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VASCULAR ENDOTHELIAL GROWTH FACTOR EXRESSION IN UTERINE CERVICAL CANCER: CORRELATION WITH CLINICOPATHOLOGIC CHARACTERISTICS AND SURVIVAL

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Aim: **This retrospective study was performed to determine the vascular endothelial growth factor (VEGF) expression in cervical cancer cells, and to examine its correlation with clinicopathologic characteristics and survival of patients.** ***Methods:*** **Seventy-five paraffin-embedded primary tumors were stained immunohistochemically for VEGF expression, which was analysed semiquantitatively.** ***Results:*** **The significant correlation between VEGF expression and stages of disease, as well as pelvic lymph node metastasis was observed. There were determined a negative correlations between VEGF expression in tumor cells and both overall and disease-free survival.** ***Conclusion:*** **VEGF expression in human cervical cancer may be used as a diagnostic parameter in the clinic. Our results are in accordance with literature data showing association of VEGF overexpression in tumor with a poorer patient survival.**
Key Words: **human cervical cancer, vascular endothelial growth factor, immunohistochemistry, survival.**

Cervical cancer is one of the most common gynaecological tumors, both in incidence and morbidity, with the incidence rates of 19.6 per 100.000 and the death rates of 8.8 per 1000,000 in Ukraine in 2008 [1]. Almost 40% of patients died at first years after treatment that is mainly caused by the development of pelvic recurrence and distant metastases.

It is well known that tumor aggressiveness is strong associated with tumor angiogenesis which is induced by many factors producing both by tumor and stromal cells. Among the inductors of angiogenesis the most effective is the vascular endothelial growth factor (VEGF) [2, 3]. It was shown the correlation between level of tumor neovascularization and time to recurrence as well as poor clinical outcome in patients with many types of malignancies (gastric cancer, oesophagus carcinoma, ovarian cancer, breast carcinoma, colorectal cancer, lung cancer, etc.) [6–16]. It was determined the association of unfavourable prognosis for patients with strong expression of VEGF induced by tumor hypoxia that is considered as a key factor of tumor aggressiveness and malignant progression [13, 17–19]. At the same time there are controversial data concerning the dependence on unfavourable prognosis for patients with cervical cancer and VEGF expression in tumor cells.

The aim of this study was to determine the association between VEGF expression in tumour cells and clinical outcome in patients with uterine cervical cancer.

75 patients with cervical cancer, who had been operated in the National Cancer Institute (Ministry of Health of Ukraine) from 1993 to 2008 were included into the retrospective study. All patients underwent an panhysterectomy of the 3rd type. No patient received chemotherapy or radiation prior to surgery. The study was approved by a medical Ethics Committee. The clinical and pathological profiles of the patients are presented in Table 1.

Table 1. Clinicopathologic characteristics of patients

Characteristic			No. of patients (n)		No. of patients (%)	
Age (years)	< 50		51		68	
	> 50		24		32	
Stage of disease	0	Tis	2	2	2.6	2.6
	I	T1AN0M0	6	39	8	52
		T1BN0M0	33		44	
	II	T2AN0M0	14	16	18.6	21.3
		T2BN0M0	2		2.6	
	III	T1BN1M0	10	16	13.3	21.3
		T2AN1M0	6		8	
	IV	T2BN1M1	2	2	2,6	2.7
Pelvic lymph node metastasis	Negative		57		76	
	Positive		18		24	
Histologic type	Adenocarcinoma		10		13.3	
	Squamous cell cancer		65		86.7	
Differentiation	Moderate		25		33.3	
	Poor		18		24	
Recurrence	Positive		30		40	
	Negative		45		60	

Immunohistochemistry on VEGF expression was performed on deparaffinized slides using monoclonal antibody (clone 4H12) (DACO Cytomation, Denmark). The immunostained sections were observed under high-power magnification (x 200). The immunohistochemical results for VEGF positive staining were evaluated as follows: the staining in less than 20% of cells was evaluated as low level, staining in 20–50% of cells — middle level, and in 50–100% of cells — high level of VEGF expression. Moreover, the intensity of staining was scored as: 1+ — weak staining; 2+ — moderate staining; 3+ — strong staining.

Statistical analysis was conducted by standard software packages of Statistica 6.0 (StatSoft Inc., USA). The correlation analysis was performed by the Pearson [r] test. The survival curves were plotted using the Kaplan-Meier method, and the statistical significance between groups was determined by the log-rank test. For all tests, $p < 0.05$ was considered as significant.

The positive staining for VEGF was determined in 89% of investigated tumors. The weak VEGF expression was observed in 15 (20%) neoplasms, moderate —

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Abbreviations used: VEGF – vascular endothelial growth factor.

in 22 (29%), and high — in 38 (51%) (Table 2). The association of VEGF immunopositivity in tumor cells with the main clinicopathological characteristics of patients with cervical cancer are presented in Table 3.

Table 2. VEGF expression in primary tumor

VEGF expression level	No. of patients (n)	No. of patients (%)
Low and negative	15	20
Moderate	22	29
Strong	38	51

Table 3. VEGF expression level in primary tumors according to clinicopathologic characteristics

Clinicopathologic characteristics	No. of patients	No. of VEGF-positive tumor cells (%)	*p*
Menopausal status			
Pre (until 50 years)	50	58.0	> 0.05
Menopause (50–55 years)	12	63.75	> 0.05
Post (over 55 years)	13	52.9	> 0.05
Histological type and Differentiation			
Squamous cell cancer	65		
Moderately differentiated	38	53.5	> 0.05
Poorly differentiated	27	65.4	> 0.05
Adenocarcinoma	10		
Moderately differentiated	2	72.0	> 0.05
Poorly differentiated	8	85.0	> 0.05
Lymph node metastasis			
Negative	57	50.5	< 0.05
Positive	18	75.3	< 0.05
Distant metastasis			
Negative	28	57.3	> 0.05
Positive	2	90.0	> 0.05
Time to recurrence			
> 5 years	45	12.5	< 0.05
< 5 years	30	84.0	< 0.05
Total	75		

According to some recent studies one of the main prognostic factors which impacts on the aggressiveness of cervical cancer is the age of patients [20]. We have observed that number of VEGF positive tumors in women in the age up to 50 years was 58.0 %, from 50 to 55 years — 63.75%, and over 55 years (postmenopausal period) — 52.9%. So, any significant differences have not been obtained between the level of VEFG expression and the age of patients ($p > 0.05$).

The more important prognostic factor that determines prognosis for cervical cancer is the stage of disease [21]. In our study it was shown that the level of VEGF expression correlated with the stage of disease ($r = 0.52$, $p < 0.05$), in particular with T-categories ($r = 0.61$, $p < 0.05$) (Table 4). Taking into account these findings and absent of common opinion concerning correlation between VEGF expression in cervical cancer and the stage of disease [22], we suggest that further investigation needs to determine VEGF expression in cervical cancer for clarification of its association with the stages of disease.

Table 4. VEGF expression in tumors according to FIGO stages

Stage of disease		No. of patients, n (%)		No. of VEGF-positive tumor cells (%)*
FIGO	TNM			
0	Tis	2 (2.6)	2 (2.6)	$45.3^{2, 3, 4}$
I	T1AN0M0	6 (8)	39 (52)	$40.0^{2, 3, 4}$
	T1BN0M0	33 (44)		
II	T2AN0M0	14 (18.6)	16 (21.3)	$80.67^{0, 1}$
	T2BN0M0	2 (2.6)		
III	T1BN1M0	10 (13.3)	16 (21.3)	$80.0^{0, 1}$
	T2AN1M0	6 (8)		
IV	T2BN1M1	2 (2.6)	2 (2.7)	$90.0^{0, 1}$

*Statistically significant differences were noted in comparison with FIGO stages: $^0p < 0.05$ (stage 0), $^1p < 0.05$ (stage I), $^2p < 0.05$ (stage II), $^3p < 0.05$ (stage III), $^4p < 0.05$ (stage IV).

Still now there is very disputable relation between the level of VEGF expression and tumour histology [22–24]. It was observed in our study that number of tumor cells shown the VEGF immunopositivity was detected in 55.3% among 65 squamous cell carcinomas and in 68.4% among 10 adenocarcinomas ($p > 0.05$) (Table 3). The nonsignificant association between these parameters may be explained by small number of patients with adenocarcinomas.

It was not revealed significant differences between VEGF expression in tumor and the grades of differentiation ($p > 0.05$) (see Table 3). It was obtained the significant correlation between high level of VEGF expression and pelvic lymph node status ($r = 0.39$, $p < 0.05$) that is in accordance with the observations of other authors [13], but we have not observed any association between VEGF expression and the distant metastasis ($p > 0.05$).

It is known that cervical cancer aggressiveness impact on the overall survival as well as disease-free survival. We have shown, that the level of VEGF expression affected both parameters of patients' survival (Figure). It was determined that number of tumor cells expressed VEGF was 11.5% in group of patients with time to recurrence more than 5 years, and number of tumor cells expressed VEGF was 84% in a group of patients with time to recurrence less than 5 years ($r = 0.43$, $p < 0.05$).

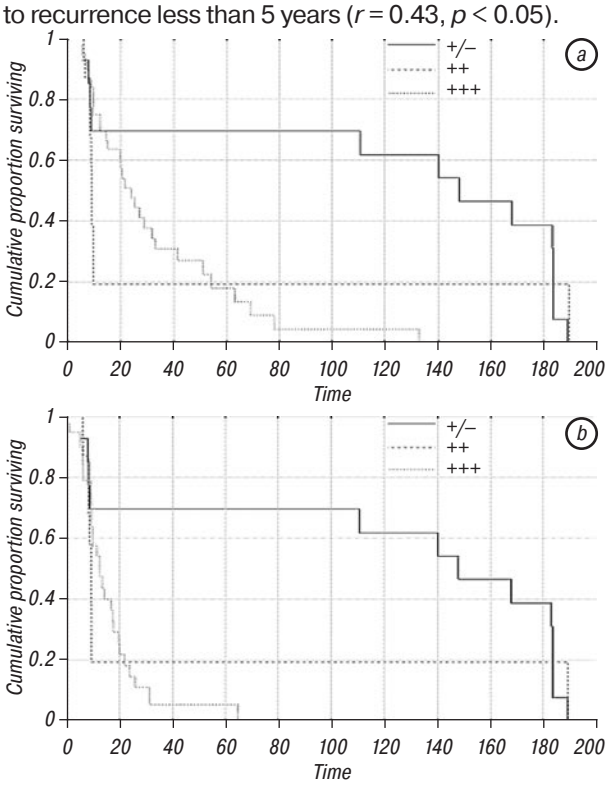

Figure. Kaplan — Meier survival curves for overall (*a*) and disease-free (*b*) survival in accordance to VEGF expression in tumor cells ($p < 0.05$)

Obtained results allowed to conclude that the level of VEGF expression in cervical cancer cells associates with the stage of disease, lymph node metastasis, and influences the disease outcome. The immunohistochemical evaluation of VEGF expression in tumor cells may be important tool to clarify the diagnosis and to personalize the treatment of patients with uterine cervical cancer.

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