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Dynamics of Proinflammatory Cytokine Levels and Their Role in the Development of Local and Systemic Effects during Progressing Cervical Cancer

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In order to evaluate the role of cytokines in the development of polymodal local and distant effects in patients with stages I-IV cervical cancer, the following parameters were measured: serum concentrations of TNF-α, IFN-γ, IL-1β, IL-6, and IL-10; levels of malondialdehyde, activities of catalase, superoxide dismutase, glutathione reductase, and glutathione-S-reductase in the plasma, erythrocyte, and cervix uteri homogenate. The expression of p53, Bcl-2, VEGF, and Ki-67 in tumor tissue was evaluated. High levels of malondialdehyde in tumor tissue and erythrocyte were paralleled by low levels of superoxide dismutase and catalase and high activities of glutathiones. Medium correlations between Ki-67, Bcl-2, and p53 and the levels of IL-6, IL-10, and TNF-α at stages Ib-IIa were detected. The results indicated that the progress of cervical cancer was associated with the neoplasm integration in the host homeostasis by using the regional and systemic cytokine functions. These effects, amplifying the biological potential of the tumor, were the most significant at stages Ib-IIa.

Key Words: *cervical cancer; cytokines*

Cytokines are produced by cells and realize short-distance regulation of cell–cell and system–system interactions; they determine cell survival, growth, functional stimulation, and apoptosis. After cytokine binding to receptors on the cell membrane, the signal is transferred to the nucleus, where the genes are activated, whose products are synthesized by the cells and regulate the above processes. This is paralleled by modulation of the local and systemic defense mechanisms. It is assumed that of all cytokines produced by the tumor, IL-1, TNF-α, and IL-6 are characterized by the most pronounced systemic effects. Their most significant effects are stimulation of the neoplasm proliferation and angiogenesis [8].

We studied the dynamics of proinflammatory cytokines (TNF-α, IFN-γ, IL-1β, IL-6, and IL-10) and their role in the development of local and systemic effects during the progress of cervical cancer (CC).

MATERIALS AND METHODS

The study was carried out in 87 patients (mean age 46 years) with newly diagnosed CC, with disease stages I-IV (according to FIGO). The measurements were carried out in tumor tissue specimens collected during surgery, blood plasma, and erythrocytes. Patients operated on for uterine myomas served as the control (specimens of visually normal cervical tissue). Donor blood served as control in studies of the blood plasma and erythrocytes. The diagnosis was verified by histological studies of biopsy specimens or operation material in all patients. The main histological variants of CC were squamous-cell cancer in 80 patients (92%), adenocarcinoma in 3 patients (3.4%), and glandular

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squamous-cell cancer in 4 patients (4.6%). By tumor differentiation the patients were distributed as follows: 21 (26.6%) patients with well-differentiated tumors, 54 (62%) with moderately differentiated, and 12 (11%) with poorly differentiated tumors.

The patients were distributed into 3 groups by the process dissemination. Group 1 included patients with the initial process (Ia), group 2 consisted of patients with locally disseminated process (Ib-IIa), and group 3 patients presented with disseminated tumor process (IIb-IV). The intensity of LPO in tumor tissue, plasma, and erythrocytes was evaluated by malondialdehyde (MDA) levels, the antioxidant system enzymatic component – by the activities of catalase, glutathione reductase (GR), glutathione-S-transferase (GT), and superoxide dismutase (SOD). Immunohistochemical evaluation of p53 and Bcl-2 expression was carried out with monoclonal antibodies, clone DO-7, IgG2b (M7001, Dako Cytomation Ltd.), and clone Bcl-2/100/D5, IgGi (NCL-Bcl-2, Novocastra). The VEGF was detected by polyclonal antibodies (Dako). Proliferative activity of the neoplasm was evaluated as the percentage of Ki-67$^+$ cells from all tumor cells. Serum concentrations of TNF-α, IFN-γ, IL-1β, IL-6, and IL-10 were measured by ELISA with the respective ELISA kits (Vector-Best-Volga). The distribution of serum cytokine levels differed from Gaussian distribution, and therefore the median served as the central characteristic and Mann–Whitney nonparametric test was used for comparisons.

RESULTS

In CC patients with stage Ia, the level of TNF-α below 16.95 pg/ml (that is, less than in the control) was found in 76% cases ($p<0.05$); at stage Ib-IIa, this level was detected in 96.5% ($p<0.05$), and at stages IIb-IV, in 100% cases ($p<0.05$).

TNF is a group of cytokines realizing their activities via the respective family of cell receptors. TNF-α modulates virtually all stages of the inflammatory reactions. This cytokine interacts with Fas membrane receptor family and the resultant complex enters the cells, which is associated with stimulation of ROS generation in mitochondria and cell damage. Hence, oxygen radicals can serve as the second messengers in TNF-α effects [6]. In addition, intracellular proteases stimulating DNases are activated, which causes DNA fragmentation and cell apoptosis. In addition to production of free radicals, TNF-α stimulates neoangiogenesis and invasive activity of tumor cells. Functional activity of *TNF* gene is demonstrated, consisting in solitary nucleotide substitutions in the noncoding part of the gene. These changes do not affect protein

TABLE 1. Dynamics of Serum Cytokines during CC Progress

Parameter		Control (*N*=25)	CC stages		
			Ia (*N*=28)	Ib-IIa (*N*=29)	IIb-IV (*N*=22)
IL-6, pg/ml	median	47.23	75.41	36.97	39.79
	quartiles	(34.55-95.62)	(41.56-57.54)	(16.00-48.42)	(33.32-36.55)
	range	(21.69-95.62)	(30.80-171.74)	(13.63-102.32)	(26.00-60.05)
IL-10, pg/ml	median	2.62	2.87	7.54	1.71
	quartiles	(0.73-4.89)	(3.16-6.87)	(6.22-9.16)	(0.05-2.51)
	range	(0.45-5.26)	(0-8.35)	(2.67-12.39)	(0-2.63)
IFN-γ, pg/ml	median	124.96	16.98	40.65	19.92
	quartiles	(1.27-290.49)	(1.67-43.17)	(3.36-117.97)	(1.65-54.53)
	range	(1.09-422.55)	(1.27-60.05)	(1.25-318.06)	(0-93.09)
IL-1β, pg/ml	median	7.98	9.53	1.65	0.61
	quartiles	(1.69-12.71)	(8.96-12.05)	(0.97-1.59)	(0.30-1.44)
	range	(1.69-12.71)	(0.73-16.37)	(0.26-2.88)	(0-1.54)
TNF-α, pg/ml	median	16.95	10.32	8.97	7.34
	quartiles	(14.37-25.59)	(0.093-18.530)	(3.98-16.03)	(0.15-14.04)
	range	(5.92-25.59)	(0-33.21)	(0-19.66)	(0-15.52)

Note. Here and in Table 2: *n* – number of samples.

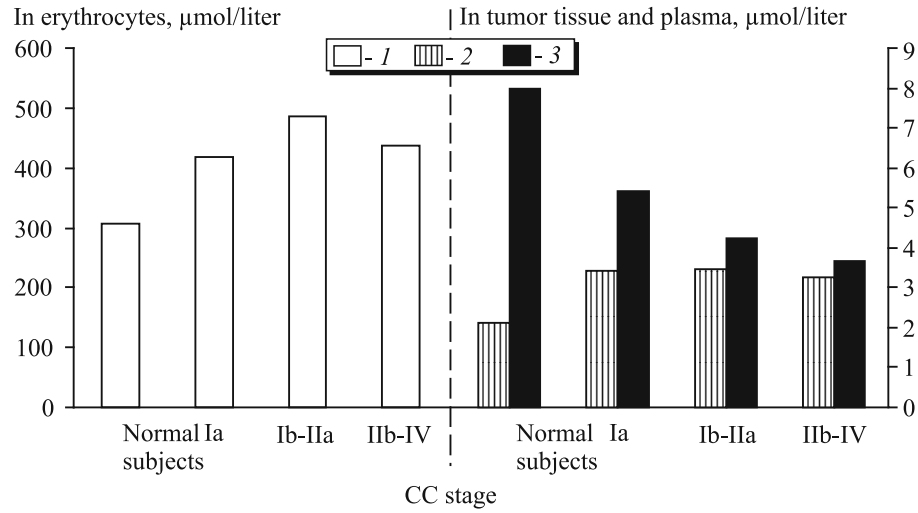

Fig. 1. MDA concentration in tumor tissue, erythrocytes, and plasma during CC progress. *1*) Erythrocytes; *2*) tumor tissue; *3*) plasma.

structure, but can modify the rate of mRNA transcription and hence, of the cytokine production, including its reduction [8,15]. The significance of these genetic variations for many diseases is discussed, but the data are contradictory. We found that low TNF-α levels in CC (below the normal) are detected at stage Ia and the trend progresses with tumor growth (Table 1). Opinions about the dynamics of serum TNF-α concentrations during the progress of malignant tumor depending on its biological characteristics vary: some authors think that cancer progress is associated with early and steady increase of TNF-α concentrations [2], while others present experimental data indicating reduction of TNF-α production during malignant tumor progress [14]. Reduction of inflammatory response with the progress of squamous-cell CC has been described [12]. According to some authors, the level of TNF-α directly correlates with detection of carcinogenic HPV and virtually does not differ in patients with squamous-cell CC and normal subjects [10,13]. Measurements of serum TNF-α in patients with cervical intraepithelial neoplasm of different degree have shown that TNF-α level virtually does not differ from the control in slight dysplasia and reduces in severe dysplasia [1]. The authors think that the reduction of TNF-α level with tumor progress in this case can

TABLE 2. Activities of Antioxidant Enzymes during CC Progress

Parameter		Control (*N*=15)	Stage Ia (*N*=21)	Stage Ib-IIa (*N*=22)	Stage IIb-IV (*N*=21)
Homogenate	GT, mmol/min/mg	15.240±0.286	19.410±1.985*	76.090±4.894*	65.460±1.826*
	GR, mmol/min/mg	5.630±0.192	7.230±0.389*	11.830±0.818*	15.44±0.33*
	SOD, arb. units	0.710±0.169	3.210±0.246*	1.910±0.177*	1.520±0.567*
	Catalase, mmol/mg	0.120±0.003	0.130±0.005	0.090±0.003	0.070±0.003
Erythrocytes	GT, mmol/min/mg	0.200±0.025	0.310±0.022*	0.380±0.018*	0.320±0.017*
	GR, mmol/min/mg	0.960±0.508	0.300±0.07*	0.30±0.05*	0.20±0.04*
	SOD, arb. units	10.00±1.15	3.20±0.77*	4.6±0.3*	6.9±1.7*
	Catalase, mmol/mg	25.3±5.97	5.3±1.06*	4.50±0.94*	7.10±1.67*
Plasma	GT, mmol/min/mg	0.0310±0.0001	0.0510±0.0054*	0.0590±0.0056*	0.0450±0.0045*
	GR, mmol/min/mg	0.040±0.015	0.080±0.064*	0.010±0.004*	0.020±0.004*
	SOD, arb. units	0.050±0.013	0.120±0.053*	0.040±0.012	0.020±0.005*
	Catalase, mmol/mg	15.240±0.286	19.410±1.985*	76.090±4.894*	65.460±1.826*

Note. *$p<0.05$ in comparison with the control.

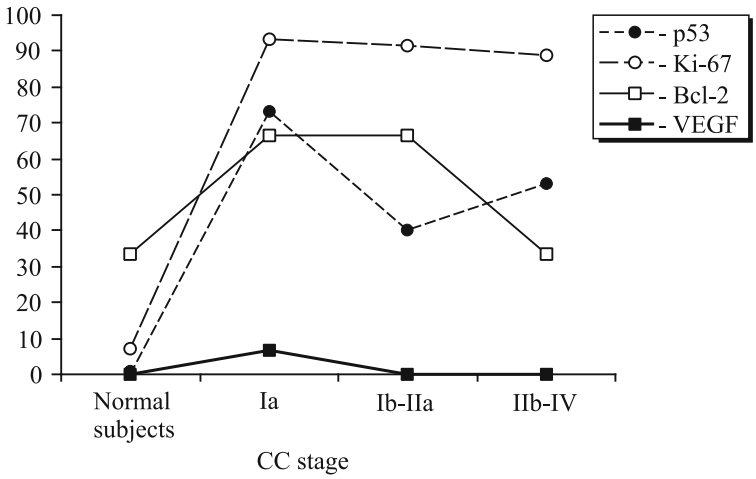

Fig. 2. Expression of Ki-67, p53, VEGF, and Bcl-2 in the primary tumor node epithelium over the course of CC progress.

indicate incompetence of the cellular component of immunity and disorders in the phagocyte functional profile, this promoting HPV persistence and neoplasm progress. We have detected a moderate correlation between TNF-α level and tumor tissue MDA in all groups (r=0.3000, r=0.3070, and r=0.3147).

Under these conditions, intensification of LPO–antioxidant system function in the tumor tissue precludes the threat of oxidative stress (Table 2, Fig. 1), but suggests the possibility of redox modulation of redox-dependent intracellular signal pathways [4,11].

Of the biological effects of IFN, its antiproliferative activity towards the neoplasm seems to be important. The effect of the cytokine on cell differentiation is dose-dependent. In high doses, it blocks cell differentiation. The data on its effects on apoptosis are contradictory: IFN-γ directly induces apoptosis and stimulates *Bcl-2* gene hyperexpression, which prevents apoptosis even under conditions of antitumor drug therapy [11]. It is assumed that Bcl-2 family proteins are involved in the regulation of the mitochondrial apoptosis pathway; Bcl-2$^+$ tumors are more aggressive by their biological potential because of their high levels of angiogenesis markers [7]. The data on IFN inhibition of proliferation of endothelial cells and fibroblasts suggest the antiangiogenic activity of the cytokine [5]. We found that the level of IFN-γ at Ia stage was below 7.0 pg/ml (that is, less than in the control) in 15 (54.5%) of 28 patients (p<0.05). At stage Ib-IIa 18 (61.5%) of 29 patients had IFN-γ levels higher than 6.8 pg/ml, but lower than in the control (p<0.05). At stage IIb-IV 18 (61.5%) of 29 patients had IFN-γ levels higher than 9.0 pg/ml but lower than in the control (p<0.05). The expression of Bcl-2 on the tumor cell membrane also increased at stage Ia, remained high at stage Ib-IIa, and decreases to the initial level under conditions of disseminated tumor process (Fig. 2). Medium correlations between Bcl-2 and IFN-γ were detected.

The effects of IL-1 and IL-10 on the cell death process were also dose-dependent [3]. Serum IL-10 levels in CC patients with stage Ia-IIb were higher than 2.6 pg/ml (surpassed the control) in 100% cases (p<0.05). The level of IL-1β below 2.9 pg/ml (lower than in the control) at stage Ib-IIa was detected in 29 (100%) patients (p<0.05). IL-6 concentration >47 pg/ml (higher than in the control) was more incident in patients with stage Ia (in 75% cases, p<0.05; Table 1). Many tumor cells produce IL-1β, and we assumed that the production of this cytokine promotes the neoplasm proliferation.

The dynamics of proliferation marker Ki-67 in the tumor epithelial tissue is presented in Figure 2. The drastic increase of Ki-67 expression moderately correlates with the levels of proinflammatory cytokines: IL-1β (r=0.543), IL-6 (r=0.714), IL-10 (r=0.600), and TNF-α (r=0.714) at stage Ib-IIa. The correlations were slight or absent at the disseminated process stage.

Virtually the entire spectrum of molecules, involved in the processing of the cytokine regulatory signals and in triggering of certain cell functions, were detected in malignant cell cytoplasm. Redox modulation of redox-dependent intracellular signal pathways was possible [5]. In order to evaluate the probability of oxidative stress and the role of Nrf2/ARE redox-sensitive intracellular signal system [6], the level of the LPO-antioxidant system functioning was evaluated. Tumor progress in CC was associated with increase of LPO levels in the neoplasm and erythrocytes (Fig. 1) in parallel with reduction of the antioxidant activities (Table 2), which suggested the probability of oxidative stress. Plasma MDA level progressively decreased with dissemination of the process (Fig. 1). The dynamics of antioxidant activities in tumor tissue at stages Ib-IIa and IIb-IV (increase of GT and GR and reduction of SOD and catalase levels) could indicate an increase in the level of superoxide anion radical, stimulating the proliferation, in the presence of a rather low level of H_2O_2,

TABLE 3. Coefficients of Correlations of Serum Cytokine Levels and Expression of Molecular Markers in Tumor Epithelial Tissue at CC Stage Ib-IIa

Parameter	IL-6	IL-10	TNF-α
Ki-67	0.7243	0.6102	0.7143
Bcl-2	0.7093	0.6000	0.7120
p53	0.7124	0.6070	0.7470

inhibiting the cell proliferation (Table 2). Correlation with Ki-67 at stage Ia was moderate (r=0.3951). This fact suggests the appearance of a cell population with active Nrf2/ARE signal system during CC progress, protecting the cell from AOS under conditions of oxidative stress [9].

The activities of proinflammatory cytokines can lead to the formation of defective antioncogenes and their inert RNA products. These are p53 (one of apoptosis inductors) and *Bcl-2* gene, its product characterized by cytoprotective activity [11]. The results of histochemical markers evaluation in the tumor are presented in Figure 2. The data suggest a significant increase of the marker levels at Ia stage of the disease. Analysis of Ki-67, Bcl-2, and p53 correlations with the levels of proinflammatory cytokines in CC detected moderate correlations with IL-6, IL-10, and TNF-α levels at stage Ib-IIa (Table 3). At stage IIb-IV the correlations were slight or zero.

Hence, the neoplasm integrates in the host homeostatic processes during CC progress due to use of the regional and systemic cytokine functions. The realization of these effects is the most significant for stimulation of the tumor biological potential at stage Ib-IIa.

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