Original Research Paper

Volume - 13 | Issue - 04 | April - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

ASSESSMENT OF EXPRESSION OF P16 IN UROTHELIAL CARCINOMA AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL FEATURES

Anushka Khanuja	PG Resident Pathology Sri Aurobindo Medical College & Post Graduate Institute
Pooja Kesharwani	PG Resident Pathology Sri Aurobindo Medical College & Post Graduate Institute
Sanjana Ahuja*	Associate professor Pathology Sri Aurobindo Medical College & Post Graduate Institute *Corresponding Author
Bela Sharda	Associate professor Pathology Sri Aurobindo Medical College & Post Graduate Institute
Amit V. Varma	Professor & Head Pathology Sri Aurobindo Medical College & Post Graduate Institute
Harshita Deep Sahu	PG Resident Pathology Sri Aurobindo Medical College & Post Graduate Institute

ABSTRACT **Background** p16 is the protein product of most commonly involved gene in bladder carcinogenesis. Therefore, an immunohistochemical study was performed to assess the expression of p16 in urothelial carcinoma and its association with histopathological features. **Methods** p16 immunohistochemistry was performed on 117 cases of urothelial carcinoma and its association with tumor grade, lamina propria and muscle layer invasion was noted. **Results** Sixty nine (59%) cases showed high grade morphology, whereas 48 (41%) showed low grade morphology. Lamina propria invasion was seen in108 (92.3 %) cases and muscle layer invasion was noted in 63 (53.9%) cases. Low expression of p16 was noted in 62 (53%) cases while high expression was seen in 55 (47 %) cases. Significant association of p16 expression was noted with tumor grade (p-value 0.00001) and muscle layer invasion (p-value 0.00001). **Conclusion** p16 is an important biomarker that can be used for prognostic stratification of patients with bladder carcinoma.

KEYWORDS : P16, Urothelial Carcinoma, Muscle Layer Invasion.

INTRODUCTION

Urothelial carcinoma is the most common type of malignancy in the urinary tract and can arise anywhere in the urothelial lining, from the renal pelvis to the urethra.[1] On the basis of cellular morphology, it is classified into low grade or high grade. On the basis of invasiveness, invasive or non-invasive.

Cyclins, cyclin dependent kinases (CDK) and their inhibitors are the key regulators of cell cycle progression and hence are the prognostic markers for survival, recurrence and progression. There are two families of CDK inhibitors, INK4 (p16, p15,p18 and p19) and CIP/KIP (p21, p27 and p57), that regulate proliferation of cells and are important for preventing tumor formation.[2]

p16 is encoded by *CDKN2A* gene (9p21.3) which prevents cell cycle progression into the S phase by inhibiting cyclin D dependent protein kinases (CDK4 and CDK6),thus, maintaining Rb in its hypo-phosphorylated state which prevents its dissociation from E2F transcription factor.[3]This protein expression is often increased in aging cells, which eventually drives cell death and apoptosis. Chromosome 9p21 is the region which is most commonly altered in the onco-pathogenesis of bladder cancer.[4]p16 is the most important gene which is either deleted or mutated in this process. p16 is a tumor suppressor gene; therefore loss of its function results in abnormal cell proliferationleading to cancer development.[5]However, the prognostic significance of p16 is still unclear. Moreover, p16 mutations or protein expression patterns have not been studied in our population in bladder cancer. In the present study, an immunohistochemical study was performed to assess the expression of p16 in urothelial carcinoma and its association with histopathological features.

MATERIALS AND METHODS

This analytical cross-sectiona lstudy was done in the Department of Pathology at a tertiary care centre, after obtaining approval from the Institutional Ethical Committee (letter no. SAIMS/RC/IEC/2021/224). Paraffin blocks of confirmed bladder tumors archived in the Department of Pathology, in the past 6 years, from January 2017 to December 2022 were retrieved and processed for immunohistochemistry with monoclonal antibodies to p16. Histomorphological reporting with all relevant clinical and radiological details was done by a consultant pathologist. One hundred one cases of transurethral resection of bladder tumors (TURBT) and 16 representative blocks of cases of cystectomy were studied. The immunohistochemical findings with the presence or loss of staining, intensity of staining and percentage of positive cells were recorded and results were interpreted accordingly. Block positivity with both nuclear and cytoplasmic staining was considered positive.

The staining intensity of tumour cells labelled by p16 were scored as follows:

- Score 0: No staining
- Score 1+: Weak
- Score 2+: Moderate
- Score 3+: Strong

Percentage of positively stained cells was scored from 0 - 100. Then the weighted score (H-score) was obtained by multiplying the percentage score by intensity score.[6-10]An H-score of less than 200 was considered as low p16 expression while an H-score of more than or equal to 200 was taken as high p16 expression.[4]

Statistical package for social sciences (SPSS 28 Trial Version) was used for data compilation and analysis. Mean and standard deviation were calculated for quantitative variables. Frequency and percentage were calculated for qualitative variables. Independent t-test was used to compare mean difference. Pearson's Chi-square test was applied to determine association. p-value of ≤ 0.05 was considered statistically significant.

RESULTS:

Out of a total of 117 cases, 48 (41%) were females and 69 (59%) were males with male to female ratio of 1.4:1. Mean age of patients was 57.4 years. Sixteen (13.7%) cystectomy cases and 101 (86.3%) TURBT cases were included in the study. Sixty nine (59%) cases showed high grade (Figure1) morphology, whereas 48 (41%) showed low grade morphology. Lamina propria invasion (Figure 2) was seen in108 (92.3 %) cases and muscle layer invasion (Figure 3) was noted in 63 (53.9%) cases. Low expression of p16 (Figure 4) was noted in 62 (53 %) cases while high expression (Figure 5) was seen in 55 (47 %) cases. Significant association of p16 expression was noted with tumor grade (p-value 0.00001; Table 1) and muscle layer invasion (p-value 0.00001 ;Table 2). Other parameters like age and sex were not associated with p16 expression.

Table 1 -Association of p16 expression with histopathologicaltumorgrade.

Histopathological Grade	High p16 Expression	Low p16 Expression	Total	P value
High	15	54	69	0.00001
	27.3%	87.1%	58.9%	
Low	40	08	48	
	72.7%	12.9%	41.1%	
Total	55	62	117	
	100.0%	100.0%	100.0%	

Table 2 – Association of p16 expresssion with muscle layer invasion.

Muscle Layer Invasion	High p16 Expression	Low p16 Expression	Total	P - Value
Absent	46	08	54	0.00001
	83.7%	12.9%	46.1%	
Present	09	54	63	
	16.3%	87.1%	53.9%	
Total	55	62	117	
	100.0%	100.0%	100.0%	

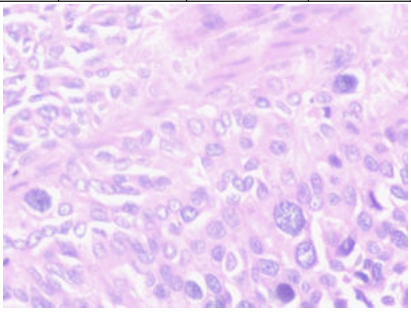

Figure 1 : H&E (40 X): High grade urothelial carcinoma showing numerous bizarre cells.

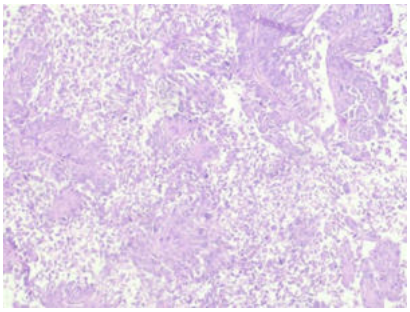

Figure 2 : H&E (10X): Tumor infiltrating the lamina propria.

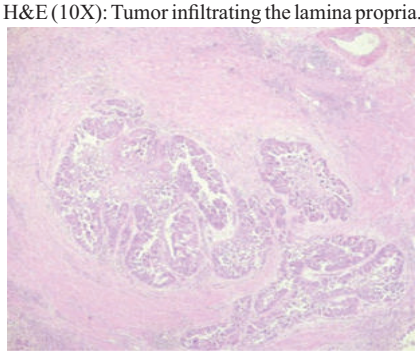

Figure 3 : H&E (4X): Tumor showing muscle layer invasion.

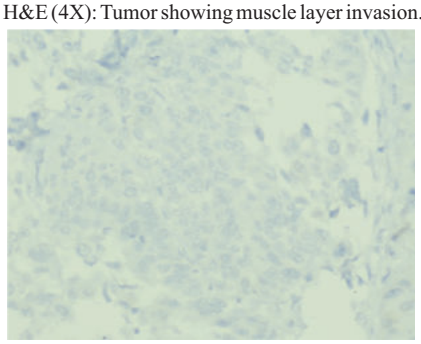

Figure 4 : IHC p16 (40X): Loss of p16 expression- H-SCORE: 0

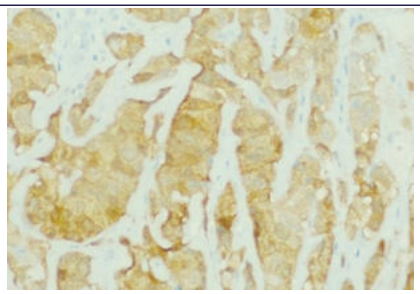

Figure 5 : IHC p16 (40X): High expression H-SCORE: 285 (Intensity: 3+ x % of Tumor cells positive for p16: 95%)

DISCUSSION

Cell cycle progression is controlled by a system of CDKs and CDKIs. Hence, reduced CDKI production causes uncontrolled cell cycling. Low expression of p16 is a known marker of adverse prognosis in many human cancers, particularly cancer of the pancreas, esophagus, head and neck.[11] Because the CDKI p16 is a tumor suppressor protein that binds to cyclin/cdk4 or cyclin/cdk6 complexes, it blocks their kinase activity and inhibits progression to the S phase of the cell cycle.[12]

In the present study, increased p16 expression was seen in low grade urothelial carcinomas (40/48; 72.7%) whereas high grade urothelial carcinomas (54/69; 87.1%) showed reduced p16 expression, which is in concordance with the study done by Ching-Hsiu Yang et al[11] wherein 91% of low grade urothelial carcinomas showed high p16 expression. Some previous studies have shown that reduced p16 expression predicts recurrence of urothelial carcinoma, [13-15] which is in concordance with observations of the present study that p16 expression correlates negatively with invasiveness and grade in urothelial carcinoma. A meta-analysis of 37 studies that included 2246 patients revealed that reduced p16 expression was linked to poor prognosis in patients of urothelial carcinoma.[16]Mutations involving p16 gene can be in the form of deletions of chromosome 9, in that case there would be complete loss of IHC expression of p16 protein.[4] Conversely, inactivated or mutated gene product is generally not easily digestible leading to abnormally high expression as it is noted in the case of p53 (another tumor suppressor gene product) which is known to be overexpressed (immunohistochemically) in endometrial and ovarian serous carcinoma. [4] In the study done by Hashmi et al,[4] significant association of high expression of p16 with poor prognostic parameters of urothelial carcinoma could be due to aberrant protein expression as a result of underlying gene mutations. In the study done by Raspollini et al,[17] strong expression of p16 was noted in 28.2% cases while 71.8% did not show p16 staining and the expression of p16 was statistically associated with disease stage (p - 0.026) but not with tumor grade or disease progression.

These observations call for further studies with larger sample size to focus the importance of p16 expression in the development/ progression of urothelial carcinoma.

CONCLUSION

p16 is an important biomarker that can be used for prognostic stratification of patients with bladder carcinoma. Molecular studies should be performed to evaluate patterns of p16 gene mutations for better understanding of disease pathogenesis in our population.

REFERENCES

1. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V. et al. EAU Guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. *Eur Urol.* 2017;71(3):447–461.
2. Ding L, Cao J, Lin W, Chen H, Xiong X, Ao H, Yu M, Lin J, Cui Q. The Roles of Cyclin-Dependent Kinases in Cell-Cycle Progression and Therapeutic Strategies in Human Breast Cancer. Int J Mol Sci. 2020 Mar 13;21(6):1960.
3. Sherr CJ, Roberts JM. Living with or without cyclins and cyclin-dependent kinases. *Genes Dev.* 2004;18:2699–2711.
4. Hashmi, A.A., Hussain, Z.F., Irfan, M. *et al.* Prognostic significance of p16 immunohistochemical expression in urothelial carcinoma. *Surg Exp Pathol* 2, 3 (2019).
5. Cairns P, Polascik TJ, Eby Y, Tokino K, Califano J et al (1995 Oct) Frequency of homozygous deletion at p16/CDKN2 in primary human tumours. Nat Genet. 11(2):210–212.
6. Cregger M, Berger AJ, Rimm DL. Immunohistochemistry and quantitative analysis of protein expression. *Arch Pathol Lab Med.* 2016;130:1026–30.
7. Remmele W, Schicketanz KH. Immunohistochemical determination of estrogen and progesterone receptor content in human breast cancer, Computer-assisted image analysis (QIC score) vs. subjective grading (IRS) *Pathol Res Pract.* 1993;189:862–6.
8. Matos LL, Stabenow E, Tavares MR, Ferraz AR, Capelozzi VL, Pinhal MA. Immunohistochemistry quantification by a digital computer-assisted method compared to semiquantitative analysis. Clinics. 2016;61:417–24.

9. Revision B. IHC Nuclear Image Analysis User's Guide, MAN-0027. 2007.
10. Han CP, Lee MY, Tzeng SL, Yao CC, Wang PH, Cheng YW et al. Nuclear Receptor Interaction Protein (NRIP) expression assay using human tissue microarray and immunohistochemistry technology confirming nuclear localization. *J ExpClin Cancer Res*. 2008;27:25.
11. Ching-Hsiu Yang, Chun-Chieh Wu, Wan-Tzu Chen, Chee-Yin Chai, Sheau-Fang Yang. Expressions of p16 and p27 in urothelial carcinoma and their prognostic value. Kaohsiung Journal of Medical Sciences. 2014; 30:453-458.
12. Rocco JW, Sidransky D. p16(MTS-1/CDKN2/INK4a) in cancer progression. Exp Cell Res. 2001;264:42-55.
13. Friedrich MG, Blind C, Milde-Langosch K, Erbersdobler A, Conrad S, Loning T, et al. Frequent p16/MTS1 inactivation in early stages of urothelial carcinoma of the bladder is not associated with tumor recurrence. EurUrol 2001;40:518-24.
14. Korkolopoulou P, Christodoulou P, Lazaris A, Thomas-Tsagli E, Kapralos P, Papanikolaou A, et al. Prognostic implications of aberrations in p16/pRb pathway in urothelial bladder carcinomas: a multivariate analysis including p53 expression and proliferation markers. EurUrol 2001; 39: 167-77.
15. Orlow I, LaRue H, Osman I, Lacombe L, Moore L, Rabbani F, et al. Deletions of the INK4A gene in superficial bladder tumors. Association with recurrence. Am J Pathol 1999;155: 105-13.
16. Gan X, Lin X, He R, Lin X, Wang H, Yan L, Zhou H, Qin H, Chen G (2016) Prognostic and Clinicopathological significance of downregulated p16 expression in patients with bladder Cancer: a systematic review and metaanalysis. Dis Markers 2016:5259602.
17. Maria Rosaria Raspollini et al. p16(INK4a) expression in urinary bladder carcinoma. Arch Ital UrolAndrol. 2006 Sep.