

Immunohistochemical Detection of CK5/6 in Bladder Tumors among Sudanese Patients

Abu Elgasim Abass Awad Elkareem¹ and Linda Omer Altayb Elhag²

College of medical laboratory science, Sudan University of Science and Technology, Khartoum, Sudan

Email: gassomy2@gmail.com¹, lindaeldosh@gmail.com²

Abstract: This is a descriptive retrospective case aimed to detect CK5/6 expression in bladder tumors using immunohistochemistry. Forty paraffin embedded blocks were randomly selected from patients samples previously diagnosed as bladder tumors (30 samples were malignant tumors (28 samples were transitional cell carcinoma and 2 samples were adenocarcinoma) and 10 samples were benign). The paraffin blocks were cut by rotary microtome and stained by immunohistochemical method for detection of CK5/6. The data obtained was analyzed using SPSS program. The patient's age ranged between 49 and 70 years with mean age of 59 years, most patients were more than 55 years representing 22(55 %) and the remaining 18(45%) patients were less than 55 years. Immunohistochemical expression of CK5/6 was revealed positive result in 20/30 samples and negative result in 10/30 samples in malignant tumors, while all benign tumors showed positive result for CK5/6 (10/10), with significant statistical association between CK5/6 expression and histopathology diagnosis of bladder tumors ($P < 0.05$). This study concludes that CK5/6 expression is associated with benign forms of bladder tumors.

Keywords— Bladder tumors; CK5/6; Immunohistochemistry

1. INTRODUCTION

Bladder cancer is a disease in which malignant cells grow in the tissues of the bladder. When bladder cancer is found at an early stage, it is treatable. Many bladder cancers are found at this stage. However, bladder cancer often returns, even when it is found at an early stage⁽¹⁾.

Bladder cancer (BC) is the 11th most commonly diagnosed cancer in the world. Incidence rate (per 100,000 person-years) is 8.9 for men and 2.2 for women. Worldwide, BC is the 14th leading cause of cancer deaths; age-standardized mortality rate (per100.000 person-years) was 3.3 for men versus 0.9 for women in 2008. BC incidence and mortality rates vary across the countries due to differences in risk factors⁽²⁾.

Risk factors are age, cigarette smoking, excessive use of certain pain medications, treatment with alkylating agent chemotherapy drugs, family history of bladder cancer, exposure to hair dye, urologic conditions such as urinary tract infections and urinary stasis, infection with schistosoma, dietary factors-a diet low in fruits and vegetables, exposure to arsenic in drinking water⁽³⁾.

Bladder cancer is examined by physical examination and medical history, cystoscopy, bladder biopsy, urine cytology, FISH and imaging tests⁽¹⁾.

Bladder cancer is treated by surgery, immunotherapy, chemotherapy and radiation therapy⁽³⁾.

Cytokeratin 5/6 are intermediate sized basic keratins. In normal tissue, CK 5/6 are mainly expressed in keratinizing (epidermis) and non-keratinizing (mucosa), squamous epithelium, as well as in basal myoepithelial cell layer prostate, salivary gland and breast⁽⁴⁾.

Urothelium is a stratified epithelium, which demonstrates similarities with squamous cell carcinoma, resulting in markedly overlapping immunoprofiles. One characteristic is the expression of CK 5/6 and other squamous

association marker, CK5/6 is expressed in 65% to 97% of benign urothelial tumors, 35% to 60% of urothelium carcinoma⁽⁵⁾.

The CK staining pattern and intensity varied between well differentiation and poorly differentiation transitional cell carcinoma. In low grade papillary transitional cell carcinoma, the CK5/6 positive cell were observed at the basal layer of the papillary, where as in high grade transitional cell carcinoma, tumor cell were diffusely positive for CK5/6 in some cases⁽⁴⁾.

2. Materials and methods:

2.1 Materials:

Archived tissue block of bladder tumors were selected for this study.

2.2 Study design

This is hospital based analytical retrospective case control study aimed to detect CK5/6 expression in bladder tumors.

3. Methods:

3.1 Sample processing:

Section to be stained were cut at 3µm thickness by rotary microtome, mounted in positively charged glass slides and put at 60°C oven for 30 minutes.

3.2 Immunohistochemical stain

Immunohistochemical staining was carried out using indirect streptoavidin- biotin immune peroxidase technique. Tissue sections (3µm) were deparaffinized in xylene and rehydrated in graded alcohol (100%, 90%, 70%, and 50%) slide were incubated for 10 minutes in 0.3 % hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed by using PT link water path with citrate buffer (pH 6.8).

The slides then were treated with anti CK5/6 primary antibody for 30 minutes. Then section were incubated in biotinylated secondary antibody for 15 minutes then washed in phosphate buffer saline (pH7.4), incubated in streptavidin-

HRP (horseradish peroxidase) for 15 minutes, washed in phosphate buffer saline (pH 7.4), incubated in diaminobenzidine tetra hydrochloride (DAB) substrate solution, washed in running water, counterstained in Mayer's hematoxylin stain for one minute. Dehydrated, cleared and mounted in DPX mounting media ⁽⁶⁾.

3.3 Result interpretation:

All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining. Detection of more than five cells with cytoplasm per one field considered as positive result.

3.4 Data analysis:

Data analysis was done using SPSS 20 computer program. Frequencies mean and Chi –square test values were calculated.

3.5 Ethical consideration:

Samples were collected after taking ethical acceptance from hospital administration.

4. Results

The age of study population range between 49 and 70 years with mean age of 59 years, and standard deviation 8.1.

Most patients were more than 55 years representing 22 (55%) and the remaining 18 (45%) were less than 55 years (Table 1).

The study includes forty samples, 30 samples were malignant and 10 samples were benign. The diagnosis of malignant samples was transitional cell carcinoma 28 samples and adenocarcinoma 2 samples (Table 2).

CK 5/6 positive expression was detected in 20/30 of malignant samples, while 10/30 samples showed negative expression, while all benign samples (10/10) showed positive expression for CK5/6. This result showed significant association (P. value =0.035) (Table 3).

Table (1): Distribution of age group among the study population

Age group	Frequency	Percentage
Less than 55 years	18	45%
More than 55 years	22	55%
Total	40	100%

Table (2): Distribution of histopathological diagnosis among the study population

Histopathological diagnosis	Frequency	Percentage
Benign	10	25
Transitional cell carcinoma	28	70
Adenocarcinoma	2	5
Total	40	100%

Table (3): Relation between histopathological diagnosis of bladder tumor and CK5/6 expression

Histopathological diagnosis	CK5/6 expression		P. value
	Positive	Negative	
Benign	10	0	0.035
Malignant	20	10	
Total	30	10	

5. DISCUSSION

The present study involves 40 cases of bladder lesions for immunohistochemical staining by cytokeratin 5/6. Regarding the age group of study population, the study revealed that most of patients were more than 55 years, indicating that older patients are more susceptible to bladder cancer due to change in gene expression and hormonal change by age. This result is compatible with Yoshiyuki *et al.* ⁽⁷⁾, who reported that risk of developing bladder cancer increases with age. Also agree with Hayam *et al.* ⁽⁸⁾, who reported that number of the patient was increased with age. This result also agree with Arshad *et al.* ⁽⁹⁾, who reported that the older patients the higher risk throughout his life.

The histopathological diagnosis of the study population revealed that more frequent type was transitional cell carcinoma, this result is compatible with Arshad *et al.* ⁽⁹⁾, who reported that (28 /30) cases of malignant tumors were diagnosed as transitional cell carcinoma. Also combatable with Irfan *et al.* ⁽¹⁰⁾, who reported that (20/25) cases of malignant lesions were diagnosed as transitional cell carcinoma. Also agree with Silvia *et al.* ⁽¹¹⁾, who reported that 90% of cases are transitional cell carcinoma.

The expression of CK 5/6 revealed that (20/30) of malignant lesions showed positive expression and (10/30) showed negative expression, and all benign cases of bladder lesion showed positive expression for CK 5/6 because CK5/6 normally present in the basal layer of the bladder tissues. This relation showed significant association (P.value =0.035), this result is compatible with Mahul *et al.* ⁽⁵⁾, who reported that (22/25) of malignant lesions showed negative expression, and all benign lesions of bladder showed positive CK5/6 expression. Also agree with Peiguo *et al.* ⁽⁴⁾, who reported that all malignant lesions showed negative expression, and positive rate of CK5/6 in benign bladder lesions was 100%.The appearance of positive among malignant may be due to samples may contain benign tissue.

6. CONCLUSTION

The study we conclude that CK5/6 expression is associated with benign forms of bladder tumors.

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