P53 and PCNA overexpression in papillary urothelial tumors: an immunohistochemical study

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التعبير المفرط لعامل (P53) وعامل التكاثر النووي في أورام المثانة الانتقالية الحليميه (دراسة مناعية نسيجية كيميائية)

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الملخص:

الهدف: لدراسة التعبير المناعي لعامل p53 وعامل التكاثر النووي PCNA في ورم المثانة الانتقالي ألحليمي وعلاقتهما مع مختلف درجات التمايز لهذا الورم.

الطريقه: تم الحصول على خمس وثلاثين نموذج لنسيج المثانة الانتقالي ألحليمي مثبته بالفورمالين ومطمورة بالبارافين للفترة مابين ٢٠٠٩-٢٠١٠ تم اجراء هذه الدراسه في فرع الامراض ، كلية الطب في جامعة الكوفه. كما تم الحصول على ١٠ نماذج لنسيج المثانة الحميد (التهاب المثانه) حيث استخدمت كمجموعه قياسيه. استخدمت طريقة ABC لتحديد التعبير المناعي النسيجي لعاملي p53 و PCNA . حيث تم احتساب ٢٠٠خلية ورميه وتم استخراج النسبه المئويه للخلايا الموجبه الصبغه وتم التعبير عن هذه النسبه من خلال حساب معدل عامل التأشير labeling index.

النتائج: أظهرت النتائج ان التعبير المفرط لعامل p53 كان موجبا في ٢٠ حاله (LI 27.4%) في حين ان عامل PCNA كان موجبا في ٢٤ حاله (LI 4802%) وارتباطهما بعلاقة ايجابيه مقارنة بنسيج المثانه الحميد ($p<0.05$). بالنسبه لعامل p53 ، لم يظهر نسيج المثانه الحميد أي نتائج موجبه في حين كانت شدة ظهوره في ورم المثانة الحليمي كالتالي: ورم المثانه الحليمي الحميد نتيجه واحدة موجبة من اصل ٥ حالات، ورم المثانة قليل الدرجه السرطانيه ٥ من اصل ١٢ حاله، سرطان المثانه الحليمي واطئ الدرجة ٨ من اصل ١١ حاله وسرطان المثاني الحليمي عالي الدرجه ٦ من اصل ٧ حالات. في حين كانت شدة ظهور PCNA كالتالي: نسيج المثانه الحميد (LI 3.5%)، ورم المثانه الحليمي الحميد (LI 25%) ورم المثانة الحليمي الحميد واطئ الدرجه(LI31.6%) ، سرطان المثانه الحليمي واطئ الدرجه (LI63.3%)، سرطان المثانة الحليمي عالي الشده (LI 69%).

المناقشات: من خلال النتائج السابقة نستنتج ان زيادة مؤشر التكاثرالذي اظهره عامل التكاثر النووي PCNA هو صفه مميزة لسرطان المثانة الانتقالي الحليمي وكذلك لورم المثانه الحليمي الحميد واطئ الدرجة. في حين كان ظهور عامل P53 صفه مميزه لسرطان المثانة الانتقالي الحليمي بدرجتيه الواطئة والعالية.

Abstract

Objective:

The aim of the present study was to evaluate the expression of P53 protein and PCNA and their correlation with different papillary grades of urothelial tumors according to WHO/ISUP classification.

Methods:

Thirty five cases of urothelial tumors and their corresponding paraffin blocks from 2009-2010 were submitted in this study in the Department of Pathology, College of Medicine, Kufa University. Ten biopsies of benign urothelium (cystitis) were considered as control group. ABC method was used to determine the expression of p53 and PCNA in these cases, Two hundred cells were counted and the percentage of cells positive for p53 and PCNA (labeling index [LI]) was counted. Immunohistochemical positivity was defined as strong, homogenous nuclear staining.

Statistically Chi square, Fisher exact probability and correlation co-efficient tests were used by the help of SPSS version 10.

Results:
P53 expression was detected in 20 cases of TCC with mean LI of 27.4%, while PCNA were detected in 24 samples out of 35 with mean LI of 48.2% with significant levels of expression between malignant and benign urothelium ($P<0.05$).
Regarding p53 expression, none of the benign lesions show positive results, while it was observed in 1 of 5 for papilloma with mean labeling index LI of 2% and 5 of 12 for LMP with LI 14.3%. In contrast, p53 was a feature of papillary carcinoma it expressed in 8 of 11low- grade and 6 of 7 high grade papillary urothelial carcinoma with mean LIs of 36.8% and 53.4% respectively.
PCNA positivity was as follow: benign urothelium (mean LI 3.5%), papilloma(mean LI 25%), LMP tumors (mean LI31.6%), low grade papillary carcinoma(mean LI 63.3%), and high grade papillary carcinoma(mean LI 69%). PCNA expression separate benign urothelium and papilloma from tumors of LMP, low and high grade papillary carcinoma.
Conclusion:
An increased in proliferative index as demonstrated by immunohistochemical staining for PCNA is most often seen in papillary carcinoma and tumors of LMP. While p53 positivity is mainly a feature of low grade and high grade papillary carcinoma.
Key wards: P53, PCNA: proliferating cell nuclear antigen, LMP: low malignant potential. LI: labeling index.

Introduction

Bladder cancer is the most common malignancy occurring worldwide & a major cause of morbidity & mortality. The incidence of bladder cancer continues to increase; in 2008, bladder cancer was diagnosed in 68,000 patients and was the proximal cause of 14,000 deaths in the U.S, it accounts for 6.5% of all cancers with highest incidence in industrialized countries[(1)]. Approximately 90% of bladder tumors are of epithelial in origin, the majority corresponding to transitional cell carcinoma [(2)]. In Iraq, bladder cancer is recorded as the second most common carcinoma in males, & the ninth in females according to the Iraqi cancer registry [(3)].

Several classification systems of bladder transitional cell carcinoma have been proposed over the years. These represent attempts at grading the increasing degrees of architectural & particularly cytologic disarrays of a single tumor type.

The World Health Organization/International Society of Urolopathology (WHO/ISUP) consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder was developed in 1998 to create "a universally acceptable classification system for bladder neoplasia that could be used effectively by pathologists, urologists, and oncologists."

This system consists from 12 diagnostic entities encompassing both neoplastic and reactive flat and papillary lesions. The types of papillary neoplasms in this classification are the following: *papilloma; papillary neoplasms of low malignant potential; low-grade papillary carcinoma;* and *high –grade papillary carcinoma [(4)]*.

At diagnosis, most of bladder tumors are superficial (stage Ta or T1); 70% of them are papillary noninvasive, and the remaining 30% manifest with early stromal invasion [(5, 6)].

The p53 is a tumor suppressor gene that maps to human chromosome 17p13.1 [(7)]. The product of this gene is a cellular phosphoprotein that has shown to have tumor suppressive properties [(8)]. Compared with wild type protein, mutant p53 protein is more

stable with prolonged half-life and more likely to detect by immunohistochemical analysis [9].

Regardless of the mechanism for nuclear reactivity, accumulation of the protein is indicative of a change in the cell state, and detection of this change by immunohistochemistry has been shown to aid the diagnosis of malignant disease [10].

Mutations involving p53 gene have been found in a wide variety of malignancies including urothelial carcinoma, immunohistochemical positivity for p53 protein have been found in 40%-60% of urothelial carcinomas [11-12].

Transitional cell carcinoma of the bladder show variable biologic potential & kinetic features (proliferation & apoptosis). Several proliferation markers (mitotic count, silver-stained nucleolar organizer regions, immunohistochemical staining with Ki-67, proliferating cell nuclear antigen, and bromodeoxyuridine labeling index) have been tested on TCCs with strong correlations among them and with tumor grade, but not with stage [13].

Proliferating cell nuclear antigen (PCNA) serves as a processivity factor (sliding clamp), encircling DNA at sites of replication and repair. The sliding clamp constitutes a highly symmetric assembly of three identical subunits. Each subunit consists of two domains [14].

PCNA was originally classified as an essential component of the eukaryotic chromosomal DNA replisome, sub sequent studies , however, have revealed its striking ability to interact with multiple partners, which are involved in several metabolic pathways, including DNA repair, translation DNA synthesis, DNA methylation, chromatin remodeling and cell cycle regulation. PCNA in mammalian cells thus appear to play a key role in controlling several reactions through the coordination and organization of different partners [15]. PCNA is known to interact with many components of the cell's replication and signaling machinery and in this context could facilitate exchange of DNA repair enzymes that recognize a common DNA intermediate [16].

The aim of the present study was to evaluate the expression of P53 protein and PCNA and their correlation with different papillary grades of urothelial tumors according to WHO/ISUP classification.

Materials and Methods

Samples of the papillary entities described in WHO/ISUP classification system were collected from review of routine hematoxylin-eosin glass slides from the archives of the major hospitals and some of the private laboratories in Najaf and Hilla governorates, in the middle of Iraq, their corresponding blocks of formalin fixed, paraffin- embedded urothelial biopsies were retrieved from the archives & four Mm thick sections were mounted on positively charged microscope slides for batched immunohistochemical staining with antibodies for p53 (1:5o Dako corporation, code no. M 7001) and PCNA (1:200, Dako corporation, code no. M0879). A standard avidin-biotin peroxidase technique was applied.

Forty five bladder biopsies were involved in this study; these biopsies included benign urothelium (cystitis) (n =10); papilloma (n=5); papillary neoplasm of low malignant potential (n= 12); papillary urothelial carcinoma, low grade (n= 11); and papillary urothelial carcinoma, high grade (n=7).

To get the average positive level of each case, four microscopic fields of 40X magnification were selected which included two representative fields of considerable

immunoreactive cells. Two hundred cells were counted and the percentage of cells positive for p53 and PCNA (labeling index [LI]) were calculated. Immunohistochemical positivity was defined as strong, homogenous nuclear staining.

Positive and negative controls were processed with each run of immunostaining for both p53 and PCNA.

Statistical analysis: SPSS for windows 10.0 software was used in statistical analyses. Data were expressed as means and the relationships between studied variables were assessed by using non-parametric Fisher's exact probability test. A P-value ≤ 0.05 was considered as statistically significant.

The strength of association between two variables were assessed using correlation –co-efficient test, correlation with always between -0.1 and +0.1. If the correlation is positive, we have positive relationship.

Results

In the current study 35 cases of papillary urothelial carcinoma were included these involve *papilloma; papillary neoplasms of low malignant potential; low-grade papillary carcinoma;* and *high –grade papillary carcinoma*, In addition to 10 samples of benign urothelium (cystitis).

Expression of p53 was detected in 20cases with mean labeling index of 27.4% with significant value of expression ($p< 0.05$), while PCNA was expressed in 24 cases out of 35 with mean LI of 48.2%, with significant level of expression ($p< 0.05$) as compared with benign urothelium.

Benign urothelium: none of the 10 cases which included in this study show immunoreactivity to p53, while the immunohistochemical staining for PCNA were limited and involving the basal layers mainly, the mean labeling index LI was 3.5%. Table 2.

Papilloma: papillomas are uncommon lesion in the new WHO/ISUP classification, one out of five cases show positive staining for p53 with mean LI of 2%. Table1, while there is increased in the proliferative activity as detected by PCNA expression (mean LI 25%, median 25%, range 0-60%) however this difference is not significant from that of benign urothelium ($P>0.05$). Table 3.

Papillary neoplasms of low malignant potential: in these neoplasms p53 mean LI was 14.3, median 0, range 0-45% without significant difference with papilloma ($p>0.05$) & significant difference with benign urothelium ($p<0.05$). Table 3. While PCNA expression was slightly higher than in papilloma without significant statistical difference (mean LI 31.6%, median 35%, range 0-70%).

Papillary urothelial carcinoma, Low grade: in low grade papillary carcinoma p53 expression (mean LI 36%, median 40%, range 0-80%) which effectively discriminate them from benign urothelium, while PCNA expression was (mean LI 63.3%, median 72%, range 5-90%) with significant difference with benign urothelium, papilloma and papillary neoplasms of LMP. Table 3.

Papillary urothelial carcinoma, high grade: in this neoplasm p53 expression was common (mean 53.4%, median 60%, range 0-90%), with significant difference with benign urothelium, papilloma & papillary neoplasms of LMP. The same statistical significance was detected with PCNA expression in this grade of urothelial carcinoma. Table 3.

Table1. Percentage of p53 positive cells in benign urothelium & different papillary entities of WHO/ISUP classification.

Grade	Mean LI%	Median LI%	Range LI%
Benign urothelium	0	0	0
papilloma	2	0	0-10
Papillary urothelial carcinoma of LMP	14.3	0	0-54
Papillary urothelial carcinoma, low grade	36.8	40	0-80
Papillary urothelial carcinoma, High grade	53.4	60	0-90

Table2. Percentage of PCNA positive cells in benign urothelium & different papillary entities of WHO/ISUP classification.

Grade	Mean LI%	Median LI%	Range LI%
Benign urothelium	3.5	0	0-20
papilloma	25	25	0-60
Papillary urothelial carcinoma of LMP	31.6	35	0-70
Papillary urothelial carcinoma, low grade	63.3	72	5-90
Papillary urothelial carcinoma, High grade	69	75	35-100

Table3. Level of significance for p53 and PCNA expression in different papillary entities of WHO/ISUP classification using Fisher exact probability test.

Lesion	P53	PCNA
Benign vs. papilloma	NS	NS
Benign vs. papillary neoplasm of LMP	0.039	0.008
Benign vs. papillary carcinoma, low grade	0.00014	0.00103
Benign vs. papillary carcinoma, high grade	0.00226	0.00056
Papilloma vs. papillary neoplasms of LMP	NS	NS
Papilloma vs. papillary carcinoma, low grade	0.017	0.088
Papilloma vs. papillary carcinoma, high grade	0.032	0.045
Papillary neoplasm of LMP vs. papillary carcinoma, low grade	NS	0.0411
Papillary neoplasm of LMP vs. papillary carcinoma, high grade	0.041	0.05
Papillary carcinoma, low grade vs. papillary carcinoma, high grade	NS	NS

*NS= not significant p value >0.05.

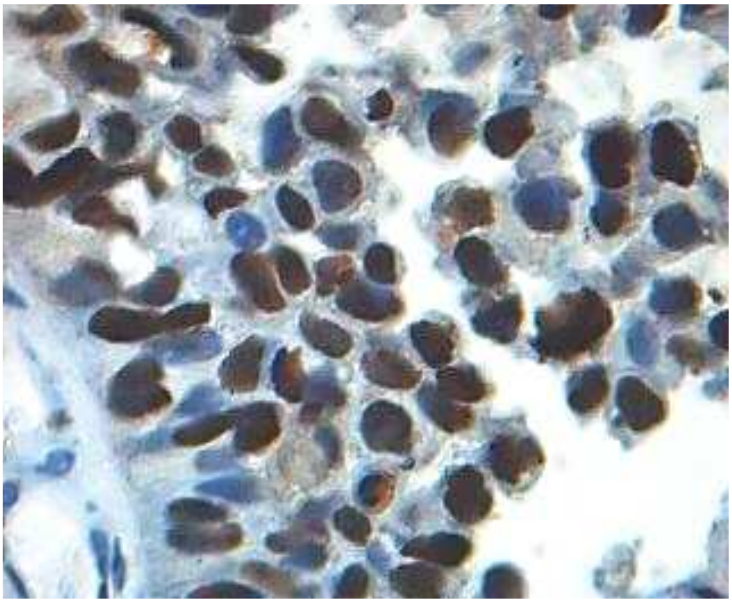

Figure1. Increased p53 expression in papillary urothelial carcinoma of high grade (400x)

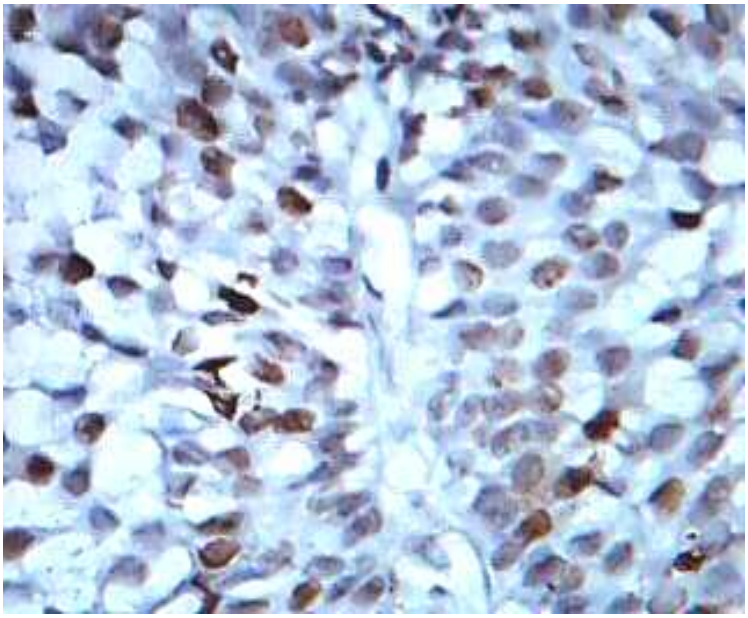

Figure2. Increased proliferative activity as expressed by PCNA in papillary urothelial carcinoma (low grade) (400X)

Discussion

The WHO/ISUP classification of urothelial neoplasms represents a consensus that resulted from the recognized need among pathologists, urologists and oncologists for a universally acceptable and clinically useful classification of bladder neoplasia [(4)]. Most of the cases at the time of the diagnosis are papillary lesion and in most of these papillary lesions the diagnosis is straight forwarded and the features to be determined are related to proper classification and evaluation of the stage. Occasionally there could be problems in the differential diagnosis with lesions that can mimic a papillary

process(papillary cystitis, fragmented or tangentially cut flat lesions) or papillary lesions that are not, strictly speaking urothelial(nephrogenic adenoma and conmyloma accuminatum) [17].

Cytogenetic and molecular studies have shown the existence of a strong relationship between urothelial carcinoma and alterations involving specific chromosomes. It has been determined that chromosomal loss and inactivation of tumor suppressor genes play a significant role in the development and progression of these tumors [7-18].

Many studies demonstrate a positive correlation between tumor expression of p53 and pathologic indicators of progression in urothelial carcinoma, including high grade and stage [19-20]. Fujimoto et al [11] reported that the incidence of p53 mutation is much higher in invasive and high grade urinary cancers than in superficial and low grade tumors. Likewise Hemal et al [21] found that p53 positivity correlated well with the grade and stage of the disease, the same findings were observed by Teng et al [22] and Harano et al [23]. While Lydia et al [24] demonstrate that tumors with loss of bcl-2 positivity and overexpression of p53 and ki67 had unfavorable prognosis; however in multivariate analysis, they had no independent prognostic value. Although these studies demonstrate that p53 expression was a feature of high grade malignancy; Cordon-Cardo et al [25] demonstrate that in activation of p53 promote tumorigenesis in human bladder cells and it deletion is an early event in the process of carcinogenesis.

In the present study, p53 expression was a feature of papillary carcinoma (low and high grades). The staining intensity revealed a statistically significant separation of papillary carcinoma of low grade from benign urothelium & papilloma ($p<0.05$), the same statistical significance applied to papillary carcinoma of high grade. Where the p53 expression does not discriminate between papillary carcinoma of low and high grades ($p>0.05$). These findings are in agreement with earlier studies, which have demonstrated the association of p53 expression with tumor grade. P53 expression does not discriminate papilloma and papillary carcinoma of low malignant potential as separate pathological entities ($P>0.05$), these findings agree with Stephen et al [26] who revealed the same statistical significance. While statistically significant observation were seen between tumor of LMP and urothelial carcinoma of high grade ($p=0.041$). Although this significance didn't seen between urothelial carcinoma of low grade and tumor of LMP, but there is subjective increase in p53 LIs between them. Table1.

Studies have consistently revealed that cell proliferative activity correlates with the growth of many human neoplasms, including urothelial carcinoma [27]. Many studies have shown an association between cell proliferation as expressed by PCNA and tumor grade, stage, and prognosis in bladder carcinoma [28, 29-30]. While Takeshi et al [31] and Offener et al [32] showed that a high PCNA index correlated with p53 expression and a significantly worse prognosis.

In the present study, there is linear relationship between PCNA and p53 in papillary urothelial tumors as expressed by correlation-co-efficient test (0.975) which is agreed with the previously mentioned studies.

Our study also demonstrate increase in the proliferative activity as expressed by nuclear immunoreactivity with PCNA monoclonal antibody as the tumor grade increase with significant discriminatory power between benign urothelium and tumors of LMP and with that of papillary carcinoma(low and high grades) ($p <0.05$) Table 3, although there is no statistically significant difference in PCNA labeling indices of benign urothelium and papilloma and between papilloma and carcinoma of LMP ($p>0.05$), but there is subjective increase in the proliferative activity as detected by median values. Table 2.

This study clearly showed that many benign lesions including the papillary once and tumors of LMP may be separated from papillary carcinoma by their

immunohistochemical staining properties. Comparison with staining patterns noted in previous studies for papillary carcinoma was difficult because grading in the previous studies may have been based on other classification systems.

In conclusion, p53 and PCNA stains may be used as adjuncts to routine histologic sections in the diagnosis of urothelial biopsies or it could used to support histologic impressions or may be employed to suggest the correct diagnosis in problematic cases.

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