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RESEARCH ARTICLE

Expression of Endoglin in Bladder Carcinoma of Iraqi Patients

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Abstract

Angiogenesis is an important process for tumor growth in almost all malignancies. Endoglin (CD105) is a membrane glycoprotein, part of the TGF beta receptor complex, which strongly expressed on the surfaces of cancerous cells. It has a vital role in angiogenesis, tumor growth, survival and metastasis of cancer cells to other locations in the body. Methods: This study designed to evaluate the role of endoglin immune expression in the development of bladder carcinoma by using monoclonal antibody, 137 biopsies of bladder carcinoma were collected from patients and 42 normal bladder tissues were collected from forensic autopsy. Results: 118 (86.13%) out of 137 tumors and 27 (64.26%) of normal tissues gave positivity with different scores while other biopsies expressed negative staining pattern. Score +3 showed the highest frequency among the tumor tissues while normal bladder tissues expressed score+1 as the highest one. Conclusion: endoglin is a marker of angiogenesis closely associated with malignant potential and bladder carcinoma development.

Keywords: *Endoglin, Angiogenesis, Urinary bladder Carcinoma (UBC)*

Introduction

Bladder cancer is the most common malignancy of the upper urinary tracts, that is an excellent example of malignancy arising from the combination of environmental carcinogens and various genetic Angiogenesis is the formation process of new blood vessels from preexisting vessels, which controlled by endogenous factors secreted by tumor cells. It is essential for tumor growth and transition from a benign state to a malignant one. Tumors display considerable variation in the patterning and properties of angiogenic blood vessels [1, 2]. Endoglin is an integral membrane highly glycosylated protein produced by various cells, which encoded by the gene located on chromosome 9 composed of 15 exons.

It has a low expression level in the resting endothelial cells, but ot is highly expressed in the endothelial cells at sites of active angiogenesis such as tumor vessels, inflamed tissues [3, 4].Endoglin is the part of TGF-ß receptor complex, which known as a hypoxia inducible transmembrane glycoprotein that plays a dual and a paradoxical effect on the cancer. Endoglin has a crucial role in the modulation of TGF-ß receptor signaling, which mediates cellular localization, migration, morphology, proliferation and cluster formation, therefore, making it an important protein for tumor growth, survival and metastasis of cancer cells to other locations in the body [5, 6].Up regulation of endoglin was observed in the endothelium of UBC when compared with normal urothelium, and was associated with intravesical recurrence. Patients with high expression of endoglin in the primary tumor tissue had 2.67 fold risk of intravesical recurrence than those with low expression [7, 8].

Materials and Method

Samples Collection

One hundred and thirty-seven biopsies collected from patients after cystoscopy or radical cystectomy while 42 normal bladder biopsies had been collected from forensic autopsy. Samples fixed in 10% formalin and embedded in paraffin blocks. Slides were prepared from tissues embedded in the paraffin blocks then stained by hematoxylin and eosin before examined by the histopathologist to determine the degree of

tumors differentiation. After that, 5 µm thickness sections were made from each paraffin embedded block and fixed on positively charged slides to be subjected to immunohistochemistry procedures for detection of endoglin.

Detection of Endoglin by Immunohistochemistry Technique

Before proceeding with the staining protocol, the slides must be deparaffinized. Incomplete removal of paraffin can cause poor staining of the section. Sections were deparaffinized in three changes of xylene for 4 min of each then rehydrated by immersing the slide sequentially for 3 min. in each of xylene, absolute ethanol, 90%EOH, 70%EOH, 50%EOH**,** tap water and PBS buffer. The slides were kept in the buffer until being ready to perform antigen retrieval. Most formalin-fixed tissues require an antigen retrieval step before immunohistochemically staining can proceed.

This is due to the formation of methylene bridges during fixation, which cross-links proteins and therefore masks antigenic sites. Buffer heat- -induced epitope retrieval was performed by placing a steel holder that carries the slides in Sodium Citrate buffer (pH=6) for endoglin then placing the container in a microwave histoprocesser at (850 w) for 20 min.

After cooling for 10-15 minutes at (4°C), the slides were removed and washed by wash buffer for 10 minutes. Immunohistochemistry is a method for demonstrating the presence and location of antigen in tissue sections by using antibodies that are highly specific to recognize only the target antigen by sequential reaction of a specific primary antibody to its corresponding antigen.

The specific antibody is located by a biotin-conjugated secondary antibody. This step is followed by the addition of a streptavidin-enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody, secondary antibody, and streptavidin-enzyme complex are then visualized with an appropriate substrate /chromogen.

Finally, the slides were dehydrated and mounted with DPX and coverslip. Then, slides were examined by compound light microscope 10X, 20X and 40X. Results were compared with positive controls which were determined according to the leaflet. Positivity was assessed semi- quantitatively by the intensity and percentage of staining. Score was determined for endoglin according to following scale when membrane of the cell has been stained with a brown color. i-Score 0 (negative): (none of the cells revealed positively for the marker)ii-Score 1 (weak positive (+1): number of positive cell represents 10% or less of total (few scatter ≤10%) iii-Score 2 (moderate positive (+2): the positive cells 11≤30%.iv-Score 3(strong positive (+3): the positive cells31 ≤50 %.v-Score 4 (very strong (+4): the positive cells more than 50% [9, 10].

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to determine the effect of the difference factors in study parameters. Chi-square test was used to significant compare between percentages in this study [11].

Results and Discussion

Referred to histopathologist diagnosis, all tumors have been identified as transitional cell carcinoma. The risk of bladder cancer increases with age and the mean age at diagnosis was 66.5 year rang from (44-85) and 63.2year range (45-81) UBC patients and control respectively. The age group (61-70) year had the maximum number of UBC patients while no patient was recorded in the age group less than 40 years. Such findings declared that the older individual was more susceptible and had a great chance of bladder carcinoma.

This result agreed with the result reported by Al-Biaty (2015) who recorded > 60 years which was the highest risk group for bladder cancer. Bladder carcinoma incidence was strongly related to age. About 9 out of 10 people with UBC were older than 55 years, with the highest incidence rates being in elderly men and women.

Age-specific incidence rates rise gradually in ages 50-54 of both males and females, with a sharper rising in males from age 60-64, to peak in both sexes in the 85+ age group [13, 14].Stage and grade for each tumor were identified according to WHO. Pathological evaluation showed that most of the bladder carcinoma presented with the high grade,

eighty two (59.9%) tumors characterized by high grade and 55(40.1%) low grade while according to stage of tumors 19 (13.9%) Ta, 40 (29.2%) T1 invade subepithelial connective tissue, 45 (32.8%) T2 invade muscle, 25(18.3) T3 invade perivesical tissue and 8 (5.8%) T4 invade another organ as shown as Table (1).

Table 1: Identification of Urinary Bladder Carcinoma according to WHO

Grade	Stages					Total N (%)
	Ta N (%)	T1 N (%)	T2 N (%)	T3 N (%)	T4 N (%)	
Low	19 (13.9)	35 (25.5)	1 (0.7)	DZADDC -	-	55 (40.1)
High	-	5 (3.7)	44 (32.1)	25 (18.3)	8 (5.8)	82(59.9)
Total	19(13.9)	40(29.2)	45(32.8)	25(18.3)	8(5.8)	137(100)

N: number %: percentage

This result agreed with other researchers who recorded that transitional cell carcinoma was the most common form of bladder cancer which including non-muscle invasive, muscle invasive, and metastatic lesions [15]. Nearly10 % of bladder carcinoma was squamous cell or adenocarcinoma. Approximately 70% of the patients had non muscle invasive and the remaining 30% had muscle-invasive tumors. Stage and grade are very important to determine treatment because the non-muscle-invasive tumors should be treated totally different from muscle-invasive tumors [16].

In the non-muscle-invasive disease, TURBT paired with adjuvant intravesical chemotherapy or immunotherapy is the choice treatment while in the muscle invasive disease, cystectomy is the most appropriate curative option [17]. On the other hand, overall rate of recurrence of non-muscle invasive bladder tumor was ranged from 60% to 70%, and the overall rate of progression is 20% to 30% [18].

The positive immunoexpression of endoglin protein was significantly higher in UBC patients in comparison with control. The positive staining expressed by membrane of 118(86.13%) tumors tissues and by 27(64.29%) of normal urothelium tissues with different staining scores, while negative staining was observed in 19(13.87%) of tumors and15 (35.71%) of normal tissues with significant differences ($p \leq 0.01$) as shown in Table (2) and Figures (1, 2). CD105 is weakly expressed in normal tissues, but it is strongly expressed in tumor endothelia of the lung [19].

Endoglin is highly expressed in the plasma membrane of the proliferating endothelial cell of tumor vessels, including those of lung, breast, bladder, gastric, liver, renal cell and ovarian cancers [20].The endoglin expression level correlates with endothelial cell proliferation, and often used as a marker of tumor angiogenesis and a potential therapeutic vascular target in oncology.

Table 2: Endoglin Expression in UBC and Control

Group	Endoglin Expression			Total		P-Value
	Positive	Negative				
	N (%)	N (%)		N (%)		
UBC	118(86.13)	19(13.87)		137(76.54)		0.0001 **
Control	27(64.29)	15(35.71)		42(23.46)		
Total	145(81)	34(19)		179(100)		

N: number %: percentage UBC: Urinary bladder Carcinoma

In terms of score, tissues of UBC patients reflect a high percentage of positive staining with score + ++ (33.58%) followed by score ++ (24.09 %) then Score ++++ (22.63%) and score + gave the lowest percentage of positive staining (6.5%) while represented the highest frequency (45.24%) in normal tissues with significant differences as shown in Table (3) but also noticed some tumors had necrosis thus membrane of the cancerous cells not expressed the endoglin clear.

Table 3: Frequency of Endoglin Scores in UBC and Control

Score	UBC	Control	Total
	N (%)	N (%)	N (%)
Score 0	19(13.87)	15(35.71)	34(18.99)

(Negative)			
Score +	8(5.84)	19(45.24)	27(15.08)
Score ++	33(24.09)	8(19.05)	41(22.91)
Score +++	46(33.58)	0	46(25.70)
Score++++	31(22.63)	0	31(17.32)
Total	137(100)	42(100)	179(100)
** (P<0.01).			

N: number %: percentage UBC: Urinary bladder Carcinoma

Endoglin expression was increased in the invasive carcinoma of the bladder. Results in Table (4) showed higher positive expression of endoglin in muscle invasive stages than expression in non invasive stages. Muscle invasive carcinoma scored +++ (32.2%) then score ++++ (22.88%) as the highest frequency while non invasive reflect score ++ (21.19%) as most dominant score.

Table 4: Positive Expression of Endoglin according to Tumor Stage

Stage	Score				Total N (%)
	+	++	+++	++++	
	N (%)	N (%)	N (%)	N (%)	
Non invasive (Ta-T1)	5 (4.24)	25(21.19)	8(6.78)	4(3.39)	42(35.60)
Invasive (T2-T3-T4)	3(2.54)	8(6.78)	38(32.20)	27(22.88)	76(64.40)
Total	8(6.78)	33(27.97)	46(38.98)	31(26.27)	118(100)
P value	0.802 NS	6.58 **	8.61 **	6.89 **	9.17 **

** (P<0.01); NS: not significant

Results showed a significant statistical difference between positive expression in the bladder carcinoma characterized by high grade 77 (56.20)% and low grade 41 (29.93)% while negative expression in high grade 5(3.65%) and 14(10.22%%) in low grade as shown in Tables (5) &(6). Results agreed with Saroufim and his colleagues who reported that positive expression of CD105 in tumoral cells was found to be significantly and directly correlated to high-grade tumors, and more advanced stages [21].

Table 5: Association between endoglin expression and UBC grade

Grade	Endoglin Expression		Total
	Positive	Negative	N(%)
	N (%)	N(%)	
Low	41(29.93)	14(10.22)	55(40.1)
High	77(56.20)	5(3.65)	82(59.9)
Total	118(86.13)	19(13.87)	137(100)
P Value	**8.41 ****	**2.93 NS**	**7.36 ****

** (P<0.01); NS: not significant

Table 6: Scores of Endoglin positive expression and UBC grade

Grade	Endoglin expression Scores				Total
	+	++	+++	++++	
	N(%)	N(%)	N(%)	N(%)	N(%)
Low	5(4.23)	24(20.34)	9(7.63)	3(2.54)	41(34.74)
High	3(2.54)	9(7.63)	37(31.36)	28(23.73)	77(65.26)
Total	8(6.78)	33(27.97)	46(38.98)	31(26.27)	118(100)
P value	0.802 NS	5.02 *	8.71 **	7.46 **	9.16 **

* (P<0.05), ** (P<0.01)

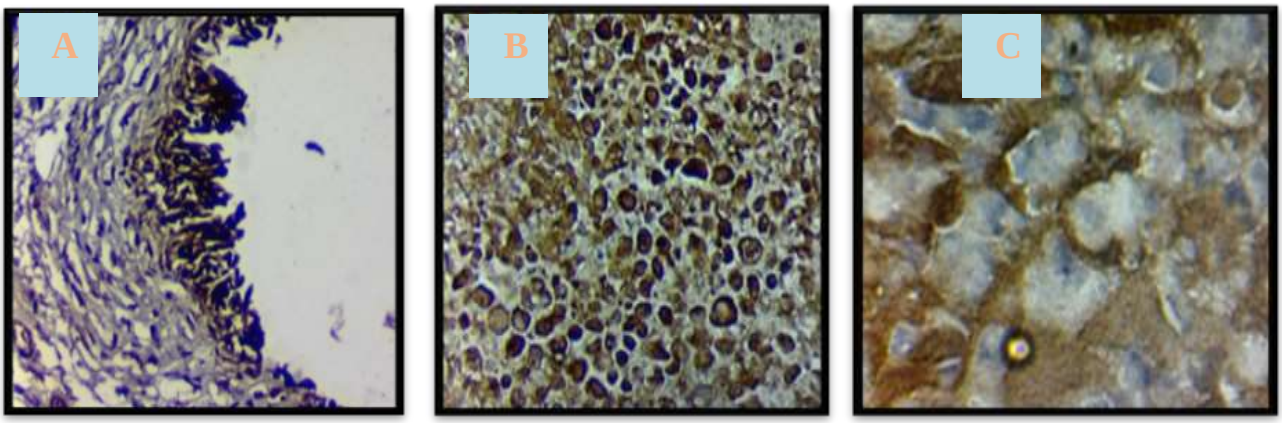

Figure 1: A: Positive Immunexpression of endoglin of normal urothelium (10 X);B:Positive Immunoexpression of endoglin (Invasive Stage: T2, High Grade, Score ++++) section under 10X C: Section under 40 X

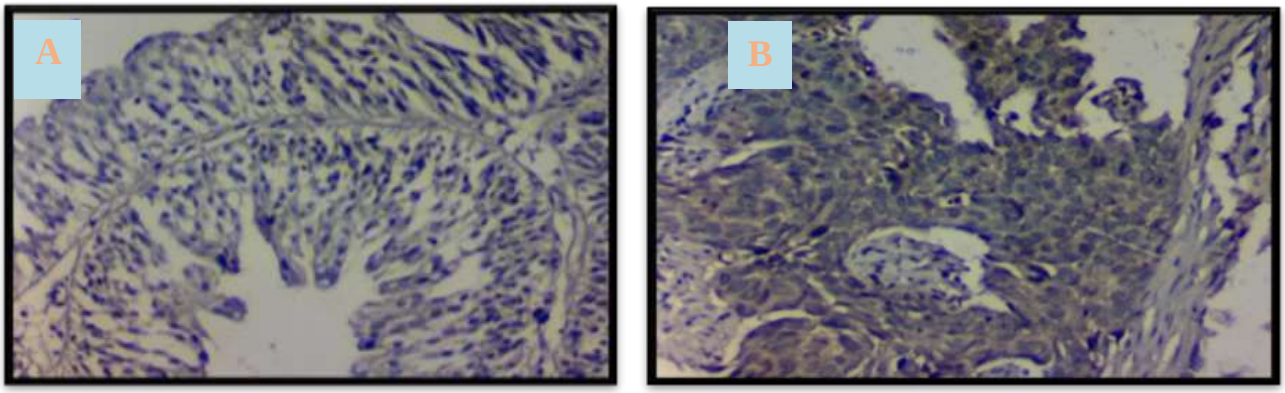

Figure 2: Negative Immunoexpression of endoglin A: Non Invasive Stage: T1, Low Grade. B: Invasive Stage: T2, High Grade (10X)

CD 105 is a membrane localized glycoprotein that modulates angiogenesis by regulating cellular proliferation, differentiation, and migration. CD105 is more closely associated with malignant potential and it is an independent predictor for tumor progression and survival with no or only weak expression in blood vessels of most normal urothelium tissues [22, 24].

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