ORIGINAL ARTICLE DOI:https://doi.org/10.52206/jsmc.2023.13.4.812 OPEN ACCESS

P16 Expression in Grading of Urothelial Carcinoma

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ABSTRACT

Background: P16 is an important biomarker studied in patients of cancer. Association of p16 expression with the nature of urothelial carcinoma may prove useful in the future for targeted therapies.

Objective: To evaluate the frequency p16 expression in patients diagnosed with urothelial cell carcinoma and correlation with clinic pathological parameters.

Material and Methods: A cross-sectional study was conducted using nonprobability convenience sampling technique at histopathology department from December 2022 to May 2023 by recruiting 98 biopsy specimens from patients diagnosed with urothelial cell carcinoma. Immunohistochemical analysis for p16 expression and their clinicopathological correlations were the parameters recorded.

Results: Mean age of the participants was 63.37 ± 7.34 years with72(73.5%) males and 26(26.5%) females. Immunohistochemistry studies revealed low expression of p16 in 81(82.7%) tissue specimens while 17(17.3%) revealed high expression of p16. Tumor grading in patients showed 61(62.2%) specimens with low grade morphology while 37(37.8%) specimens were of high-grade tumors. Clinicopathological correlations with p16 frequency of expression revealed that high expression of p16 was associated more with higher grades of tumor (p-0.013) as compared to low grades of tumor. Similarly, tumors with invasion of lamina propria or muscular propria had significantly high expression of p16 in comparison of non-invasive tumors with a p-value of <0.05.

Conclusion: High expression of p16 in urothelial carcinoma is more frequently associated with higher grade of tumor, invasion of lamina propria or muscular propria which may lead to poor prognosis. This may prove beneficial for molecular studies and targeted therapies in the future.

Keywords: Biomarkers, Tumor, Urinary bladder neoplasm

This article may be cited as: Sajjad A, Basharat S, Javaid F, Safdar A, Mahmood R, Khattak MT, et al. P16 Expression in Grading of Urothelial Carcinoma. J Saidu Med Coll Swat 2023;13(4):180-185. DOI:https://doi.org/10.52206/jsmc.2023.13.4.812

INTRODUCTION

Urothelial carcinoma is the most common histological type of bladder cancer with a high risk of morbidity and mortality. Higher predisposition of urothelial carcinoma in male patients is thought to be linked with increased exposure to carcinogens. Urothelial carcinomas have a greater propensity to invade and metastasize the surrounding structures resulting is morbidity and mortality.[1]

Out of all the bladder cancers diagnosed 90% are urothelial carcinomas. Risk factors which can predispose to urothelial carcinoma can be genetic, environmental, occupational, dietary, infection, chemicals, gender, ethnicity and socioeconomic factors.[2] Advances in the field of diagnostic histopathology has led to the use of several tumor markers to predict the behavior and prognosis of bladder cancer. Interaction of cyclin dependent kinase and their inhibitors play a vital role in regulation of cell cycle. P16 is a cyclin Dependent kinase inhibitor and any mutation, methylation or deletion of p16 can lead to uncontrolled activity of cyclins leading to rapid growth and multiplication of cancerous cells.[3,4]

The discovery of novel marker like p16 have enabled the health care workers for prompt screening in carcinomas with increasing prevalence and complicated clinical behavior. The utility of useful markers can serve as effective diagnostic and therapeutic agent for the treating physicians and surgeons to commence treatment earlier in the course of invasive diseases. Several trials have been conducted to evaluate the role of p16 as a prognostic tool in predicting the prognosis of several neoplasms.[5] Variable expression of p16 in different carcinomas has paved a way to predict the behavior and invasiveness of the tumor. For instance, expression of p16 have been found to be associated with good prognosis and lesser invasiveness in head and neck tumors.[6] Loss of p16 tumor marker on immunohistochemistry in esophageal tumors revealed invasiveness and metastasis leading to poor prognosis and poor outcomes.[7]

The relation of p16 gene mutation and immunohistochemistry staining of p16 is dubious. However, it is thought that p16 mutations lead to

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Received: September 05th, 2023 - 1st Revision: September 26th 2023
2nd Revision: December 08th 2023 - Accepted: December 10th, 2023

increased expression of p16 as it is overproduced or develops resistance to breakdown resulting in overexpression on immunohistochemistry (IHC) and increased tumorigenesis. Human papilloma virus (HPV) is a known cause of cervical cancer and urothelial carcinoma, the utility of IHC overexpression of p16 is a surrogate marker of cervical cancer associated with HPV. In case of HPV associated urothelial cell carcinoma the overexpression of p16 was not evident and hence proposed that p16 is not reliable to be used as a surrogate marker for the detection of HPV associated urothelial carcinoma.[8]

Merkel cell carcinoma, small cell carcinoma, uterine and urothelial carcinoma have been studied for the expression of p16 and the prognostic role of p16 in all these carcinomas cannot be negated. However, the expression of p16 in different carcinomas is variable and requires further research for validation. This paper aims at evaluating the frequency of p16 expression on immunohistochemical studies in patients diagnosed with urothelial cell carcinoma and correlation with clinicopathological parameters.

MATERIAL AND METHODS

After approval of the ethical review committee of institute on 27th October 2022 under ERC # RMI/RMI-REC/ Article Approval / 75, this cross-sectional study was conducted at histopathology department from 1st December 2022 to 31st May 2023. Calculation of sample size was performed using WHO sample size calculator by employing a 5% level of significance, 80 % power of test and an estimated prevalence of 3%[9] bladder cancer.

We included a total of 98 participants in our study employing non-probability consecutive sampling technique. On the basis of history examination and laboratory investigations patients diagnosed with urothelial carcinoma underwent histopathological screening of the biopsied tissues. All samples were subject to assessment for clinicopathological parameters like tumor grading, invasion of lamina propria and muscular propria which were recorded on a Performa. Patients of either gender diagnosed with urothelial cell carcinoma were prospectively recruited in the study. Patients who had carcinomas other than urothelial cell carcinoma were excluded from this study.

Immunohistochemistry

Tissue specimens from patients diagnosed as cases of urothelial cell carcinoma were received in sterile containers by histology department. Specimens were formalin fixed and embedded in paraffin which were examined by a classified histopathologist after recording demographic characteristics of the patients. All specimens were stained with eosin and Hematoxylin as per the standard protocol of our laboratory. Morphological characteristics of the tumor tissue including grade of the tumor, invasion of lamina propria and muscular propria were the recorded parameters. Deparaffinization using xylene followed by hydration with decreasing concentration of alcohol. As per the manufacturer's instructions immunohistochemistry studies were performed for p16 staining in all the specimens using antibody CIN tec P16INK4a clone E6H4 (manufacturer-Cell marque, manual staining). Known cases of HPV associated squamous cell carcinoma with nuclear and cytoplasmic positivity were taken as positive controls. Nuclear and cytoplasmic staining was assessed in all tissue specimens under microscopic guidance and details recorded. The staining intensity of the cells for no staining, weak, intermediate or strong staining was recorded as 0, +1,+2 and +3. Similarly, the percentage of stained cells ranging from minimum of 0 to 100 was recorded. Using these two recorded parameters Histochemical scoring (H-scoring) was done utilizing nuclear positivity by multiplication of the two variables. For instance, a tumor with Intermediate intensity of staining (+2) with 100% staining of cells will have an H-score of 2 x 100 = 200 while a tissue specimen with strong staining in 100% cells will have an H-score of 3 x 100 = 300. The final H-score was recorded as low expression of p16 if it was = 200 and high expression of p16 for tumors with H-score of >200.

Data analysis and interpretation was performed by using SPSS version 23. Categorical variables like gender, grades of p16 expression, tumor grade and invasion of the lamina propria and muscular propria were presented with frequencies and percentages while mean and standard deviation was calculated for scale variables like age in years. Chi square test was applied for categorical and independent t-test was used for continuous data with a p-value of =0.05 considered as significant. Data was presented using figures and tables.

RESULTS

Total 98 specimens of patients diagnosed with urothelial cell carcinoma underwent histopathological screening out of which 72(73.5%) were males and 26(26.5%) were females with a mean age of 63.37 ± 7.34 years. Immunohistochemistry studies revealed low expression of p16 in 81(82.7%) tissue specimens while 17(17.3%) revealed high expression of p16. Fig-1 shows the frequency of p16 expression among participants. Tumor grading in patients showed 61(62.2%) specimens with low grade morphology while 37(37.8%) specimens were of high-grade tumors. Demographic and pathological characteristics are shown in Table-I. Clinicopathological correlations with p16 frequency of expression revealed that high expression of p16 was associated more with higher grades of tumor (p-0.013) as compared to low grades of tumor. Similarly, tumors with invasion of lamina propria or muscular propria had significantly high expression of p16 in comparison of non-invasive tumors with a p-value of <0.05 as shown in Table 02.

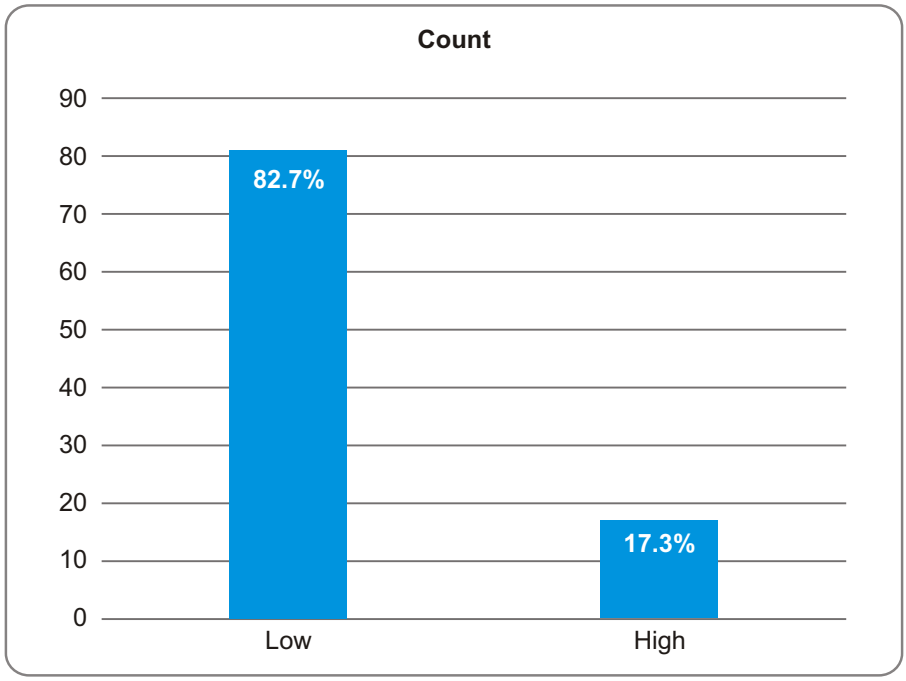


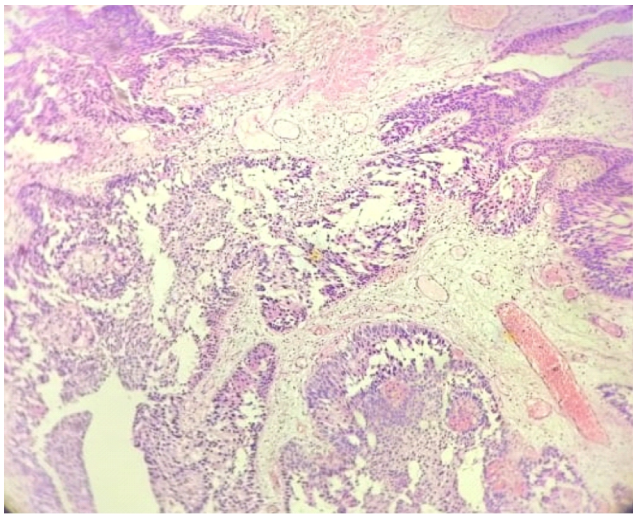
Figure 01. Percentage p16 expression among participants.

Table 01. Demographic and pathological characteristics of patients (n=98)

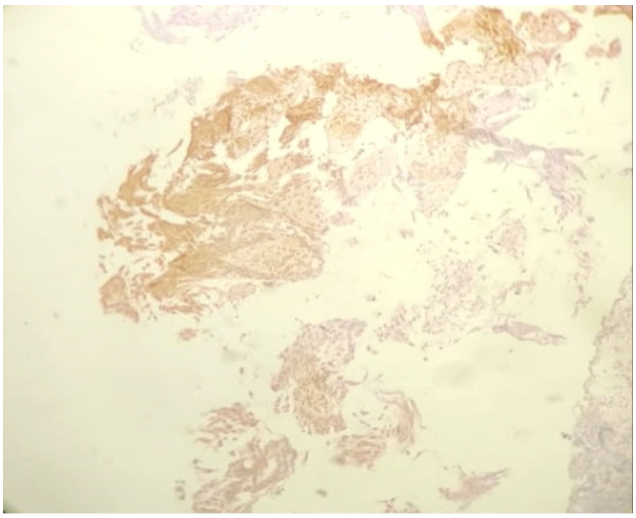
Variables	Subgroups	n(%)
Gender	Males	72(73.5%)
	Females	26(26.5%)
Age in years (Mean ± S.D)		63.37 ± 7.34
Tumor grade	Low grade	50(51%)
	High grade	48(49%)
Invasion of muscular propria	Cannot be assessed	29(29.6%)
	Present	19(19.4%)
	Absent	50(51%)
Invasion of Lamina Propria	Present	27(27.6%)
	Absent	71(72.4%)

Table 02. P16 immunohistochemistry and clinicopathological correlations (n=98)

Variables		Low expression n(%)	High expression n(%)	p- value
Gender	Males	60(74.1%)	12(70.6%)	0.767
	Females	21(25.9%)	05(29.4%)	
Age group	=50years	03(3.7%)	02(11.8%)	0.170
	>50years	78(96.3%)	15(88.2%)	
Tumor grade	Low grade	46(56.8%)	04(23.5%)	0.013
	High grade	35(43.2%)	13(76.5%)	
Invasion of muscular propria	Cannot be assessed	26(32.1%)	03(17.6%)	<0.01
	Present	09(11.1%)	10(58.8%)	
	Absent	46(56.8%)	04(23.5%)	
Invasion of lamina propria	Present	17(21%)	10(58.8%)	0.002
	Absent	64(79%)	07(41.2%)	



Invasive urothelial carcinoma, high grade



p16 diffuse positivity in high grade urothelial carcinoma

DISCUSSION

In our research the expression of p16 was evaluated and it was concluded that high expression of p16 on immunohistochemistry was more frequently associated with tumors of high grade, invasion of lamina propria or muscular propria. This significant association predicts the poor prognosis of patients which requires prompt intervention. Several trials have been conducted for the evaluation of p16 in urothelial cell carcinoma and a variable pattern of expression has been observed. For instance, in a study conducted by Hashmi AA et al, results were similar as ours and high expression of p16 in urothelial cell carcinoma was significantly observed in tumors exhibiting invasion of muscular propria or lamina propria.[10] Moreover, high expression of p16 was linked with poor prognosis and a logistic regression analysis revealed that patients exhibiting low expression of p16 have a lower risk of mortality as compared to participants with high expression of p16. In a similar study which was conducted to evaluate p16 and p27 expression in patients with urothelial cell carcinoma revealed different results.[11] Yang CH et al concluded that high expression of p16 in urothelial cell carcinoma was not associated with tumor invasiveness or high-grade. P16 is cyclin D kinase inhibitor having genes located at chromosome 9.

In another study which was conducted to evaluate the genetic alterations in p16 genes it was concluded that p16 chromosome deletion was most seen in Taiwanese patients with upper tract urothelial carcinoma and high levels of p16 was observed in patients with evidence of nodal metastasis with a likelihood of poor prognosis in the later stage.[12] The diagnostic and prognostic utility of p16 was observed in several carcinomas by Carneiro FP et al. P16 overexpression was found in 88% of all the carcinomas including cervical, ovarian breast and lung carcinomas.[13] P16 overexpression was observed in different histological tumors like small cell carcinoma of lung, squamous cell cervical carcinoma and urothelial carcinoma.[13] These results stress the importance of p16 immunohistochemistry studies for patients presenting with metastatic tumors which can help the health care workers to predict the behavior of metastatic tumors earlier in the course.[14]

The novel marker is effective indicator for differentiating a dysplastic cervical lesion from benign tumors. All tumors with high grade intraepithelial lesions revealed 100% p16 positive staining on immunohistochemistry analysis as compared to benign conditions, signifying a superior role as tumor marker for morphological differentiation of the tumor.[15] Hence a wide range of conclusions, prognostic implications, histological differentiation of tumors and way forward to molecular studies and targeted therapies can be employed by examining immunohistochemistry for p16.[16,17]

Several modalities are in practice and under research with regards to a special emphasis on utility of p16 as a tumor marker.[18] The emerging role of p16 as a tumor marker served an important role in the stratification of our patients in regards of tumor progression. This can prove helpful in commencing early intervention and targeted therapies for better outcomes with decreased morbidity and mortality in patients of urothelial cell carcinoma. Novel therapies are under research which include antibodies targeted against p16 expression which can help seize the mitotic activity of the cancerous cells while protecting the normal cells.[19] Such innovations in the field can revolutionize and decrease the morbidity and mortality in a number of tumors that express p16.[20]However, it is proposed that cells may develop resistance against such therapies and a number of complications may be seen in patients treated with such antibodies. Nonetheless, these therapies are promising in the time to come for the better outcome of cancer patients.

Limitations of the Study

Our research included a small number of patients, and this was a single center study. The follow up of patients may have been useful for giving additional information. Effect of treatment on p16 expression and its association with other biomarkers would have been more conclusive.

CONCLUSION

High expression of p16 in urothelial carcinoma is more frequently associated with higher grade of tumor, invasion of lamina propria or muscular propria which may lead to poor prognosis. This may prove beneficial for molecular studies and targeted therapies in the future.

Acknowledgements

We are grateful to our professors who always support and guide us. And we are very obliged to our technical staff who helped us in the technical work despite their heavy workload.

Ethical Consideration

This study was approved by the ethical board of Rehman Medical Institute, Peshawar, Pakistan.

Funding

There is no role of any funding agency in this study.

Conflict of Interest

Authors declare no conflicts of interest.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

AUTHOR'S CONTRIBUTION

The following authors full fill authorship criteria as per ICMJE guidelines;

Sajjad A, Basharat S:	Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.
Javaid F, Safdar A:	Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.
Mahmood R, Khattak MT:	Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.
Khan IM:	Analysis and interpretation of data, critical revision,final approval, agreed to be accountable for all the work.

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