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Original Article

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Expression of CD44 Protein and Cytokeratin 20 in Non Bilharzial and Bilharzial Bladder Carcinoma

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ABSTRACT

Background/Aim: Carcinoma of the urinary bladder is one of the most common cancers world wide. It is the fourth most common malignancy in males and the ninth most common malignancy in females. CD44 is a family of cell-surface transmembrane glycoproteins that serve as receptors for hyaluronate and bind extracellular matrix components. CD44 plays a definite role in cell– cell and cell– matrix interactions. Thus, its down-regulation would facilitate loss of cell – cell cohesion, detachment from the basement membrane, and subsequent infiltration of the underlying tissues. It is suggested that the expression of CD44 is associated with differentiation and prognosis in bladder carcinoma. Cytokeratins (CKs) are a family of proteins that form the intermediate filament cytoskeleton of epithelial cells. Cytokeratin 20 (CK20) has been proposed as a marker of neoplastic change as well as a predictor for progression of urothelial carcinoma. The aim of the work is to study the immunohistochemical expression of CD44 and CK20 in carcinoma of the urinary bladder and correlate the immunohistochemical expression of CD44 and CK20 with different prognostic parameters including the grade and stage of the studied tumors.

Materials & methods: The studied 71 specimen were subjected to the ordinary H&E staining and immunohistochemical staining for CD44 and CK20.

Results: Correlative studies between CD44 and CK20 expression with different prognostic parameters including the grade and stage of the studied tumors revealed statistically significant correlation between CD44 and CK20 expression and the tumor grade and stage of urothelial carcinoma cases. No relation was found between the expression of CD44 and CK20 and the presence of Bilharziasis.

Conclusions: Loss or reduction of CD44 immunoreactivity and increasing CK20 positivity were significantly associated with increasing tumor grade and stage in the studied cases of urothelial carcinoma and to each other, so, they have combined utility in predicting the behaviour and prognosis of urothelial carcinoma, with no significant difference in their expression between non Bilharzial and Bilharzial bladder carcinomas.

Keywords: CD44, Cytokeratin 20, Non Bilharzial, Bilharzial Bladder Carcinoma.

INTRODUCTION

Carcinoma of the urinary bladder is one of the most common cancers world wide. It is the fourth most common malignancy in males and the ninth most common malignancy in females[1].

Transitional cell carcinoma is the most common histologic type; as it represents more than 90% of primary urinary bladder carcinoma. Other histologic types include sqamous cell carcinoma, which constitutes less than 3%, and adeno-

carcinoma which constitutes less than 2% of primary carcinoma of the urinary bladder besides other rare types as small cell carcinoma[2].

In Egypt, carcinoma of the urinary bladder accounts for 20.6% of all tumors, constituting 31.7% of male and 5% of female cancers. At the National Cancer Institute, Cairo University, a higher total frequency was reported, namely 27%, according to the cancer pathology registry. The peak age of diagnosis is usually 50±5 years with a male to female ratio of 5:1 [3].

There are several known and potential risk factors of bladder carcinoma. Cigarette smoking and occupational exposure to aromatic amines are the most important among them [4].

Cellular adhesion molecules are important participants in cell to cell interactions, and interactions between cells and components of the extracellular matrix. Several large subfamilies of cellular adhesion molecules are known to exist [5].

CD44 is a family of cell-surface transmembrane glycoproteins that serve as receptors for hyaluronate and bind extracellular matrix components such as collagen, laminin, chondroitin sulphate and fibronectin [6].

It is known that CD44 plays a definite role in cell– cell and cell– matrix interactions. Thus, its down-regulation would facilitate loss of cell – cell cohesion, detachment from the basement membrane, and subsequent infiltration of the underlying tissues [7].

Many experimental studies have shown a significant association between the tumor grade, stage and progression and the decrease or loss of the expression of CD44 suggesting that CD44 expression is associated with differentiation and prognosis in bladder carcinoma [8].

The cytokeratins (CKs) family is involved in cell structure and differentiation. Their expression is generally confined to epithelia and their neoplasms, CK20 is consistently detected in human intestinal mucosa, urothelium, and Merkel cells and its expression is restricted to their carcinomas [9].

It has been suggested that CK20 expression can be a tool for distinguishing CIS from other papillary urothelial neoplasms, and also can predict malignant potential in low-grade transitional cell tumors, and therefore CK20 can be useful in defining treatment strategies for patients with these tumors [10].

It is suggested that the pattern of CK20 staining is a useful adjunct to morphology in the diagnosis of different urothelial neoplasms [11]. CK20 has been proposed as a marker of neoplastic change as well as a predictor for progression of urothelial carcinoma [12].

AIM OF THE WORK

The aim of the work is to study the immunohistochemical expression of CD44 and CK20 in carcinoma of the urinary bladder and correlate the immunohistochemical expression of CD44 and CK20 with different prognostic parameters including the grade and stage of the studied tumors.

MATERIALS & METHODS

This study was carried out on 71 biopsies of primary bladder carcinomas. These cases were collected from the archives of the Pathology Department of the Faculty of Medicine, Tanta University and from private laboratories.

Tissue specimens were in the form of TUR (27 specimens) and cystectomy (44 specimens). The cases were categorized into 3 groups.

Group I: Cases of urothelial carcinoma (48 cases).

Group II: Cases of squamous cell carcinoma (20 cases).

Group III: Cases of adenocarcinoma (3 cases).

Clinico-pathological evaluation: Collection of patients' data including age, gender, clinical presentation and recurrence from the patients' files was done.

Histopathological assessment: All the biopsies were fixed in neutral buffered formalin 10 % for 24 hours, transformed into paraffin blocks,

sliced into 3-5µm sections, and mounted on glass slides. By ordinary H&E staining, sections were reviewed to assess the histologic diagnosis and to detect the grade, the stage of bladder carcinomas, changes of adjacent mucosa if present, and association with Bilharziasis. Grading was performed according to the WHO classification of bladder tumors[13].

For cases of squamous cell carcinoma: Grading was performed according to the grading system of Gringon [14]. Staging of the studied tumors was done according to (AJCC) the American Joint Committee on Cancer TNM classification[15].

Immunohistochemical staining: Immuno-histochemical staining was performed using the streptavidin-biotin method as described by Taylor et al. (2002) [16]. The examination was performed on 3-5µm sections from 10% formalin fixed, paraffin embedded tissue blocks for the evaluation of CD44 and CK20 expression. The Antibodies used are mouse monoclonal antibodies for CD44 (code MS-668) and CK20 (code MS-377), Lab Vision Corporation.

Controls: In each staining session section from a tonsil was used as a positive control for CD44, in which CD44 expressed membranous staining, and section from a case of colonic carcinoma known to be positive for CK20 was used as a positive control for CK20, in which cytoplasmic CK20 staining was expressed. As negative controls, tonsillar and colonic carcinoma sections were processed in the above mentioned sequence, but the primary antibodies of CD44 and CK20 were not added respectively and instead phosphate buffered saline (PBS) was used in this step.

Interpretation of CD44 stained sections: Each slide was carefully examined through out the tumor area. CD44 positivity was indicated by brown coloration of the cell membrane of the tumor cells.

Immunoreactivity was quantified by counting the number of positively stained tumor cells per 10 high power fields (10 H.P.F.s), results were expressed as the percentage of positively stained tumor cells.

The intensity of staining was graded from 0 to 3 and expressed as weak, moderate and strong intensity. The scoring system used in the assessment of CD44 immunoreactivity is shown in Table "1" [17].

Interpretation of CK20 stained sections: CK20 positivity was indicated by brown coloration of the cytoplasm of the tumor cells. The pattern of expression was classified in to four categories [7]:

1-Normal pattern restricted to staining of only superficial cells as in normal urothelium.

2- Absent staining.

3- Focal pattern, in which less than 10% of tumor cells show positive staining.

4- Diffuse pattern, in which more than 10% of tumor cells show positive staining.

For statistical analysis, the former two patterns were classified as CK20 negative group, while the last two patterns were classified as CK20-positive group.

Statistical analysis: The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. For quantitative data, the range, mean and standard deviation were calculated. For qualitative data, the number and percent distribution was calculated. Chi-square was used as a test of significance and when found inappropriate, Fisher exact test was used. Significance was adopted at $P<0.05$ for interpretation of results of tests of significance[18].

RESULTS

Clinical data of the studied cases (Table 2): Out of the 71 studied cases, 49 cases (69%) were males and 22 cases (31%) were females, their ages ranged between 35 and 80 years with the mean age (57.5 years). Male to female ratio was 2.2:1.

Results of group I (Cases of urothelial carcinoma): This group included 48 cases (67.6%) diagnosed as urothelial carcinoma, 35 cases of them (72.9%) were males and 13 cases (27.1) were females, their ages ranged between

35 and 80 years with mean age 57.5 years, the male to female ratio was 2.7:1 (Table 2).

1-Histopathological findings (Table 3): Cases were categorized according to the WHO classification into 12 cases (25%) of non-infiltrating urothelial carcinoma, and 36 cases (75%) of infiltrating urothelial carcinoma.

Grading of the studied cases (Table 4): The studied cases of urothelial carcinoma were classified into:

- Fourteen cases (29.1%) of low grade urothelial carcinoma.

-Thirty four cases (70.9%) of high grade urothelial carcinoma.

Staging of studied cases (Table 5):

●Ten cases (20.8%) were **Ta**

●Two cases (4.2%) were **Tis**

●Six cases (12.5%) were **T1**

●Sixteen cases (33.3%) were **T2a**

●Fourteen cases (29.2%) were **T2b**

2-Immunohistochemical evaluation of CD44 immunostaining:

Correlation between CD44 expression and the histopathological type of the studied cases of urothelial carcinoma (Table 6): Out of the studied 48 cases of urothelial carcinoma, 19 cases (39.6%) were CD44 positive, they included 5 cases (41.7%) of non-infiltrating urothelial carcinoma [Fig. 1] and 14 cases (38.9%) of infiltrating urothelial carcinoma [Fig. 2].

Out of the studied 48 cases 29 cases (60.4%) were CD44 negative, they included 7 cases (58.3%) of non-infiltrating urothelial carcinoma [Fig. 3] and 22 cases (61.1%) of infiltrating urothelial carcinoma [Fig. 4].

P-value was >0.05 denoting that CD44 expression was not significantly correlated with the histopathologic type of the studied cases of urothelial carcinoma.

Correlation between CD44 expression and the grade and stage of the studied cases of urothelial carcinoma is showed in Table 7. P-value was <0.05 denoting that CD44 expression significantly decreased gradually with increasing tumor grade and stage.

Results of group II (Cases of squamous cell carcinoma "SCC"): This group included 20 cases (28.2%) diagnosed as squamous cell carcinoma. 14 cases (70%) were males and 6 cases (30%) were females with a male to female ratio 2.3:1, the ages of cases in this group ranged between 40 and 74 years with mean age 57 years (Table 2)

1-Histopathological findings:

Grading of the studied cases:

Grade I: Included 4 cases (20%), **Grade II:** Included 10 cases (50%) and **Grade III:** Included 6 cases (30%).

Staging of studied cases:

●Three cases (15%) were **T1**

●Eight cases (40%) were **T2a**

●Nine cases (45%) were **T2b**

Immunohistochemical evaluation of CD44 immunostaining: Out of the studied 20 cases of squamous cell carcinoma, CD44 was positive in 18 cases "90%" [Fig. 5], while two cases (10%) were negative for CD44.

Correlation between CD44 expression and the grade and stage of the studied cases of squamous cell carcinoma is showed in Table 8. P-value was >0.05 denoting that CD44 expression was not significantly correlated with the tumor grade and stage in the studied cases of squamous cell carcinoma.

Results of group III (Cases of adenocarcinoma): This group included 3 cases (4.2%) diagnosed as primary adenocarcinoma of the urinary bladder. Patients were females and their ages ranged between 53 and 65 years with mean age 59 years (Table 2).

Immunohistochemical results of CD44 immunostaining: All the studied three cases of adenocarcinoma were CD44 negative [Fig. 6].

Studying CD44 positivity according to the histologic type of the studied groups (Table 9):

- CD44 was positive in 39.6% of urothelial carcinoma cases and in 90% of squamous cell carcinoma cases, while all the 3 studied cases of adenocarcinoma were CD44 negative. P-value was <0.05 denoting that CD44 expression was significantly correlated with the histologic type of the studied tumors.

Results of cases associated with Bilharziasis:

Out of the studied 71 cases of bladder carcinoma, there were 19 cases (26.8%) associated with Bilharziasis manifested by the presence of Bilharzia ova surrounded by inflammatory cellular reaction.

Immunohistochemical results of CD44 in cases associated with Bilharziasis (Table 10): In the studied 19 cases of Bilharzial bladder carcinoma, 12 cases (63.2%) were CD44 positive including 10 cases (83.3%) of urothelial carcinoma and 2 cases (16.7%) of squamous cell carcinoma. P-value was >0.05 denoting non significant association between CD44 expression and the presence of Bilharziasis in bladder carcinoma.

Immunohistochemical evaluation of CK20 immunostaining:

Results of group I (Cases of urothelial carcinoma):

1- Correlation between CK20 expression and the histopathologic type of the studied cases of urothelial carcinoma (Table 11):

CK20 was positive in 32 cases (66.7%) out of the studied 48 cases of urothelial carcinoma, they included 5 cases (41.7%) of non-infiltrating urothelial carcinoma [Fig. 7&8] and 27 cases (75%) of infiltrating urothelial carcinoma [Fig. 9&10].
On the other hand, 16 cases (33.3%) were CK20 negative, they included 7 cases (58.3%) of non-infiltrating urothelial carcinoma and 9 cases (25%) of infiltrating urothelial carcinoma.

P-value was >0.05 denoting that CK20 expression was not significantly correlated with the histopathologic type of the studied cases of urothelial carcinoma.

Correlation between CK20 expression and the tumor grade and stage of the studied cases of urothelial carcinoma is shown in Table 12. P-value was <0.05 denoting that CK20 expression increased significantly with increasing tumor grade and stage.

Results of group II (Cases of squamous cell carcinoma "SCC"):

All the studied 20 cases (100%) of squamous cell caecinoma were CK20 negative [Fig. 11].

Results of group III (Cases of adenocarcinoma):

All the studied three cases (100%) of adenocarcinoma were CK20 positive [Fig. 12].

Studying CK20 positivity according to the histologic type of studied groups (Table 9):

CK20 was positive in 66.7% of urothelial carcinoma cases and in all the studied three cases of adenocarcinoma, while 100% of squamous cell carcinoma cases were CK20 negative. P-value was <0.05 denoting that CK20 expression was significantly correlated with the histologic type of the studied tumors.

Immunohistochemical results of CK20 immunoreactivity in cases associated with Bilharziasis (Table 10): Out of the studied 19 cases of Bilharzial bladder carcinoma, CK20 was positive in 12 cases (63.2%) including, 11 cases (91.7%) of urothelial carcinoma and one case (8.3%) of adenocarcinoma.

Correlation between CD44 and CK20 in cases of urothelial carcinoma (Table 13): In the studied cases of urothelial carcinoma, 10 cases (20.8%) were positive for CD44 and CK20, 7 cases (14.6%) were negative for CD44 and CK20, 22 cases (45.8%) were positive for CK20 and negative for CD44 and 9 cases (18.8%) were positive for CD44 and negative for CK20. P-value was <0.05 denoting a significant correlation between CD44 and CK20 expression in cases of urothelial carcinoma.

Table (1): The scoring system used in the assessment CD44 immunoreactivity

Percentage of positive cells	Staining intensity	Intensity score
None	Negative	None
1-30%	Mild	+1
31-60%	Moderate	+2
More than 61%	Strong	+3

Table (2): Age and gender of the studied cases of bladder carcinoma

Histologic type	Gender				M:F ratio	Age range	Mean age	S.D.
	Male	%	Female	%				
Urothelial carcinoma (n=48)	35	72.9	13	27.1	2.7:1	35-80	57.5	10.25
Squamous cell carcinoma (n=20)	14	70	6	30	2.3:1	40-74	57	10
Adenocarcinoma (n=3)	-	-	3	100	-	53-65	59	11.5
Total (n=71)	49	69	22	31	2.2:1	35-80	57.5	12.54

Table (3): Distribution of cases of urothelial carcinoma according to their histopathologic type

Histopathologic type	N. of cases	Percentage of cases
Non-infiltrating urothelial carcinoma	**12 (2*)**	**25 %**
-Non-infiltrating papillary urothelial neoplasm of low malignant potential	3	6.3 %
- Non- infiltrating low grade papillary urothelial carcinoma	4 (2*)	8.2 %
- Non-infiltrating high grade papillary urothelial carcinoma	3	6.3 %
- Carcinoma in situ	2	4.2 %
Infiltrating urothelial carcinoma	**36 (12*)**	**75 %**
-Pure infiltrating urothelial carcinoma	26 (10*)	54.1 %
- Infiltrating urothelial carcinoma with squamous differentiation	6 (2*)	12.5 %
- Infiltrating urothelial carcinoma with glandular differentiation	2	4.2 %
- Plasmacytoid variant	2	4.2 %
Total	**48**	**100**

* Cases of urothelial carcinoma associated with Bilharziasis.

Table (4): Grading of the studied cases of urothelial carcinoma

Urothelial carcinoma	N. of cases	Percentage of cases
Low grade:	**14**	**29.1%**
▪ Papillary urothelial carcinoma of low malignant potential	3	6.3 %
▪ Non- infiltrating low grade papillary urothelial carcinoma	4	8.2 %
▪ Infiltrating urothelial carcinoma	7	14.6 %
High grade :	**34**	**70.9 %**
▪ Non-infiltrating high grade papillary carcinoma	3	6.3 %
▪ Carcinoma in situ	2	4.2 %
▪ Infiltrating urothelial carcinoma	19	39.5 %
▪ Infiltrating urothelial carcinoma with squamous differentiation	6	12.5 %
▪ Infiltrating urothelial carcinoma with glandular differentiation	2	4.2 %
▪ Infiltrating urothelial carcinoma with plasmacytoid differentiation	2	4.2 %

Table (5): Staging of the studied cases of urothelial carcinoma

Staging	N. of cases	Percentage of cases
Ta (non- infiltrating papillary)	10	20.8%
Tis (carcinoma in situ)	2	4.2%
T1(invasion of the lamina propria)	6	12.5%
T2a (invasion of the superficial muscle)	16	33.3%
T2b (invasion of the deep muscle)	14	29.2 %
Total	48	100

Table (6): Correlation between CD44 immunoreactivity and the histopathological type of the studied cases of urothelial carcinoma

Urothelial carcinoma	Total	CD44 positive		CD44 negative	
		N.	%	N.	%
-Non-infiltrating:	**12**	**5**	**41.7**	**7**	**58.3**
Papillary urothelial carcinoma of low malignant potential	3	1	33.3	2	66.7
non- infiltrating low grade papillary carcinoma	4	3	75	1	25
non- infiltrating high grade papillary carcinoma	3	1	33.3	2	66.7
carcinoma in situ	2	-	0	2	100
-Infiltrating:	**36**	**14**	**38.9**	**22**	**61.1**
infiltrating urothelial carcinoma	26	10	38.5	16	61.5
urothelial carcinoma with squamous differentiation	6	4	66.7	2	33.3
urothelial carcinoma with glandular differentiation	2	**-**	**0**	2	**100**
plasmacytoid variant	2	**-**	**0**	2	**100**
Total	**48**	**19**	**39.6**	**29**	**60.4**
X2			0.533		
P			0.081		

Table (7): Correlation between CD44 expression and the grade and stage of the studied cases of urothelial carcinoma

Grade / **CD44**	Low-grade urothelial carcinoma (n=14)		High-grade urothelial carcinoma (n=34)		Total (n=48)	
	N.	%	**N.**	%	**N.**	%
Positive CD44	9	64.3	10	29.4	19	39.6
Negative CD44	5	35.7	24	70.6	29	60.4
X^2			10.791			
P			0.002*			

Stage / CD44	Ta (n=10)		Tis (n=2)		T1 (n=6)		T2a (n=16)		T2b (n=14)		Total n=48	
	N.	%	**N.**	%	**N.**	%	**N.**	%	**N.**	%	**N.**	%
Positive CD44	5	50	-	0	2	33.3	5	31.3	4	28.6	18	37.5
Negative CD44	5	50	2	100	4	66.7	11	68.7	10	71.4	30	62.5
X²							15.117					
P							0.003*					

* Significant

Table (8): Correlation between CD44 immunoreactivity and the grade and stage of the studied cases of squamous cell carcinoma

Grade / CD44	I (n=4)		II (n=10)		III (n=6)		Total (n=20)	
	N.	%	**N.**	%	**N.**	%	**N.**	%
Positive CD44	2	50	10	100	6	100	18	90
Negative CD44	2	50	0	0	0	0	2	10
X^2					2.335			
P					0.479			
Stage / CD44	T1 (n=3)		T2a (n=8)		T2b (n=9)		Total (n=20)	
	N.	%	**N.**	%	**N.**	%	**N.**	%
Positive CD44	2	66.7	7	87.5	9	100	18	90
Negative CD44	1	3.33	1	12.5	9	0	2	10
X^2					2.335			
P					0.479			

Table (9): Correlation between CD44 and CK20 immunoreactivity and the histologic type of the studied groups

Histologic type / CD44	CD44 + ve		CD44 – ve		Total	
	N.	%	**N.**	%	**N.**	%
Urothelial carcinoma	19	39.6	29	60.4	48	67.6
Squamous cell carcinoma	18	90	2	10	20	28.2
Adenocarcinoma	0	0	3	100	3	4.2
X^2			6.468			
P			0.005*			
Histologic type / CK20	**CK20 + ve**		**CK20 – ve**		**Total**	
	N.	%	**N.**	%	**N.**	%
Urothelial carcinoma	32	66.7	16	33.3	48	67.6
Squamous cell carcinoma	0	0	20	100	20	28.2
Adenocarcinoma	3	100	0	0	3	4.2
X^2			5.488			
P			0.004*			

* Significant

Table (10) Relation between CD44 and CK20 immunoreactivity and the association with Bilharziasis

CD44 / **Bilharziasis**	**CD44 + ve**		**CD44 – ve**	
	N.	**%**	**N.**	**%**
Bilharzial bladder carcinoma	12	63.2	7	36.8
Non Bilharzial bladder carcinoma	34	65.4	18	34.6
X^2			**0.030**	
P			**0.862**	
CK20 / **Bilharziasis**	**CK20 + ve**		**CK20 – ve**	
	N.	**%**	**N.**	**%**
Bilharzial bladder carcinoma	12	63.2	7	36.8
Non Bilharzial bladder carcinoma	34	65.4	18	34.6
X^2			**0.030**	
P			**0.862**	

Table (11): Correlation between CK20 immunoreactivity and the histopathologic type of the studied cases of urothelial carcinoma

Histopathologic type	**Total**	**CK20 positive cases**		**CK20 negative cases**	
		N.	**%**	**N.**	**%**
-Non-infiltrating urothelial carcinoma:	**12**	**5**	**41.7**	**7**	**58.3**
Papillary urothelial carcinoma of low malignant potential	3	0	0	3	100
Non- infiltrating low grade papillary carcinoma	4	2	50	2	50
Non- infiltrating high grade papillary carcinoma	3	2	66.7	1	33.3
Carcinoma in situ	2	1	50	1	50
-Infiltrating urothelial carcinoma	**36**	**27**	**75**	**9**	**25**
Infiltrating urothelial carcinoma	26	23	88.5	3	11.5
Urothelial carcinoma with squamous differentiation	6	0	0	6	100%
Urothelial carcinoma with glandular differentiation	2	2	100%	0	0
Plasmacytoid variant	2	2	100%	0	0
Total	**48**	**32**	**66.7**	**16**	**33.3**
X2			3.421		
P			0.093		

Table (12): Correlation between CK20 immunoreactivity and the tumor grade and stage of the studied cases of urothelial carcinoma

Grade / **CK20**	Low-grade (n=14)		High-grade (n=34)		Total (n=48)	
	N.	%	**N.**	%	**N.**	%
Positive CK20	8	57	24	70.6	31	64.6
Negative CK20	6	43	10	29.4	17	35.4
X^2	12.581					
P	0.031*					

Stage / **CK20**	Ta (n=10)		Tis (n=2)		T1 (n=6)		T2a (n=16)		T2b (n=14)		Total n=48	
	N.	%	**N.**	%	**N.**	%	**N.**	%	**N.**	%	**N.**	%
Positive CK20	4	40	1	50	4	66.7	12	75	13	93	37	77.1
Negative CK20	6	60	1	50	2	33.3	4	25	1	7	11	22.9
X^2	11.127											
P	0.002*											

* Significant

Table (13): Correlation between CD44 immunoreactivity and CK20 immunoreactivity in the studied cases of urothelial carcinoma

CD44 (n=48)	**CK20(n=48)**			
	+ve (n=32)		**-ve (n=16)**	
	N	%	**N**	%
+ve (n=19)	10	20.8	9	18.8
-ve (n=29)	22	45.8	7	14.6
X^2	13.554			
P	0.004*			

* Significant

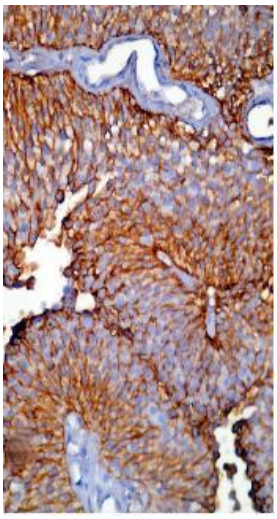

Fig. (1): Papillary urothelial neoplasm of low malignant potential showing positive CD44 membranous immuno-reactivity score "+3" (streptavidin biotin x200).

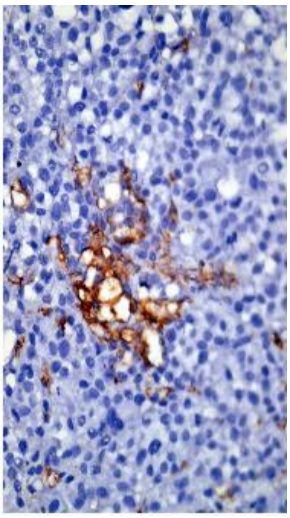

Fig. (2): High grade infiltrating urothelial carcinoma showing positive CD44 membranous immunoreactivity score "+1" (streptavidin biotin x200).

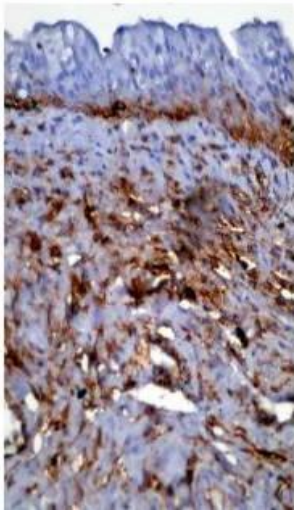

Fig. (3): Urothelial carcinoma in situ showing negative CD44 immuno-reactivity score "0" in the neoplastic cells. The residual non neoplastic basal cell layer and the stromal cells show positive reaction (streptavidin biotin x200).

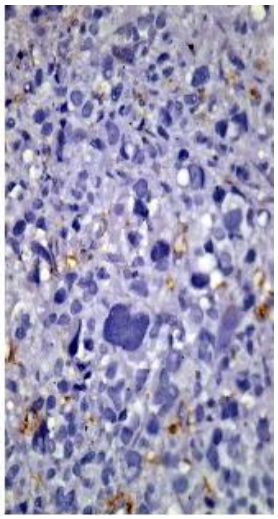

Fig. (4): High grade infiltrating urothelial carcinoma showing negative CD44 membranous immuno-reactivity score "0" (streptavidin biotin x400).

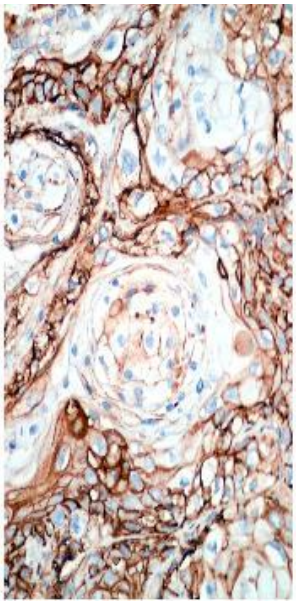

Fig. (5): Squamous cell carcinoma (grade II) showing positive CD44 membranous immunoreactivity score "+3" (streptavidin biotin x200).

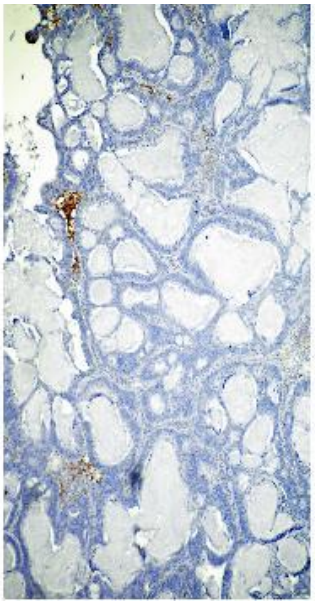

Fig. (6): Adenocarcinoma showing negative CD44 immunoreactivity score "0" (streptavidin biotin x100).

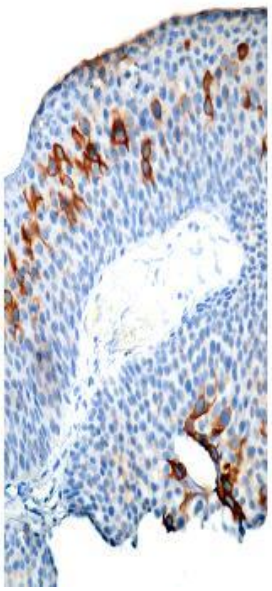

Fig. (7): Non infiltrating low grade papillary urothelial carcinoma showing focal CK20 cytoplasmic immuno-reactivity (streptavidin biotin x200).

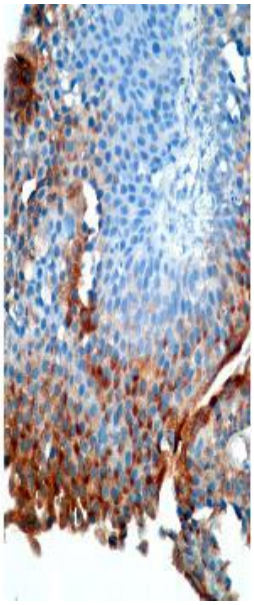

Fig. (8): Non infiltrating high grade papillary urothelial carcinoma showing diffuse CK20 cytoplasmic immuno-reactivity (streptavidin biotin x200).

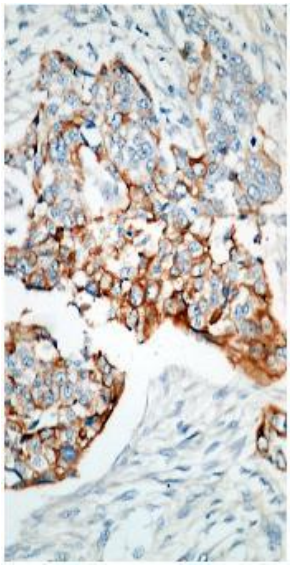

Fig. (9): High grade infiltrating urothelial carcinoma showing positive CK20 cytoplasmic immuno-reactivity (streptavidin biotin x200).

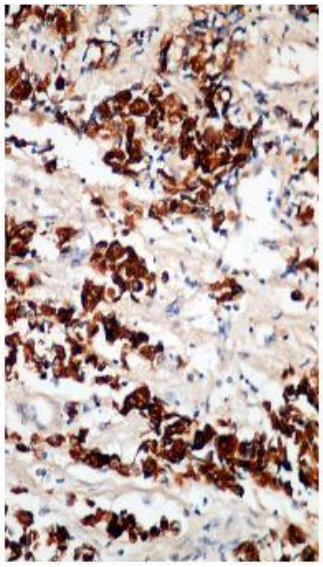

Fig. (10): Plasmacytoid variant of infiltrating urothelial carcinoma showing positive CK20 cytoplasmic immunoreactivity (streptavidin biotin x200).

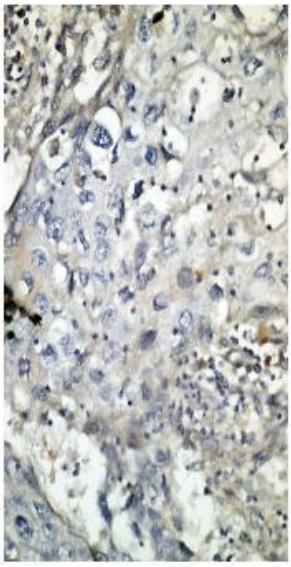

Fig. (11): Squamous cell carcinoma grade III showing negative CK20 immunoreactivity (streptavidin biotin x400).

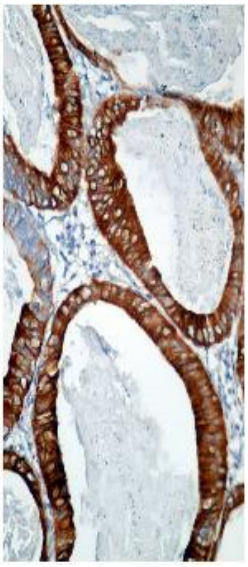

Fig. (12): Adeno-carcinoma showing positive CK20 cytoplasmic immuno-reactivity (streptavidin biotin x200).

DISCUSSION

Bladder carcinoma is the fourth most common malignancy in males and the ninth most common malignancy in females [(1)].

The present study included 71 cases of urinary bladder carcinoma, with male to female ratio 2.2:1. In a study made by Hussein et al. (2002) [(19)], the ratio was 4:1, while Gadalla et al. (2004) [(17)] reported that in their study, male to female ratio was 5:1, however, Desai et al. (2000) [(7)], found in their study a higher male to female ratio as they found it to be 11:1. Nineteen (27%) of the studied cases were associated with Bilharziasis.

On studying the relationship between CD44 immunoreactivity and the histological type of urothelial carcinoma, it was found that 41.7 % of non-infiltrating tumors were CD44 positive, versus 38.9% of infiltrating tumors without statistical significance (P>0.05), which agreed with a study made by Desai et al. (2000) [(7)], who found that 45.5 % of non-infiltrating tumors were CD44 positive, versus 36.3% of infiltrating tumors.

Concerning the relation between CD44 immunoreactivity and the grade of the tumor, the present study revealed that in urothelial carcinoma, 29.4% of high grade urothelial carcinoma cases were CD44 positive while 64.3 % of low grade urothelial carcinoma cases were CD44 positive.

On the other hand, in cases of squamous cell carcinoma, 50% of grade I tumors were positive for CD44, while 100% of grade II and grade III tumors were positive for CD44.

So, the present study revealed a statistically significant inverse relationship between the tumor grade of urothelial carcinoma and CD44 positivity (P<0.05), but not reaching statistical significance in squamous cell carcinoma (P>0.05), the latter may be due to the relative small number of cases in this study.

These results agreed with those reported by Gadalla et al. (2004) [17] , and Desai et al. (2000) [7], who found that in cases of urothelial carcinoma, the expression of CD44 in low grade tumors (65%) was significantly higher than those detected in high grade tumors (32%). Gadalla et al. (2004) [17] also found no significant association between the grade of the tumor and the positivity rates of CD44 in cases of squamous cell carcinoma.

As regards to the relationship between CD44 immunoreactivity and the tumor stage, in urothelial carcinoma, all the studied cases of carcinoma in situ "Tis" were CD44 negative, and four cases out of 14 cases (28.6%) of tumors that invaded the deep muscle (T2b) were CD44 positive.

The results of CD44 expression in carcinoma in situ in this study were in agreement with a study made by Rodney (2003) [20] and Mckenney et al. (2003) [21], who reported that CD44 immunoreactivity was not observed in cases of CIS. In some of our CIS cases, a residual non-neoplastic basal cell layer was present beneath the CIS that showed expression of CD44.

In cases of squamous cell carcinoma, 66.7% of (pT1) tumors were CD44 positive, and 100% of deep muscle invasive tumors (T2b) were CD44 positive.

So, there was a statistically significant inverse relationship in the present study between CD44 positivity and tumor stage in cases of urothelial carcinoma ($P<0.05$), however in cases of squamous cell carcinoma the relation was not statistically significant ($P>0.05$), which may be also due to relative small number of cases in this study.

These results coincided with the findings of Sugino et al. (1996) [22] and Gadalla et al. (2004) [17]. On the other hand, Desai et al. (2000) [7] mentioned that CD44 expression did not show statistically significant correlation with the tumor stage.

Stavropoulos et al. (2001) [23] assessed the expression of CD44 in superficial bladder tumors, reporting that the detectable loss of CD44 expression in these tumors may be a useful predictor of tumor progression.

The correlation of CD44 expression with the tumor grade and stage can be explained on the grounds that CD44 plays a definite role in cell-cell and cell-matrix interactions [24], thus, its down-regulation would facilitate loss of cell-cell cohesion, detachment from the basement membrane with subsequent infiltration of the underlying tissues[7].

On comparing between CD44 immunoreactivity in urothelial and squamous cell carcinoma of the urinary bladder, the present study revealed an increased CD44 reactivity in squamous cell carcinoma, as 90% of squamous cell carcinoma cases versus 39.6% of urothelial carcinoma cases were immunopositive for CD44 with a statistically significant difference ($P<0.05$). These findings were in agreement with those reported by Gadalla et al. (2004) [17], who reported that in squamous cell carcinoma, CD44 positivity rates were higher (93.5%) than urothelial carcinoma (48.5%).This is may reflect the potential aggressiveness of the squamous cell carcinoma.

In the present study, no relationship was found between CD44 expression and the presence of Bilhariziasis. Although its positivity was detected in 63.2 % of Bilhariziasis associated tumors and in 65.4% of non Bilharizial cases, the difference was of no statistical significance ($P>0.05$), these findings were in agreement with those reported by Gadalla et al. (2004) [17].

The second marker studied in this work was CK20, which is consistently detected in human intestinal mucosa, urothelium, and Merkel cells, and its expression is restricted to their carcinomas [9].

On studying the relationship between CK20 immunoreactivity and the histological type of urothelial carcinoma, we found that 41.7% of non-infiltrating tumors were CK20 positive, versus 75% of infiltrating tumors without statistical significance ($P>0.05$), which agreed with a study made by Desai et al. (2000) [7], who found that 58 % of non-infiltrating tumors were CK20 positive versus 71% of infiltrating tumors.

All cases of squamous cell carcinoma were CK20 negative, which agreed with the findings reported by Jason et al. (2003) [25] and Ramos et

al. (2003) [26]. All cases of adenocarcinoma were CK20 positive, similar to the findings reported by Wang et al. (2001) [27], who mentioned that CK20 positivity was observed in 94% of their studied cases of adenocarcinoma.

Concerning the relation between CK20 immunoreactivity and the grade of the studied tumors, the present study revealed that in urothelial carcinoma, 70.6% of high-grade urothelial carcinoma cases were CK20 positive, while 57% of low grade urothelial carcinoma cases were CK20 positive. So, the present study revealed a statistical significant relationship between the tumor grade of urothelial carcinoma and CK20 positivity ($P<0.05$). These results coincided with those reported by Desai et al. (2000) [7], who reported that a higher frequency of CK20 positivity was observed with increasing tumor grade and the reasons for this relation at this time was still unknown.

Similar findings had been reported by Shahin and Farag (2004) [28], who mentioned that 38% of low-grade tumors showed CK20 positivity, while 78% of high grade tumors were CK20 positive ($P=0.001$). On the other hand, Buchumensky et al. (2000) [29] demonstrated that no correlation was found between CK20 immunoreactivity and the tumor grade.

As regards to the relationship between CK20 immunoreactivity and the tumor stage, the present study revealed a statistically significant increase in CK20 positivity with increasing tumor stage in cases of urothelial carcinoma ($P<0.05$), as 40% of non-infiltrating papillary tumors (Ta), 50% of cases of urothelial carcinoma in situ (Tis), and 93% of tumors that invaded the deep muscle (T2b) were CK20 positive.

In accordance with the results of the present study, Desai et al. (2000) [7] reported that a higher frequency of CK20 positivity was observed with increasing tumor stage ($P= 0.003$). These results coincided also with those reported by Shahin and Farag (2004) [28]. However, our results did not agree with Jason et al. (2003) [25], who reported that as tumors increased in stage, the expression of CK20 decreased.

The present study revealed that CK20 positivity was detected in 66.7% of cases of urothelial carcinoma, 0% of squamous cell carcinoma cases and 100% of cases of adenocarcinoma, with a statistically significant difference ($P<0.05$), which agreed with the findings reported by Ramos et al. (2003) [26], who reported that all cases of squamous cell carcinoma in their study were CK20 negative, and 97% of their studied cases of adenocarcinoma were CK20 positive.

In the present study, 63.2 % of Bilharzial bladder carcinoma were positive for CK20, while 65.4% of cases of non Bilharzial bladder carcinoma were positive for CK20 without statistically significant difference ($P>0.05$). These findings agreed with Klein et al. (2000) [30], who reported that 58% of Bilharzial bladder carcinomas were positive for CK20 versus 55% of cases of non-Bilharzial bladder carcinoma.

In conclusion, Loss or reduction of CD44 immunoreactivity and increasing CK20 positivity were significantly associated with increasing tumor grade and stage in the studied cases of urothelial carcinoma and to each other, so, they have combined utility in predicting the behaviour and prognosis of urothelial carcinoma, with no significant difference in their expression between non Bilharzial and Bilharzial bladder carcinomas.

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الملخص العربى

دراسة تعبير سى دى44 و سيتوكيراتين20 فى سرطان المثانة البولية غير المصاحب والمصاحب للبلهارسيا

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قسم الباثولوجيا–كلية الطب–جامعة طنطا

يعد سرطان المثانة البولية واحدا من أكثر أنواع السرطانات شيوعا على مستوى العالم فهو يعد رابع أكثر أنواع الأورام الخبيثة شيوعا فى الرجال و التاسع فى السيدات. تضم عائلة ال سى دى44 مجموعة من الجليكوبروتينات التى تمر عبر الغشاء الخلوى ،والتى تلعب دورا كمستقبلات لمادة الهيالورونات، كما تعمل على تماسك الأجزاء المختلفة للمواد خارج الخلوية. ويلعب ال سى دى44 دورا محوريا فى التفاعلات التى تحدث بين الخلايا وبعضها وبينها وبين المواد المحيطة بها، فعند تناقصه يقل التماسك بين الخلايا وبعضها و بينها وبين الغشاء القاعدى للخلايا مما يساعد على انتشار الورم.ومن المرجح أن التعبير المناعى الهستوكيميائى ل سى دى44 يرتبط بدرجة تميز الورم وبالمعايير الإنذارية المختلفة لسرطان المثانة البولية. أما سيتوكيراتين20 فهى عائلة من البروتينات تشكل الهيكل الخيطى الوسطى للخلايا الطلائية. ومن المرجح أن سيتوكيراتين20 يمكن أن يكون نذيرا للتغيرات السرطانية ولتقدم الورم فى سرطان المثانة البولية.

الهدف من البحث : دراسة التعبير المناعى الهستوكيميائى ل سى دى44 وسيتوكيراتين20 فى سرطان المثانة البولية وربط ذلك بالمعايير الإنذارية المختلفة متضمنة درجة ومرحلة الورم فى الأورام المدروسة.

مواد وطرق البحث: تمت صباغة 71 من حالات سرطان المثانة البولية بصبغة الهيماتوكسيلين والإيوسين وبالصبغات المناعية ل سى دى44 وسيتوكيراتين20 .

النتائج: أوضحت الدراسة أن هناك ارتباط ذو دلالة بين التعبير المناعى الهستوكيميائى ل سى دى44 و سيتوكيراتين20 والمعايير الإنذارية المختلفة متضمنة درجة ومرحلة الورم فى حالات سرطان الخلايا الظهارية المبطنة للمثانة البولية ، ولم توجد علاقة بين تعبير كل من سى دى44 و سيتوكيراتين20 ووجود البلهارسيا.

الخلاصة: وجد أن فقد أو نقص التعبير المناعى الهستوكيميائى ل سى دى44 وزيادته ل سيتوكيراتين20 له ارتباط ذو دلالة بزيادة درجة ومرحلة الورم كما أن تعبير كل منهما له ارتباط ذو دلالة بتعبير الآخر ولذلك فإن لهما فائدة مشتركة فى التنبؤ بسلوك وتقدم الأورام السرطانية للخلايا الطلائية المبطنة للمثانة البولية، ولم يوجد فارق فى تعبيرهما بين السرطانات غير المصاحبة للبلهارسيا والمصاحبة لها.