

## Microvessels density in relation to prognostic parameters (grading and staging) in transitional cell carcinoma of urinary bladder (Immunohistochemical study)

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### **Abstract:**

**Background:** Tumor stage, histologic grade, and regional lymph node status are currently used to obtain prognostic information about bladder cancers. However, additional prognostic indicators are needed to aid clinicians in selecting patients who would benefit most from specific therapies. A majority of studies assessing the prognostic value of measuring tumor angiogenesis (i.e., measurement of tumor microvessel densities) have found a positive association between increasing microvessel densities and worsening prognosis.

**Purpose:** We explored the relationship between established prognostic indicators and the extent of tumor-associated angiogenesis in patients with invasive transitional cell carcinoma (TCC) of the bladder.

**Methods:** Tumor angiogenesis were assessed by calculating standard microvessels density (MVD) identified by using immunostaining of endothelial cells with murine antihuman monoclonal antibody recognizing CD34 on endothelial cells Each slide was first scanned on low magnification (X10) to identify the four areas with highest density of microvessels (hot spots); each spots then was evaluated at high power magnification (X40) and the number of stained vessels per high power field (X40) were determined. The final MVD score was obtained from these four field and MVD was determined and calculated.

**Results:** We found that there is significant correlation between microvessels and tumor grade ( $P<0.5$ ), (Table 3), and also significant correlation of microvessels density with staging of the bladder carcinoma, (Table 4).

**Conclusion :**Neovascularization (angiogenesis) is very important for establishment and progression of tumor , so assessment of tumor vascularization whether by counting the microvessels density or other method may be of value regarding predicting further management and prognosis.

### **Introduction:**

Transitional cell carcinoma of the urinary bladder comprise about 90% of primary tumors of the urinary bladder. Both genetic and environmental factors play a role in their development (such as chemical factors as it is more common in those handle petrochemicals) and their incidence is increased in smokers and arylamines (1,2,3,4); also this is true for aniline dye (5,6), auramine , phenacetin and cyclophosphamide (7,8,9); schistosoma hematobium also associated with increased incidence of transitional cell carcinoma and squamous cell carcinoma (10,8); most of the cases occur in patients over the age of 50 years (11).

Angiogenesis is the development of new vessels from pre-existing vessel, is involved in the growth , maintenance and metastases of most solid tumors. It is essential to meet the metabolic requirements for tumor progression and play a major role in the

development of metastases in a variety of clinical malignancies (12,13). In the urinary bladder, the angiogenesis has been reported to be a neoplastic characteristic (14,15); so we assess the microvessels density in a series of carcinoma to evaluate angiogenesis as prognostic markers and to correlate it with other important prognostic markers which are the staging and grading; several groups have investigated the prognostic significance of estimate of tumor angiogenesis in urinary bladder carcinoma, although these studies (16,17,18,19,20,21) used slightly different methods; they support that a highly vascular bladder carcinoma behaves more aggressive than a carcinoma with a low vascular density. The process by which neovascularization occurs, can be quantified immunohistochemically by determining the microvessels density (MVD). Increased MVD not only supply the tumor with more oxygen and nutrient, but also provide a ready portal for metabolic spread, as angiogenic vessels tend to be leaky (22).

#### **Material and method:**

This retrospective study is carried out in the department of pathology and forensic medicine in university of Kufa, using 55 cases of different bladder lesions which are selected randomly from a laboratory in Najaf, all are formalin-fixed paraffin-embedded tissue blocks, two sections were taken from each block each 4 micron thick, one section was stained immunohistochemically for endothelial cells of the blood vessels by antihuman monoclonal antibody CD34 class II, clone QBEnd -10, Code No. /code/code-Nr.M7165, Edition/ Ausgabe 01.03.04, Dako cytometry, recognizing CD34 on endothelial cells of the blood vessels, and the other was stained with hematoxylin and eosin stain for comparative morphological assessment. Antigen unmasking was performed by micro waving the slides 3 times for 5 minutes in citrate buffer (PH 6.0). After washing in PBS, the primary antibody was applied for one hour at room temperature. For detection system we use LSAB2 (DAKO, K0675) with the secondary antibody as biotinylated link (30 minutes) followed by streptavidin-peroxidase (30 minutes). After every step, the slides were washed in PBS (PH 7.4).

The reaction was detected by incubating with DAB for 5 minutes. After counterstaining with hematoxylin, the slides were dehydrated, clarified and mounted. There were 42 male and 13 female, their ages range from 50 years old to more than 69 years old. The staging of the tumor was achieved according to the TNM staging system as follows: Ta (superficial tumor); T1 (tumor invading the subepithelial connective tissues); T2 (tumor invading the detrusor smooth muscle); grading evaluation was performed according to the World Health Organization (WHO) criteria; there were 24 cases grade II, 31 cases grade III, 18 cases Ta, 6 cases T1, 31 cases T2. All the cases are paraffin-embedded tissues, immunohistochemical staining was performed using the standard LSAB method.

Tumor angiogenesis was assessed by calculating standard microvessels density. MVD was identified by using immunostaining of endothelial cells with murine antihuman monoclonal antibody recognizing CD34 on endothelial cells, each slide was first scanned at low magnification (X10) to identify the four areas with highest density of microvessels (hot spots); each spot then was evaluated at high power magnification (X40) and the number of stained vessels per high power field (X40) were determined. The final MVD score was obtained from these four fields and MVD was determined and calculated, as shown in figure 1 which demonstrates MVD in grade II TCC, and also in figure 2 which reveals MVD in grade III TCC.

**Statistical analysis:**

ANOVA or t-test were used for testing correlation between MVD with grading and staging of the tumor. This is carried out by the help of SPSS version 10 statistical package using Chi square test at level of significance  $\alpha \leq 0.05$ .

**Results:**

There were 42 male and 13 female , there ages ranges from 50 years old to more the 69 years old (table 1). The staging of the tumor were achieved according to the TNM staging system as follow: Ta (superficial tumor); T1 (tumor invading the subepithelial connective tissues); T2 ( tumor invading the detrusor smooth muscle); grading evaluation was performed according to the world Health Organization (WHO) criteria; there were 24 cases grade II, 31 cases grade III, 18 cases Ta, 6 cases T1, 31 cases T2 ( table 2 & 3). In table 2, we found that there is significant correlation between microvessels and tumor grade and t with ( $P<0.5$ ), (table 3) while table 4 show significant correlation between microvessels density and staging of the bladder carcinoma ( $P<0.05$ ).

**Table 1: Age and gender distribution of the patient included in this study.**

Gender	Age groups			
	50-60	61-69	>69	total
Male	12	21	9	42
Female	3	4	6	13

**Table 2: The value of microvessels density in relation to grading of the tumor.**

Grading	microvessels density/HPF			
	<20	20-50	>50	total
Grade II	12	10	2	24
Grade III	8	10	13	31

( $P<0.5$ )

**Table 3: The value of microvessels desity in relation to staging of bladder carcinoma .**

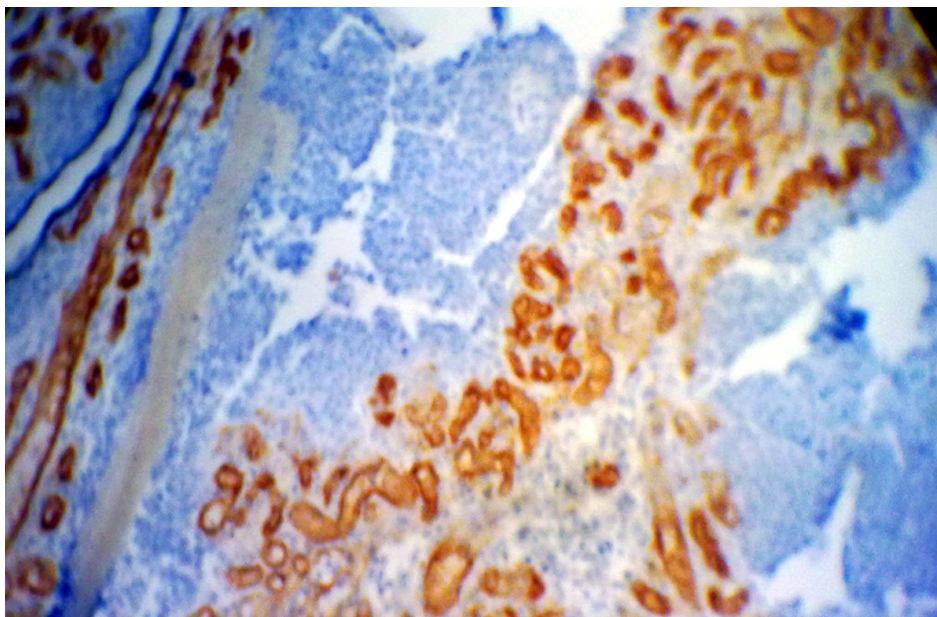
Staging	Microvessels density /HPF			
	<20	20-50	>50	total
<b>Ta</b>	9	6	3	18
<b>T1</b>	1	5	0	6
<b>T2</b>	7	10	14	31

( $P<0.5$ )

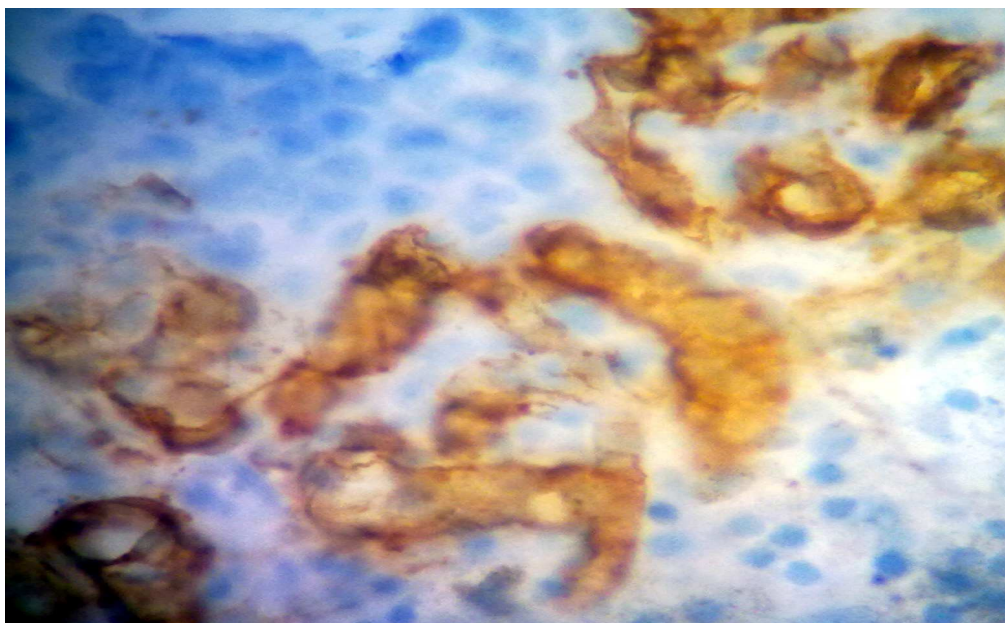
**Table 4: The value of microvessels density in relation to staging of bladder carcinoma .**

Staging	microvessels density /HPF			
	<20	20-50	>50	Total
<b>stage 0a</b>	9	6	3	18
<b>stage I</b>	1	5	0	6
<b>stage II</b>	7	10	14	31

( $P<0.5$ )



**Figure 1:showing small vascular channels stained with CD34 in grade II transitional cell carcinoma of urinary bladder (X10).**



**Figure 2:showing small vascular channels stained with CD34 in grade III transitional cell carcinoma of urinary bladder (X40).**

### **Discussion**

At present only the tumor stage and grade are recognized cardinal prognostic factors used in the management of invasive bladder cancer (26). However, with the development of new modalities there is always a need for additional prognostic markers; for a marker to be of use it should provide more information than those currently available. The tumor vascularity is also important as a prognostic factor and significantly related to survival (27). The quantification of angiogenesis may allow the selection of patients with a better prognosis who perhaps benefit from more aggressive adjuvant therapy; similarly it may be possible to avoid such aggressive therapy in those patients where adjuvant therapy may provide little benefit in terms of prognosis.

In our study we found a significant correlation between microvessels density with staging and grading of bladder carcinoma ( $p < 0.05$ ), this is agreed with Vasilios et al (25), while Martin et al found no difference in MVD between pTa and pT1 (24) and this is also true for Frank Reiher et al. Stavropoulos et al found that MVD differed significantly between primary and recurrent tumors probably due to neovascularization after previous manipulation. (23). We think that because of this association between microvessels density with grading and staging so the assessment of vascularity might be useful in both predicting patient outcome and selecting those who would benefit from more aggressive therapy.

### **Conclusion:**

Neovascularization (angiogenesis) is very important for establishment and progression of tumor, so assessment of tumor vascularization whether by counting the microvessels density or other method may be of value regarding predicting further management and prognosis.

**Recommendation:**

Further studies using larger sample for better evaluation and using prospective studies for evaluation of tumor angiogenesis as an independent prognostic factors in bladder carcinoma, and also studying the effect of antiangiogenic factor on both biological progression of the disease and its effect on prognosis.

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