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# THE SIGNIFICANCE OF P53 IHC AS A MARKER IN DIAGNOSIS AND PROGNOSIS IN LOW GRADE AND HIGH GRADE UROTHELIAL CANCER

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**Abstract**

**Background** :
One of the most important approach for the diagnosis and determination of patient prognosis in urothelial cancer is the microscopical classification of papillary carcinoma . Overlap in the extent of the tumor may leads to some difficulties in the diagnosis of the tumor , so it is illogical to depend on morphology alone.

**Objective** :
We aimed in this paper to to evaluate P53 as an immunohistochemical marker and compared with pathological findings for the diagnosis and prognosis of urothelial cancer.

**Methods**:
Our study was conducted in Ghazi Al-Hariri hospital. Baghdad , Iraq , during the period between March and September, 2021 . Samples from Urinary bladder were collected from 35 patient with urothelial carcinoma who had undergone surgery. Biopsy samples referred to histopathology department. Immunohistochemical Staining of P53 was performed and correlated to the histopathological indices of the tumor.

**Results:**
Out of 35 cases , 20 patients were diagnosed as a low grade and 15 patients as a high grade urothelial carcinomas . P53 specificity was 87% , while the sensitivity was 46% . There was a positive correlation noted between immunohistochemical findings and tumor grade . Out of 15 cases of high grade tumor , 11 cases had strong positive p53 staining , three cases had weak staining , and one case of high grade was negative . Out of twenty cases of low grade tumor , 15 cases were weak staining for p53 stain, three cases had strong p53 stain, and two cases were negative.

**Conclusion**: Addition of p53 immunohistochemical staining to the morphology can be helpful in the diagnosis and prognosis of patient with urothelial carcinomas .

**Keywords:** urothelial cancer, p53, grade , immunohistochemistry.

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Introduction

Urothelial cancer is one of the commonest malignancies worldwide . About 336,000 new cases being diagnosed yearly. This malignancy can occurs in both sexes and at any age but it predominantly affects middle and old age males. There are many risk factors participate in the development of urothelial carcinoma , the most common one is smoking. In smokers, the risk of urothelial carcinoma is higher than that in general population (3 -5-fold).

The incidence showed a declining approximately 1.6 fold after discontinuation of smoking (1-7). The role of cigarette smoking in the development of bladder cancer is still not clear. There are many carcinogenic factors have been discovered in tobacco (e.g , arylamine, oxygen free radicals and 4-aminobenzyle) (8- 12). Other responsible factors like drugs (e.g , analgesia, cyclophosphamide ), chemicals ,diet rich in fat, and arsenic-containing water, also reported as causative agents. additionally , chronic infections (schistosomiasis), fungi, radiation and stones might be predisposing factors (13). Hematuria is the predominant presenting symptom . Twenty percent of patients with gross hematuria have been diagnosed as urothelial cancer , while this diagnosed was given for about 10% of patients with microscopic hematuria (14-17) .

According to World Health Organization (WHO) , urothelial carcinomas are classified into low and high grades (18-20) . This classification which is generally accepted (25,30), is basically depend on the degree of anaplasia which is characterized by "hypercellularity, hyperchromasia, loss of cell polarity and loss of cell differentiation from the basement membrane to the cell surface, nuclei pleomorphism, variation in cell size, abnormal mitosis, chromatin pattern, , and giant cell formation" (21-24). From the clinical point of view, for each tumor type there is a specific treatment line and eventually this will affect patient prognosis . One of the common problems in the diagnosis of urothelial carcinomas is the overlap between the grades of the tumor, therefore, the precise microscopical diagnosis of the carcinoma is not all the time straight forward. So, we need additional methods to distinguish between low and high grades of the tumor.

The aim of our study was to accurately assess the tumor grade by using P53 immunohistochemistry (IHC) in correlation with the morphological findings to affirm the diagnosis and eventually select a specific management line and better follow-up approach .

**Material and Methods**

This study was conducted in Ghazi Al-Hariri teaching hospital , Baghdad , Iraq during the period from March to September, 2021. Samples Selected and taken from 35 patients with bladder cancer who underwent surgery. Biopsy samples referred to histopathology department. Demographic information was recorded. The biopsy samples were selected , 20 low grade and 15 high grade cases were included. P53 IHC staining and the histopathologic findings were assessed (in relation to the tumor grade wether low or high). Immunohistochemical Staining and P53 expression was completed "by using the KIT (anti- p53) polyclonal antibody (1:500,DAKO,carpinteria,CA) with 4-µm cut sections thickness of tissue. Deparaffinization, rehydration , antigen unmasking by boiling and then using of 3% H2O2 for 20 minutes to inhibit internal peroxidase activity".

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P53 positive and negative cut-off points were categorized according to the intensity of staining and suitability of cells (percentages of cytoplasmic and nuclear staining) as follows :
Positive staining : cell staining >5% .
Mild Staining : cell staining 5-10 % .
Strong staining : cell staining > 50% .
Moderate staining in between these ranges (5) .
Statistical Analysis : “Statistical significance was determined by Fisher’s and Chi-square analysis for categorical variables". "categorical data were described as frequency (%) with a 95% confidence interval in 5 normal groups. Chi-square test was also used to analyse the difference between the indices".

**Results**

Out of 35 patients , 30 patients were males and 5 patients were females. The mean age was 55.6 ± 11.6 year.
Table-1 showes the relation ship between the intensity of P53 staining and the tumor grade .
Out of 15 cases of high grade tumor, 11 cases had a strong positive p53 staining , three cases had a weak staining, and one case was negative.
Out of twenty cases of low grad tumor, 15 cases had a weak staining for p53 stain, three cases had a strong p53 staining , and two cases had a negative staining . Furthermore, there was no statistically significant linkage noted between the tumor depth of invasion and P53 expression (Table 2).
P53 specificity was 87% with a low sensitivity (46%) comparing with low and high morphological parameters of the tumor. Positive predictive value was 72.5% and negative predictive value was 65.6%. So; P53 intensity of expression were very important for urothelial carcinomas diagnosis, meanwhile; negative results were not determinant .

**Discussion**

P53 tumor suppressor gene mutation have been reported in many types of malignancies . In urothelial cancer ، P53 overexpression is thought to be linked to worse prognosis. Inspite of a large number of reports , still there is a confusion regarding this subject, P53 immunohistochemical staining was performed on biopsy samples after making slides and staining. According to the results, P53 specifity was 87% for the low- and high-grade papillary carcinomas, and the sensitivity was 46%. In addition, there were a strong association between the immunohistochemical stain and the grade of the tumor, but there was no a statistically significant correlation noted with the muscle depth of invasion . Many previous studies in the same field investigate the diagnostic and prognostic role of P53 in urothelial carcinomas . In Toll et al. study, "invasive and non-invasive cases of papillary carcinoma were immunohistochemically stained for Ki67, P53, E-Cadherin, and CK20" and they reported that, there was no a statistically significant difference noted between the invasive and non-invasive tumors concerning IHC staining (31).
In Roychowdhury et al. study, "P53 staining was performed on high and low grade papillary carcinoma cases. They found that the expression of P53 plays an important role in suppression.of neoplastic

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transformation by cell cycle arrest or apoptosis so it work as tumor suppressor gene". (32). Additionally, Anadi et al. reported in their study that "P53 marker was strongly associated with the expression of high- and low-grade tumors" (33). Also , Shim et al. found that "immunohistochemically analysed the Ki67, P53, and CK20 markers. They observed that tumor grade and IHC results were significantly associated only for Ki67" (34).

Furthermore ; Mumtaz et al. Reported "a significant association between the grade of the tumor and IHC results (P53 and CK20 staining in both high and low-grade papillary carcinomas)" (5).

In Rajcani et al., study , "IHC analyses of Ki67, HMWCK, and P53 in cases of bladder carcinoma and chronic bladder inflammation showed a significant correlation between tumor grade and Ki67 and HMWCK markers". Additionally , they reported a positive P53 in premalignant changes of chronic inflammation (3) .

## Conclusion

According to our study which was consistent with most of previous reports , p53 specifity was 87% with low sensitivity (46%) comparing with low and high morphological parameters of papillary carcinoma . So ; positive values and the intensity of staining are important for the diagnosis and prognosis of urothelial carcinomas , but their negative values are not determinant.

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## Conflict of Interest

No conflict of interest was reported.

Table 1: Expression of P53 with the grade of the tumor.

| **Tumor grade** | **P53 expression** | | | **P value** |
|---|---|---|---|---|
| | Negative | Weak positive | Strong positive | |
| High grade | 1 | 3 | 11 | |
| Low grade | 2 | 15 | 3 | 0.001 |
| Total | 3 | 18 | 14 | |

Table-2 : Expression of P53 in relation to muscularis propria invasion.

| **P53 expression** | **muscularis propria invasion** | | | **P value** |
|---|---|---|---|---|
| | **Present** | **Absent** | **Muscularis propria not present** | |
| **Negative** | **1** | **2** | **0** | |
| **Weak positive** | **6** | **10** | **2** | |
| **Strong positive** | **4** | **9** | **1** | **0.23** |
| **Total** | **11** | **21** | **3** | |

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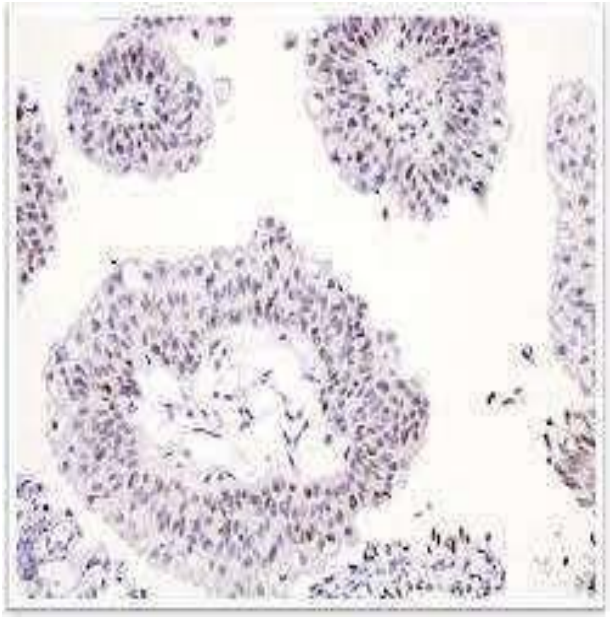

Figue-1: P53 IHC in low grade urothelial carcinoma (mild nuclear staining,10x)

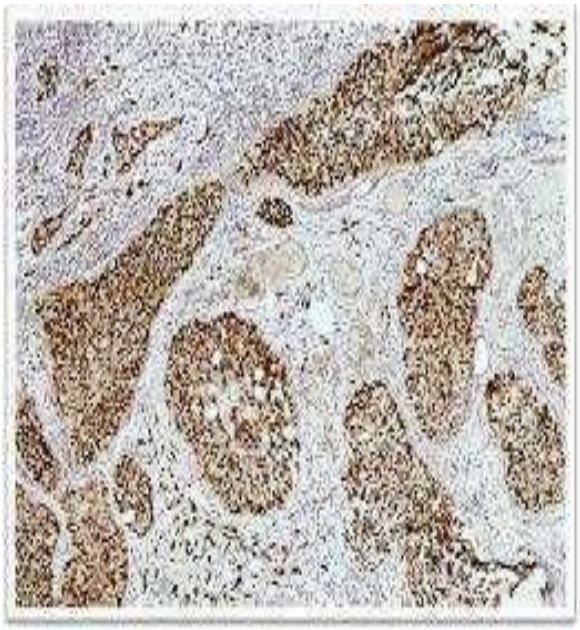

Figure-2: P53 IHC in High grade papillary urothelial carcinoma ( strong nuclear stain,10x)

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