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MOLECULAR MARKERS OF VEGF, TNF-α AND TNF-β IN BLADDER CANCER PATIENTS AT STAGE $T_3N_0M_0$ AND THEIR RELATIONSHIP WITH THE DEGREE OF TUMOR NEOPLASIA

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Abstract

17 bladder cancer patients, stage $T_3N_0M_0$ (main group), were included in the study. There were 10 men (mean age 58.2 ± 6.2 years) and 7 women (mean age 59.5 ± 2.4 years). Clinical data of 12 healthy individuals were used as a control. According to statistic data, the average level of VEGF in bladder cancer patients urine was 246.55± 6.90 pg/ml which significantly exceeded this indicator in the control group (129.21± 7.60 pg/ml), the difference was statistically significant At bladder cancer diagnosis the sensitivity and specificity of the urinary level of TNF-α was low and amounted to 30% and 20%, respectively, and the level of TNF-β was even lower - 25% and 20%, respectively, which is not representative for the pathology under study.

Key words: carcinoma; urinary bladder; genotype; growth factor; necrosis factor; polymorphism; differentiation.

At the moment, a lot of data have been accumulated confirming the participation of growth factors, in particular, VEGF (vascular endothelial growth factor) and TNF (tumor necrosis factor), responsible for the mitogenic activity of cells, in the development and progression of malignant neoplasms. Therefore, they are promising objects when using target therapy [1, 3, 4].

VEGF is a glycoprotein and is one of the most important stimulators of angiogenesis in various healthy and cancerous tissues. VEGF performs its functions through a tyrosine kinase receptor located in the membrane of endothelial cells [5].

Angiogenesis is one of the main factors in the survival and spread of tumor cells. A family of endothelial vascular growth factors - VEGF - plays a key role in the formation of a new vascular network during tumor development. The appearance of new vessels contributes to the progression of the disease, increasing the tumor growth rate and its ability to metastasize. Assessment of tumor angiogenesis is important for predicting the course of the disease and prescribing chemotherapy for many malignant tumors [6].

Tumor necrosis factor alpha (TNF-α) was identified in the blood serum of mice. As an endogenous tumoricidal agent, it is produced by mononuclear macrophages activated under the action of endotoxin. Its characteristic feature is high selective toxicity in relation to tumor cells [2]. The tumoricidal factor does not have species specificity and does not have a damaging effect on healthy cells.

TNF-α and TNF-β present two close (similar) proteins (approximately 30% of amino acid residues are homologous) and show similar activity to the inflammatory reaction, immune and tumor processes. TNF- β or lymphotoxin was detected in the lymph nodes of immunized rats. The source of TNF- α is an activated macrophage, and TNF- β is an activated T - cell. Due to the same specific TNF cell surface receptors, both factors cause lysis of lymphoma cells, necrosis of sarcoma induced by methylcholanthrenoma, activate polymorphonuclear lymphocytes, and exhibit antiviral activity.

In the literature, we did not find information about the significance of VEGF, TNF-α and TNF-β markers in the diagnosis and prognosis of the neoplastic process in patients with bladder cancer in the T3 stage, and this is the subject of present study.

The purpose: to determine the molecular markers of VEGF, TNF-α, and TNF-β in patients with bladder cancer in the $T_3N_0M_0$ stage and establish their relationship with the degree of neoplasia G.

Task

1. To identify the difference in the ratio of molecular markers VEGF, TNF- α and TNF- β in patients with bladder cancer and healthy individuals.

2. To investigate the relationship between markers VEGF, TNF- α, and TNF - β with the degree of neoplasia in patients with $T_3N_0M_0$ bladder cancer.

Materials and methods

17 patients with bladder cancer stage $T_3N_0M_0$ (main group) were included in the study. Among them there were 10 men and 7 women. The mean age of men was 58.2 ± 6.2 and women 59.5 ± 2.4 years. As a control, clinical data of 12 healthy individuals were used. The study was approved by the Lviv National Medical University Bioethics Commission and in accordance with the principles of the WMA Declaration of Helsinki. All patients signed informed consent to participate in the study, conducted standard general clinical studies, verified HIR and PD in accordance with the regulatory documents of Ukraine requirements.

Primers for VEGF amplification were taken from the work of Lee et al. (2005, DOI 10.1158/1055-9965); PCR conditions were selected experimentally. To determine the G + 405 C polymorphism, amplified fragments with a length of 273 p. n. subjected to restriction digestion with BsmFI endonuclease. Restriction products were fractionated in a 2.2% agarose gel with ethidium bromide and visualized in UV light. Pathomorphological examination was carried out on a "Prima Star" light-optical microscope, the preparations were stained with hematoxylin-eosin, viewed at magnifications of 100 and 400 times

Results and their discussion

According to statistic data, the average level of VEGF in bladder cancer patients urine was 246.55± 6.90 pg/ml which significantly exceeded this indicator in the control group (129.21± 7.60 pg/ml), the difference was statistically significant ($p = 0.04$). Detailed statistical characteristics of VEGF indicators in the patient under study are given in Table 1.

When studying the levels of VEGF in the urine of patients with neoplasia grades G1, G2, G3, it was found that in the mentioned subgroups of patients, the average value of VEGF was 236.41 ± 7.83 pg/ml, 245.76 ± 7.79 pg /ml and 250.77 ± 7.24 pg/ml, respectively.

As it follows from the data obtained, a low degree of G1 neoplasia was detected a little more often - in 7 out of 17 patients (41.17%), G2 was diagnosed in 35.29% of patients, and G3 grade was detected in 23.52 % of bladder cancer patients. It should be emphasized that due to the retrospective nature of the study, the above data do not fully reflect the actual epidemiological situation regarding the frequency of bladder cancer neoplasia degrees, stage $T_3N_0M_0$.

Table 1

The level of VEGF in bladder cancer patients, stage $T_3N_0M_0$

Group/subgroup	Number of patients		Average level of VEGF($VEGF_{aver}$) ±Δ, pg/ml	95% CI for $VEGF_{aver}$ pg/ml	Median, pg/ml	min, pg/ml	max, pg/ml
	Abs	%					
$T_3N_0M_0$	17	100	246.55 ± 6.90	240.18-250.12	247.55	148.63	282.87
$T_3N_0M_0$, G1	7	41.17	236.41±7.83	230.77-246.19	241.75	144.66	278.70
$T_3N_0M_0$, G2	6	35.29	245.76±7.79	239.81-251.79	256.25	184.39	280.66
$T_3N_0M_0$, G3	4	23.52	250.77±7.24	241.51-257.59	132.15	150.79	283.60
control	12	-	129.21±7.60	120.66-139.71		0	149.51
$P_{1, control}$	-	-	0.04	-	-	-	-
$P_{2, control}$	-	-	0.05	-	-	-	--
$P_{3, control}$	-	-	0.04	-	-	-	-
$P_{4, control}$	-	-	0.02	-	-	-	-
$P_{2,3}$	-	-	0.54	-	-	-	-
$P_{2,4}$	-	-	0/32	-	-	-	-
$P_{3,4}$	-	-	0.16	-	-	-	-

Table 2

Distribution of bladder cancer patients, stage $T_3N_0M_0$ according to the degree of neoplasia, G

Stage	Degree of neoplasia					
	G1		G2		G3	
	Abs	%	Abs	%	Abs	%
$T_3N_0M_0$, n =47	7	41.17	6	35.29	4	23.52

The data regarding the levels of TNF-α in the urine of bladder cancer patients, stage $T_3N_0M_0$ had no fundamental differences from the results obtained during the study of stages T1 and T2. In bladder cancer patients the average level of TNF-α in the urine was 362.61±5.76pg /ml, and in the control group it was 352.68 ± 6.75 pg/ml, this difference was not statistically significant ($p>0.05$).

When determining the levels of TNF-α in patients with different degrees of neoplasia, it was found that in subgroups of patients with G1, G2, G3, the average values of this marker in urine were 360.23 ± 5.87pg/ml, 362.04 ± 6.18 pg/ml and 363.27± 7.55 pg/ml, respectively, which are shown in Table 3 along with detailed statistics.

Table 3

The level of TNF-α in patients with PCM stage $T_3N_0M_0$

Group/subgroup	Number of patients		Average level of TNF-α (TNF-α_{av})±Δ, pg/ml	95% CI for TNF-α_{av}), pg/ml	Median, pg/ml	Min, pg/ml	Max, pg/ml
	abs	%					
$T_3N_0M_0$	17	100	362.61±5.67	358.58-363.44	361.8	351.23	374.29
$T_3N_0M_0$, G1	7	41.17	360.23±5.87	357.49-362.99	360.33	350.55	368.51
$T_3N_0M_0$, G2	6	35.29	362.04±6.18	357.22-364.5	361.84	351.35	373.28
$T_3N_0M_0$, G3	4	23.52	363.68±6.75	357.73-366.32	365.4	350.44	376.32
Control	12		352.68±6.75	350.26-350.26	350.99	342.82	365.23
P1, control	-	-	0.51	-	-	-	-
P2, control	-	-	0.61	-	-	-	-
P3, control	-	-	0.68	-	-	-	-
P4, control	-	-	0.56	-	-	-	-
P2,3	-	-	0.51	-	-	-	-
P2,4	-	-	0.64	-	-	-	-
P3,4	-	-	0.55	-	-	-	-

Table 4

The level of TNF-β in bladder cancer patients, stage $T_3N_0M_0$

Group/subgroup	Number of patients		Average level TNF–β (TNF-β_{av})±Δ, pg/ml	95% CI for TNF- β_{aver}, pg/ml	Median, pg/ml	Min, pg/ml	Max, max/ml
	Abs	%					
$T_3N_0M_0$	17	100	37.88±4.77	36.55-39.4	38.46	31.12	44.34
$T_3N_0M_0$, G1	7	41.17	36.21±4.45	34.34-38.88	36.70	31.12	45.66
$T_3N_0M_0$, G2	6	35.29	37.25±4.50	36.4-39.02	38.71	30.15	44.71
$T_3N_0M_0$, G3	4	23.52	41.01±4.73	36.92-43.03	40.71	31.99	46.67
Control	12	-	33.22±4.32	30.11-35.42	33.32	29.03	39.34
P_1 control	-		0.45	-	-	-	-
P_2 control	-		0.38	-	-	-	-
P_3 control	-		0.25	-	-	-	-
P_4, control	-		0.18	-	-	-	-
$P_{2,3}$	-		0.63	-	-	-	-
$P_{2,4}$	-		0.71	-	-	-	-
$P_{3,4}$	-	-	0.32	-	-	-	-

According to the data of the study conducted, in bladder cancer patients, stage $T_3N_0M_0$, the average level of TNF-β in urine was 37.88 ± 4.77 pg/ml, while this indicator in the control group was 33.22 ± 4.32, the difference was not statistically significant ($p>0.05$).

In bladder cancer patients, neoplasia grade G1, the average level of TNF-β in urine was 36.21±4.45 pg/ml, with grade G2 – 37.25 ±4.50 pg/ml, and with grade G3 tumor neoplasia – 41 ,01±4.73pg/ml

Conclusions

1. According to the results of the statistical analysis, in bladder cancer patients stage $T_3N_0M_0$, the average level of VEGF in urine was 246.55 ± 6.90 pg/ml and significantly exceeded this indicator in the control group, which was 129.21± 7.60 pg/ml, this difference was statistically significant.

2. At bladder cancer diagnosis, the sensitivity and specificity of the urinary TNF-α level were low and amounted to 30% and 20%, respectively, and the TNF-β level was only 25% and 20%, respectively, which is not representative for this pathology.

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Conflict of interests

The Authors declare no conflict of interests