



Expressional Evaluation of Androgen Receptor in Transitional Cell Carcinoma of Urinary Bladder Patients

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ABSTRACT

The study was undertaken to evaluate the possible role of Androgen Receptor (AR) in relation to tumor grade, age, sex and urinary bladder cancer risk. Expression of AR detected by immunohistochemistry in 125 Transitional Cell Carcinoma and 100 control cases. Expression of AR was noticed in 28.8% cases. AR increased with increasing tumor grade. Expression of AR was seen to be significantly higher in male in the age group ≥ 50 years ($p < 0.05$). AR expression was found to be associated significantly with the tumor grades, age and sex suggesting that AR may be used as prognostic markers in the treatment of urinary bladder carcinomas.

Keywords: Androgen receptor; smoking; transitional cell carcinoma; urinary bladder; IHC;

1. INTRODUCTION

Urothelial carcinoma is approximately three times more common in men than women (Madeb and Meesing, 2004). Although these differences have been primarily attributed to

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differences in exposure to smoking and industrial chemicals it is evident now that genetic, anatomic, societal, and hormonal factors also play a role. Cigarette smoke (CS) is a major risk factor which causes the development of many cancers, including cancers of kidney, bladder, and lung (Shishodia and Aggarwal, 2004). Results from recent prospective studies suggest that cigarette smoking may act as an initiator of urinary bladder carcinogenesis (Samanic et al., 2006). However, the exact mechanism by which cigarette smoking induces bladder cancer remains unclear but might be due to the carcinogens in cigarette smoke which causes carcinogenesis by forming DNA adducts that result in transitional mutations (Kadlubar, 1995). Earlier investigators showed that p53 overexpression/mutation and smoke are associated with bladder carcinoma (Zhang et al., 1994).

Another protein, the androgen receptor, has been found in a wide range of tissues and its expression is regulated by testosterone, dihydrotestosterone (DHT). Overexpression and mutation of AR have been found in hormone-dependent tumors such as human prostate, breast and testicular cancers (McPhaul, 1995; Isola, 1993; Noronha, 1986). The expression of estrogen receptor (ER) and progesterone receptor (PR) plays an important role during development, growth, tumorigenesis and progression of several malignancies (Lamb et al., 2005; Zhuang et al., 1997). In this study, we evaluated the possible role of AR in relation to tumor grade, age and urinary bladder cancer risk.

2. MATERIAL AND METHODS

2.1 Study Population

Total numbers of 125 histopathologically confirmed cases of Transitional cell carcinoma (TCC) of urinary bladder and 100 cases of control referred to the departmental pathology lab of MAMC, New Delhi were taken. One hundred twenty five cases of Transitional cell carcinoma were classified according to the WHO grading system by experienced pathologists in three grades: Grade I, Grade II and Grade III. Keeping the marker profile in view the cases were further divided according to age into two groups: less than 50 years & ≥ 50 years.

2.2 Immunohistochemical Analysis

Formalin fixed paraffin-embedded tissue blocks were cut in 5 microns thick serial sections. The sections were deparaffinized, rehydrated and rinsed in phosphate buffer saline (PBS). An immunohistochemical assay for AR was performed on consecutive paraffin sections using streptavidin-biotin method (Christian et al., 2009). Monoclonal mouse anti-human antibodies were used as primary antibodies for AR (Clone AR 441, Lab Vision, USA). After antigen retrieval, slides were incubated with primary antibody, followed by secondary biotinylated antibody. Sections were washed in PBS and then incubated with streptavidin peroxidase. Finally chromogen Diaminobenzidine (DAB) was used and sections were counterstained with haematoxylin.

2.3 Scoring Method

The slides were scored independently by one of the co-authors without reference to any clinical or pathological information. Tumors were classified as AR negative (i.e., low expression) if less than or equal to 10% of cells displayed positivity. AR (i.e., high expression) if greater than 10% of cells were positive (Freedman et al., 1996). A total of 5-6

fields from each tissue section were chosen, and 100 cells from each field were counted at final magnification at 400X. With every batch of staining a positive and negative control were used to verify the standard of staining.

2.4 Statistical Analysis

Chi square (χ^2) test was performed to find out the possible correlation between hormonal receptors and other clinical parameters. Statistical significance was defined as $P < 0.05$.

3. RESULTS

A total of 125 cases (104 male and 21 female) of histopathologically confirmed cases of Transitional cell carcinoma (TCC) and 100 cases of control (75 male and 25 females) were assessed. The age of the patients ranged from 24 – 80 years with a mean age of 55 years, whereas in control group it was 14 to 55 years with mean being 38 ± 14 years.

The incidence of carcinoma of urinary bladder was predominantly seen among males 83.2 % patients as compared to the 16.8 % females, giving the ratio 4.5:1 (male v/s females). The maximum number of controls was in < 40 years age group (80%) followed by 40-55 years (20%). However, when the control group was divided into males and females, the mean age was 38 ± 15 years for males and 40 ± 10 years for females. The sex ratio of this group was 4:1. Of the 125 cases studied, a peak incidence of carcinoma of urinary bladder was seen in the age group of 50-70 years. There were 76% (95) cases in the age group of 50-80 yrs., 24% (30) in the age group of 24-50 yrs. It was observed that out of these 38, 47 and 40 had grade I, grade II and grade III carcinoma respectively. Cases and controls did not differ significantly in sex or age distribution.

3.1 AR Expression in Bladder Cancer

Nuclear immuno reaction for AR was considered positive and found in 36 cases (28.8%) of TCC (Figure 1). Out of 104 males 36 (34.6%) were AR positive, whereas all the females were AR negative (Table 1).

Table 1. Expression pattern of AR in TCC cases

Clinical parameters of TCC		AR positive Cases	
		Total Cases	Percentage positivity
Tumor Grades	Grade I	06	15.7%
	Grade II	11	23.4%
	Grade III	19	47.5%
	Total Cases	53	28.8%
Sex	Male	36	34.6%
	Female	00	00%
Age	<50 years	06	14.2%
	≥50 years	30	36.1%

AR= Androgen Receptor; TCC=Transitional Cell Carcinoma

It was found that AR was positive in 6 case (15.7%), 11 cases (23.4%) and 19 cases 47.5% in grade I, grade II, and grade III, respectively.

The TCCs cases were divided according to sex and age. In males, the expression of AR was significantly higher (44.110%) in ≥ 50 years as compared to < 50 years of age (18.75%) with p value < 0.05 (Table 2). When AR expression (% positive cells) was calculated it was found that mean of expression in grade I cases was 15%, in grade II was 33% and in grade III was 60%. The differences in expression pattern between the consecutive grades were statistically significant ($p < 0.05$). There was no AR expression in control cases.

Table 2. Relationship between AR, sex and age

Sex	Age (years)	AR Positivity			P- Value
		Total cases	Positive cases	Percentage	
Male	<50	32	06	18.75%	(p<0.05)
	≥ 50	68	30	44.110%	
Female	<50	10	00	00%	-----
	≥ 50	15	00	00%	

AR= Androgen Receptor

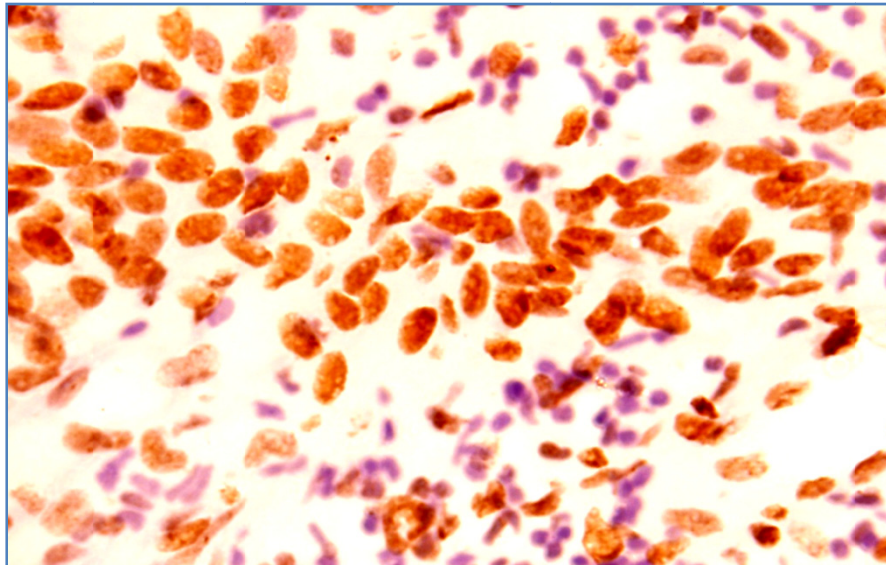


Fig. 1. Transitional Cell Carcinoma showing expression of AR in nuclei (X 400)

4. DISCUSSION

The cancer of urinary bladder is one of the most common cancers among male (Madeb and Meesing, 2004). In our study the peak incidence of bladder cancer was observed in the age group of 50-70 years. The reasons for this remain unclear, but it might be due to the

cumulative effects of long time exposures to carcinogens, the failings of DNA repair mechanisms and aging.

In our study of 125 cases with Transitional cell carcinoma of urinary bladder and 100 controls were analysed in AR. There is no evidence that Androgen Receptor (AR) is expressed in normal urinary bladder. It was only detected in lamina propria and smooth muscle of ureter and urinary bladder in mice (Zhuang et al., 1997). We also failed to detect AR expression in normal urinary bladder or indicating no or undetectable level of expression. This low or undetectable level of AR expression is in deep contrast to high expression of AR in the transitional cell carcinoma of urinary bladder. This finding is also in confirmation with the other investigations (Isola, 1994). The expression pattern was not only high (28.8%) but also showed that expression pattern of AR was rising from grade I to grade II and further to grade III. Our finding indicates there is a strong positive correlation between AR expressions with tumor grade (Grade I- 15.7%, Grade II- 23.4.6% and Grade III- 47.5%). The pattern of AR expression in our findings has certain notable features. When AR expression was analyzed according to sex and age it was found that TCC in females showed no AR expression whereas, among males it was 34.6% ($p < 0.05$). The AR expression in males of 50 years and above age group was 44.11% which was significantly higher than in males less than 50 years (18.75%). There are studies made on AR in bladder cancer (Noronha et al., 1994), but this kind of pattern has not been reported earlier. However, this difference is might be due to the tobacco chewing, cigarette smoking, industrial chemicals (Zhang et al., 1994) and alcohol intake are higher in men than women; it is also known that men acquire these habits earlier than women and older patients correlates with long latency period required for carcinogenic induction by environmental mutagens. Mechanism of carcinogenesis and tumorigenesis may be associated with molecular alterations of genes involved in control of cell proliferation and other functions.

5. CONCLUSIONS

AR expression was found to be associated significantly with the tumor grades, age and sex suggesting that AR may be used as prognostic markers in the treatment of urinary bladder carcinomas.

REFERENCES

- Bolenz, C., Ashfaq, R., Shahrokh, F.S. (2009). Estrogen and progesterone hormonal receptor expression in urothelial carcinoma of the bladder christian. *Eur. Urol.*, 56, 891-1104.
- Freedman, A.N., Michalek, A.M., Marshall, J.R., et al. (1996). The relationship between smoking exposure and p53 overexpression in colorectal cancer. *Br. J. Cancer.*, 73(8), 902-908.
- Isola, J.J. (1993). Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. *J. Pathol.*, 17, 31-35.
- Kadlubar, F.F., Badawi, A.F. (1995). Genetic susceptibility and carcinogen- DNA adduct formation in human urinary bladder carcinogenesis. *Toxicol. Lett.* 82-83, 627-632.
- Lamb, C.A., Helguero, L.A., Giulianelli, S., et al. (2005) Antisense oligonucleotides targeting the progesterone receptor inhibit hormone-independent breast cancer growth in mice. *Breast Cancer Res.*, 7, 1111 – 1121.

- Madeb, R., Meesing, E.M. (2004). Gender, racial and age differences in bladder cancer incidence and mortality. *Urol. Oncol.*, 22, 86-92.
- McPhaul, M.J. (1995). Mutations in the androgen receptor gene that cause androgen resistance. In: Weintraub, B.D., Ed. *Molecular Endocrinology, Basic Concepts and Clinical Correlations*. New York, Raven Press, 411-423.
- Noronha, R.F.X., Rao, B.R. (1986). Sex hormone receptors in localized and advanced transitional cell carcinoma of urinary tract in humans. *Urol.*, 28, 401-403.
- Samanic, C., Kogevinas, M., Dosemeci, M., Malats, N.R. (2006). Smoking and Bladder Cancer in Spain: Effects of Tobacco Type, Timing, Environmental Tobacco Smoke, and Gender. *Cancer Epidemiol. Biomarkers Prev.*, 15, 1348-54.
- Shishodia, S., Aggarwal, B.B. (2004). Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates activation of cigarette smoke-induced nuclear factor (NF)-kappaB by suppressing activation of I kappa B kinase in human non-small cell lung carcinoma: correlation with suppression of cyclin D1, COX-2, and matrix metalloproteinase-9. *Cancer Res.*, 64, 5004-5012.
- Zhang, Z.F., Sarkis, A.S., Cordon-Cardo, C., et al. (1994). Tobacco smoking, occupation, and p53 nuclear overexpression in early stage bladder cancer. *Cancer Epidemiol. Biomarkers Prev.*, 3, 19-24.
- Zhuang, Y.H., Blauer, M., Tammela, T., et al. (1997). Immunodetection of androgen receptor in human urinary bladder cancer. *Histopathol.*, 30- 55.

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