

## IMMUNOREACTIVITY OF PROLIFERATING CELL NUCLEAR ANTIGEN IN TRANSITIONAL CELL CARCINOMA OF URINARY BLADDER

ABDUL HASEEB KHAN, SAEED AKHTAR KHAN  
NASEER AHMAD CHAUDRHY AND MUHAMMAD TAYYAB  
*Department of Pathology, Postgraduate Medical Institute, Lahore*

*This is a descriptive study designed to evaluate Proliferating cell nuclear antigen (PCNA) in transitional cell carcinoma. A total of fifty per urethra resected bladder tumour samples (TUR BT) were collected from Mayo Hospital and Services Hospital, Lahore and processed for H&E and PCNA stain. The grading of tumours were made on H&E stain. While Proliferating cell nuclear antigen labeling index was recorded for each case. The mean PCNA labeling index was significantly higher in grade III when compared with tumours of grade II. ( $p < 0.001$ ) carcinoma. Similarly mean PCNA labeling index was significantly higher ( $p < 0.05$ ) in patients having duration of symptoms up to 3 month when compared with the patients having longer duration of symptoms. The mean PCNA labeling index had significantly higher value in high grade tumours as compared to low grade tumours ( $p < 0.001$ ). Although determination of PCNA labeling index is costly yet it has significant role in tumour grading.*

Tumours of urinary bladder are one of the leading malignancies in the developed countries.<sup>1</sup> The incidence is also high in Northern Africa and Western Asia. The world wide estimate of carcinoma of urinary bladder of new cases is 261,000 annually.<sup>2</sup> Amongst these, more than 50,000 cases and an estimate of 9500 deaths have been predicted in United States alone.<sup>3</sup> The incidence is three times more in males as compared to females and twice as common in whites as compared to blacks.<sup>4</sup>

Carcinoma of urinary bladder accounts for 2% of all malignant tumors and approximately 7% of all urinary tract malignancies in males. Transitional cell carcinoma constitutes the vast majority of bladder cancers.<sup>5</sup> It is the second most common malignancy in the genitourinary tract.<sup>6</sup>

Carcinoma of urinary bladder is the 8<sup>th</sup> common malignancy in Pakistan. It is the fourth common malignancy in men and its incidence is 5.4% in North West Pakistan. It has frequency of 5.75% among men and 1.61% among women.<sup>7,8,9</sup>

Recently, a series of unique proteins that may serve as useful antigenic markers have been isolated from actively dividing cells, like ki-67 and proliferating cell nuclear antigen. The proliferating cell nuclear antigen (PCNA), also known as Cyclin, is a nuclear protein of 36 kd whose level of synthesis correlate directly with rate of cellular proliferation and DNA synthesis.<sup>10</sup> Monoclonal antibodies have recently been generated to PCNA/Cyclin.<sup>11</sup> Monoclonal antibodies to PCNA/Cyclin (PC 10) have been shown to label the proliferating cell in vitro as well as in alcohol fixed, paraffin embedded tissue.<sup>12</sup>

The PCNA labeling index in urinary bladder cancer may prove to be new objective and quantitative assay of the biological potential or individual tumour. The new immunohistochemical method using anti-PCNA antibodies (PC 10) to study tumour cell kinetic has overcome many of inherent difficulties of conventional auto radiographic technique as well as Bromodeoxyuridine labeling index.<sup>13</sup>

So anti PCNA monoclonal antibodies have been found to be useful tool and good proliferative marker.<sup>14</sup> PCNA expression has been assessed as prognostic indicator in renal cell carcinoma.<sup>15</sup> Positive correlation between PCNA immunostaining and AgNOR has been observed in histological grading of gastrointestinal stromal tumours.<sup>16</sup>

Similarly a significant difference was observed between grade II and grade III in carcinoma of urinary bladder when two parameters AgNOR and PCNA were used. A directly proportional relationship was also demonstrated between each of PC 10 and AgNOR scores and the clinical stage. So PC 10 and AgNOR scores may be important prognostic indices in carcinoma of urinary bladder.<sup>17</sup>

As PCNA staining have been used successfully for evaluation of different malignancies.<sup>18,19</sup> This study was carried out to evaluate PCNA Immunoreactivity in different grades of transitional cell carcinoma.

### MATERIALS AND METHODS

Fifty sample of Transurethraly resected urinary bladder tumour (TUR BT) were collected in 10% formalin solution from Mayo Hospital and Services Hospital Lahore These formalin fixed tissues

were processed in automatic processor for H&E staining<sup>20</sup> and PCNA staining using the commercially available reagents of DAKO Corporation, USA, according to manufacturer instructions. The grading of tumour was made on H&E stain.

**MICROSCOPIC INTERPRETATION**

The average labeling index was expressed as percentage ratio of total labelled cells to the total number of cells counted.<sup>13</sup>

**RESULTS**

The proliferating cell antigen labeling index in transitional cell carcinoma ranged from 2% to 39% with the mean±SD value of 19.12±8.10. Mean PCNA labeling index decreased as the ages of the patients increased. Comparison of mean PCNA labeling index in different age groups is table 1. Mean PCNA labeling index was significantly higher in grade III when compared with tumours of grade II (table 2). Similarly mean PCNA labeling index was significantly higher (p<0.05) in patients having

duration of symptoms up to 3 month when compared with the patients having longer duration of symptoms (table 3).

**Table 1:** Mean PCNA labeling index in different age groups.

Age groups (years)	No of cases	Mean PCNA labeling index ± SD
40 – 49	5	25.80 ±10.03
50 – 59	18	19.33 ±11.06
60 – 69	22	17.77 ±9.51
70 – 79	5	17.60 ±11.76
Total	50	19.12 ± 8.10

p=NS

**Table 2:** Comparison of Grade of transitional cell carcinoma with mean PCNA labeling index.

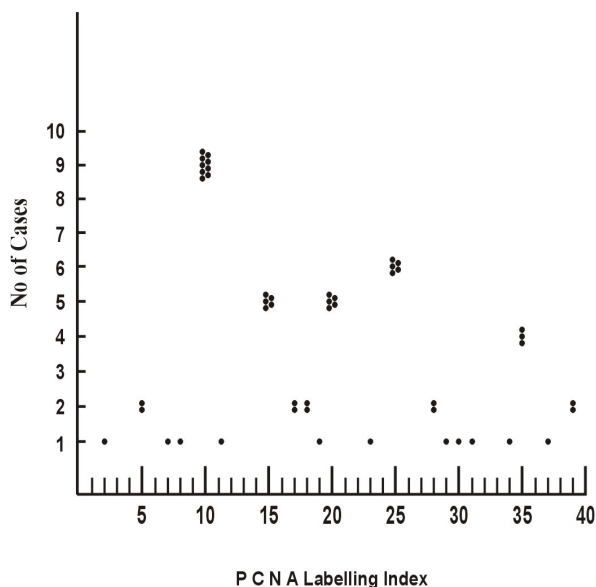
Grade	No of cases	PCNA labeling index Mean ± SD
II	34	16.61 ±9.98
III	16	24.43 ± 7.01

P< 0.001

**Table 3:** Comparison of Mean PCNA labeling index with duration of symptoms.

Duration of Symptoms (months)	No of cases	PCNA labeling index Mean ± SD
1–3	18	22.16 ±12.20
4–12	32	15.90 ±11.0
Total	50	19.12 ± 8.10

P< 0.05



**Fig 7** Proliferating Cell Nuclear Antigen (P C N A ) Labelling Index In Transitional Cell Carcinoma

**DISCUSSION**

Carcinoma of urinary bladder accounts for 2% of all malignant tumours and approximately 7% of all urinary tract malignancies in males.<sup>5</sup> It is the second most common malignancy in the genitourinary tract.<sup>6</sup> Smoking and exposure to a variety of chemical compounds are main known factors promoting the onset of carcinoma of urinary bladder.<sup>21</sup> Immunostaining of the PCNA provides important information about cell kinetic and is performed on routinely obtained formalin fixed paraffin embedded materials.<sup>18</sup>. PCNA plays an essential role in nucleic acid metabolism in all eukaryotes. The PCNA protein interacts with a large number of proteins. These proteins can be divided into two groups, the first contain proteins that have a known enzymatic activity, the second contain regulatory proteins that are included in cell cycle progression.<sup>22</sup> The PCNA gene codes for a protein that is necessary for cellular DNA synthesis and cell cycle progression.<sup>22</sup>The ratio of cyclin/DNA remain constant during S phase.<sup>23</sup>

Normal epithelium has distinct PCNA pattern of expression which is confined to basal cell layer while dysplatic urothelium and carcinoma in situ showed irregularly distributed pattern of PCNA immunoreactive nuclei in all the layers. In tumour cells complexes, the pattern of PCNA immunoreactivity was different in papillary and primary infiltrating transition cell carcinoma.<sup>24</sup>

The PCNA immunostaining is useful and reproducible method of assessing one aspect of cellular proliferative activity. It has some advantages over flow cytometry in that it maintains tissue integrity and morphologic relationship.<sup>25</sup>

The percentage of tumour cells with positive staining for PCNA was found to correlate well with histological grading.<sup>26</sup> The results have been shown good relation between PCNA and histopathological grade of tumour.<sup>27</sup> The fraction of PCNA positive nuclei ranges between 0% to 100%. In WHO grade I tumours, only occasional cells are positive for PCNA where as nearly all the nuclei are positive in WHO grade III tumours. This indicates that the cell proliferation can be assessed by PCNA/Cyclin immunostaining in transitional cell carcinoma.<sup>28</sup>

In our study, cells of transitional cell carcinoma showed immunoreactivity for PCNA. Only five cases had PCNA labeling index of less than 10% with mean  $\pm$  SD value of  $5.40 \pm 3.22\%$  (Fig 1). The rest of cases had mean PCNA labeling index  $\pm$  SD  $22.84 \pm 8.97\%$  which was statistically higher ( $p < 0.01$ ) than the above group. These findings are similar to that of Chen et al.<sup>29</sup> in which they reported mean PCNA labeling index  $\pm$  SD, of  $12.58 \pm 12.33\%$  in superficial tumours and  $34.55 \pm 21.89\%$  in invasive tumours.

All above studies suggest that proliferating cell nuclear antigen labeling index in carcinoma of urinary bladder may prove to be an objective and quantitative assay of biological aggressiveness and may provide significant prognostic information.

The PCNA labeling index had significantly higher value in high grade tumours as compared to low grade tumours.<sup>30,31</sup> Similarly the PCNA labeling index in our study was significantly higher ( $p < 0.001$ ) in grade III tumours when compared with grade II tumours (Table 2). This pattern clearly indicates that PCNA labeling index can be used in grading of tumours of urinary bladder.

Similarly the mean PCNA labeling index has shown significant difference ( $p < 0.05$ ) in tumours having shorter duration of symptoms (1–3 months) as compared to tumours having longer duration of symptoms (table 3).

Our studies **conclude** that PCNA labeling index has significant role in tumour grading and future studies may prove it as reliable parameter in grading of transitional cell carcinoma and thus helpful in follow up of patients of transitional cell carcinoma.

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