Immunohistochemical Cytokeratine 20 over Expression in Urinary Bladder Carcinoma

Maather Baqer Hussein Al-Harmoosh*y; Msc, Shoroq Mohammad Abbas Al-Temimi*; M.Sc

**Department of pathology / College of Medicine / University of Al- Qaddissia`/ Diwaniyah / Iraq*

Abstract

This study was conducted to estimate the over expression of CK 20 protein in human bladder carcinoma (transitional cell carcinoma in comparison to squamous cell carcinoma) and to show its possible correlation to the pathological parameters (grade and stage) of TCC cases . We evaluated the available tissue blocks of 50 patients with bladder carcinoma lesions (40 with TCC & 10 cases with SCC) who had referred to Al-diwaniya Hospital between January 2007 and December 2010.The mean age of the patients was 55 years (range, 33 to 77 years). The TCC were classified according to grades into grade I, II and III in 20 (50%), 10 (25%), and 10 (25%) cases, respectively and according to stages into Ta in 10 (25%), T1 in 10(25%), T2 in 10(25%) and T3 in 10(25%). From 40 cases of TCC, A total of 28 (70%) patients were positive for over expression of CK20 and no expression were found in cases SCC. High histological grades of the TCC were associated with decrease expression of CK 2 .There were 20 (100%) in GI , 5 (50%) in GII , 3 (30%) in GII (*P* = .0000) while CK20 expression decreased as the tumor stages increased , it was 10 (100%) in Ta , 8 (80%) in T1 , 6 (60%) in T2 , 4 (40%) in T3 , and there is a statistically significant correlation with the stages of TCC of bladder (p=0.0229) .

Aim of the Study: This study was conducted to estimate the over expression of CK-20 protein in human bladder TCC in comparison to bladder SCC lesions and to show its possible correlation to the pathological parameters of TCC like (grade and stage) .

Key ward: - Bladder TCC, Bladder SCC, Cytokeratin 20, IHC

الخلاصه

يهدف البحث إلى دراسة التعبير المناعي النسيجي الكيميائي لجين أل cytokeratine 20 كمؤشر للتغيرات الجينية الطارئة في ألخلايا السرطانية لسرطان المثانة الانتقالي والحرشفي والمقارنة بين التعبير المناعي النسيجي الكيميائي لجين أل cytokeratine 20 في كل منهما ولمعرفة ترابط هذا التغير مع ثوابت أخرى في مرضى سرطان المثانة الانتقالي مثل درجة تمايز الورم ودرجة انتشاره . تمت دراسة 50 عينة من سرطان المثانة في مختبرات مستشفى الديوانية التعليمي للفترة منذ بداية كانون الثاني 2007 وحتى كانون الأول 2010 , تم جمع 40 عينة من مرضى مصابين بسرطان المثانة الانتقالي و10 عينات من سرطان المثانة الحرشفي , تراوحت أعمارهم بين 33 و 70 سنه مع معدل عمر 55 سنه ،وقد قورنت المجموعتان مع بعض . أظهرت الدراسة المناعية النسيجية أن تعبير أل- cytokeratine 20 كان موجبا في 70% من سرطانالمثانة الانتقالي ولا يوجد أي تعبير ل- cytokeratine 20 في سرطان المثانة الحرشفي , .كان تعبير ال- cytokeratine 20 أقل في الأورام ذات التمايز الضعيف(الدرجة الثالثة) من سرطان المثانة الانتقالي عنه في الأورام ذات التمايز القوي والمتوسط (الدرجة الأولى والثانية) وأقل في السرطان المنتشر (T2 &T3) عنه في السرطان الغير منتشر(T1 &Ta) كما انه توجد علاقة واضحة لتعبير ل- cytokeratine 20 مع درجة تمايز الورم ودرجة انتشاره (قيمة ألفا < 0.05). مما يدل على ان جين أل-cytokeratine 20 يلعب دور أساسي في تقييم حالة سرطان المثانة الانتقالي المستقبلية وانتشاره.

Introduction

Each year in the United States, nearly 56,000 new cases of bladder cancer are

diagnosed and approximately 12,000 people die from this disease [1]. The prevalence of bladder cancer in the United States is estimated at almost 500,000 cases. Almost twice as many cases of bladder cancer occur in men as in women, with cigarette smoking its leading cause [2]. Other risk factors include exposure to industrial carcinogens and chronic infection with Schistosomiasis haematobium. The most common cell type of bladder cancer is transitional cell carcinoma, although adenocarcinomas, squamous cell carcinomas and sarcomas also occur[3]. In the Western hemisphere, the vast majority of bladder tumors are transitional cell carcinomas (TCC). However, individuals with spinal cord injury are at increased risk of developing squamous cell carcinoma (SCC) of the bladder [4]. Although the molecular pathways leading to SCC remain to be determined, chronic infection and inflammation have been implicated in the etiology of this disease. Bladder cancer is staged on the degree of tumor invasion into the bladder wall [5]. Carcinoma in-situ (Tis) and Stages Ta and T1 are grouped as superficial bladder cancers because they are restricted to the inner epithelial lining of the bladder and do not involve the muscle wall. Of the "non-muscle invasive" tumors, Stage Ta tumors are confined to the mucosa, while Stage T1 tumors superficially invade the lamina propria. T1 tumors are regarded as being more aggressive than Ta tumors [6]. Invasive tumors (Stages T2, T3 and T4) extend into the muscle (Stage T2) and into the perivesical fat layer beyond the muscle (Stage T3), with metastatic tumors (Stage T4) involving local nodes or distant organs. The cellular morphology of superficial bladder tumors is graded on the degree of cellular differentiation. The grading consists of well-differentiated (Grade 1), moderately differentiated (Grade 2) and poorly differentiated (Grade 3) tumors. Grading of cell morphology is important for establishing prognosis, as Grade 3 tumors are the most aggressive and the most likely to become invasive[3].

Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue. The term "cytokeratin" began to be used in the late 1970s [7]. There are two types of cytokeratins: the acidic type I cytokeratins and the basic or neutral type II cytokeratins. Cytokeratins are usually found in pairs comprising a type I cytokeratin and a type II cytokeratin. Basic or neutral cytokeratins include CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8 and CK9. Acidic cytokeratins are CK10, CK12, CK 13, CK14, CK16, CK17, CK18, CK19 and CK20. The cytokeratins cannot be divided into low versus high molecular weight solely based on their charge. Expression of these cytokeratins is frequently organ or tissue specific. As an example, CK7 is typically expressed in the ductal epithelium of the genitourinary (GU) tract and CK20 most commonly in the gastrointestinal (GI) tract. Histopathologists employ such distinctions to detect the cell of origin of various tumors [8]. The subset of cytokeratins which an epithelial cell expresses depends mainly on the type of epithelium, the moment in the course of terminal differentiation and the stage of development. Thus this specific cytokeratin fingerprint allows the classification of all epithelia upon their cytokeratin expression profile. Furthermore this applies also to the malignant counterparts of the epithelia (carcinomas), as the cytokeratin profile tends to remain constant when an epithelium undergoes malignant transformation. The main clinical implication is that the study of the cytokeratin profile by immunohistochemistry techniques is a tool of immense value widely used for tumor diagnosis and characterization in surgical pathology [10]. Cytokeratin 20 (CK20) is expressed in relatively few tissue types, including normal urothelium, gastrointestinal mucosa and Merkel cells [8, 9]. It is also expressed in TCC but is generally

absent in squamous tumors of multiple tissue types, including SCC of the bladder. [10].

Material and Method

We evaluated the available tissue blocks of 50 patients with urinary bladder lesions (40 with TCC & 10cases with SCC) who had referred to Al-diwaniya Hospital between January 2007 and December 2010, The mean age of the patients was 55 years (range, 33 to 77 years). Three micrometer thick sections were prepared from paraffin-embedded tissue blocks and stained by hematoxylin-eosin method. Tumor grade was then determined using the World Health Organization and the TCC were classified according to grades into grade I, II and III in 20 (50%), 10 (25%), and 10 (25%) cases, respectively and according to stages into Ta in 10 (25%), T1 in 10(25%) , T2 in 10(25%) and T3 in 10(25%). A manual avidine–biotin-peroxidase complex proced-ure was used in the immunohistochemical analysis (Dako Cytomation, Copenhagen, Denmark). Tumors were scored by two independent observers based on the percentage of tumor cells staining positive within each tumor. Tumors were scored as positive for CK20 when more than 30% of the cells expressed these proteins[13]. Data were analyzed using the SPSS software and the chi-square was used.

Results

CK20 staining was positive in 28(70%) of 40 specimens with TCC histology , But non of the 10 SCC specimens exhibited this protein ,so there is a statistically significant correlation to type of bladder tumors (p=0.0000 ,table 1, figure 1 and 2) . According to grades of TCC of bladder ,the expression of CK20 was 20 (100%) in G1 , 5 (50%) in G2 , 3 (30%) in G3 , so CK20 expression decreased as the tumor grades increased and there is a statistically significant correlation to grades of TCC of bladder (p=0.0000,table 2).
According to stages of TCC of bladder ,the expression of CK20 was 10 (100%) in Ta, 8(80%) in T1 , 6(60%) in T2 , 4(40%) in T3, so CK20 expression decreased as the tumor stages increased and there is a statistically significant correlation to stages of TCC of bladder (p=0.0229 ,table 3) .

Table 1. Expression profile in TCC and SCC by CK20 immunohistochemistry.

Type of tumors	-VE	+VE	Total	χ	P
TCC	12 (30%)	28 (70%)	40 ()	15.9091	0.0000
SCC	10 (100%)	0	10 ()		

Table 2. Expression profile in TCC according to grades of tumors.

Grades	-VE	+VE	Total	χ	P
I	0	20(100%)	20	18.0952	0.0000
II	5(50%)	5(50%)	10		
III	7(70%)	3(30%)	10		

Table3. Expression profile in TCC according to stages of tumors.

Stages	-VE	+VE	Total	χ	p
Ta	0	10(100%)	10	9.5238	0.0229
T1	2(20%)	8(80%)	10		
T2	4(40%)	6(60%)	10		
T3	6(60%)	4(40%)	10		

Discussion

We evaluated the expression profiles of CK20 in bladder cancers from patients with TCC and SCC in this population display distinct patterns of expression of this protein .CK20 is a member of a family of cytoskeletal associated intermediate

filaments and is normally expressed in the umbrella cell layer of the bladder [11]in dysplastic urothelium ,localized expression is lost and CK20 is observed throughout the urothelium [12].

In our presented study, we observed diffuse cytoplasmic staining of CK20 in most of the TCC specimens, these results agree with [13] those found 21(75%) stained positive for CK20 in TCC. Also our result agrees with [14] those found CK20 expression in TCC to be between 29 and 89%. ([14)(15)].

While CK20 was not expressed in SCC these finding agree with those of other studies [13, 15- 16], so absence of CK20 in squamous metaplastic lesions implies that this biomarker is not expressed when urothelial cells are transformed to the squamous phenotype [17].

According to grades of TCC, CK20 expression decreased with increasing grades of tumors these results agree with [14], those found CK20 was expressed in all 7 (100%) of the G1 TCC, in 6 (40%) of the 15 G2 TCC and in 1 (25%) of the 4 G3 TCC, because CK20 is preferentially expressed in well –differentiated tumors, this variability in the reported frequency of CK20 expression in TCC has been attributed to sample selection [14].

According to stages of TCC ,CK20 expression decreased with increasing stages of tumors these results agree with[13] those found in 3 (100%) of Ta TCC ,in 11 (79%) of the 14 T1 TCC ,in 3 (60%) of the 5 T2 TCC and in 2 (50%) of the 4 T3 TCC ,so CK20 expression decreased as the tumour stage increased . The expression of this tumor marker appear to be dependent on the tumour type ,grades and stages of tumor ,the expression of CK20 in superficial TCC lesions has been associated with lower rates of tumor recurrence [18].

Conclusion

This study demonstrates that the histologic phenotypes of squamous and tranitional cell tumours are reflected in their expression of this protein and is modified further as tumours progress in grades and stages, CK20 appears to be a useful marker for TCC cells .we plan to extend the study of this marker with other markers include another group of patients with more advanced disease.

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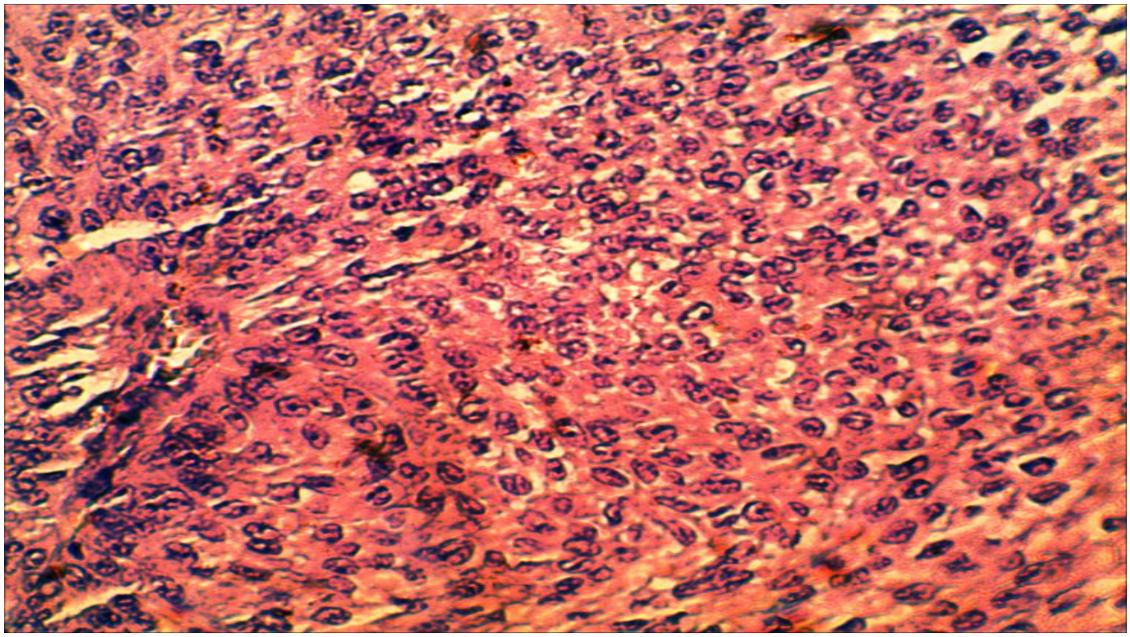
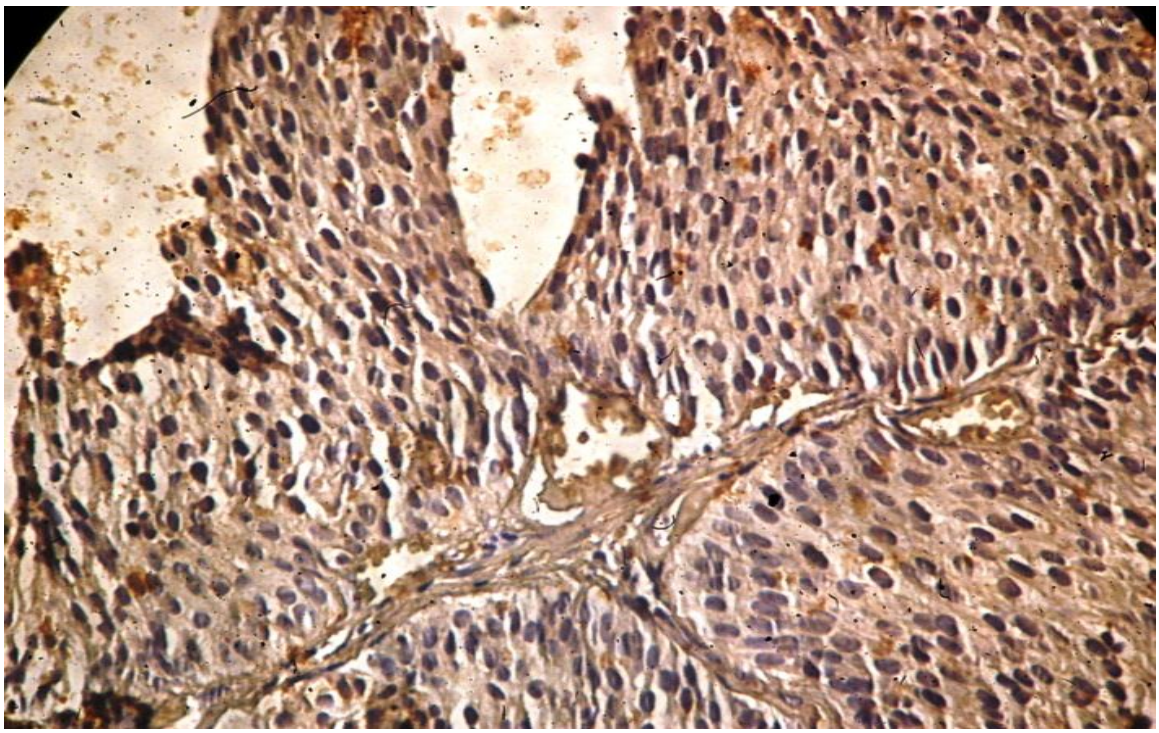
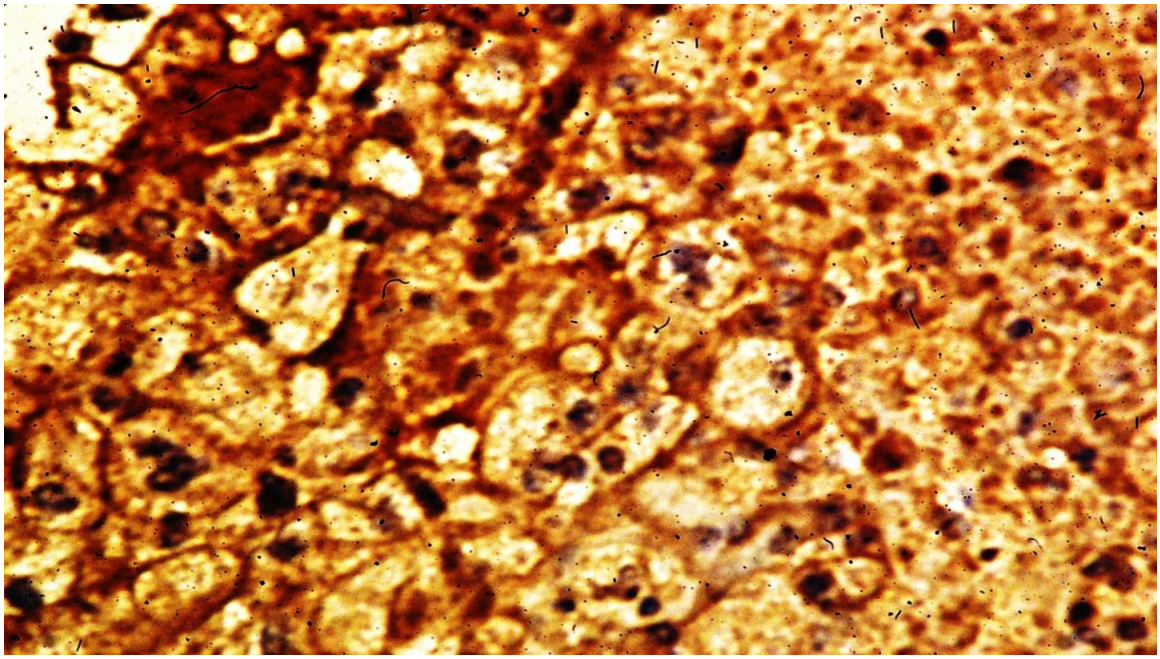


(a)

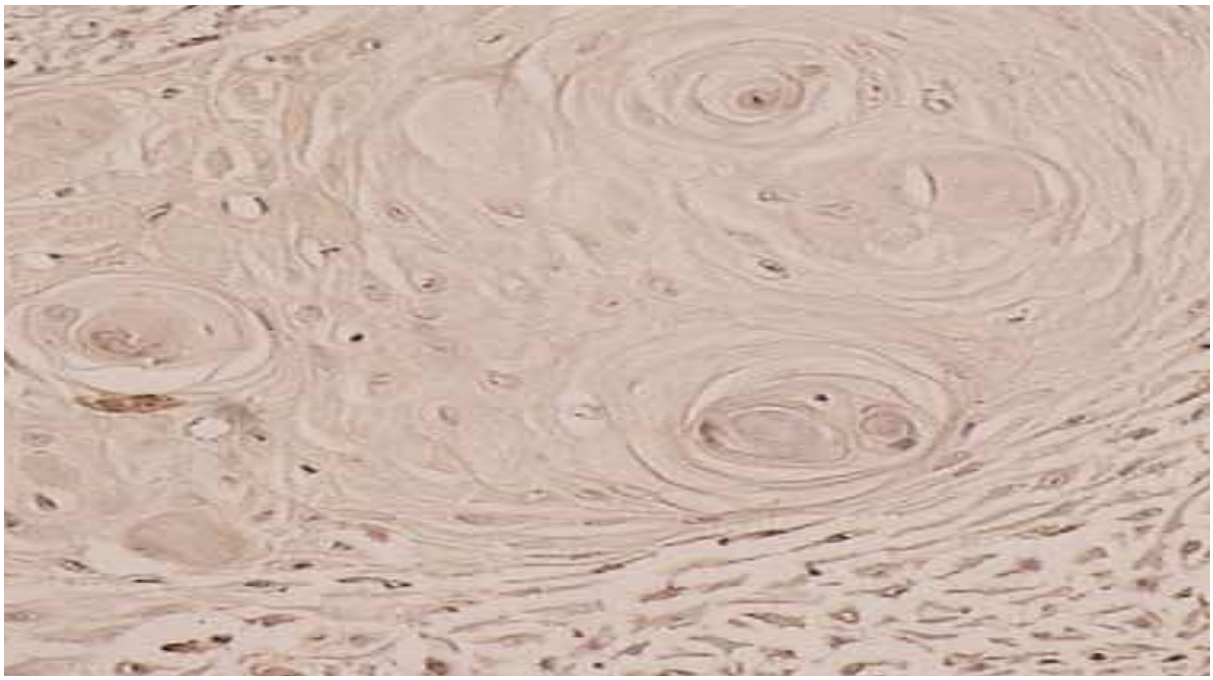


(b)

Figure 1. transitional cell carcinoma , negative (A) , positive cytokeratine expression (B)



(a)



(b)

Fig. 2. Cytokeratin 20 expression in transitional cell (A) and squamous cell (B) carcinomas of the bladder.

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