



Analysis of Clinicopathological Parameters and Ki 67 Immunoexpression in Urothelial Neoplasms of Bladder

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ABSTRACT

Background: Mitotic count is one of the most important determinant of proliferative capacity of any tumour. Immunohistochemical expression of ki 67 is an alternative and effective way of determining the growth fraction. This study was conducted to assess the expression of Ki 67 proliferative marker in bladder urothelial tumors, and to correlate proliferative markers with histopathological grading & staging, thereby helps in identifying high risk patients for disease progression and recurrence who may thus benefit from perioperative systemic chemotherapy.

Methods: This study was conducted in department of pathology in collaboration with department of urology in the period of I year from March 2018-March 2019. Results was evaluated on paraffin embedded tissues of bladder neoplasm specimens by immunohistochemical methods and correlated with clinical parameters.

Result: In present study, high grade tumours have over expression of Ki 67 and graded as 3+ and 4+. Low grade tumours have low expression and have grades 1+and 2+. Out of 30 cases,40% (12 cases) show 4+ and 27% show (8 cases)3+, both being high grade tumors. Whereas, 27% (8 cases) have 1+ and 7% (2 cases) have 2+, both being low grade tumours). p value is highly significant which is p<0.001.

Conclusion: Expression of Ki 67 does correlate with grade and stage of the tumour thereby on prognostic significance. Hence ki 67 may be used as marker to predict aggressive behaviour of bladder neoplasms and also to differentiate high grade and low grade tumours.

Keywords: Bladder Neoplasms, Ki 67, Mitotic Count, Proliferative Markers.

Introduction

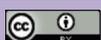
Bladder cancer is one of the common neoplasm of lower urinary tract in the present era, which has its own significant morbidity and mortality rates in the society. Hence, it has a great impact on the economic burden of health care systems. Treatment of bladder cancer is very much costlier. Parameters like tumour grading, size, staging alone have minimal role to specify progression, disease recurrence and treatment response. Till now, there are no prognostic markers identified for bladder neoplasms apart from grading and staging.

Many TURBT biopsies do not include muscle in the specimen provided, so invasion cannot be predicted histopathologically⁽²⁾. In such cases, markers which can detect invasion and confirm higher grade of the tumor will serve as the best guide for patient treatment and survival. Cell proliferation is recently evaluated for biological aggressiveness of bladder tumours. Mitotic count is a most appropriate marker to identify proliferative status in a cancer⁽³⁾. In those days, proliferation of tumour cells are difficult to measure, though they are clinically significant. Bladder tumours grow rapidly and have doubling time around 70days and hence recurrence occurs very rapidly.

Ki 67 is a monoclonal antibody developed against Hodgkins disease derived cell line L428 and it is seem to interact with nucleus expressed in cells that undergo proliferation. Several authors have published Ki 67 indices in wide range of human tumours like Non Hodgkins lymphoma, prostate, breast and lung carcinomas. It seems to co-relate with aggressive nature of these tumours^(4,5). Ki 67 being a nuclear protein that is expressed by cells undergoing proliferation, it can be identified immunohistochemically.⁽⁶⁻⁹⁾ Due to availability of effective chemotherapy for bladder tumours, there is a great need for accurate prognostic and predictive markers. Therefore, they will help in selecting appropriate patients who are suitable for early intervention and spares those patients from morbidity due to adjuvant therapies. Our study is aimed at considering the use of Ki 67 staining in urothelial neoplasms of bladder and their correlation with tumour grading and staging.

Materials and Methods

This is a Prospective study undertaken in the Department of Pathology for a period of 1 year. All TURBT specimens with malignant histology are included in the study. 30 TURBT specimens were subjected into study and were



evaluated further. Two sections are taken for each case. One for immunohistochemistry and other for routine hematoxyline and eosin staining. Sections for IHC were cut at four micron thickness on to coated slides which are then incubated at 58 degree celcius overnight. Initial sections were stained with hematoxylin and eosin stain.

IHC was done by Two step indirect technique by using ki 67 antibody. Lymph nodes are used as positive control. Antigen retrieval was done by placing slides in microwave in Tris EDTA buffer solution at Ph 9. DAB was used as a chromogen and hematoxylin used as a counter stain.

The entire sections in a slide is examined under high power objective for the presence of positive immunoreactivity. Tumour cells are read positive if there is golden brown nuclear staining of the neoplastic cells. Semiquantitative scoring was carried out. The intensity of Ki 67 nuclear staining is assessed and graded according to Adams et al,2000⁽¹¹⁾ as depicted in table 1. Then ki 67 grading and histopathological grading is compared and statistically evaluated.

For Ki 67 labelling index each slide is examined under high power and hot spot is selected. Hot spot is an area with highest immunostained nuclei. 1000 tumour cell nuclei is counted and the labelling index is expressed as percentage of positive cells. Caution should be expressed while interpreting Ki 67 positivity as any proliferating cell such as lymphocytes can be positive. Hence hot spot should always be selected after meticulous comparison with routine H&E sections. All data are analysed with a statistical software package (SPSS, version 16.0 for windows).

Result

The study was performed on 30 cases of bladder urothelial carcinomas. Male to female ratio is 5:1. The mean age for males is 67 yrs and for females is 47 yrs. Males >60 years constituting 63% is the predominant population having bladder neoplasms in our study. Initial clinical presentation in most of the patients is painless hematuria (77%). Other symptoms are dysuria (13%) and urgency (10%).

Histopathologic grading and staging done and they are distributed as depicted in table no.2. Immunohistochemical analysis with ki 67 was studied and correlated with clinical and morphological parameters.

In present study, high grade tumours have over expression of Ki 67 and graded as 3+ and 4+. Low grade tumours have low expression and have grades 1+ and 2+. As depicted in fig 4, out of 30 cases, 40% (12 cases) show 4+ and 27% show (8 cases) 3+, both being high grade tumors. Whereas,

27% (8 cases) have 1+ and 7% (2 cases) have 2+, both being low grade tumours.

Out of 30 cases, Ta stage tumors which are non invasive tumors have very low expression of Ki 67 compared to T1 and T2 stage which are invasive. Out of 5 Ta stage tumors 4 cases are 1+. Out of 6 T1 tumors 4 cases are 3+ and 2 cases are 4+. Out of 19 T2 stage tumors 9 are 4+, 4 are 3+, 2 are 2+ and 4 are 1+ as depicted in fig 6. All 5 cases which had lymphovascular invasion had higher Ki 67 score that is 4+. Statistical analysis showed highly significant between Ki 67 proliferation index, tumour grade ($p < 0.001$) and tumour stage ($p < 0.001$).

In our study out of 20 smokers, 4 cases (13%) were low grade and 16 cases (53%) are high grade. Among non smokers, 6 cases (20%) were low grade and 4 cases (13%) are high grade. Chi square analysis of variables is found to be statistically significant i.e. p value is < 0.05 .

Discussion

Bladder cancers are emerging as commonest cancer worldwide, whose incidence is rising recently due to habits of tobacco usage and occupational exposure. Many biomolecular pathways are involved in the occurrence of urothelial neoplasms. Around 50 -70% of patients who are muscle-invasive will eventually, may develop metastasis or recurrence after treatment. Annual death rate due to bladder cancer is 12,000. Even though pathologic staging and grading is an important predictor of tumour progression but its value in determining clinical outcome is very limited. Hence, an independent predictor will enable us to identify individuals who are at increased risk and may benefit from chemotherapy⁽¹⁴⁾.

Uncontrolled cell proliferation is considered to be an important step in tumorigenesis. Ki 67, a nuclear protein is a marker of cell proliferation which is present during G1, S, G2 and M phase of cell cycle. Ki 67 can be detected immunohistochemically in formalin fixed, paraffin embedded tissues. Ki 67 predicts clinical outcome in several other tumours like breast, lung, cervix and prostate tumors. More recently, many investigators have found Ki 67 as an independent predictor of clinical outcome and response to therapy in bladder cancers.

Transitional cell carcinoma is the commonest variant which accounts for 90% of bladder neoplasms in the literature. In our study, all patients (100%) had TCC. Age and gender wise distribution of bladder neoplasm's are shown in table 1. Only 33% (10 out of 30) of the patients are < 60 years and 67% (20 out of 30) are > 60 years. Only one female patient had lowest age of presentation i.e. at 28 years. Among 30 patients 83% (25) are males and 17% (5) are females.

Table:1

Ki 67 nuclear staining	grade
<10% positivity	1+
<25% positivity	2+
25-50% positivity	3+
>50% positivity	4+

Table 2: Clinicopathological Parameters.

Clinicopathological parameters		No of cases	%
AGE	<60 YRS	10	33
	>60YRS	20	67
SEX	MALE	25	83
	FEMALE	5	17
SMOKING HISTORY	SMOKERS	20	80
	NON SMOKERS	10	20
MUSCLE IN TURBT	INCLUDED	21	70
	NOT INCLUDED	9	30
MUSCLE INVASION	PRESENT	19	63
	ABSENT	11	27
TUMOUR GRADE	LOW	10	33
	HIGH	20	67
TUMOUR STAGE	Ta	5	17
	T1	6	20
	T2	19	63
LV INVASION	PRESENT	5	17

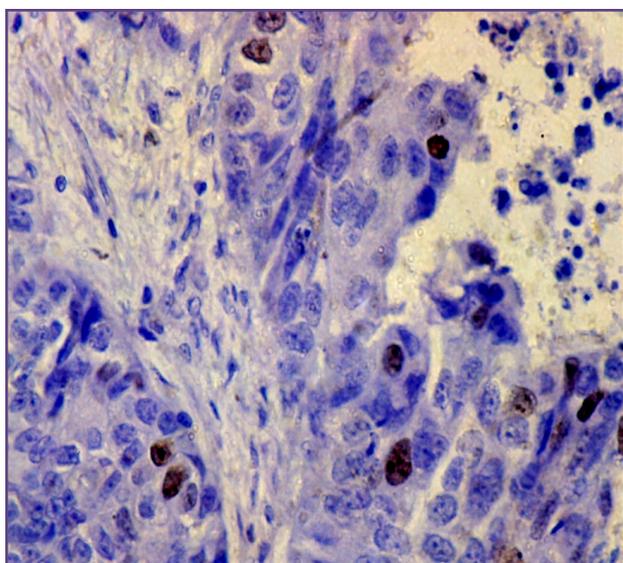


Fig. 1: IHC : KI 67 showing nuclear positivity with score 1+(40X).

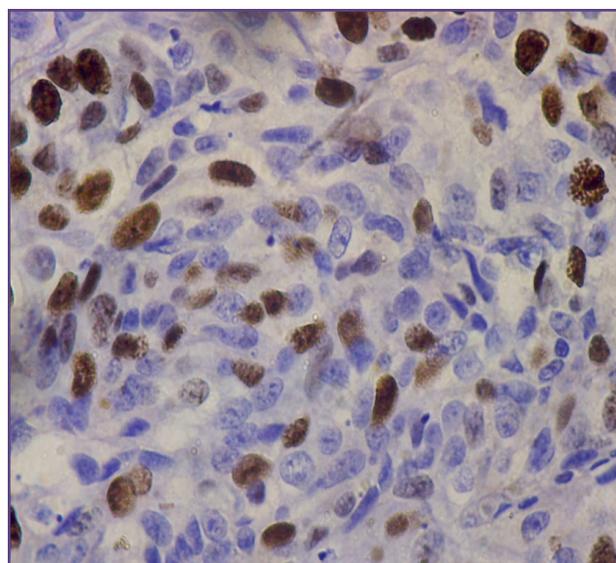


Fig. 2: IHC-KI 67 score 2+(40X).

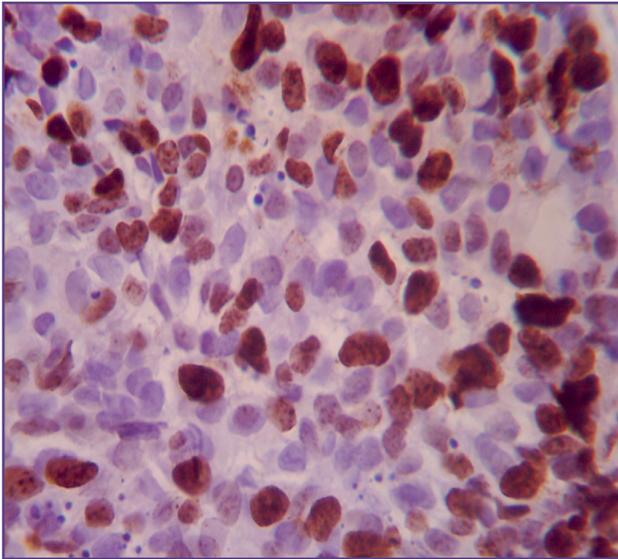


Fig. 3: IHC:Ki 67 score 3+(40X).

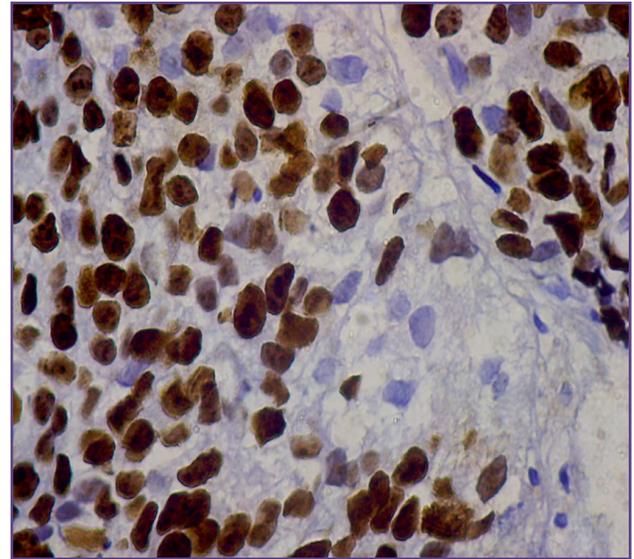


Fig. 4: IHC:Ki 67 score 4+(40X).

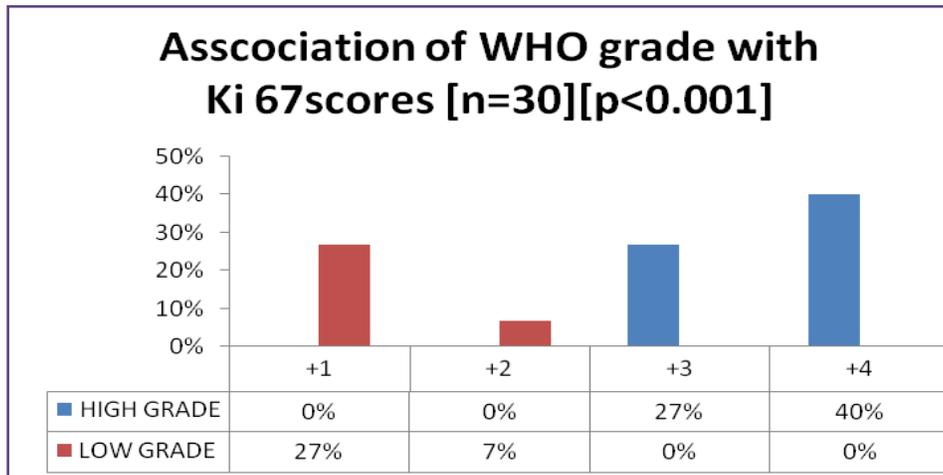


Fig. 5: Ki 67 scoring and WHO grade.

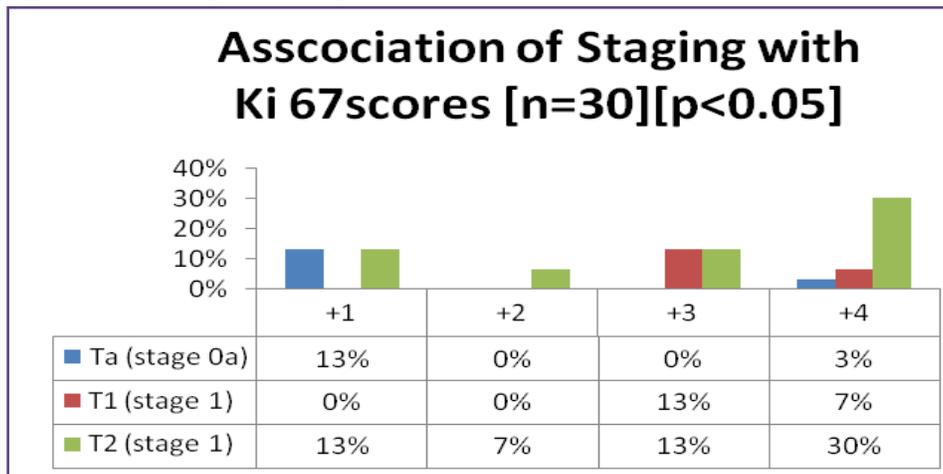


Fig. 6: Ki 67 score & staging.

The mean age of patients was 64 ± 4.1 years (range 28 -85 years) and male to female ratio is 5:1. This is in accordance with study conducted by Paraggupta et.al (2009) in which mean age was 60.2 ± 4.4 and male to female ratio is 8.6:1⁽²⁰⁾. Similar studies conducted by Alaa S. Jumma(2011) and M. Enache et al(2012) showed mean age of presentation to be 60 yrs and male to female ratio ranges from 3:1 to 5:1^(7,9).

In our study, 77% (23 out of 30) of the patients presented with painless hematuria. Their increased incidence may be due to lack of screening of microscopic hematuria. Remaining 13% (4 out of 30) presented with dysuria and 10% (3 out of 30) with urgency. This is similar to studies conducted by Paraggupta et.al (2009) in which 97% of patients presented with painless hematuria^(20,12).

Patients were subjected to cystoscopy and then location, number and nature of tumour were recorded. Then transurethral resection of bladder cancer (TURBT) was performed. In the received thirty biopsy specimens around 30% (9 out of 30) did not have muscularis propriae in them (chart-5). This is similar to Paraggupta et.al((2009) in which 34.5% of the patients did not have detrusor muscle in their TURBT specimens²⁰. Also this is concordance with Maruniak et al(2002) study, which states muscularis propria was absent in 40-50% of TURBT specimens. This leads to understaging in many patients⁽²⁾.

Total of 63% showed muscle invasive disease at time of presentation. Remaining 27% of the patients are non-muscle invasive. Since, patients arrive at the later stage to the hospital, many are found to be muscle invasive. This is similar to study conducted by Alaa S Jumaa(2011)⁷ in which 57% of patients constituted muscle invasive disease and in study conducted by M.Enache et.al(2012) 52%constituted T2-T4 disease⁽⁹⁾.

Cigarette smoking is one of the risk factor which causes increased incidence of bladder tumours, at least 4-5 times than the normal population in both males and females. In our study, history of smoking is more in males (66%) and all females are non smokers. In the study by paraggupta et.al (2009) incidence among male smokers (78% vs 22%) are higher compared to females⁽²⁰⁾.

In our study 20 cases(67%) are high grade tumors and 10(33%) cases are low grade tumors. All 30 cases were subjected to Ki 67 immunostaining and was graded according to Adams et al (2000) from 1+ to 4+⁽¹¹⁾. The results obtained are, 40% (8 out of 20) of high grade tumors are 3+ and 60%(12 out of 20) of high grade tumors are 4+. Among low grade tumors 80%(8 out of 10) are graded as 1 + and

remaining 20%(2 out of 10) have score of 2+. From these results we can say that all high grade tumors had higher proliferation rate and had score of 3+ and 4+, whereas low grade tumors have score of 1+ and 2+(fig 5). Thus tumor grade and Ki 67 scoring correlated well in our study with p value being highly significant($p < 0.001$). This is similar to study conducted by Alaa S. Jumaa (2011) in which 33%(7 out of 21) of high grade cases showed 3+ and 66%(14 out of 21) of cases showed 4+. Whereas in low grade tumours, 75% (21 out of 28) of cases showed 1+ and 25%(7 out of 28) showed 2+. This study also concluded that Ki 67 scores correlates with tumour grading⁽⁷⁾.

Another study conducted by M. Enache et.al(2012) states that Ki 67 index was positive in 72%(32 cases) with maximum in moderately and poorly differentiated grades and p value was statistically significant($p < 0.001$). Another study by Goyal et.al(2014) and Vitalymargulis et.al (2009), states that Ki 67 scoring have stronger correlation with tumor grading and hence it may serve as a reliable indicator of clinical outcome^(1,6,9).

In the present study, we can make out that Ta stage tumors have very low Ki 67 expression (4 out of 5) and was given score 1+. Whereas T1 and T2 stage tumors have high expression of Ki 67 and was graded as 3+ and 4+. Thereby, stage correlated well with Ki 67 scoring and it is statistically significant ($p < 0.05$)(fig 6). Similar studies conducted by Alaa S Jumaa (2011) states that T2 stage tumours have higher expression of Ki 67 with score of 4+ and Ta, T1 tumours have lower expression with score 1+⁽⁷⁾. These results were also consistent with studies conducted by Nakopoulol et al(1998), Lei C.X. et al(2010), and Pich A et al (2002) which states that Ki 67 expression correlates with grade and stage of tumor. Hence Ki 67 serves as a prognostic marker in indicating biologic aggressiveness of the disease.^(3,14,15)

In the present study, 5 out of 30 cases (16%) showed lymphovascular invasion. All these 5 cases had Ki 67 scoring of 4+ and they are found statistically significant ($p < 0.05$). This is in concordance with Vitaly Margulis et al(2006) in which Ki 67 over expression was associated significantly with lymphovascular invasion. Thus, Ki 67 helps in identifying patients with worst clinical outcome⁽⁶⁾.

In the present study, in patients less than age 60 years there is equal distribution of low and high grade tumours (50% Vs 50%). whereas as age advances > 60 yrs, there is increased incidence of high grade tumours (25% Vs 75%). This is similar to study by paraggupta et al(2009) in which

younger individuals present with lower grade and stage of tumours compared to elderly patients⁽²⁰⁾. This is actually contrast to common nature of malignancy in which biological behavior of malignancy is very aggressive in younger individuals.

In our series, males have increased incidence of high grade tumours compared to females and also smokers had higher occurrence of high grade tumours compared to non smokers. Possible explanations are, males have increased risk of exposure to many environmental and dietary exposures and also smoking may increase additional mutations in urothelium, thus causing high grade disease.

Conclusion

These observations emphasize the use of Ki 67, in addition to tumour grade and stage to predict the clinical outcome of patients and thereby helps in identifying high risk patients. These patients are frequently followed up and may benefit by additional adjuvant therapies. Clinical importance of this marker prompts multi institutional prospective studies with large sample size. Awareness among public is needed as they ignore hematuria symptoms and presents to the clinicians at advanced stage of the disease. Reason behind aggressive tumors in elderly is not known and hence research should be carried out further.

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