World Journal of Medical Sciences 7 (4): 255-259, 2012
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2012.7.4.1109

Vascular Endothelial Growth Factor: Correlation with the Different Stages of Papillary Bladder Tumors

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Abstract: To evaluate the difference in immunohistochemical expression of vascular endothelial growth factors (VEGF) among the different stages of papillary bladder tumors, to assess the potentiality of VEGF expression as a risk factor in tumor stage progression in superficial papillary urinary bladder tumors, to examine the ability of this marker in identifying the invasion of muscularis propria when uncertain or equivocal, and to assess if anti-VEGF drugs could be introduced as adjuvant therapy in superficial bladder papillary tumors and precursor lesions. Fifty cases of papillary bladder tumors of different stages were collected from various labs and institutes and stained for VEGF immunostain. The VEGF immuno-expression was seen to be gradually increased with tumor progression, with significant difference between the papillary bladder neoplasm of low malignant potential (PUNLMP) group and other groups (*P* value 0.034), and insignificant difference between the Ta and T1 groups (*P* value = 0.36). Diffuse strong positive staining was statistically significantly associated with muscle invasion (T2- staging), (*P*~0.012). VEGF could be added as a risk factor for disease progression in cases of papillary bladder neoplasms, moreover, in cases of absent mucularis propria or uncertain invasion diffuse strong positivity of the tumor cells could be helping factor in muscle invasion prediction.

Key words: Immunohistochemical · VEGF · PUNLMP · Tumor · Urinary bladder

INTRODUCTION

Bladder cancer had been for years the most important type of cancer in Egypt due to its etiological relationship to urinary Schistosomiasis; an endemic disease in Egypt since ancient history. With control of Schistosomiasis, the incidence of squamous cell carcinoma decreased. Nonetheless, the overall incidence of cancer bladder did not decrease too much due to the increasing incidence of transitional cell carcinoma that is mainly associated with smoking; now a major risk factor for bladder cancer in Egypt [1]. According to the Egyptian National Cancer Institute registry of 2003/2004 urinary bladder cancer ranks the third among all cancers, with transitional cell carcinoma representing about 64% of cases. In the same registry it was highlighted that there were trends for more superficial and papillary tumors and less invasive stages if compared with the old series [2]. Papillary urothelial tumors represent a heterogeneous group with different potentiality and expected outcomes; about 60% of the superficial tumors (Ta & T1) will recur after transurethral resection, and about 10% of them will progress to invasive and/or metastatic tumors and are therefore potentially lethal [3].Several classification systems for grading papillary urothelial neoplasms have been proposed in an attempt to more precisely predict recurrence and progression in this group of patients. The most recent available WHO/ISUP classification of tumors of urinary tract inputs a broad spectrum of urological pathology with detailed morphologic definitions and creates the category of papillary urothelial neoplasm of low malignant potential (PUNLMP) with negligible risk of progression [4].

Much research on superficial bladder tumors has been devoted to identifying risk factors for recurrence and progression. The most important factors identified to date appear to be clinical and histopathological variables, e.g. stage, grade, size, multi-centricity, recurrence at 3 months after initial transurethral resection, or bladder neck involvement [5]. However, reliable predictors of

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tumor recurrence and progression for individual patients, which could optimize treatment and follow-up schedules based on specific tumor biology, are yet to be identified [6]. Besides, there is absence of validated biomarkers in urothelial carcinoma which are able to forecast and stratify responses to emerging targeted therapies [7]. Vascular Endothelial Growth Factors (VEGFs) are classes of proteins that regulate vascular development in embryos and angiogenesis in adult mammals after sustaining an injury or notably in cancerous tumors [8]. Numerous studies highlighted the increased expression of VEGF in cancer cells resulting in tumoral angiogenesis, providing the tumor with the network of blood vessels needed to grow and expand. VEGF expression is stimulated by hypoxia [9], a common characteristic of most newly formed tumors, and genetic mutations such as K-ras and p53, extremely common mutations present in a majority of cancers [10]. In urothelial tumors, VEGF elevated expression in tumor biopsies as well as its levels in urine or serum have been reported to be independent prognostic and predictive factors of recurrence and disease progression [11].

In this study we evaluate the difference in immunohistochemical expression of VEGF among the different stages of papillary urothelial carcinomas of the bladder, to examine the ability of this marker in identifying the invasion of muscularis propria when uncertain and its role as risk factor in tumor stage progression in superficial papillary bladder tumors, thus; anti-VEGF drugs could be introduced as adjuvant therapy in superficial bladder papillary tumors and precursor lesions. For all disease stages this may help in identifying patients who might benefit from anti-angiogenic therapeutic approaches.

MATERIALS AND METHODS

Fifty cases of papillary bladder tumors blocks were collected from various institutes and labs, reviewed for their diagnosis and classified according to WHO/ISUP classification of tumors of urinary tract (2004) into:

- Five cases of papillary urothelial neoplasm of low malignant potential.
- Ten cases of papillary non invasive urothelial carcinoma (pTa), low grade
- Eight cases of papillary non invasive urothelial carcinoma (pTa), high grade.
- Fifteen cases of papillary urothelial carcinoma with positive lamina propria invasion and absent muscle invasion (pT1).
- Twelve cases of papillary urothelial carcinoma with positive muscle invasion (pT2).

The avidin-biotin-peroxidase complex method was used for immunohistochemical detection. Briefly, 5-Fm-thick histological sections from formalin-fixed paraffin-embedded blocks of tumor tissue were dewaxed in xylene, rehydrated through graded alcohols, immersed in 10 mM Tris and 0.5 M EDTA, pH 9.0 and microwaved twice for 5 minutes each. Subsequently, the sections were incubated with 0.3% H_2O_2 for 30 minutes to block endogenous peroxidase activity. The sections were then incubated overnight with the primary antibodies monoclonal mouse clone VG1, code M 7273, DAKO). Nonspecific binding was blocked by incubating the sections for 30 minutes with Blocking Solution. Detection was carried out using the labVision System kit with diaminobenzidine as chromogen. Counterstaining was performed with hematoxylin Harris [12].

According to Santos *et al.* [12] VEGF positivity was indicated by the presence of cytoplasmic or membrane brown staining. VEGF-positive cases were defined when (>50% of cells) were stained. The staining intensity was also recorded. The lining endothelium of the normally scattered blood vessels acted as internal positive control [12].

Statistical Analysis: Comparison of the study variables between all pathological types in the present study was done using the likelihood chi square test. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, and USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) statistical program. Efficacy is expressed by: sensitivity (true positive/true positive +false negative), specificity (true negative/true negative and false positive), positive predictive value (true positive/true positive + false positive) and negative predictive value (true negative/ true negative + false negative)

RESULTS

The study included (50) cases of papillary bladder carcinoma of different stages classified according to their stage as mentioned before. The cases included nine females and 41 males, with the age ranging from 43 up to 72 years old. The results of VEGF immuno-staining was presented in Table 1. Generally the immuno-expression of VEGF increased gradually with the tumor progression

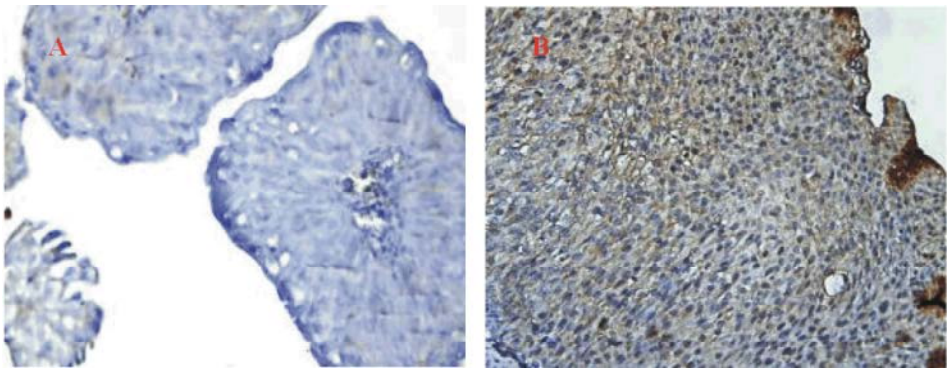

Fig. 1: (A): Papillary bladder neoplasm of low malignant potential showed negative staining of the tumor cells. (B); diffuse strong cytoplasmic and/or membranous staining for VEGF in a case of invasive papillary bladder carcinoma with positive muscle invasion.

Table 1: The results of VEGF immunostaining

		VEGF EXPRESSION			
		+ve		-ve	
Tumor stage	No. of cases	No.	%	No.	%
PUNLMP5	5	0	0	5	100
Ta, low grade	11	4	36.4	7	63.6
Ta high grade	7	3	42.9	4	57.1
T1	15	7	46.7	8	53.3
T2	12	12	100	0	0
Total	50	26		24	

from the earliest stages (PUNLMP) to the stage of the muscle invasion (T2); all the cases of PUNLMP showed negative immuno-expression to VEGF (less than 50% of cells). The immunostained cells showed weak positivity (Fig.1a). Cases of non invasive urothelial carcinoma, low grade showed positive staining in 4 of them (36.4%), and negative results in the rest of them (63.6%). The noninvasive urothelial carcinoma, high grade cases showed positive staining in 42.9% of cases and negative staining in 57.1%. The positive cases in both categories of (Ta) were 38.9%, and showed variable degrees of intensity from weak to moderate. Cases of T1 stage showed positive staining (more than 50% of tumor cells) with moderate to strong intensity in 7 cases (46.7%) of the cases, while the rest of the cases (53.3% of the cases) showed negative staining (less than 50% of tumor cells). At stage T2 (positive muscle invasion) all the twelve cases reacted positively for the VEGF (100%) with strong expression in all cases (Fig.1b). The difference between the VEGF expression in the PUNLMP group and other groups was statistically significant (P value 0.034), while difference between the Ta and T1 groups was insignificant (P value =0.36). Diffuse strong positive staining was statistically significantly associated with muscle invasion (T2- staging), (P~0.012), with sensitivity 100%, negative predictive value 100%, specificity 63.2%, and positive predictive value 46.2 %.

DISCUSSION

Bladder cancer ranks as the third cancer in Egypt according to most registries; transitional cell carcinoma is one the major subtypes. Many classifications were proposed in order to arrange these tumors in classes according to their behavior and prognosis; however the most recent and widely accepted is that of the WHO/ISUP classification of tumors of urinary tract (2004) in which papillary neoplasm are graded from hyperplasia, tumors of low malignant potential, noninvasive tumors low grade and high grade (Ta), tumors with lamina propria invasion (T1) and muscularis propria invasion (T2). For superficial bladder cancers, the appropriate treatment is not straightforward, and the risk of tumor recurrence/progression must be balanced against the toxicity of the intra vesical treatment (IVT). It must be emphasized that bladder preservation with effective TUR and IVT is clearly preferable to radical surgery only if equivalent efficacy is proven. As Angiogenesis is a crucial pathogenic mechanism for this type of urothelial carcinoma and so is a potential therapeutic target [13], chemotherapy regimens combined with anti-angiogenic endothelial-specific drugs may induce remarkable responses. VEGF immuno expression is therefore examined in different stages of papillary urothelial carcinoma. The study included 50 cases of papillary urothelial carcinoma; Five cases of papillary urothelial neoplasm of low malignant potential, ten cases of papillary non invasive urothelial carcinoma (pTa), low grade, eight cases of papillary non invasive urothelial

carcinoma (pTa), high grade, fifteen cases of papillary urothelial carcinoma with positive lamina propria invasion and absent muscle invasion (pT1), twelve cases of papillary urothelial carcinoma with positive muscle invasion (pT2).

In our study we noticed that the category of PUNLMP is not commonly diagnosed in various institutes and labs. In our multi institutional search we hardly found 5 cases; this may reflect the limited acceptance of the Egyptian pathologists to this diagnostic category. Many would prefer to address these cases under the category of (low grade noninvasive urothelial carcinoma) to ensure that the patient will receive the best treatment options and will be followed up more strictly. Similar attitude was recorded by Jones and Cheng [14]. Those authors found that the presence of the PUNLMP category is controversial; they also recommended that cases of PUNLMP are to be treated similarly to patients with low grade, non invasive urothelial carcinoma. Maclennan *et al.* [15] stated that this terminology in practice is of questionable validity and utility. In both studies new biomarkers and molecular tests are recommended to validate this category. According to the current study, all the cases of this category, PUNLMP, showed negative staining for VEGF. In spite of the limited number of cases included, the obtained results reflect the indolent character and the excellent expected behavior of this group, when diagnosed strictly by its criteria. This justifies, as Pan *et al.* [16] mentioned, removal of the label of carcinoma and designating it instead as a neoplasm, thus making the appropriate clinical follow up is recommended as a convenient line of management [17]. In our results, tumors of Ta, low grade and high grade, as well as cases of T1 stages showed variable ascending positivity percentages; 36.4%, 42.9% and 46.7% respectively, with ascending intensities, however the difference between them was insignificant ($P > 0.05$). On the other hand, all the cases of the T2 group (cases with positive muscle invasion) showed strong diffuse positive staining for the marker with a significant difference from the Ta and T1 cases ($P < 0.05$). The notable increase in the VEGF scoring and intensity reflects the expected increase in tumor angiogenesis during the disease progression which may be stimulated by hypoxia as the tumor increase in size and depth. This role of VEGF in tumors angiogenesis in urothelial tumors was also stated by Bellmunt and Guix [18], and although some authors denied any correlation between VEGF and tumor stage and its grade [19, 20]. However others agreed with our results and stated that VEGF was higher in advanced stages and reported an association between VEGF immuno-expression and established clinicopathologic features in urothelial carcinomas [21]. As well, Inoue *et al.* [22] believed in the presence of such correlation and based on that their proposed model of treatment of urothelial carcinoma. Keeping with these results we proposed that VEGF may be added to other angiogenesis related markers suggested by Shaitia *et al.* [23], which are altered in cancer bladder cases and therefore could be a target for therapy. Similarly, Matsushita *et al.* [24] agreed that VEGF becomes a biomarker for bladder cancer and found that increased VEGF levels can result in increased vascular permeability and interstitial fluid pressure, possibly impairing the delivery of chemotherapy and that is why they suggest adding anti-VEGF to chemotherapy regimens for urinary bladder cancer cases.

The differential expression of VEGF among Ta and T1 cases in the current study and other studies may reflect the individual potentiality of each tumor for progression and may be its potential ability for recurrences; such hypothesis is to be examined on follow up studies with long term analysis. In the current study the strong diffuse staining for VEGF was statistically associated with muscle invasion, with sensitivity 100% and negative predictive value100%, which may suggest that consulting VEGF positivity may be of some concern in questionable cases or small biopsies. Thus, for each individual case, the negativity for VEGF could exclude muscle invasion, however, the positivity could not document it (specificity 63.2%, and positive predictive value 46.2 %) in all cases the clinical and cystoscopic correlation is mandatory.

CONCLUSION

VEGF immuno-expression was according to our results associated with tumor progression in various stages of papillary bladder tumors, the negativity of cases of PUNLMP reflected its potential indolent behavior, the gradual increase in the positivity scoring and intensity among cases of Ta, T1 and T2 confirmed a role played by VEGF during the disease progression and suggest performing such immunostain for progression and recurrence risk assessment, for cases of superficial tumors, for such cases anti-VEGF therapy could be, therefore recommended as adjuvant therapy based on the results of the VEGF immunostaining for each individual case. The 100% sensitivity of the association between strong diffuse staining and positive muscle invasion may also suggest the VEGF immunostaining testing for questionable cases with taking into consideration being a good negative test.

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