Expression of Vascular endothelial growth factor (VEGF) in squamous bladder cancer with different grades of differentiation (Immunohistochemical study)

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التعبير المفرط لعامل نمو البطانة الوعائية (VEGF) في سرطان المثانة البولية الحرشفي وعلاقتهما بدرجة التمايز النسيجي (دراسة مناعية نسيجية كيميائية) ،

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الملخص

الأهداف: من اجل تقييم مغزى التعبير المفرط لعامل نمو البطانة الوعائية في سرطان الخلايا الحرشفي للمثانة البولية وعلاقته مع درجة التمايز.

الطريقة: تم الحصول على ٢٠ عينة مثبتة بالفورمالين ومطمورة بالبارافين لمرضى مصابين بسرطان الخلايا الحرشفي للمثانة البولية. كما تم الحصول على ١٠ نماذج لنسيج المثانة الخالي من الأورام (والمصابة بالالتهاب) حيث استخدمت كمجوعة قياسية. استخدمت طريقة ABC لتحديد التعبير المناعي النسيجي لعامل VEGF.

النتائج والمناقشات: أظهرت ألنتائج أن التعبير النسيجي المناعي لـVEGF كان موجبا في ٨ حالات (٤٠%) من اصل ٢٠ حالة لسرطان الخلايا الحرشفي للمثانة البولية بينما لم نلاحظ أي تعبير نسيجي لـ VEGF في حالات التهاب المثانة البولية الحميد ($P<0.05$). إن زيادة شدة إظهار VEGF (score+4) سجلت المستويات الأعلى وإنها ترتبط بعلاقة ايجابية معنوية مع درجة التمايز مشيرة إلى إن سرطان الخلايا الحرشفي للمثانة البولية موجبة VEGF هي عدوانية حيويا وتتواجد أكثر تكرارا في درجة التمايز الثالثة. إن تردد إظهار VEGF وشدته لا ترتبط بعلاقة ايجابية معنوية مع درجة التمايز مشيرة إلى إن سرطان الخلايا الحرشفي للمثانة البولية موجبة VEGF هي لا تزال موضع جدل حول أهميتها كعامل قياسي لعواقب سرطان الخلايا الحرشفي للمثانة البولية .

الاستنتاج: أن VEGF يلعب دور مهم في تولد و نشأة سرطان الخلايا الحرشفي للمثانة البولية ويسند الدليل عن دوره في تكاثر الورم ونموه ودرجة تمايزه.

Abstract

Objective: The present investigation was designed to estimate the immunohistochemical expression of Vascular Endothelial Growth Factor (VEGF) in squamous cell carcinoma of urinary bladder in correlation to the grade of differentiation as a prognostic parameters and to determine whether the expression is correlated with the increased incidence of this type of cancer.

Methods: Formalin fixed, paraffin-embedded blocks from 20 (15 male and 5 female) patients with squamous cell carcinoma of urinary bladder were submitted in this study in the Department of Pathology, College of Medicine, Kufa University. A group of 10 patients with benign bladder lesions (cystitis) was included in this study as a control group. The Avidin-Biotin Complex (ABC) method was employed for immunohistochemical detection of VEGF. Statistically Chi square and Correlation-Regression (R) tests were used by the help of SPSS, using the P value significance at level equal or less than 0.05 and R value at level 0.3.

Results: VEGF immuno-expression was positive in 40% of squamous cell carcinoma of urinary bladder but was negative in all benign bladder lesions (cystitis) with significant difference (P<0.05). VEGF immunostaining was positively correlated with grade of squamous cell carcinoma of urinary bladder but the findings were not statistically significant (P<0.05).

Conclusion: These findings support the role of VEGF in the carcinogenesis of squamous cell carcinoma of urinary bladder regarding its biological behavior and aggressiveness, and thus VEGF overexpresion could be considered as a poor prognostic parameter in squamous bladder cancer. No positive correlation has been noticed between immunohistochemical expression and the high incidence of squamous cell carcinoma of urinary bladder.

Introduction

It is well established that bladder cancer is an aggressive and potentially fatal malignancy, reported as the 7th most common cancer worldwide, the 4th most common cancer in men, and the 8th most common cancer in women. (1) Primary squamous cell carcinoma of urinary bladder is a malignant neoplasm derived from bladder urothelium with pure squamous phenotype. (2) It is accounted as the second common cancer of the urinary tract after transitional cell carcinoma accounting with an average incidence around 5% among urinary bladder carcinomas. (3) Almost all squamous cell cancers are already advanced and muscle infiltrative at the time of diagnosis. (4)

Angiogenesis play a crucial role in tumor growth, progression and invasive capacity because it enables delivery of oxygen and nutrients. Among the angiogenic cytokines which can be secreted by some tumor cells and involved in angiogenic switch, vascular endothelial growth factor (VEGF) is the principle factor involved in this process. (5)

VEGF or vascular permeability factor (VPF) is produced by a number of different cell types in response to various stimuli. It belongs to the gene family that includes placental growth factor (PLGF). VEGF isoforms are VEGF121, VEGF165, VEGF189, and VEGF206 while the VEGF family includes: VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D, and VEGF-E. VEGFA has a molecular weight of 34-46 kDa and contains 232 amino acids. Its location is designated as being 6p12 and alias is VEGFA. (6)

VEGF has emerged as the central regulator of the angiogenic process in physiological and pathological conditions especially as poor prognostic indicator for the tumor aggressiveness and patient's survival. (7)

The present study aimed to clarify whether there is a correlation between VGEF over-expression and the grade of differentiation of squamous cell carcinoma of urinary bladder and to assess whether such a correlation could be used as a prognostic factor for the early diagnosis of malignancy.

Materials and Methods

Between January of 2006 and December of 2007, a twenty (15 male and 5 female) formalin fixed paraffin embedded samples from patients with primary squamous cell carcinoma of urinary bladder were collected from the major hospital and private laboratories in the Najaf area in Iraq. All biopsies were classified, according to modified WHO classification into three grades; in which we can see that 18(90%) of cases were recorded as grade III and only 2(10%) as grade II while none as grade I. The age range of patients was from 56 to 87 years, with a mean age of 65 years. A group of 10 patients

with benign bladder lesions (cystitis) were used as control samples. The Avidin-Biotin Complex (ABC) method was employed for immunohistochemical detection of VEGF.

The character for positive immunostaining is brown cytoplasmic precipitate for VEGF, while its intensity was assessed by counting the percentage of stained cells in hundred malignant cells. The immunostaining was calculated as the percentage of immunoreactive cells per total number of malignant cells. The intensity is scored zero when negative (none of the malignant cells revealed a specific cytoplasmic immunostaining for VEGF), score 1 is decided when 5-10% of malignant cells, score 2 when less than 25%, score 3 when 25-50%, and score 4 when more than 50% of tumor cells revealed positive immunostain for VEGF (Figure 1 and 2). [8]

Results were statistically analyzed by the help of SPSS version 15 software statistical package using P value at level of significance equal or less than 0.05, and correlation test (R at a significant level of 0.3).

Results:

In all sections of benign control lesions (cystitis), none of the ten examined samples revealed cytoplasmic immunostaining for VEGF. Among the malignant samples, overexpression was detected in 8(40%) of samples of squamous cell carcinoma of urinary bladder. This difference was statistically significant ($p<0.05$) (Table 1).

Table 2 shows the distribution of positive immunostaining for VEGF in different grades of malignant sample of squamous cell carcinoma of urinary bladder according to the modified WHO classification. The VGEF over-expression shows a positive percentage of 50% of grade II and 39% of grade III. No statistically significant correlation or differences among the three grades were found ($P>0.05$, $R<0.3$).

Table 3 revealed that most of the positive cases was detected in 6 out of 8 cases (75%) scored as 4, while minority (25%) scored as 3 without significant correlation or difference for the intensity of VEGF immunostaining among different grades ($P>0.05$, $R<0.3$).

Table 1: Immunohistochemical expression of VEGF in benign and malignant bladder tissues.

Type of Tissue	Immunostaining of VEGF				Total	
	Positive		Negative			
Benign (Cystitis)	0		10	100%	10	33%
Malignant (SCC)	8	40%	12	60%	20	67%
Total	**8**	**27%**	**22**	**73%**	**30**	**100%**

(P<0.05)

Table 2: Immunostaining of VEGF in relation to grade of tumor.

Grade	Immunostaining of VEGF		Total
	Positive	Negative	

Grade I	0		0		0	
Grade II	1	50%	1	50%	2	10%
Grade III	7	39%	11	61%	18	90%
Total	**8**	40%	**60**	25%	**20**	**100%**

(P>0.05, R <0.3)

Table 3: Correlation between intensity of VEGF and grade of tumor

Grade	**Score**					**Total**
	0	**+1**	**+2**	**+3**	**+4**	
Grade I	0	0	0	0	0	0
Grade II	1(50%)	0	0	0	1(50%)	2(10%)
Grade III	11(61%)	0	0	2(11%)	5(28%)	18(90%)
Total	**12(60%)**	**0**	**0**	**2(10%)**	**6(30%)**	**20(100%)**

(P>0.05, R <0.3)

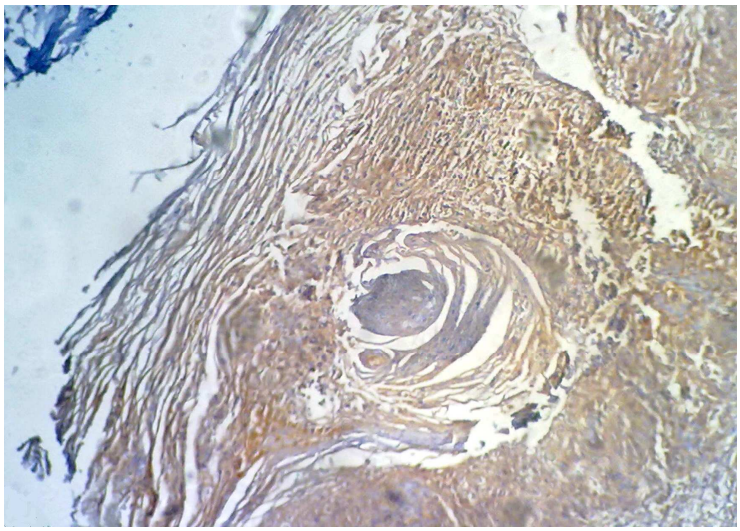

Figure 1: Primary squamous bladder cancer showing diffuse strong (score +4) VEGF immunostaining (20X).

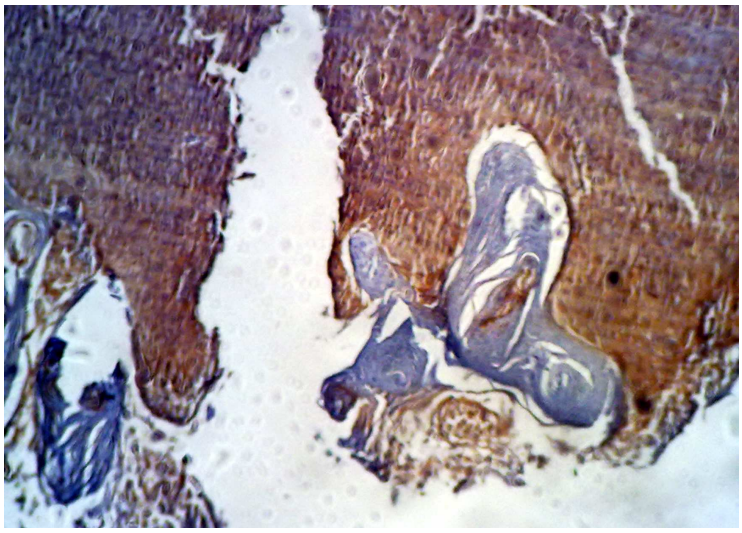

Figure 2: primary squamous bladder cancer showing diffuse strong (score +4) VEGF immunostaining (10X).

Discussion:

In general, there is a progressive increase in the incidence and death rates from cancer over the world including urinary bladder cancer which is regarded as one of the commonest ten cancers. In Iraq, urinary bladder cancer is well known to be associated with schistosomaiasis. The overall increase in the occurrence of bladder cancer worldwide represents a great challenge and necessity for thorough investigations which may help in both prevention and early detection of bladder cancer.

Recently, many advances in the biology of bladder cancer have been introduced, and many researches have been focused on various aspects of carcinogenesis underlying the development of bladder cancer. A simple attempt to open continuous line of urinary bladder research has been established at the Department of Pathology of the College of Medicine in Kufa University.

Angiogenesis is a central part of many normal homeostatic processes and nonneoplastic diseases. Regarding malignant neoplasia, it is now evident that tumours have a very limited capacity to grow without vascular support; therefore, formation of blood vasculature is obligatory step to sustain the influx of essential nutrients to the cancer mass. [9]

Molecular players of angiogenesis have been characterized since the early years of angiogenic studies, and one of the most prominent stimulating growing factors is certainly the vascular endothelial growth factor family. The most prominent member of this family, vascular endothelial growth factor (VEGF, VEGF-A) is the foremost controller of physiological and pathological angiogenesis. [10]

VEGF (vascular endothelial growth factor) is a homodimeric, disulfide-linked glycoprotein involved in angiogenesis which promotes tumor progression and metastasis. It exhibits potent mitogenic and permeability inducing properties specific for the vascular endothelium. [11]

Bladder tumors are characterized by markedly increased angiogenesis when compared to the normal urothelium from which they are derived. Thus, (VEGF) is a

crucial growth factor mediating tumor angiogenesis and its expression has been associated with advanced grade, stage and recurrence. [12]

Our samples were qualified as primary (pure) squamous cell carcinoma of urinary bladder, while those with primary transitional cell carcinoma with variable degrees of squamous differentiation were identified carefully and excluded.

The statistical analysis of our data revealed negative VEGF immunostaining in all cases of benign lesions (cystitis) which is significantly different compared with malignant samples (Table 1). This observation go ahead almost in parallel with the results of previous investigators. This finding agrees with studies reported by Wang S *et al.,* (2000) [13], and Ching-Chiang Yang *et al.,* (2004) [14], While Nilay S *et al.,* (2006) [15] showed VEGF immunostaining in 4% of normal bladder but also with significant difference from malignant tissue ($P < 0.05$).

Conversely, 40% of the malignant samples expressed a frank positive cytoplasmic immunostaining for VEGF. The percentage of the positive detection of VEGF immunoreaction in squamous cell carcinoma has been shown to be a reliable biomarker of tumor angiogenesis and progression (Table 1). [16]

By analyzing the positive detections of immunoreaction for VEGF according to the grade groups we can see that most detection were recorded in the higher grade (grade III) as 7(87.5%) out of 8 positive cases were among the grade III group. Although the previous data were statistically showing a high percentage of detection rate among grade III, we should notice that most 90% of our study group already falling in such category, and only 10% were classified under grade II while none were grade I. Therefore, we couldn't relay strongly on such conclusion in spite of presence of some studies support our results in more or less agreements (Table 2). [17]

The majority of VEGF positive immunostaining was found in samples with higher intensity (score +4), which in turn detected in most of the presented cases of squamous cell carcinoma of urinary bladder as it is found in 6(75%) out of 8 positive detection. On the other hand, the positive VEGF immunostaining of low intensity (score +1 and score +2) was found to be absolutely nil. While score 3 were recorded in only 2(25%) out of 8 positive cases (Table 3).

Moreover, a linear increase in VEGF over-expression was noticed when the grade of the tumor was considered. Again, the intensity appears to increase with grade of tumor, although there is no significant difference was found ($P>0.05$) (Table 3). Still, we couldn't find a published paper handling such relation between the intensity and the grade of squamous cell carcinoma of urinary bladder. However, as the tumor becomes more advanced there is greater gene expression due to either nonsense mutations, insertion of bases or, to a lesser extent, deletions that will give rise to truncated proteins. Similarly, a high proportion of VEGF over-expression was noticed in moderate and poorly differentiated bladder cancer cells but not in well differentiated cells which showed much less immunostaining expression.

In conclusion, altered VEGF protein was found to be significantly expressed in higher grades squamous cell carcinoma of urinary bladder which indicate the biological aggressiveness of the tumor and as we know the liability of tumor metastasis increase linearly with increasing angiogenic effect of the tumor in which the VEGF immunoreaction is one of the strongest indicator.

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