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ORIGINAL PAPER

Immunohistochemical expression of androgen receptors in urothelial carcinoma of urinary bladder. Is it significant? Experience from coastal India

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Summary ***Background: Bladder carcinoma (BC) ranks second among the genitourinary cancers worldwide. Influence of androgens and expression of androgen receptors in neoplasms are recent findings which were implicated in the development of BC. We aimed to study androgen receptor (AR) expression in bladder urothelial neoplasms and correlate its expression with grade and stage of the tumor.***
Methods: Immunohistochemistry (IHC) was done on samples collected in a tertiary care hospital over one year consisting of 71 urothelial BC and 20 non-neoplastic urothelial conditions. Two pathologists graded the IHC and nuclear staining was considered as positive expression.
Results: AR was expressed in 23.9% (17/71) of bladder urothelial neoplasms. AR was expressed in 25.7% and 22.3% of high and low-grade tumors and 25% and 22.3% of non-muscle-invasive and muscle-invasive BC. AR expression had no significant correlation with gender, age (> 50 years), muscle invasion or grade. AR expression was significantly absent in non-neoplastic conditions (p = 0.018).
Conclusions: AR has varied expression in BC and it is relatively lower in this study population.

KEY WORDS: Bladder cancer; Urothelial neoplasms; Androgen receptor expression.

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INTRODUCTION

Bladder cancer (BC) ranks second among the genitourinary cancers worldwide with over 12 million cases annually (1, 2). About 95% of bladder tumors are of epithelial type and the diagnosis is mainly dependent on cellular dysplasia and muscle invasion. Metastasis is a late phenomenon in BC (3). The occurrence of BC is higher in men than in women because men are more exposed to industrial chemicals and cigarette smoke, which contain amines. However, even in the absence of these carcinogens, men are more prone to BC. In this context, *androgen receptor* (AR) could be proposed as another potential reason for the difference (4).
Androgens were discovered in 1936 and are steroidal hormones that are secreted by adrenal cortex, testes, and ovary. AR gene in humans is present on the X chromosome (2). There is a significant role of androgens and their receptors in male bladder development and functioning. AR has also been shown in urothelium and bladder submucosa. It also regulates the storage of urine and other functions of the urinary tract. Activation of AR correlates with progression of growth of *urothelial cancer* (UC) (2). Hence we aimed to study AR expression in bladder urothelial carcinomas and assess its association with stage and grade of the disease.

METHODS

The *Institutional Ethics Committee* approved the study. This retrospective study included UCs from the urinary bladder received in the Pathology department of a tertiary care center of coastal south India between January 2013 and December 2018. UC were classified as per the *World Health Organization classification* 2016 (9). The urothelium in bladder biopsies of non-neoplastic conditions served as controls. Cases in which paraffin blocks were not available or had inadequate tissue for *immunohistochemistry* (IHC) were excluded.
The samples underwent routine processing with formalin fixation and were embedded in paraffin. Three microns thickness sections were used for IHC. IHC was performed using anti-AR [*Ready-to-use mouse monoclonal primary antibody kit (BioGenex)*] on the appropriate tumor blocks following the manufacturer's instructions. The secondary antibody used was *Dako REAL EnVision/HRP (Labeled polymer, Code K5007)* against rabbit and mouse primary antibodies. The AR expression was considered positive based on the German immunoreactive score as shown in Table 1 (11).
A descriptive analysis of data was done using the software package SPSS version 21.0. Cross tables were generated to compare the neoplastic and non-neoplastic groups and associations between the groups were analyzed using the Chi-square test and Student t-test. *Odds ratio* (OR) was calculated to establish the association between AR and urothelial carcinomas. A p-value < 0.05 was considered statistically significant.

Table 1.
AR expression calculated by German immunoreactive score.

Percentage of immunoreactive cells	Proportion score	Intensity of staining	Intensity score	Final score = Proportion score x intensity score	Final interpretation
0%	0	Negative	0	0-1	Negative (0)
1-10%	1	Weak	1	2-4	Weakly positive (1+)
11-50%	2	Moderate	2	6-8	Moderately positive (2+)
51-80%	3	Strong	3	9-12	Strongly positive (3+)
81-100%	4				

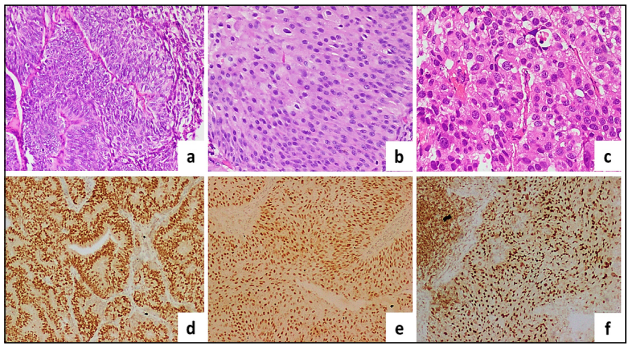
Results

During the study period, we received 220 bladder biopsies or specimens from transurethral bladder resections. Out of them 71 specimens of UCs were included in the study based on the inclusion and exclusion criteria. The mean age at presentation of UC cases was 62 years (age range: 36-91 years) with a male predominance (63 men and 8 women). High grade tumor was seen in 50.7% (35/71) of cases and muscle invasive UC in 38% (19/71) of tumors (Table 2, Figure 1a-c).

Table 2.
Clinical-pathological features of UC cases (n = 71).

Variables	Cases (n)	Percentage (%)
Gender		
Male	63	88.7%
Female	8	11.2%
Age in years		
< 50	7	90.1%
> 50	64	9.9%
Muscle invasiveness		
Absent	44	62%
Present	19	38%
Histologic grade		
High	35	50.7%
Low	36	49.3%

Figure 1.
AR expression in urothelial carcinoma.
a. Urothelial carcinoma showing papillary pattern (HE stain, 10x);
b. Urothelial carcinoma – low grade (HE stain, 40x);
c. Urothelial carcinoma – high grade (HE stain, 40x);
d-e. Immunohistochmical expression of AR receptors in urothelial carcinomas (AR IHC stain, 10x).



Immunohistochemical AR nuclear expression was seen in 23.9% of the cases (n = 17) Figure 2.
AR expression was not associated with age of the patients, gender, grade and stage of the tumor (Table 3).
Positive AR expression was seen in 23.9% of neoplastic samples (Figure 1d-e) while AR expression was completely absent in non-neoplastic lesions. This was statistically significant (p = 0.018) (Table 4).

Figure 2.
Distribution of androgen receptor expression in UC.

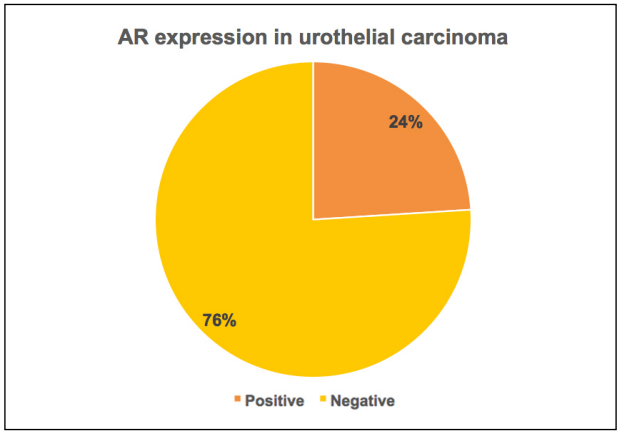


Table 3.
Association of AR expression with age, gender, grade and stage.

	AR Expression				P-value
	Negative	Positive	Positive	Positive	
	0	1+	2+	3+	
Age (years)					
< 50	5 (71.4%)	2 (28.6%)	0 (0%)	0 (0%)	0.09
> 50	49 (66.2%)	3 (4.1%)	9 (12.2%)	13 (17.5%)	
Gender					
Men	48 (76.2%)	4 (6.3%)	8 (12.6%)	3 (4.7%)	0.85
Women	6 (75%)	1 (12.5%)	1 (12.5%)	0 (0%)	
Grade					
Low	28 (77.8%)	2 (5.5%)	6 (16.7%)	0 (0%)	0.2
High	26 (74.2%)	3 (8.5%)	3 (8.5%)	3 (8.5%)	
Muscle invasion					
Non invasive	33 (75%)	3 (6.8%)	7 (15.9%)	1 (2.3%)	0.5
Invasive	21 (77.8%)	2 (7.4%)	2 (7.4%)	2 (7.4%)	

Table 4.
AR in neoplastic and non-neoplastic urothelial lesions.

	AR expression		
	Negative	Positive	Total
Neoplastic	54	17	71 (78%)
Non-neoplastic	19	0	19 (22.0%)
Total	73 (100%)	17(100%)	90 (100%)

Discussion

Androgens are considered to have a pivotal role in urothelial carcinogenesis. AR expression had a significant impact on modern oncological breast cancer treatment. In this study, immunohistochemical expression of AR was analyzed in the urothelial neoplasms. Expression of AR was seen in 23.9% of the cases of urothelial neoplasms. A study conducted by *Mir et al.* (13) showed AR expression in 12.9% of bladder tumors, *Miyamoto et al.* (11) in 42% of tumors and *Boorjiana et al.* (14) in 53.1%. The rate observed in this study is within a similar range with differences in the expression in urothelial neoplasm that could be attributed to ethnicity, sample sizes, antibody clones, IHC techniques and scoring methodologies.

An interesting, subtle yet significant observation in this study was the variation in AR expression between non-neoplastic and neoplastic urothelial lesions. AR expression was completely absent in non-neoplastic lesions whereas it was positive in 23.9% of neoplastic lesions. *Mashhadi et al.* had previously reported 22% of AR-positivity in cases with no expression in the controls (10). A meta-analysis with five studies conducted by *Chen et al.* (15) showed negative correlation between expression of AR and BC predisposition.

Data from studies by *Izumi et al.* suggested a low expression of AR ($p = 0.02$) in BC as compared to non-neoplastic urothelial tissues (16).

AR expression was noted in 25.7% of high grade BC and 22.3% of low-grade BC. Tumor grade was not associated statistically with AR expression. *Ide et al.* showed significant androgen loss in BC with higher grade compared with lower grades ($p < 0.001$) (17). Data from a study conducted by *Miyamoto et al.* showed lower expression of AR in high-grade BC (36%) compared to the low grade tumours (55%; $p = 0.0232$) (11).

This brings forward a potential utility of AR IHC of bladder lesions as a marker to exclude benign nature if expressed. AR IHC could be helpful in the differential diagnoses between basal cell hyperplasia or transitional metaplasia versus a low-grade urothelial carcinoma. The validation of these results is an area for further research. Furthermore, AR expression in other malignancies like lymphoma or other varieties of carcinoma versus urothelial carcinoma could be evaluated.

In the present study there was a downregulation of AR expression with muscle invasion. AR expression was seen in 22.3% of MIBC and 38.9% of NMIBC. *Mir et al.* showed expression of AR in 9% of NMIBC as compared with 15.1% of MIBC ($p = 0.059$) (13). *Miyamoto et al.* also reported lower expression of AR in MIBC (33%) compared to NMIBC (51%; $p = 0.018$) (11). *Wagih et al.* and *Szabados et al.* also showed similar results (18, 19).

A therapeutic implication of AR expression in urothelial carcinomas could be the use of AR inhibitors to prevent UC growth in presence of androgens and to prevent chemotherapy resistance (20).

Limitations of our study are absence of data on treatment and follow-up and incomplete data on progression and recurrence. Few of our cases were excluded due to non-availability of the tissue in the block for IHC. This also reduced the sample size of our study.

Conclusions

AR has varied expression in BC and it is relatively lower in this study population. The expression of AR in bladder cancer had no significant correlation with gender, age (50 years), muscle invasion or grade of the tumor. AR expression was downregulated in MIBC albeit without any statistical significance, thereby precluding its role in targeted therapy.

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