

Original Research Article

IMMUNOHISTOCHEMICAL EVALUATION OF P63 EXPRESSION IN THE GRADING OF UROTHELIAL NEOPLASMS

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ABSTRACT

Background: Urothelial carcinoma (UC) is the most common malignancy of the urinary bladder and demonstrates wide morphological and biological variability. Interobserver variability is still a problem, despite the fact that tumor grading is essential for prognosis and treatment choices. It has been suggested that p63, a nuclear transcription factor involved in epithelial development, might be used as an additional marker to grade urothelial neoplasms. The aim is to evaluate the immunohistochemical expression of p63 in varying grades of urothelial neoplasms and to assess its correlation with histopathological grade and clinicopathological parameters.

Materials and Methods: This laboratory-based prospective and retrospective observational study included 50 cases of primary urothelial carcinoma diagnosed between July 2015 and June 2019 at the Department of Pathology, Dhanalakshmi Srinivasan Medical College and Hospital. Formalin-fixed paraffin-embedded tissue sections were subjected to immunohistochemical staining using mouse monoclonal antibody against p63 (clone 4A4). Nuclear staining in more than 10% of tumor cells was considered increased expression, while less than 10% was considered decreased expression. Statistical analysis was performed using SPSS version 21.0, and Chi-square test was applied with $p < 0.05$ considered significant.

Results: High-grade urothelial carcinoma constituted 62% of cases, while 38% were low grade. Increased p63 expression was predominantly observed in low-grade tumors (64.3%), whereas decreased expression was mainly associated with high-grade tumors (95.5%). A statistically significant inverse correlation was found between p63 expression and tumor grade ($\chi^2 = 18.662$, $p = 0.000$). No significant association was observed between p63 expression and age or tumor stage.

Conclusion: p63 expression shows a significant inverse correlation with histological grade of urothelial carcinoma. Decreased expression is associated with high-grade tumors, suggesting its utility as an adjunct immunohistochemical marker in grading urothelial neoplasms. Larger studies with long-term follow-up are recommended to further establish its prognostic significance.

Keywords: Urothelial carcinoma; p63; Immunohistochemistry; Histological grading; Bladder cancer; Tumor differentiation; WHO/ISUP classification; Muscle invasion; Prognostic marker.

INTRODUCTION

Urothelial carcinoma (UC) is the most common malignancy of the urinary bladder and can arise

anywhere along the urothelial lining from the renal pelvis to the distal urethra. It accounts for more than 90% of bladder cancers and represents a significant global health burden.^[1-3] According to global cancer

statistics, bladder cancer is among the ten most common cancers worldwide, with a marked male predominance, being three to four times more frequent in men than women.^[5,6] The incidence increases with advancing age and is most commonly diagnosed in individuals over 50 years, with a median age in the seventh decade.^[4]

Several environmental and genetic risk factors contribute to the development of urothelial carcinoma. Cigarette smoking is the most well-established etiological factor and is responsible for nearly half of bladder cancer cases.^[5] The risk is further increased by occupational exposure to benzidine, beta-naphthylamine, petrochemicals, paint products, rubber industries, and aromatic amines.^[6] Other contributory factors include long-term catheterization, recurring UTIs, and chronic discomfort from bladder stones. Another factor is genetic susceptibility, which includes mutations in N-acetyltransferase 2 (NAT2) and polymorphisms in glutathione S-transferase genes (GSTM1, GSTT1).^[7] About 20% of patients who appear with painless gross hematuria go on to receive a bladder cancer diagnosis, making it the most prevalent presenting symptom in clinical settings. From non-invasive papillary tumors to highly invasive carcinomas with the potential to spread, the illness spectrum is broad. Urothelial neoplasms can be generally categorized histologically as papillary and flat lesions. Non-invasive papillary lesions are divided into four categories by the World Health Organization/International Society of Urological Pathology (WHO/ISUP) classification system (2016): papilloma, low-grade urothelial carcinoma, high-grade urothelial carcinoma, and papillary urothelial neoplasm of low malignant potential (PUNLMP).^[8] Cytological atypia and architectural pattern are used to grade. High-grade tumors are linked to aggressive behavior and a higher risk of invasion and metastasis, whereas low-grade tumors often have a favorable prognosis with high recurrence but low advancement rates. Even among seasoned pathologists, grading of urothelial neoplasms is linked to considerable interobserver heterogeneity despite established morphological criteria. Finding trustworthy immunohistochemistry markers that are correlated with tumor grade and biological behavior is therefore crucial.

Encoded on chromosome 3q27–29, p63 is a nuclear transcription factor that is a member of the p53 gene family. It is essential for the growth and upkeep of epithelium. p63 is crucial for basal cell proliferation in the normal urothelium and is highly expressed in the basal and intermediate cell layers.^[9] TAp63 and ΔNp63 variants with different biological roles are produced by alternate promoter use and splicing of the TP63 gene, which results in various isoforms. TAp63 mainly acts as a tumor suppressor, activating death receptor pathways and mitochondrial apoptotic mechanisms to promote cell cycle arrest and apoptosis. On the other hand, by preventing apoptosis and encouraging cell division, ΔNp63 can have an

oncogenic effect. This dual function adds to p63's intricate behavior during carcinogenesis.

The expression patterns of p63 in urothelial neoplasms change according to the invasiveness and grade of the tumor. According to a number of studies, high-grade invasive carcinomas usually show decreased or lost p63 expression, while low-grade papillary urothelial carcinomas show retained or enhanced p63 expression.^[9] It is believed that enhanced biological aggressiveness and tumor dedifferentiation are related to this reduction of p63 expression.

A possible prognostic marker that correlates with known prognostic factors like tumor stage and grade is impaired p63 expression. Evaluation of p63 expression may offer further diagnostic and prognostic information, especially in situations where morphological grading is difficult, as treatment choices for bladder cancer are mostly dictated by tumor grade and stage.

Immunohistochemical assessment of p63 may be used as a supplementary method in the stratification of urothelial neoplasms, considering the important clinical consequences of tumor grading in deciding on treatment options and forecasting recurrence and progression. Therefore, in order to better understand p63's involvement in tumor biology and its potential value in regular diagnostic practice, it is imperative to research its expression across various grades of urothelial malignancies.

Aims and objectives

Primary Objective:

- To evaluate the immunohistochemical expression of p63 in varying grades of urothelial neoplasms.

Secondary Objectives:

- To assess the correlation between p63 expression and histopathological grade of urothelial carcinoma.
- To evaluate the association of p63 expression with morphological parameters in urothelial neoplasms.
- To determine whether altered p63 expression can serve as an indicator of tumor aggressiveness.

MATERIALS AND METHODS

Source of Data: The source of data included histopathologically confirmed cases of primary urothelial neoplasms diagnosed in the Department of Pathology at Dhanalakshmi Srinivasan Medical College and Hospital (DSMCH). Cases were retrieved from the departmental archives for the period July 2015 to June 2019. Hematoxylin and eosin (H&E)-stained slides and tissue blocks that were formalin-fixed and paraffin-embedded (FFPE) were acquired. Clinical information was gathered from pathology reports and case sheets, including age, sex, and surgical operation type. After receiving clearance from the DSMCH Institutional Ethics Committee, the study was started.

Study Design: This was a laboratory-based prospective and retrospective observational study focusing on the immunohistochemical expression of p63 in urothelial neoplasms and its correlation with histopathological grading.

Study Location: The study was conducted in the Department of Pathology, Dhanalakshmi Srinivasan Medical College and Hospital (DSMCH).

Study Period: The study was carried out between April 2018 and September 2019.

Sample Size: A total of 50 cases of primary urothelial carcinoma fulfilling the inclusion criteria were included in the study.

Inclusion Criteria

1. Cystoscopy-guided biopsy, transurethral resection of bladder tumor (TURBT), and cystectomy specimens diagnosed as urothelial neoplasm.
2. Availability of adequate archival tissue in formalin-fixed paraffin-embedded blocks for immunohistochemical analysis.

Exclusion Criteria

1. Benign urothelial lesions.
2. Non-urothelial carcinomas.
3. Recurrent tumors.
4. Insufficient tissue blocks.
5. Unsatisfactory or equivocal immunostaining due to technical factors.

Procedure and Methodology: Primary urothelial carcinoma cases satisfying the selection criteria were retrieved from departmental archives. All H&E slides were reviewed to reconfirm the diagnosis and classify tumors according to WHO/ISUP grading criteria into low-grade and high-grade urothelial carcinoma. Histopathological parameters such as architectural pattern, cytological atypia, and depth of invasion were evaluated. Immunohistochemistry (IHC) for p63 was performed on all selected cases.

Sample Processing: All tissue specimens had been fixed in 10% neutral buffered formalin and routinely processed. Sections of paraffin blocks, each 4

microns thick, were cut out and placed on positively charged slides. Antigen retrieval was carried by using heat. Mouse monoclonal antibody against p63 (clone 4A4, PathnSitu; ready-to-use) was used to incubate the sections. Diaminobenzidine (DAB) was used as the chromogen in the PolyExcel Horse Radish Peroxidase (HRP) polymer system for detection. Brown staining of the nucleus was seen as positive. To provide quality control, prostate tissue served as a positive control in every staining session.

Immunohistochemical Evaluation of p63: For assessment of p63 expression, at least 500 tumor cells were evaluated in high-power fields (400×) in areas showing maximum staining.

Only strong nuclear staining was considered positive.

Scoring criteria were as follows:

- <10% of tumor cells showing nuclear staining → Decreased expression
- >10% of tumor cells showing nuclear staining → Increased expression

The p63 expression pattern was then correlated with histopathological grade (low grade vs high grade urothelial carcinoma).

Data Collection: Clinical details (age and gender) and histopathological parameters (tumor grade and stage) were recorded in a master chart. p63 immunohistochemical expression was documented and correlated with tumor grading to determine its association with tumor differentiation.

Statistical Analysis: The collected data were coded and entered into Microsoft Excel and analyzed using SPSS (Statistical Package for Social Sciences) version 21.0.

- The association between p63 expression and tumor grade was assessed using the Chi-square test.
- Correlation of p63 expression with age and gender was also analyzed.
- A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1: Distribution of Histological Grades of Urothelial Carcinoma (n = 50)

Histological Grade	Number of Cases (n)	Percentage (%)
Low Grade	19	38%
High Grade	31	62%
Total	50	100%

[Table 1] shows the distribution of histological grades among the 50 cases of urothelial carcinoma included in the study. With 31 instances (62%), high-grade urothelial carcinoma made up the majority, while low-grade carcinoma accounted for 19 cases (38%). This suggests that high-grade cancers are more common in the current research sample.

Majority of low-grade tumors showed increased p63 expression, whereas most high-grade tumors showed decreased p63 expression. The association between p63 expression and histological grade was statistically significant (p < 0.05).

[Table 2] illustrates the correlation between p63 expression and histological grade. Only one instance (4.5%) had decreased p63 expression (<10%) among the 19 low-grade cancers, whereas 18 cases (64.3%) had increasing expression (>10%). In contrast, 10 instances (35.7%) had increasing p63 expression and 21 cases (95.5%) had decreased p63 expression among the 31 high-grade cancers. All things considered, 28 instances (56%) had increased expression and 22 cases (44%) had decreased expression. There is an inverse relationship between p63 expression and histological grade, as seen by the

statistically significant link between p63 expression and tumor grade ($\chi^2 = 18.662$, $p = 0.000$).

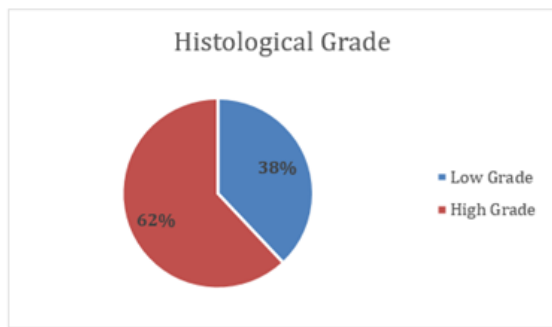


Figure 1: Histological Grade

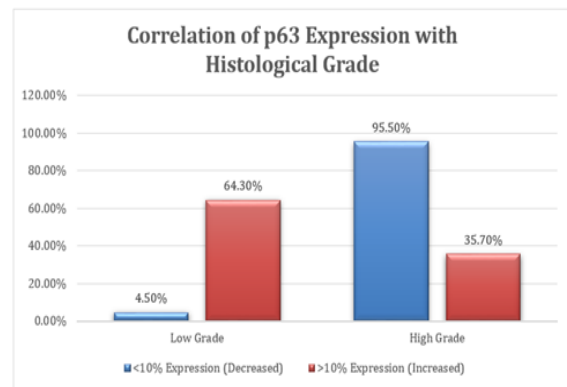


Figure 2: Correlation of p63 Expression with Histological Grade.

Table 2: Correlation of p63 Expression with Histological Grade

Histological Grade	<10% Expression (Decreased)	>10% Expression (Increased)	Total	Statistical Inference
Low Grade	1 (4.5%)	18 (64.3%)	19	$\chi^2 = 18.662$ $p = 0.000$
High Grade	21 (95.5%)	10 (35.7%)	31	
Total	22 (44%)	28 (56%)	50	Significant

Table 3: Correlation of p63 Expression with Age Groups

Age Group	<10% Expression	>10% Expression	Total	Statistical Inference
20–40 yrs	0 (0%)	2 (7.1%)	2	$\chi^2 = 2.227$ $p = 0.328$
40–60 yrs	10 (45.5%)	9 (32.1%)	19	
>60 yrs	12 (54.5%)	17 (60.7%)	29	Not Significant
Total	22	28	50	

p63 expression did not show a statistically significant association with age group.

[Table 3] presents the correlation between p63 expression and different age groups. Out of the 50 cases, 29 (58%) were older than 60, 19 (38%) were between 40 and 60, and 2 (4%) were between 20 and 40. There was no discernible trend in the distribution of decreased and increased p63 expression across all age groups. Age and p63 expression did not significantly correlate, according to statistical analysis ($\chi^2 = 2.227$, $p = 0.328$).

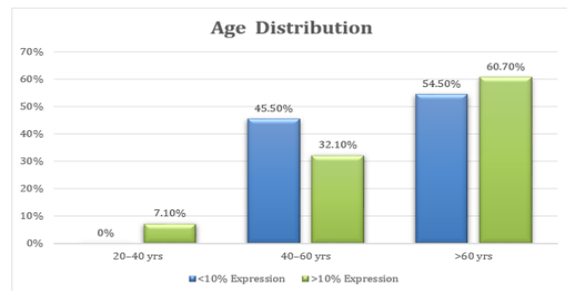


Figure 3: Age Distribution.

Table 4: Correlation of p63 Expression with Stage of Tumor (n = 22*)

*Muscle tissue included specimens only

p63 Expression	Ta/T1	T2 & Above	Total	Statistical Inference
<10%	4 (57.1%)	9 (60%)	13	$\chi^2 = 0.016$ $p = 0.899$
>10%	3 (42.9%)	6 (40%)	9	
Total	7	15	22	Not Significant

p63 expression did not show a statistically significant correlation with tumor stage.

[Table 4] shows the correlation between p63 expression and tumor stage in 22 muscle tissue-included specimens. Of them, 15 cases were T2 and above, and 7 cases were Ta/T1 stage. Four Ta/T1 cancers (57.1%) and nine T2 and higher tumors (60%) showed decreased p63 expression. Three instances (42.9%) of Ta/T1 and six cases (40%) of T2 and higher cancers showed increased expression. There was no statistically significant correlation between tumor stage and p63 expression ($\chi^2 = 0.016$, $p = 0.899$).

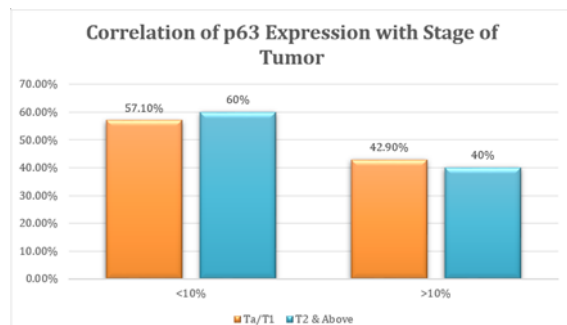


Figure 4: Correlation of p63 Expression with Stage of Tumor

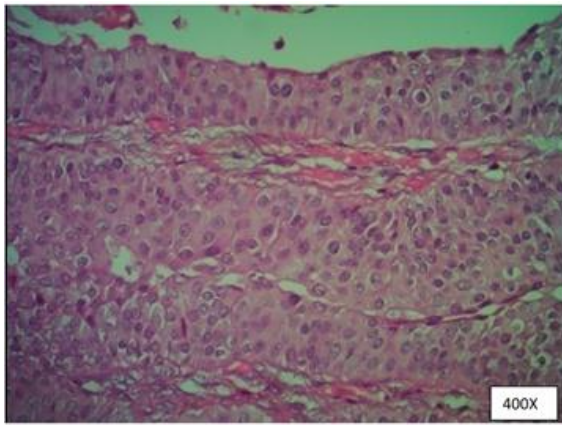


Figure 5: Low grade urothelial carcinoma H&E

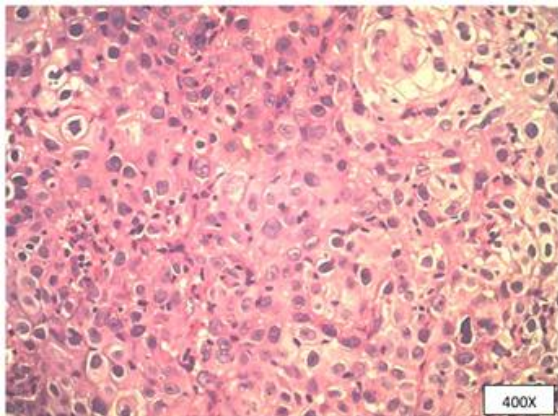


Figure 6: High grade urothelial carcinoma H&E

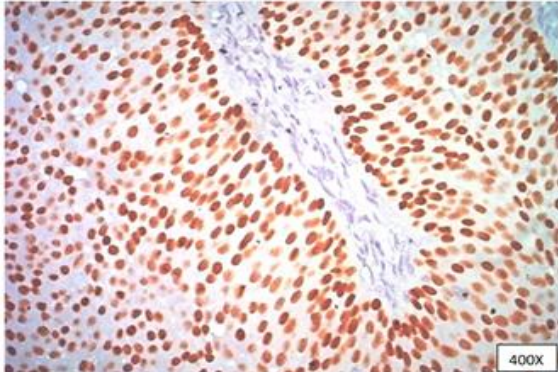


Figure 7: Increased expression (>10%) of p63 in low grade urothelial carcinoma

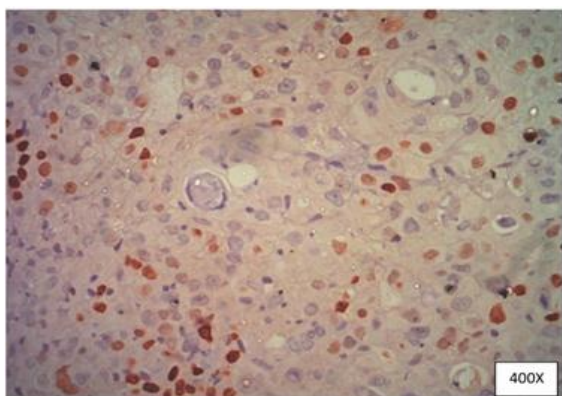


Figure 8: High grade urothelial carcinoma -decreased (< 10%) expression of p63

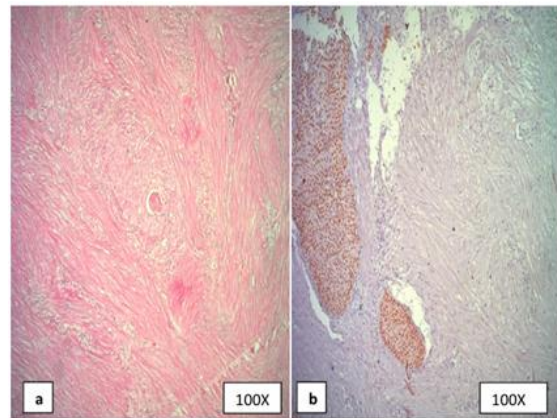


Figure 9: Low grade urothelial carcinoma - muscle invasion a) H&E b) IHC expression of p63 in tumour cells.

DISCUSSION

The present study was undertaken to evaluate the immunohistochemical expression of p63 in urothelial carcinoma and to determine its correlation with histological grade and other clinicopathological parameters such as age and tumor stage. Fifty instances of primary urothelial cancer were examined in this investigation. [Table 1] indicates that the majority of cases (62%, n=31) were high-grade urothelial carcinoma, whereas 38% (n=19) were low-grade tumors. Institutional investigations have shown similar patterns, with high-grade lesions predominating, perhaps as a result of delayed presentation to tertiary care facilities.^[10,11] This study's main goal was to evaluate the relationship between histological grade and p63 expression. Of the 50 cases, 28 instances (56%) exhibited increased expression (>10%) of p63, whereas 22 cases (44%) showed reduced expression (<10%). Histological grade and p63 expression were shown to be significantly correlated (Table 2, $\chi^2 = 18.662$, $p = 0.000$).

P63 expression was higher in the majority of low-grade urothelial carcinomas (64.3%, n=18) and lower in the majority of high-grade carcinomas (95.5%, n=21). Tumor dedifferentiation and aggressive biological activity are linked to loss of p63 expression, according to this statistically noteworthy inverse connection. Elnashar et al. and Sayed Abdel Raheem and Afaf T. published similar results, showing that high-grade tumors have lower p63 expression than low-grade lesions.^[12,13]

In stratified epithelia, including the urothelium, p63, a nuclear transcription factor that is a member of the p53 gene family, is essential for basal cell maintenance and epithelial differentiation.^[14] The majority of p63 expression in normal urothelium is seen in basal and parabasal cells. When basal cell differentiation is disrupted and a poorly differentiated phenotype develops in high-grade malignancies, p63 expression is lost or reduced.^[14,15] Consequently, a reduction in p63 expression might be a sign of the advancement of a tumor. Given the strong correlation

shown in [Table 2], p63 may be a helpful supplementary immunohistochemical marker for differentiating between low- and high-grade urothelial carcinoma, especially in instances with difficult morphology. These results are in line with earlier research that emphasized p63's function as a differentiation marker in urothelial cancer.^[12,13]

[Table 3] assessed the relationship between p63 expression and age. There was no statistically significant correlation between p63 expression and age groups ($\chi^2 = 2.227$, $p = 0.328$). Despite the fact that most instances were seen in individuals over 60, there was no discernible age-related variation in the p63 expression pattern. Elnashar et al. observed similar findings, finding no significant correlation between p63 expression and demographic factors.^[12] These results imply that tumor biology is better represented by p63 expression than by patient-related variables. 22 muscle tissue biopsy specimens were evaluated for tumor stage and invasiveness. Of them, seven were non-muscle invasive and fifteen were muscle invasive. 60% of muscle-invasive tumors and 57.1% of non-muscle-invasive tumors had reduced p63 expression, as seen in [Table 4]. But according to $\chi^2 = 0.016$, $p = 0.899$, this connection was not statistically significant. Koyuncuer published similar results, finding no significant relationship between tumor invasiveness and p63 expression.^[15]

These findings show that although p63 expression has a strong correlation with histological grade, it is not a reliable indicator of tumor stage or muscle invasion. Since high-grade cancers were more likely to invade muscles than low-grade tumors, tumor grade itself is still highly correlated with invasive potential. Prior research has documented comparable findings concerning grade and invasiveness.^[11,16] Overall, the results of this investigation show a strong negative relationship between the histological grade of urothelial cancer and p63 expression [Table 2]. Low-grade cancers had increased p63 expression, whereas high-grade lesions primarily displayed reduced expression. Nevertheless, there was no discernible correlation seen between p63 expression and either tumor stage [Table 4] or age [Table 3]. As a result, p63 is a helpful supplementary immunohistochemistry marker for urothelial neoplasm grading. Its expression is not indicative of invasive potential, but rather of tumor differentiation. It is advised to do larger research with longer follow-up to elucidate its predictive value.

CONCLUSION

The present study evaluated the immunohistochemical expression of p63 in urothelial carcinoma and its correlation with histological grade and clinicopathological parameters. Males and individuals older than 60 had higher rates of urothelial cancer. Most of the instances were high-grade tumors. Low-grade urothelial carcinoma was mostly found to have increased p63 expression,

whereas high-grade tumors were primarily found to have reduced expression. Tumor grade and p63 expression were shown to be statistically inversely correlated, suggesting that aggressive behavior and tumor dedifferentiation are reflected in the decrease of p63 expression. Nevertheless, neither the tumor's muscle invasiveness nor the patient's age were significantly correlated with p63 expression. According to these results, p63 is a helpful supplementary immunohistochemical marker for classifying urothelial neoplasms and might help detect tumors that are physiologically aggressive. To confirm its therapeutic usefulness and prognostic value, further trials with longer follow-up are needed.

Limitations of the study

The present study has certain limitations. The sample size was relatively small ($n = 50$), which may limit the generalizability of the findings. Because the study was limited to one tertiary care facility, it might not be representative of the general community. The statistical analysis of the relationship between p63 expression and muscle invasiveness may have been impacted by the fact that only 22 patients had enough muscle tissue to determine the tumor stage. It was not possible to evaluate the prognostic importance of p63 expression in terms of recurrence, progression, and overall survival since the study lacked long-term follow-up data. Furthermore, there was no analysis of interobserver variability in immunohistochemical interpretation or histological grading. Additionally, no molecular correlation investigations were conducted. The clinical and prognostic importance of p63 expression in urothelial neoplasms should be further confirmed by larger multicentric investigations that include molecular analysis and long-term follow-up.

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