Original Article

ERA'S JOURNAL OF MEDICAL RESEARCH VOL.10 NO.1

10.24041/ejmr2023.2

Ki-67 LABELLING INDEX IN UROTHELIAL NEOPLASM

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Received on : 08-02-2023
Accepted on : 11-05-2023

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ABSTRACT

Urothelial neoplasms can be classified as non-invasive & invasive lesions. The major prognostic factors are degree of differentiation and depth of invasion in urothelial tumors. Ki-67 is a non- histone nuclear protein marker of cell proliferation which is encoded by MKi-67 gene in humans. This study evaluates and correlate immunohistochemical Ki-67 expression in Non-invasive & Invasive urothelial neoplasm. This retrospective study is done in the Department of Pathology of L.N. Medical College and J.K. Hospital, Bhopal. Urothelial Neoplasm cases diagnosed from January 2019 to January 2022 were included, & relevant clinico-pathological data and their Ki-67 Labelling Index was evaluated & correlated. Total 60 cases were studied, 30 had invasive carcinoma whereas, 30 had non-invasive neoplasm. Ki-67 expression (>13%) was seen maximum in invasive cancers i.e 40% cases, whereas (<13%) was seen maximum in non-invasive neoplasms i.e 38.3%. A statistically significant expression of Mean Ki-67 Labelling Index was observed that increased from papilloma to PUNLMP, Non- Invasive urothelial neoplasm Low grade & High grade in non-invasive urothelial neoplasm ($p<0.001$), and from lamina propria invasive to muscle invasive urothelial cancers ($p=0.013$). A Higher tumor proliferation of greater than 13% was significantly related to greater tumor size ($p=0.04$). Ki-67 labelling index being a measure of tumor proliferation is related to tumor histological grade. Large urothelial tumor size was associated to high Ki-67 LI and was not strongly associated with age and gender. Therefore Ki-67 expression can be used as Diagnostic & Prognostic marker in urothelial tumors.

KEYWORDS: Ki-67, Urothelial Neoplasm, Labelling Index

INTRODUCTION

The recent classification of urothelial tumors can be subdivided into Urothelial Papilloma, Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP), Non-invasive Papillary Urothelial Carcinoma Low Grade and High-grade and Infiltrating Urothelial Carcinoma.(1) Non- invasive urothelial neoplasm accounts for major proportion of all bladder tumors. The major prognostic factors are degree of differentiation and depth of invasion in urothelial tumors.(2) However recurrence, aggressiveness and progression can be indicated by tumor cell proliferation.(3) Ki-67 is a non- histone nuclear protein marker of cell proliferation which is encoded by MKi-67 gene in humans.(2) This study evaluates and correlate immunohistochemical Ki-67 expression in Non-invasive urothelial neoplasm (Urothelial Papilloma, PUNLMP, Low Grade and High Grade non-invasive Papillary Urothelial Neoplasm) and Invasive urothelial carcinoma (Lamina Propria Invasion, Muscle Invasion).

MATERIAL AND METHODS

This retrospective study done in the Department of Pathology of LN Medical College and J.K. Hospital, Bhopal. Urothelial Neoplasm cases diagnosed from January 2019 to January 2022 were included, & relevant clinico-pathological data regarding invasive and non-invasive urothelial neoplasm were taken and Ki-67 Labelling Index was evaluated.

INCLUSION CRITERIA

All histopathologically diagnosed cases of urothelial neoplasm in LNMC & RC.

EXCLUSION CRITERIA

History of prior chemotherapy, radiotherapy, hormonal therapy and/or any other malignancy.

This study included 60 cases of urothelial neoplasm with their clinical data. Re-evaluation of histomorphological diagnosis was done on retrieved paraffin blocks and slide. Immunohistochemical staining was performed using manual technique with

primary BIOGENEX Fremont CA mouse clonal antibody (catalogue number AM 297-5M) clone MIB 1. Positive control were included in each batch of staining. Ki-67 labelling index was stratified into low and high expression according to percentage of positive nuclei as <13 % & >13 % respectively. (11) All the data was analysed in SPSS version 25.0 2017.

RESULTS

Retrospective analysis of 60 urothelial neoplasm cases were evaluated in the present study. Total 60 cases were studied, 30 had invasive, 30 had non-invasive cancer. Ki-67 expression high (>13%) was seen maximum in invasive cancers i.e. 40%, whereas low (<13%) was seen maximum in non-invasive cancers i.e. 38.3%. Higher tumor proliferation of grater than 13% was related to greater tumor size (*p=0.04*). Majority of patients were males i.e (n=53). Maximum patients were ≥65 years of age. 30 patients had non-invasive urothelial neoplasm classified as Papilloma (8.3%), PUNLMP (10%), Low grade (16.7%), High grade (15%). 30 patients had Invasive cancers categorized as Lamina propria invasion (31.7%) & Muscle invasion (18.3%). (Table 1) A strong positive correlation on Pearson's correlation test between low and high Ki-67 labelling index versus Non-invasive urothelial neoplasm (Pearson's correlation coefficient of 0.80 and 0.69 respectively), and Invasive urothelial neoplasm (Pearson's correlation coefficient of 1 and 1 respectively). (Table 2) On comparison of mean Ki-67 labelling index (ANNOVA test for equality of means) in non-invasive and invasive neoplasm at 95% CI, a statistically significant expression was observed that increased from papilloma to PUNLMP, Low grade & High grade in non-invasive urothelial neoplasm (*p<0.001*), and from lamina propria invasive to muscle invasive urothelial cancers (*p=0.013*). (Table 3)

S NO.	PATIENT VARIABLES	Cases (N=60)	Ki-67 Labelling Index Low(<13%)	Ki-67 Labelling Index High(>13%)	CHI SQUARE	P VALUE	RESULT (S-significant NS-not significant)
1	**AGE**						
	<65 years	26(43.3)	23(38.3)	3 (5)	0.0007	*0.98*	NS
	≥65 years	34(56.7)	30 (50)	4 (6.7)			
2	**SEX**						
	Male	53(88.3)	48 (80)	5(8.3)	0.1617	*0.69*	NS
	Female	7(11.7)	6 (10)	1(1.7)			
3	**TUMOR SIZE**						
	<3 cm	38(63.3)	35(58.3)	3 (5)	6.432	***0.04***	S
	3 to 5cm	12 (20)	10(16.7)	2(3.3)			
	>5cm	10 (16.7)	6 (10)	4(6.7)			
4	**NON-INVASIVE UROTHELIAL NEOPLASM**	30(50)	23(38.3)	7(11.7)			
	Papilloma	5(8.3)	4 (6.7)	1 (1.7)	2.599	*0.46*	NS
	PUNLMP	6(10)	5 (8.3)	1 (1.7)			
	Low Grade	10(16.7)	8 (13.3)	2 (3.3)			
	High Grade	9(15)	5 (8.3)	4 (6.7)			
5	**INVASIVE UROTHELIAL CARCINOMA**	30 (50)	6 (10)	24 (40)			
	Lamina propria Invasion	19 (31.7)	6 (10)	13 (21.7)	2.001	*0.99*	NS
	Muscle Invasion	11 (18.3)	1 (1.7)	10 (16.7)			

Table 1: Immunohistochemical Expression of Ki-67 Labelling Index (Low <13% & High >13%) In Urothelial Neoplasms & Its Clinico-Pathological Parameters.

	Cases (N=60)	Ki-67 Labelling Index		Ki-67 Labelling Index	
		Low (<13%)	High (>13%)	Low (<13%)	High (>13%)
				R* value	R* value
NON-INVASIVE UROTHELIAL TUMOR	30	23	7	0.808452083	0.69310328
PAPILLOMA	5	4(80%)	1		
PUNLMP	6	5(83.3%)	1		
LOW GRADE	10	8(80%)	2		
HIGH GRADE	9	5(55.6%)	4		
INVASIVE UROTHELIAL CARCINOMA	30	6	24	1	1
Lamina propria Invasion	19	6(31.5%)	13		
Muscle Invasion	11	1(81.8%)	10		

**R- PEARSON'S CORRELATION CO-EFFICIENT (between ±0.5 and ±1 it is strong positive correlation, between ± 0.30 and 0.49 is moderate/medium correlation, ≤±0.29 is weak correlation , 0 means no correlation)*

Table 2: Correlation Between Ki-67 MIB1 (Low and High) Expression and Urothelial Tumors (Invasive And Non-invasive) By Pearson's Correlation Coefficient Test

	Cases (N=60)	Ki-67 Labelling Index	95% CI	P value	RESULT (S-significant, NS-not significant
NON-INVASIVE UROTHELIAL CARCINOMA	30(50%)				
Papilloma	5(8.3%)	2.90	0.91 to 4.87	***<.001****	YES
PUNLMP	6(10%)	2.94	1.52 to 4.36		
Low Grade	10(16.7%)	4.64	2.50 to 6.77		
High Grade	9(15%)	17.22	7.10 to 27.34		
INVASIVE UROTHELIAL CARCINOMA	30(50%)				
Lamina propria Invasion	19(31.7%)	5.07	3.81 to 6.33	***<.013****	YES
Muscle Invasion	11(18.3%)	13.08	7.04 to 19.12		

***Mean comparison by ANNOVA Test ; IT test for equality of means , CI-confidence interval*

Table 3: Mean Ki-67 Labelling Index in Invasive and Non-invasive Urothelial Cancers

DISCUSSION

Most tumors including urothelial carcinomas features mutations in cell cycle regulatory genes and uncontrolled cell proliferation.(4) Ki-67 LI assessed immunohistochemically cell growth fraction and aggressiveness of malignancy due to abnormal cell proliferation and disruption in cell cycle. Ki-67 nuclear antigen is encoded by gene on chromosome 10 in proliferating cells only.(4,5) In the present study immunohistochemical low and high Ki-67 expression was taken with cut off value of 13 % nuclear positivity as it is more extrapolative in relation to tumor progression in previous studies.(4,6)

In this study, we evaluated and correlated Ki-67 LI of urothelial neoplasm (n= 60 cases) with various clinico-pathological variables (age, gender, tumor size

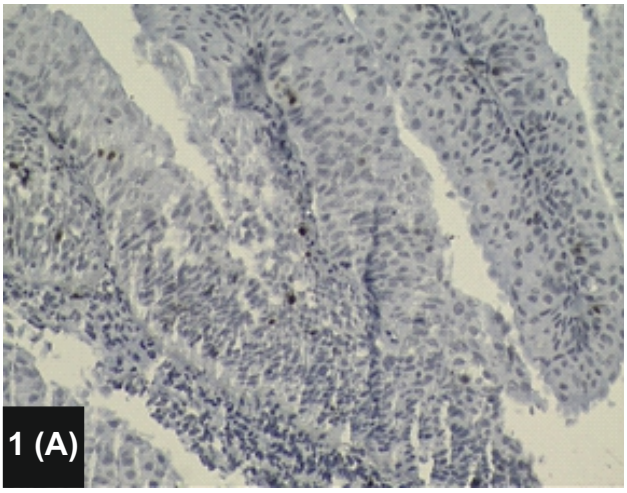
,tumour grade and depth of invasion).

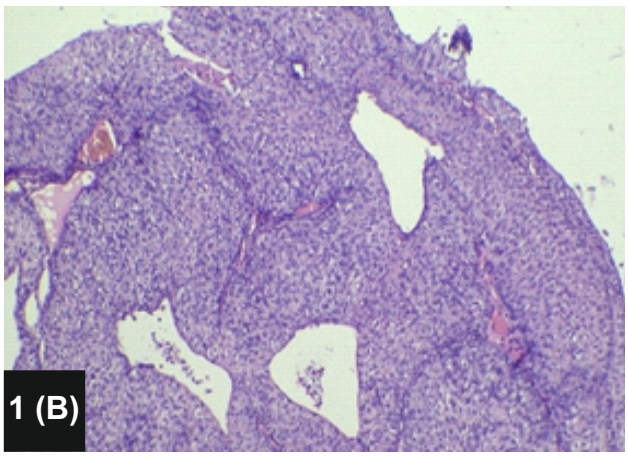
The urothelial neoplasm was more in males (n =53 cases) and in higher age greater than 65 years (n=34 cases). A comparable results of gender distribution in Urothelial Neoplasm was attained in a study by Thakur et al (7) and age distribution was found in the result of other studies by Gupta et al (8) and Biswas RR et al. (9)

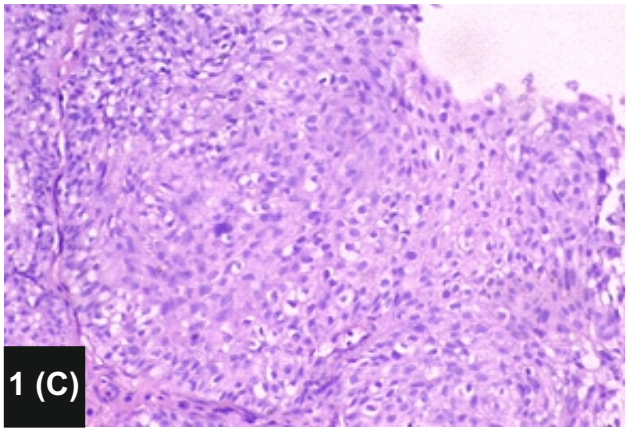
A high and low Ki67 labelling index was correlated in urothelial tumors and its clinicopathological parameters. This study shows maximum high Ki-67 expression (>13%) in invasive cancers i.e. 40%, whereas low (<13%) was seen maximum in non-invasive cancers i.e. 38.3% and high tumor proliferation (>13%) determined by Ki-67 overexpression was related to greater tumor size. Age and gender were not statistically significantally correlated with Ki-67 LI. This result was analogous with studies done by Lujia Wang et al.(10) Quintero, et al.(11)

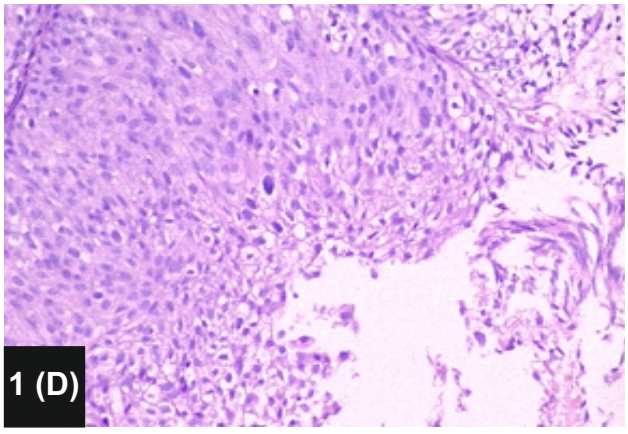
On comparison of mean Ki-67 labelling index in non-invasive and invasive neoplasm, a statistically significant expression was observed that increased from papilloma to PUNLMP, from Low grade to High grade in non-invasive urothelial neoplasm, and from lamina propria invasion to muscle invasion in urothelial neoplasm. The above outcome was in consonance with study done by Quintero, et al., which showed that high tumour proliferation suggested by Ki-67 labelling index could be independent predictor of stage progression in urothelial tumors.(11) Study done by Badawia B Ibrahim et al., Stec, R et al., showed significant correlation between Ki-67 LI expression with histopathological differentiation of urothelial tumor which is in concordance with our study.(2,12)

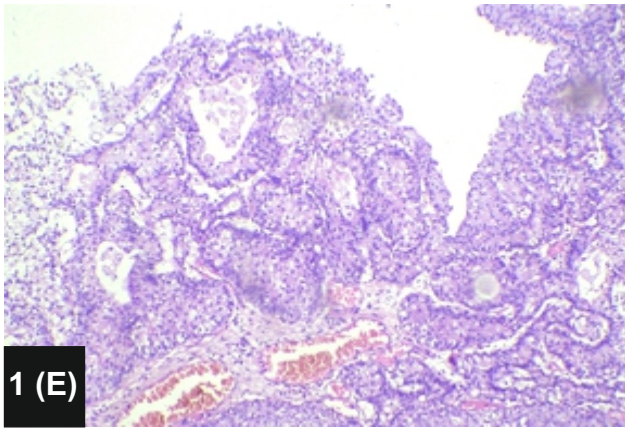
A statistically significant correlation was seen of pathological stage (pT) and expression of Ki-67 by Rehman P et al. However the morphological variants were not significantly associated with it, which correspond with our study.(13)

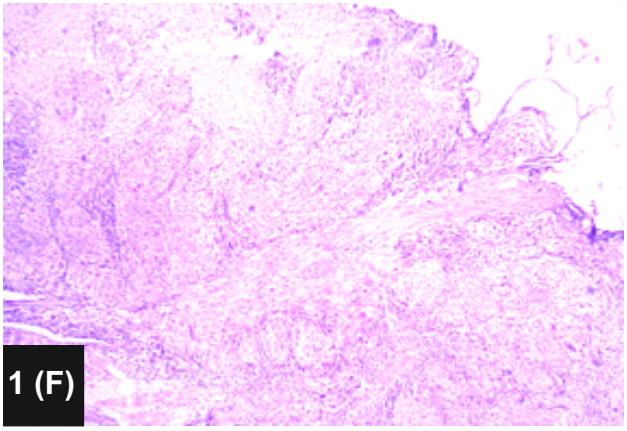


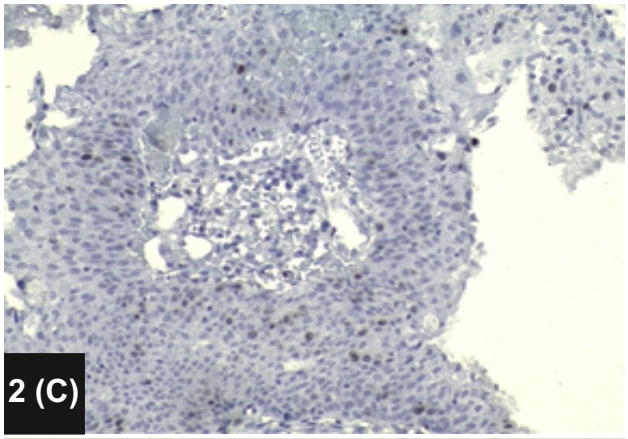












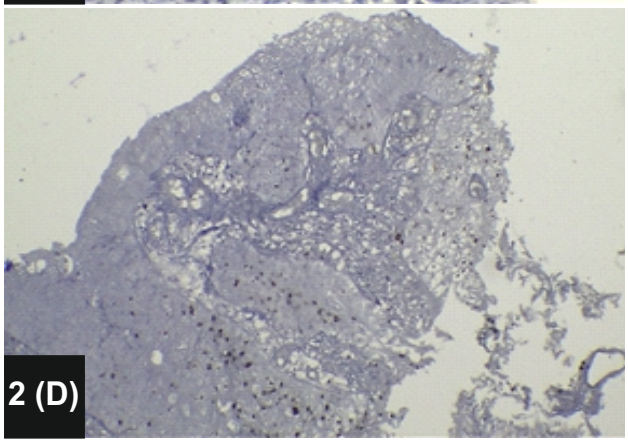
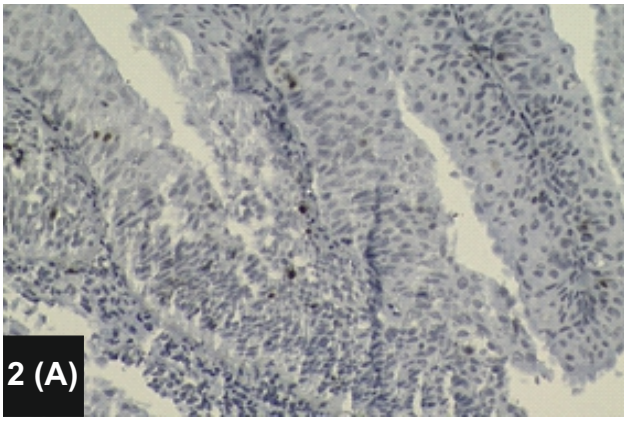
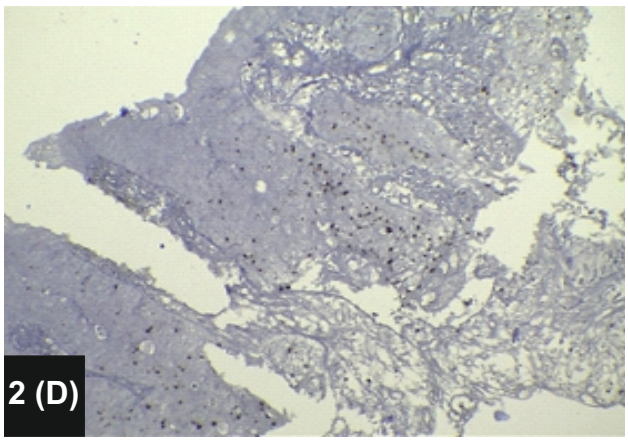
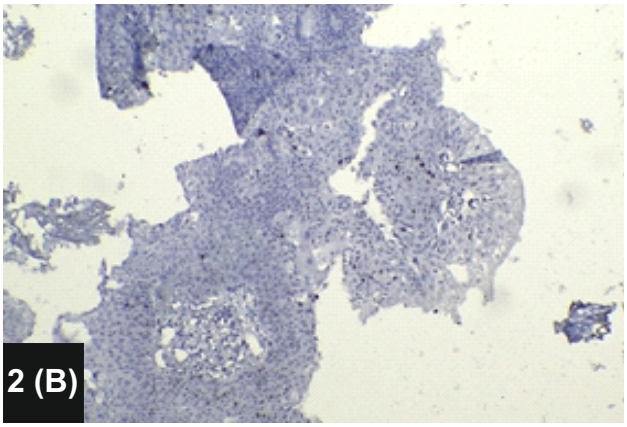


Fig. 1: Photomicrograph H&E Staining, 40X (A) Papilloma showing discrete papillary structures, hierarchical branching but without fusion, (B) PUNLMP showing epithelial lining of fibrovascular cores which is thicker than normal urothelium with preserved polarity and no atypia. (C) Non-Invasive Urothelial Tumor Low Grade showing mild cytologic atypia with regard to polarity, nuclear size, shape and chromatin (D) Non-Invasive Urothelial Tumor High Grade showing disordered architecture and cytologic pleomorphism (E) Invasive Urothelial Carcinoma showing invasion into the lamina propria (F) Invasive Urothelial Carcinoma showing deep invasion of the detrusor muscle.







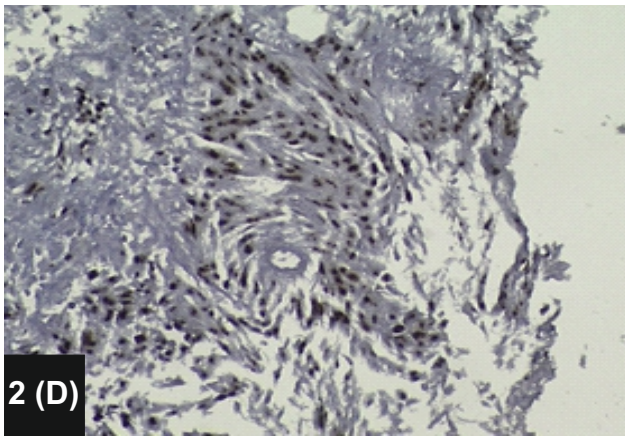


Fig. 2: IHC, 100X (A) Papilloma showing very low expression of Ki-67 (B) PUNLMP showing low expression of Ki-67 (C) Non-Invasive Urothelial Tumor Low Grade, showing low expression of Ki-67 (D) Non-Invasive Urothelial Tumor High Grade, showing intermediate expression of Ki-67 (E), (F) Invasive Urothelial Carcinoma showing high expression of Ki-67

CONCLUSION

Ki-67 labelling index being a measure of tumor proliferation is related to tumor histological grade. Large urothelial tumor size was associated to high Ki-67 LI and was not strongly associated with age and gender. Therefore Ki-67 expression can be used as Diagnostic & Prognostic marker in urothelial tumors.

ABBREVATIONS

PUNLMP- Papillary Urothelial Neoplasm of Low B Malignant Potential
LI - Labelling Index
LP - Lamina Propria
IHC - Immunohistochemistry

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How to cite this article:

Kaur R., Gupta P., Agarwal V., Mathur A. Ki-67 Labelling Index In Urothelial Neoplasm. Era J. Med. Res. 2023; 10(1): 13-18.