Cytokeratin 5/6 expression in bladder cancer: association with clinicopathologic parameters and prognosis

Atif Ali Hashmi, Zubaida Fida Hussain, Muhammad Irfan, Muhammad Muzzammil Edhi, Sarah Kanwal, Naveen Faridi and Amir Khan*

Abstract

Objectives: Well differentiated keratinized squamous component as a part of urothelial carcinoma can be easily appreciated; however non-keratinizing squamous differentiation closely resembles urothelial differentiation. In addition prognostic significance of CK 5/6 expression in the absence of apparent squamous differentiation is still unclear. Therefore, in the present study we aimed to evaluate the frequency of CK 5/6 expression in 127 cases of urothelial carcinoma and its prognostic significance in loco-regional population.

Results: Positive CK5/6 expression was noted in 6.3% (8 cases) and 13.4% (17 cases) revealed focal positive CK 5/6 expression. On the other hand, 80.3% (102 cases) showed negative CK5/6 staining. Significant association of CK5/6 expression was noted with tumor grade and muscularis propria invasion, however no significant association was noted with overall and disease free survival. On the basis of the results of our study we can conclude that CK5/6 is an independent prognostic biomarker in urothelial carcinoma and therefore can be used in the prognostic stratification of the patients with bladder cancer.

Keywords: Urothelial carcinoma, Bladder cancer, Cytokeratin 5/6, CK5/6, Deep muscle invasion

Introduction

Bladder cancer is among one of the most common malignancy in males worldwide and its incidence is even higher in developing countries owing to certain endemic infections [1, 2]. Urothelial carcinoma is the most common histologic subtype of bladder cancer, while Squamous cell carcinoma is seen in association with bladder stones and schistosomiasis. Muscle invasion is one of the most important prognostic factors in bladder cancer; as it necessitates radical therapy and poor 5 year disease free survival [3, 4]. While, well differentiated keratinized squamous component as part of urothelial carcinoma can be easily appreciated; non-keratinizing squamous differentiation closely resembles urothelial differentiation. On the other hand, WHO/ISUP don’t recommend routine use of immunohistochemical markers to identify squamous differentiation in urothelial carcinoma. Conversely, markers of squamous differentiation like p63, p40, CK5/6 can be positive in urothelial carcinoma [5]. In addition prognostic significance of CK 5/6 expression in the absence of apparent squamous differentiation is still unclear. Therefore, in the present study we aimed to evaluate the frequency of CK 5/6 expression in urothelial carcinoma and its prognostic significance in loco-regional population.

Main text

Total 240 diagnosed cases of urothelial carcinoma specimens were selected from records of pathology department. All patients underwent surgeries at Liaquat National hospital, Karachi from January 2010 till December 2014 over a period of 5 years. The study was approved by research and ethical review committee of Liaquat National Hospital and informed written consent...
was taken from all patients at the time of surgery. Hema-
toxin and eosin stained slides and paraffin blocks of all
cases were retrieved and new sections were cut when
necessary. Slides of all cases were reviewed by two senior
histopathologists and pathologic characteristics like his-
tologic type, tumor grade, lamina propria invasion, mus-
cularis propria invasion were evaluated. Clinical records
of 61 patients were available and are thus reviewed from
institutional records to evaluate history of radiation and
chemotherapy and recurrence status. Moreover, rep-
resentative tissue blocks of 127 cases were available for
CK5/6 immunohistochemistry.

CK5/6 IHC was performed by using FLEX Monoclonal
Mouse Anti-human Cytokeratin 5/6, clone D5/16 B4 (Lot
No. 20042129) by DAKO envision method according to
manufacturers protocol on 127 cases of urothelial carci-
noma (on representative tissue blocks). Results of IHC
staining were interpreted by two senior histopatholo-
gists with more than 5 years experience of reporting his-
topathology and immunohistochemistry and they were
blinded by other histopathological features of the tumors.
For quantification, at least 1000 cells were counted in
10 HPFs (40 ×). Intermediate to strong cytoplasmic and
membranous staining in more than 10% of tumor cells
was considered positive. Weak to intermediate staining
in <10% was taken as focal positive, while no staining was
considered as negative (Fig. 1).

Recurrence status and follow-up were evaluated by
reviewing hospital medical records. Overall survival
was taken as time from surgical excision till death or last
follow-up and disease free survival was defined as time
between surgical excision and local recurrence or distant
metastasis, death or last follow-up.

All cases of primary urothelial carcinoma were
included in the study. Cases of squamous cell carci-
noma or those cases of urothelial carcinoma showing
divergent differentiation (including squamous different-
ation) were excluded from the study.

Statistical package for social sciences (SPSS 21)
was used for data compilation and analysis. Mean
and standard deviation were calculated for quantita-
tive variables. Frequency and percentage were calcu-
lated for qualitative variables. Chi square was applied
to determine association. Student t test or Mann
Witney test were applied to compare difference in
means among groups. Survival curves were plotted
using Kaplan–Meier method and the significance of
difference between survival curves were determined
using log-rank ratio. P value ≤ 0.05 was taken as
significant.

Mean age of patients was 63.23 + 13.9 years with male
to female ratio of 3:1. 95.8% specimens were of tran-
surethral resections. 50.8% (122 cases) were of high
grade morphology, whereas 49.2% (118 cases) showed

![Fig. 1 CK5/6 expression in bladder carcinoma](image)
low grade histology. Lamina propria invasion was seen in 30.4% (73 cases), while muscularis propria invasion was noted in 22.9% (55 cases). Mean follow up of patients involved in the study was 22.0 + 13.74 months and recurrence was seen in 45.9% (28 cases) as presented in Table 1.

Positive CK5/6 expression was noted in 6.3% (8 cases) and 13.4% (17 cases) revealed focal positive CK5/6 expression. On the other hand, 80.3% (102 cases) showed negative CK5/6 staining. Significant association of CK5/6 expression was noted with tumor grade and muscularis propria invasion, however no significant association was noted with lamina propria invasion and disease free survival (Table 2 and Figs. 2 and 3).

In the present study we found that CK5/6 expression is low in urothelial carcinoma in our set up; however, its positivity signifies adverse prognostic features like higher tumor grade and muscularis propria invasion.

CK5/6 is a basal cytokeratin which normally expresses in squamous epithelium and in squamous cell carcinoma. Although diagnosis of squamous cell carcinoma in bladder is restricted to those tumors which show pure squamous differentiation in the absence of any urothelial component. Conversely, advanced urothelial carcinoma can show divergent differentiation (including squamous component) in up to 50% of cases and is associated with poor disease progression [6]. Morphologic diagnosis of squamous differentiation in urothelial carcinoma is based on the presence of either intercellular bridges or presence of keratinization in the form of keratin pearls or individual cell keratinization; however non-keratinizing or poorly differentiated squamous component can closely resemble urothelial carcinoma and therefore can't be readily apparent. Gaisa et al. [7] performed IHC markers of squamous differentiation including CK5/6 and CK4/14; and found squamous differentiation in a high proportion of urothelial carcinoma without morphologic evidence of squamous differentiation. Langer et al. evaluated the prognostic value of keratin subtyping in urothelial carcinoma and revealed the prognostic impact of various cytokeratin staining in urothelial carcinoma including CK5/6.

**Table 1** Clinicopathologic features of bladder carcinoma

| Feature                                      | n (%)        |
|----------------------------------------------|--------------|
| Age (years)a                                 | 63.28 ± 13.90|
| Follow up (months)a                         | 22.00 ± 13.74|
| Gender                                       |              |
| Male                                         | 182 (75.8)   |
| Female                                       | 58 (24.2)    |
| Tissue type                                  |              |
| Transurethral resection                      | 230 (95.8)   |
| Radical cystectomy                          | 10 (4.2)     |
| Tumor grade                                  |              |
| Low grade papillary urothelial carcinoma    | 118 (49.2)   |
| High grade papillary urothelial carcinoma   | 122 (50.8)   |
| Lamina propria invasion                     |              |
| Present                                      | 73 (30.4)    |
| Absent                                       | 167 (69.6)   |
| Deep muscle invasion                         |              |
| Present                                      | 55 (22.9)    |
| Absent                                       | 140 (58.3)   |
| Can’t assessed                               | 45 (18.8)    |
| Recurrence (n = 61)                          |              |
| Yes                                          | 28 (45.9)    |
| No                                           | 33 (54.1)    |
| Survival status (n = 61)                     |              |
| Alive                                        | 52 (85.2)    |
| Expired                                      | 9 (14.8)     |

* Mean ± SD

**Limitations**

One of the major limitations of our study was that we performed only single biomarker of squamous differentiation in our study; use of multiple markers like CK5/14 and CK4/14 could increase the sensitivity of the study. However, on the basis of the results of our study we can conclude that CK5/6 is an independent prognostic biomarker in urothelial carcinoma and therefore can be used in the prognostic stratification of the patients with bladder cancer.
Table 2  Association of CK 5/6 expression with clinicopathologic features of bladder cancer

|                          | CK 5/6 n (%) | P value |
|--------------------------|--------------|---------|
| **Gender**               |              |         |
| Male                     | 8 (100)      | 77 (75.5) | 8 (47.1) | 93 (73.2) | 0.014 |
| Female                   | 0 (0)        | 25 (24.5) | 9 (52.9) | 34 (26.8) |       |
| Total                    | 8            | 102      | 17       | 127       |       |
| **Age group**            |              |         |
| ≤ 25 years               | 0 (0)        | 1 (1)    | 0 (0)    | 1 (0.8)   | 0.613 |
| 26–50 years              | 2 (25)       | 26 (25.5)| 2 (11.8) | 30 (23.6) |       |
| > 50 years               | 6 (75)       | 75 (73.5)| 15 (88.2)| 96 (75.6) |       |
| Total                    | 8            | 102      | 17       | 127       |       |
| **Tissue type**          |              |         |
| Transurethral resection  | 6 (75)       | 99 (97.1)| 17 (100) | 122 (96.1)| 0.049 |
| Radical cystectomy       | 2 (25)       | 3 (2.9)  | 0 (0)    | 5 (3.9)   |       |
| Total                    | 8            | 102      | 17       | 127       |       |
| **Tumor grade**          |              |         |
| Low grade                | 0 (0)        | 59 (57.8)| 5 (29.4) | 64 (50.4) | 0.000 |
| High grade               | 8 (100)      | 43 (42.2)| 12 (70.6)| 63 (49.6) |       |
| Total                    | 8            | 102      | 17       | 127       |       |
| **Lamina propria invasion** |            |         |
| Present                  | 5 (62.5)     | 31 (30.4)| 3 (17.6) | 39 (30.7) | 0.081 |
| Absent                   | 3 (37.5)     | 71 (69.6)| 14 (82.4)| 88 (69.3) |       |
| Total                    | 8            | 102      | 17       | 127       |       |
| **Deep muscle invasion** |              |         |
| Present                  | 5 (62.5)     | 19 (18.6)| 2 (11.8) | 26 (20.5) | 0.049 |
| Absent                   | 1 (12.5)     | 47 (46.1)| 10 (58.8)| 58 (45.7) |       |
| Can't assessed           | 2 (25)       | 36 (35.3)| 5 (29.4) | 43 (33.9) |       |
| Total                    | 8            | 102      | 17       | 127       |       |
| **Recurrence (n = 54)**  |              |         |
| Yes                      | 2 (50)       | 18 (40)  | 2 (40)   | 22 (40.7) | 1.000 |
| No                       | 2 (50)       | 27 (60)  | 3 (60)   | 32 (59.3) |       |
| Total                    | 4            | 45       | 5        | 54        |       |
| **Survival status (n = 54)** |          |         |
| Alive                    | 3 (75)       | 39 (86.7)| 5 (100)  | 47 (87)   | 0.514 |
| Expired                  | 1 (25)       | 6 (13.3) | 0 (0)    | 7 (13)    |       |
| Total                    | 4            | 45       | 5        | 54        |       |

Fisher exact test applied

P ≤ 0.05, considered as significant
Abbreviations
IHC: immunohistochemistry; WHO: World Health Organization; ISUP: International Society of Urological Pathology.

Authors’ contributions
AAH and ZFH: main author of manuscript, have made substantial contributions to conception and design of study. MI, MME and SK: have been involved in requisition of data. NF AND AK have been involved in analysis of the data and revision of the manuscript. All authors revise the manuscript. All authors read and approved the final manuscript.

Author details
1 Liaquat National Hospital and Medical College, Karachi, Pakistan. 2 Brown University, Providence, RI, USA. 3 Kandahar University, Kandahar, Afghanistan.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Please contact author, Atif Ali Hashmi (doc_atif2005@yahoo.com) for data requests.

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Not applicable.

Ethical approval and consent to participate
Ethics committee of Liaquat National Hospital, Karachi, Pakistan approved the study. Written informed consent was obtained from the patients for the participation.

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