous change thereby supporting the evidence that certain men with the AR-negative prostatic carcinoma have a worse prognosis compared to those with the AR-positive prostatic carcinoma. AR overexpression in various histological grades of prostatic carcinoma along with their association was studied as it is debated by previous studies.⁵

CONCLUSIONS

Androgen receptor (AR) positivity is different in Gleasons and histological grades of prostate carcinoma. Gleason score and AR expression have shown direct relationship with each other. AR is a diagnostically useful marker for prostate adenocarcinoma.

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