IMMUNOHISTOCHEMICAL EXPRESSION OF P16 IN LOW GRADE UROTHELIAL CARCINOMA

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ABSTRACT

Objective: To determine the frequency of Immunohistochemical expression of P16 in low grade urothelial carcinoma patients.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from May 2018 to Jul 2019.
Methodology: A total of 120 formalin-fixed and paraffin embedded blocks from patients having low grade urothelial carcinomawere included in the study and were stainedimmunohistochemically with P16 antibody. Expression of P16 was noted by two independent pathologists and nuclear stain of strong intensity was taken as positive.
Results: There were 91 (76%) males and 29 (24%) females with age range from 18-85 years (mean 67.19 ± 11.5 years) female to male ratio was 1:3. P16 stain was positive in (70.8%) and negative in 37 (31%) of low gradeurothelial carcinoma cases.
Conclusion: The *p*-16 is expressed in a significant number of urothelial carcinomas (low grade) and this marker could be used in routine practice for early identification of patients at high risk of progression to advanced stage.

Keywords: Bladder cancer, Immunohistochemistry, Low grade P16 expression, Urothelial carcinoma.

INTRODUCTION

Urothelial carcinoma of the urinary bladderis one of the most common cancers in the world[1]. It's prevalence differs across the countries worldwide and also between different regions of same country[2]. Higher prevalence of Urothelial carcinoma has been documented in Pakistan. A local multicenter hospital-based analysis revealed that urothelial carcinoma is amongst the 10 leading cancers in men with ranked variation from center to center and is estimated to account for 4-5% of male cancer-related deaths[3].

Grading of the tumor is an important prognostic factor. Prevalence of urothelial cancer (low grade) is between 70-80%. Though having good prognosis it has high recurrence rates[4]. Approximately 30% of recurrent tumors progress by invading lamina propria. Lymph node metastasis and grading arehistorically established prognostic factors but these variables are limited to assess effectively that which patient will develop recurrence/metastasis[5].

The dynamic interaction of cyclins, cyclin-dependent kinases (CDKs) and its inhibitors regulate cell cycle progression. Those participating in the G1/S phase change, such as cyclin D1 and E, and CDK-inhibitors such as P16are the best predictive indicators for survival, relapse, and advancement[6]. The P16 allele acts as a tumor suppressor at chromosome 9P21, an area which is frequently mutated in different tumors 7, by negative regulation of the G1/S phase of the cell cycle with a number of malignancies showing P16 deletion. Researchers have shown that decreased expression of the P16 gene leads to unchecked progression of the cell cycleleading to abnormal cell division and fastertumorigenesis[7,8]. Identification of P16 expression directly correlates with tumor behavior and this may help in selecting patients for early aggressive therapy[9]. Thus, elucidation of immunohistochemical expression of P16 in low grade urothelial carcinomapatients is vital for predicting the tumor progression, thus tailoring the management accordingly.

To our knowledge, very few studies have been conductedon this subject but due to alterations in sample proportions, precisions of statistics and study populations the outcomes stay indecisive. Therefore, significance of this biomarker in low grade urothelial carcinoma is still un-clear.We therefore conducted a prospective study to determine the frequency of Immunohistochemical expression of P 16 in low grade urothelial carcinomain Pakistani population.

METHODOLOGY

This was a cross-sectional study performed in department of histopathology at Armed Forces Institute of Pathology, Rawalpindi, from May 2018 to July 2019 after approval from ethics committee [FC-HSP 16-1/READ-IRB/18/647] of Armed Forces Institute of

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Pathology. A total of 120 formalin-fixed and paraffin embedded block from patients having low grade urothelial carcinoma were includedand cases of carcinoma in situ, preoperative local or systematic anticancer neoadjuvant therapy and no transurethral resection or radical cystectomy were excluded. The sample size was calculated using the OPEN EPI calculatorby taking the prevalence of low gradeurothelial cancer i.e. 80%, margin of error=5%, level of confidence=95%, then a sample of 120 patients was includedusing non probability consecutive sampling technique. Grading was estimated according to AJCC cancer staging system[10].

All cases were stained by hematoxylin and eosin stain and the diagnosiswas confirmed by two independent pathologists. Allcases included in study were stained for P16 by immunohistochemistry using CIN tec P16INK4a, E6H4™ antibody as per manufacturer's instructions and nuclear staining was assessed. Staining was scored as no staining (0), weak (1+), intermediate (2+), strong (3+), While fraction of cells staining was scored from 0-100. H score was obtained by multiplying intensity and proportion scores. Two hundred was taken as cut off point with a score in excess of 200 being taken as high P16 expression and vice versa e.g moderate staining (2+) in 100% tumor cells (2 × 100 = 200) or strong staining (3+) in more than >69% tumor cells (3 × 69 = 207). Again, the cases were classified either as (positive pattern) or (negative pattern), a positive pattern was given to an unequivocal strong nuclear stain pattern in >25% of urothelial cells in the intermediate and deep layers of urothelium. While cases with (absence of nuclear staining or with a nuclear stain pattern in <25% of urothelial cells in the intermediate and deep layers) were consideredas negative.

SPSS-21 was used for statistical analysis. Mean and standard deviation was computed for age and tumor size. Percentage and frequencywas calculated for qualitative variable like gender, location of tumor, tumor invasiveness and immunohistochemical P16 expression in urothelial carcinoma. Stratification with respect to clinicopathological data was done to determine association of clinicopathological data and P16 expression. Post stratification chi-square test was applied to see the effect of of clinicopathological data on frequency of P16 expression. The *p*-value <0.05 was considered statistically significant.

RESULTS

Out of total 120 patients of urothelial carcinoma, 91 (76%) were men & 29 (24%) were women with age range from 18-85 years (mean 67.19 ± 11.5 years). The details of clinicopathological features of Urothelial Carcinoma are illustrated in table-I. Positive P16 immunohistochemical expression was observed in 83 (69%) of the patients with Urothelial Carcinoma, as shown in table-II. High and low P16 expressions were witnessed in 20 (16.6%) and 100 (83.4%) cases, respectively, as shown in table-III.

Table-I: Clinicopathologic features of urothelial carcinoma (n=120).

Clinicopathologic Features	n (%)
Age Groups	
<25 years	01 (1%)
25-50 years	24 (20%)
> 50 years	95 (79%)
Tumor Size (cm)	
<3	59 (49%)
>3	61 (51%)
Gender	
Male	91 (76%)
Female	29 (24%)
Location of Tumor	
Urinary Bladder	59 (49%)
Kidney	25 (21%)
Ureter	36 (30%)
Invasiveness of Tumor	
Present	51 (43%)
Absent	69 (57%)

Table-II: Frequency of Immunohistochemical expression of p16 in low grade urothelial carcinoma (n=120).

P16 expression	n (%)
Positive	83 (69%)
Negative	37 (31%)

Table-III: Intensity of p16 expressionin low grade urothelial carcinoma (n=120).

P16 Expression	n (%)
Low	100 (83.4%)
High	20 (16.6%)

P16 expression showed significant association with gender and intensity of P16 expression (p=0.006 and p≤0.001 respectively). Other factorsincluding age, size andlocation of tumorwere not associated with P16 expression, as shown in table-IV.

DISCUSSION

P16 is an essential CDK inhibitor whose in activation has been implicatedas a significant event in the tumorigenesis of low-grade urothelial bladder tumors[11]. The major rationale of P16 inactivation is removal and methylation of the P16 gene at the molecular genetic level[12]. Numerous studies have inspected manifestation of P16 protein in bladder cancer immunohistochemically[11-13]. Lossof P16 has been described to ensue at variable proportions, ranging from 19-71% in

several studies[14-16]. The differences inresults may be initiated by inconsistencies in tumor cohorts (non-invasive/invasive tumors or both) and immunohistochemical methodology.

Table-IV: Association between clinicopathological features and immunohistochemicalexpression of p16 in low grade urothelial carcinoma (n=120).

Demographic Details	P16 Expression		*p*-value
	Positive n (%)	Negative n (%)	
Age			
<25 years	01 (1%)	-	0.598
25-50 years	15 (12.5%)	09 (7.5%)	
> 50 years	67 (55.8%)	28 (23.3%)	
Tumor Size (cm)			
<3	39 (32.5%)	20 (16.6%)	0.303
>3	44 (36.6%)	17 (14.1%)	
Gender			
Male	69 (57.5%)	22 (18.3%)	0.006
Female	14 (11.6%)	15 (12.5%)	
Location of Tumor			
Urinary Bladder	39 (32.5%)	20 (16.6%)	0.654
Kidney	17 (14.1%)	08 (6.6%)	
Ureter	27 (22.5%)	09 (7.5%)	
Invasiveness of Tumor			
Present	32 (26.6%)	19 (15.83%)	0.134
Absent	51 (42.5%)	18 (15%)	
P16 Expression			
High	06 (5%)	14 (11.6%)	<0.001
Low	77 (64.1%)	23 (19.1%)	

The current study determines the frequency of imunohistochemical manifestation of P16 in low grade bladder carcinoma. Our research showed high frequency of P16 expression in the low grade Urothelial Carcinomacases i.e. 69% which was consistent with Yang *et al*, that showed 61% positive P16 expression in low grade carcinomas[17]. But higher than the stated rate of P16 manifestation was noted in cases of Urothelial Carcinomain studies from Shalkhly *et al*. (41%, P16 positive), Kruger *et al*., (54%, P16 positive) Tzai *et al*. (20%, P16 positive) and Shariat *et al*. (54%, P16 positive)[18,19,20]. Similarly, a study conducted by Abdel Wahab *et al*. In which reported rate of immunohistochemical expression of P16 in low grade Urothelial Carcinoma was 70.8%[21]. The variability in results may be due to variation in sample sizes, statistical precision and study setup.

Mumtaz *et al*, revealed that low manifestation of P16 was witnessed in 86% (104 cases), whereas 14% (17 cases) showed high P16 expression which is consistent with our study that showed low expression of P16 in 83.4% (100 cases), whereas 16.6% (20 cases) revealed high P16 expression. The slight variability in results may be due to difference in inclusion criteriaas our study comprised on low grade cases[22].

Regarding the association between clinico-pathological characteristics and P16 expression, significant association was noted with gender and intensity of tumor expression and no association has been found with other parameters like size, locationand invasiveness of tumorwhich is in line with the study conducted by Yang *et al*[17]. The major limitation of this study is that this is single center study and non-probability consecutive sampling technique has been used which confine the generalizability of the results.

CONCLUSION

P16 has emerged as a crucial immunohisto-chemical marker that helps in stratification of the patients with regards to their chances of progression to higher grade. This marker could be used in routine practicefor early identification of cohort of patients at high risk and thereby early management may prevent progression to advanced stage, thus reducing morbidity and mortality. Furthermore, our results support the more prospective multicenter studies to evaluate the frequency of P16 expression in low grade urothelial carcinoma cases.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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