The assessment of proliferative activity in transitional cell carcinoma of the urinary bladder by using Ki67 overexpression and its effects on prognosis of the tumor in correlation to prognostic markers (staging and grading); (immuno-histochemical study)

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دراسة درجة الانقسام في سرطان المثانة باستخدام Ki67 ودراسة تأثيره على عواقب هذا المرض و ايجاد علاقته مع درجة الورم السرطانية ومراحل وصوله (دراسة مناعية نسيجية)

علاء صلاح جمعة

الخلاصة:

تمهيد: تعتبر سرطانات المثانة واحدة من اكثر انواع السرطان شيوعا في العالم وبالأخص في الدول المتقدمة. ان الغرض من هذه الدراسة هو ايجاد العلاقة بين اظهار المعلم النسيجي Ki67 مع الدرجة السرطانية و مراحل تقدم المرض وبالتالي ايجاد تأثيره على عواقب المرض ونتائجه المتوقعة.

النتائج: اجريت هذه الدراسة باستخدام عينات مأخوذة من ٤٩ مريضا مصابين بمرض سرطان المثانة مثبتة في الفورمالين ومطمورة في شمع البارافين وصبغت باستخدام المعلمات النسيجية لـ Ki67، ودرست علاقة اظهار الـ Ki67 مع درجة الورم السرطانية و مرحلة وصوله باستخدام التحليل الاحصائي.

الاستنتاج: ان درجة الانقسام والتكاثر للمرض والمقاسة هنا باستخدام الـ Ki67 لها علاقة طردية مع دلائل المرض التي تتعلق بعواقبه وبالتالي لها تأثير سلبي على درجة سرطانية المرض من الناحية السريرية.

Abstract:

Background: Urinary bladder carcinoma is one of the most common cancer worldwide with higher incidence in the industrialized countries. The purpose of this study is to correlate ki67 overexpression which is a marker of proliferation with prognostic parameters (grading and staging) and thereby determine its impact on tumor prognosis and clinical outcome.

Findings: Forty nine specimens of formalin-fixed , paraffin –embedded bladder cancer tissues were collected from bladder cancer patients were included in this study. Labeled streptavidin-biotin (LSAB+) complex method was employed for immunohistochemical

detection of Ki67. Ki67 overexpression score were correlated well with prognostic markers (stage and grade) of transitional cell carcinoma of the bladder. ($P<0.05$).

Conclusion: Proliferative activity of transitional cell carcinoma which was assessed here by using Ki67 overexpression as proliferative marker was correlated well with prognostic biomarkers which are grading and staging and therefore affect the prognosis adversely and indicate aggressive clinical behavior.

Introduction:

Bladder cancer is one of the most common cancer worldwide, with high incidence in industrialized countries .It arises from the urothelium forming the fifth most common cancer among American men, and approximately three quarters of all cases occur in men [(1)] .

Bladder cancer is predominantly a disease of the elderly male. The peak age of incidence is the seventh decade, and the male-to-female ratio is 3 to 1, but the most cases of TCC of bladder present over the age of 50 years, but they also occur in younger adults and children [(2)] .

The incidence is higher in American whites than American blacks, higher in Western industrialized countries than African and Asian nations, and higher in urban areas than rural areas. These trends suggest a role for industrial substances in the development of bladder cancer [(3)].

In Iraq , bladder carcinoma is recorded as the second most common carcinoma in male, and the sixth in female according to Iraqi cancer registry. The mean age of highest incidence of bladder carcinoma in Iraq that is associated with bilharzial ova is between 40 and 49 years [(3,4)].

In the United States, bladder carcinoma is the fourth most common type of carcinoma in men and the ninth most common carcinoma in women. More than 47,000 men and 16,000 women are diagnosed with bladder carcinoma each year .The number of patients diagnosed annually with bladder carcinoma increasing by 33%, one reason for its higher incidence in men is that the androgen receptor, which is much more active in men than in women, plays a major part in the development of the carcinoma [(5)].

While providing important prognostic information in transitional cell carcinoma (TCC) of the urinary bladder, current clinical and pathologic variables have a limited ability to predict tumor recurrence, progression, or patient survival. With availability of effective systemic chemotherapy, there is a clear need for accurate predictors of failure after local therapy with curative intent. Biomarkers may be helpful for selecting patients best suited for early systemic intervention and for sparing patients who have undergone cystectomy from the morbidity associated with local adjuvant or salvage radiation therapy.

Cell proliferation is a hallmark of cancer. Ki-67 is a nuclear protein expressed by proliferating cells and can be observed immunohistochemically. Nuclear Ki-67 antigen expression is a measure of cell growth fraction and hence biological aggressiveness of a malignancy [(6)]. This marker has shown promise as an independent prognosticator of patient outcome in several malignancies [(7,8,9)]. Most recently, investigations established Ki-67 antigen as an independent predictor of recurrence, progression, and response to immunotherapy in patients with nonmuscle-invasive TCC [(10.11,12,13)]. To date, few studies explored the predictive role of Ki-67 after radical cystectomy for advanced TCC [(14,15)] These studies were flawed due to small size, and they did not do subgroup

analyses to determine the significance of Ki-67 for different patient groups. We determined the association of Ki-67 expression with clinicopathologic characteristics.

Material and method

Forty nine specimens of formalin-fixed , paraffin –embedded bladder cancer tissues were collected from bladder cancer patients were included in this study. All cases were referred to Kufa school of medicine teaching hospital for histopathological evaluation from different parts of the middle Euphrates region in Iraq. The age range of patients from50yrs to 70yrs. Confirmation of histopathological diagnosis and grading of tumor were carried out after reviewing all slides before proceeding further to the immunohistochemical staining (analysis).tissue sections with thickness of 4 micron were taken from the formalin-fixed, paraffin-embedded blocks for immunohistochemistry. Labeled streptavidin-biotin (LSAB+) method was employed for immunohistochemical detection of Ki67 using monoclonal mouse anti-human Ki67 antibody (DAKO. Clone MIB-1, Ref N1633, Lot 00002106, DAKO Corp., Carpinteria, CA).The intensity of Ki67 nuclear nuclear staining was classified into score 0 (none of the cells revealed positivity for the marker); weak or mild staining (5-10% positive to tumor cells – score +1); moderate staining (<25%- score +2); strong staining (25-50%-score +3); highly strong staining (>50%-score +4) according to Adams et al , 2000[16] . All biopsies were classified in to grade I,II, & III according to WHO grading system and to according to IUSSP[26] grading system , staging were done according to the TNM staging system. The redults were statistically evaluated with Chi-squared test (at a significant level of $P<0.05$) using SSPS software.

Results:

The score of Ki67 expression in grade III TCC was +4 in 14 (fig 1&3) cases and +3 in 7 cases a while grade II cases of transitional cell carcinoma express Ki67 is score +1 in 21 of cases and score +2 in 7 cases(fig 2), the difference in Ki67 expression was statiscally significance in relation to grading of this tumor ($P<0,05$) ; this also true for Ki67 expression differences between low grade and high grade papillary tumor; as shown in table 2 and table 4.

As shown in table 3 which express in the relation between Ki67 expression in transitional cell carcinoma of the urinary bladder in respect to staging , we see that Ki67 score in stage Ta (stage 0a) was score +1 (14 cases), while the score of Ki67 overexpression in stage T1 (stage 1) was score +1 (7 cases), cases with transitional cells carcinoma of T2 (stage II) having Ki67 expression value of score +2 (7cases); score +3 (7 cases) and score +4 in (14 cases), from the above we notice significant differences in Ki67 expression in relation to staging of transitional cell carcinoma ($P <0.05$).

From the above results we notice that there is significant relation between Ki67 expression scores with grading and staging of transitional cell carcinoma which established prognostic value of Ki67 overexpression in biological behavior of the tumor.

Table 1: Age and gender distribution of the patient included in this study.

Gender	Age		
	50-60	61-70	Total
Male	21	14	35
Female	14	--	14
Total	35	14	49

Table 2: The value of Ki67 scores in relation to grading of the tumor.

	Ki67 scores				
Grading	+1	+2	+3	+4	Total
grade II	21	7	-	-	28
Grade III	-	-	7	14	21

There is significant relation (P<0.05)

Table 3: The value of Ki 67 in relation to staging of bladder carcinoma .

	Ki67 scores				
Staging	+1	+2	+3	+4	Total
Ta (stage 0a)	14	-	-	-	14
T1 (stage I)	7-	--	-	-	7
T2 (stage II)	-	7	7	14	28

There is significant relation (P<0.05)

Table 4: The value of Ki67 scores in relation to grading of the tumor.

	Ki67 scores				
Grading	+1	+2	+3	+4	Total
Low grade papillary tumorI	21	7	-	-	28
High grade papillary tumor	-	-	7	14	21

There is significant relation (P<0.05)

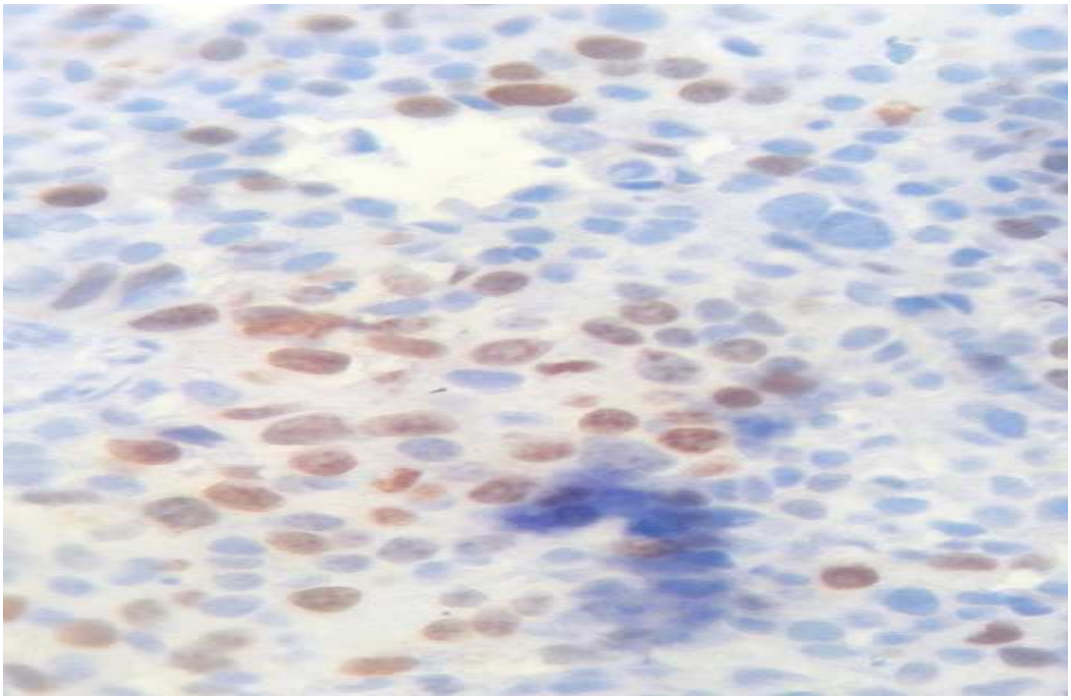

Fig1: transitional cell carcinoma stained with Ki67 score +4

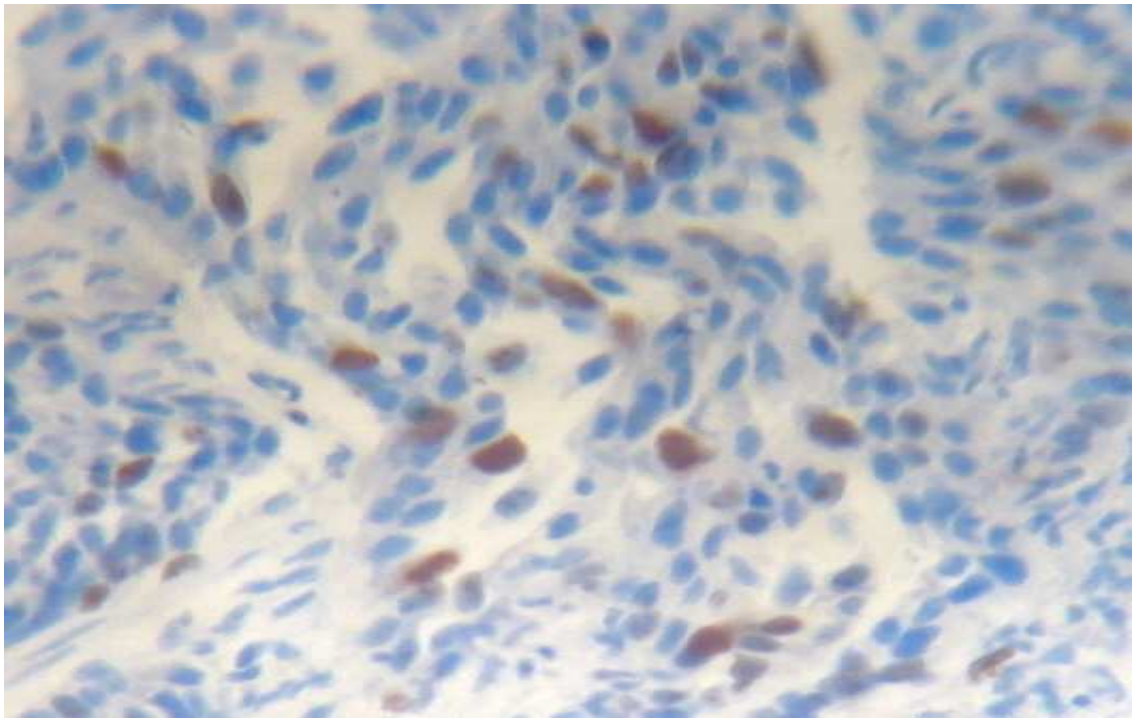

Fig2: transitional cell carcinoma stained with Ki67 score +2

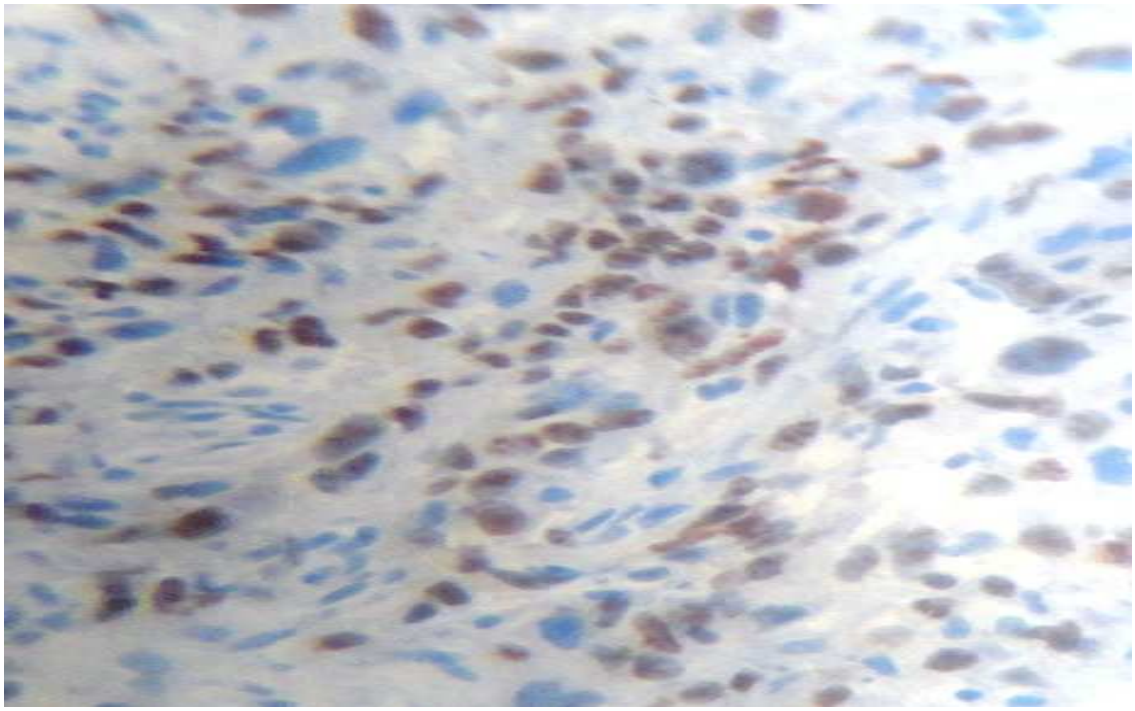

Fig 3: transitional cell carcinoma stained with Ki67 score +4

Discussion:

Approximately 50% to 60% of patients diagnosed with muscle-invasive TCC of the urinary bladder will develop metastatic progression after local therapy with curative intent, resulting in ~12,000 deaths annually (17,18). Although pathologic staging after local therapy is the most important prognosticator, its value for predicting clinical outcomes remains limited. A reliable predictor of metastatic progression would enhance our ability to identify patients who would benefit from adjuvant therapy and spare those who would not the toxicity associated with adjuvant therapies.

Several biomarkers, including proliferation-associated molecule Ki-67, have shown promise in their ability to stratify patients according to their risk for disease progression (19) . Ki-67 is an established marker of cell proliferation, present during the G1, S, G2, and M stages of the cell cycle. In addition to Ki-67 antibody, MIB-1 antibody also detects the Ki-67 antigen but can be used on formalin-fixed, paraffin-embedded tissues. Staining for Ki-67 with MIB-1 is a simple and reproducible technique for assessing cell proliferation in bladder carcinoma and can be done on a small amount of tissue taken (6) . Ki-67 expression is independently associated with clinical outcome after local therapy in several tumor types, such as breast, soft tissue, lung, cervix, melanoma, hepatocellular carcinoma, and prostate (7,8,9,25).

Recently, investigators have established Ki-67 expression as an independent predictor of disease recurrence, progression, and response to intravesical therapy in patients with nonmuscle-invasive bladder cancer (10,11,12,13).

This study found that there are clear association between ki67overexpressoin and the most important prognostic parameters that determine the biological behavior which

are the grade and stage of the tumor as ki67 expression was score +4 in T2 (stage II) in 14 cases, score +3 in 7 cases and score +2 in 7 cases out of out of 28 cases; while ki67 expression in T1 cases was score +1 and ki67 expression in Ta cases was score +1 in 14 cases out of 14 case.; and this data reflect significantdifferences in ki67 overexpression in relation to staging of the tumor ; at the same time we notice significant differences in ki67 overexpression in relation to grading of the tumor ($P<0.05$). these results reflect the importance of cellular proliferation which was assessed here by measuring overexpression of ki67 in predicting the clinical behavior of the tumor, these results are consistent with results of other study (20), and also the above results reflect the impact of ki67 expression on the prognosis of the tumor ; this consistent with results of other study (21.),(22).

The prognosis of the disease is very important denominator for predicting clinical course of the disease and therefore factor that have adverse prognostic effects and here is ki67 overexpression logically affect the survival of the patients adversely and this is compatible with the results of Pick-A et al (23)., also the results of our study denote to aggressive behavior of the tumor with increasing ki67 expression as they are correlated well with staging and grading of tumor ($P<0.05$) , this consistent with the result of vitaly et al (24).

Conclusion: Proliferative activity of transitional cell carcinoma which was assessed here by using Ki67 overexpression as proliferative marker was correlated well with prognostic biomarkers which are grading and staging and therefore affect the prognosis adversely and indicate aggressive clinical behavior.

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