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The Endoglin (CD105) Expression as a Marker of Tumour Vasculature in Urinary Bladder Tumours of Iraqi patients

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Abstract

The Endoglin CD105 is a good marker for metastatic in solid tumors . It has a role in angiogenesis process which mediates blood vessels formation. The aim of this study to evaluate CD105 immunoexpressions in bladder tumors, and to determine its relation with clinico-pathological grades in patients with UBC . The study included 50 biopsies from patients with urinary bladder carcinoma (UBC) and 15- benign bladder biopsies from patients with other bladder disorders rather than cancer (UBD). CD105 immunostaining was assessed using Envision method . Immunohistochemical (IHC) studying showed that (CD105) proteins were significantly high expressed in UBC compared with UBD patients (80% vs. 33.3 % ; $p \leq 0.01$) , and there was significant difference in the positive IHC results of this protein in correlation to clinico-pathological grades in UBC patients ($p \leq 0.01$) . we conclude that CD105 IHC expressionin bladder cancer may be consider as prognostic marker .

Keywords: Bladder cancer; angiogenesis, invasive carcinomas, CD105.

Introduction

"CD105 (endoglin) is a proliferation- associated and hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells (EC) . It is a receptor for transforming growth factor (TGF) -β1 and -β3 and modulates TGF-β signaling by interacting with TGF-β receptors I and/or II. CD105 (endoglin) is a marker of tumor microvessels and is associated with tumor progression"[1]. CD105 can spread to the blood circulation, whit an increase in its levels in patients with different types of cancer .In addition to its worse tumor metastasis correlation [2]. All malignancies studied showed that endoglin expression as determined by immunohistochemical staining was consistently associated with lower patient survival rates. This is due to the fact that increased tumor vasculature is an established

marker of poor prognosis. Furthermore, in gastrointestinal, breast, prostate, and head and neck malignancies, endoglin expression was associated with the advanced high stage disease [3],[4],[5],[6],[7]. So Endoglin expression seems to have prognostic value in a variety of solid cancers. In Iraq, the Endoglin expression in bladder tumors was not studied, and in this study the association between the Endoglin expression and UBC grades had been shown for the first time.

Materials and Methods

Fifty biopsies from patients with urinary bladder carcinoma (UBC), 43 (male) and 7 (female) with an average age 63.7 years and a ranged from 27 to 83 years, were included in this study, the patient samples were collected from AL-Yarmook Teaching Hospital, and Gazii AL-Harriri Hospital for Specialists Surgeries . The diagnosis of these tissue blocks were primarily based on the obtained histopathological records of bladder biopsy samples in hospital laboratory. Confirmatory histopathological re evaluation of each obtained tissue blocks was done by specialist pathologist . In addition 15 benign bladder biopsies from patients with urinary bladder diseases(UBD),they were 8 males and 7 females with an average age of 52 years range of (32-78) years . For each case, one representative section was stained with Hematoxylin and Eosinand the histopathological diagnosis was revised, while other sections were put on positive charged slides and stained immunohistochemically for CD105 . Immunohistochemical staining was carried out using the Novocastra TM Polymer Detection Systems (Envision technique) by using commercial kit from Novocastra, Newcastle, UK, RE7150-K , the slides were deparaffinized, rehydrated then blocked. All of the slides were treated with anti CD105 monoclonal antibody, dilution1:100 (Dako, Denemark), then incubated with a post primary block solution for 30 minutes. In the next step the slides were rinsed gently in PBS 2× 5 and tissue sections incubated with a secondary antibody Novolink TM polymer mouse and rabbit immunoglobulins) for 30 minutes, washed in PBS 2× 5 with gentle rocking. After washing, the samples were stained with diluted liquid DAB, and then counter stained with hematoxylin . Slides washed, dehydrated then mounting, and examining under light microscope at 10X,20X,40X magnification.

Results and Discussion

1-: Histopathology

Most our study patients had transitional cell carcinoma as the type of bladder carcinoma . .
Concerning the grading of the tumor , our study showed that most of patients presented with high grade G ≥ 2 tumor 32/50 (64 %),while 18/50 (36 %) were with low grade G ≤ 1 tumor (Figure 1) **.** This results indicated patients with poor differential seen in 32 cases(64%) of UBC patients , most of them was muscle invasion , this agreed with several studies done in Iraq [8],[9] , the possible explanation, most cases of muscle invasion with high grade of tumor .

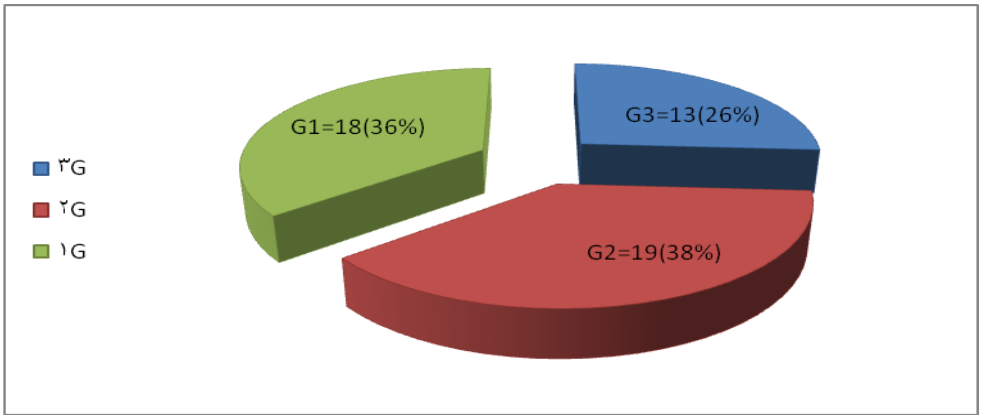

Figure (1): Pie chart showing the distribution of TCC cases according to grade of UBC

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2: CD105 IHC Scores in Bladder Patients Groups

According to the resent studies CD105 is a proliferation-associated marker of endothelial cells , and that its expression correlates strongly with cell proliferation markers in tumor endothelia[10,11]. In addition , It has a good tumor angiogenesis marker in breast cancer [12], malignant melanoma [13], and colorectal carcinoma [14].

In this study, the positive immunohistochemical expression of CD105 protein was significantly higher in UBC patients in comparison with that of UBD patients (80% versus 33.3 % ; $p \leq 0.01$) (Table 1) (Figure 2). These results are in agreement with Tanaka *et al*. (2001) who suggested that CD105 is weakly expressed in normal tissues, but it is strongly expressed in tumor endothelia of lung[15]. In terms of scores, UBC patients with the score ++ represented the highest frequency (42.5%). While, the score scatter ≤ 10 represented the highest frequency in UBD (60%) .

Table (1): CD105 expression in bladder patients groups

Study groups		CD105 EXPRESSION		Total	P value
		positive	negative		
UBC	No	40	10	50	8.528 **0.0063
	Percentage%	80	20	100	
UBD	No	5	10	15	
	Percentage%	33.3	66.7	100	
Total	No	45	20	65	
	Percentage%	69.2	30.8	100	

** (P<0.01).high significant

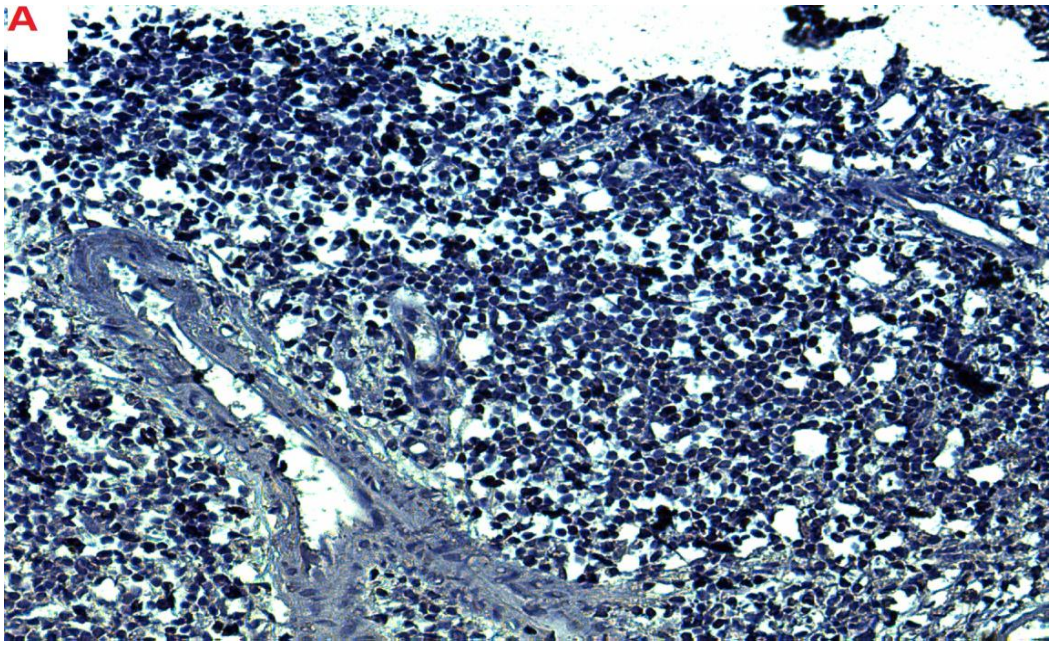

Figure (2): A : Invasive transitional cell carcinoma , poorly differential(Grade II) showing no detectible CD105 (Endoglin) immunostaining (Score 0 negative) (20X)

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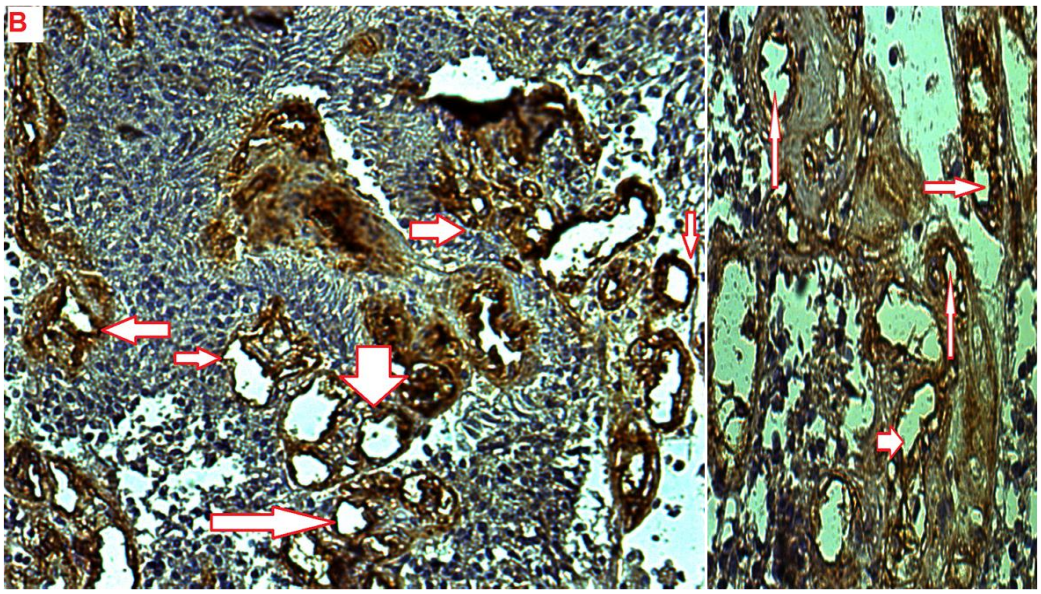

Figure (2): B:Invasive transitional cell carcinoma , poorly differential(Grade II) showing positive CD105 immunostaining (score +++ , brown) (arrow) in bladder tissues . (20 ,40X).

However, (Table 2) showed the frequency of distribution of CD105 scores in group subjects . Chi-Square test showed that there was high statistical difference ($p \leq 0.01$) between urinary bladder carcinoma and other urinary bladder disorders for CD105 IHC scores in tissue sample taken from each case . Regarding the tumor grade of UBC , CD105 was detected in 11 out of 18 of grade-1, 18 out of 19 of grade-2, and 11 out of 13 of grade-3. There was a high significant association between the grade of UBC and the immunohistochemical expression of CD105 ($p \leq 0.01$) , the majority of high grade tumor cases showed positive immunohistochemical CD105 expression 29(90.6%), while only 11 cases (61.1%) of low grade tumor showed positive immunohistochemical CD105 expression , (Table 3).

Table (2): Frequency of CD105 IHC scores in patients groups

CD105 Score	UBC		UBD		Total	
	No.	%	No.	%	No.	%
+++	12	30.0	0	0.0	12	26.7
++	17	42.5	0	0.0	17	37.8
+	9	22.5	2	40.0	11	24.4
Scatter ≤ 10	2	5.0	3	60.0	5	11.1
Total	40	88.9	5	11.1	45	100
P-value $\chi 2$ –value	---	0.005 7.742 **	---	0.0016 10.39 **	---	0.004 7.955 **

** (P<0.01).High significant

These results agree with Dales *et al*. (2003) who found CD105 expression correlated with a high risk for metastasis among all patients with breast cancer [12] , and compatible with recent studies identified endoglin CD105 expression in several solid tumor types, with the level of expression correlating with various clinicopathologic factors including decreased survival and presence of metastases [1] . However, metastasis is an important cause of death for patients with cancer and there is evidence pointing out for a role of angiogenesis in the dissemination of malignant tumors [16].

"Newproliferating capillaries have leaky basement membranes, making them more accessible to tumor cells than mature vessels. It has been demonstrated that increasing density of newly formed microvessels in growing tumors correlated closely with increasing number of tumor cells shed into the bloodstream and develop metastasis"[17].

Table (3) Association between CD105 IHC expression with tumor grade

parameter		CD150 EXPRESSION		Total	P-value $\chi 2$ –value
		positive	negative		
Grade of UBC	Low grade (G≤ 1)	11(61.1%)	7(38.9%)	18	0.0032 7.265 **
	High grade (G≥ 2)	29(90.6%)	3(9.4%)	32	
	Total	40(80%)	10(20%)	50	

** (P<0.01) high significant

Chi-square was used to compare the results of frequency distribution of CD105 scores among tumor grades of UBC and it showed a high significant correlation between each score and tumor grade (P = 0.0027) (Table 4).

Table(4) : The percentage of CD105 expression in bladder carcinoma patients in relation to the tumor grade.

CD105 IHC scor	Grade-1	Grade-2	Grade-3	% of total
0 No.	7	1	2	10
%of Total	(38.8)	(5.3)	(15.4)	(20)
Scatter ≤10 % No.	1	1	0	2
%of Total	(5.6)	(5.3)	(0.0)	(4)
10-30% (+) No.	5	4	0	9
%of Total	(27.8)	(21)	(0.0)	(18)
30-50 % (++) No.	4	8	5	17
% of Total	(22)	(42.1)	(38.5)	(34)
50-80% (+++) No.	1	5	6	12
% of Total	(5.6)	(26.3)	(46.1)	(24)
Total No.	18	19	13	50
%of Total	(36)	(38)	(26)	100.0
P value	0.0027			
X^2	8.552 **			

** (P<0.01).high significant

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