INTEGRAL ANALYSIS OF BLOOD PLASMA BIOCHEMICAL PARAMETERS AS AN OPTIMIZING DIAGNOSTIC TECHNIQUE OF EPITHELIAL TISSUE MALIGNANT NEOPLASMS

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The aim of the investigation was to assess the interaction of tumor marker level, the indices of protein and mineral metabolism, free radical oxidation in blood plasma and the tumor-forming stage in malignant processes in epithelial tissues.

Materials and Methods. Blood plasma of 73 patients with epithelial tissue malignant neoplasms and 31 apparently healthy people were studied. Blood plasma biochemical parameters were assessed using the analyzers: free-radical activity — by induced biochemiluminescence; oxidative protein modification — by the level of carbonyl derivatives; elemental analysis — using atomic emission spectrometry.

Results. Low diagnostic value of the determination of oncomarkers at early stage of the studied oncological diseases was shown. We observed the change of blood plasma biochemical parameters at early carcinogenesis stage: the albumin level reduction and the concentration increase of urea, α1- and γ-globulin fractions. Cancer patients were found to have impaired element homeostasis: Na, Fe, Cu, Li level decrease, K, P, Sr increase. We revealed the activation of free-radical oxidation and oxidative protein modification, the correlation of the intensity of these processes with the content of some elements in blood plasma. Integral analysis of blood plasma biochemical parameters increases diagnostic value of the determination of tumor markers in the detection of malignant tumors of epithelial tissues.

Key words: malignant neoplasms; tumor markers; epithelial cancer; macroelements; microelements; protein fractions; free-radical oxidation.

Currently, tumor markers in blood plasma and other biological fluids are increasingly determined for prediagnosis of solid carcinomas [1]. Tumor markers, or oncomarkers, are complex substances, which are determined in significantly higher concentrations in transformed malignant cells compared to normal ones, or produced by an organism in response to the presence of malignant cells and detected in blood and/or urine of oncological patients [2]. However, this method has a number of disadvantages. Various hepatic and renal dysfunctions can result in false increase of oncomarker level. False-high levels for prostate-specific antigen (PSA) can be temporarily revealed after prostate palpation, urological procedures, long urinary retention or in smoking patients. In addition, there should be taken into consideration the dependence of the content of some oncomarkers on a patient's age. Moreover, low sensitivity of the method in detection of early carcinogenesis stages raises doubts in advisability of tumor marker detection as a screening assay [3]. So, in breast cancer, increased levels of CA 15-3 oncomarker are found in 9% of cases at stage I, in 19% of cases — at stage II, in 38% of cases — at stage III, and in 75% — at stage IV. Increased levels of this marker occur in 5–6% of healthy women. Thus, using this marker a tumor can be detected only in 76 of 400 women, and there are 66 useless examinations per 1 effective examination.

On the other hand, the change of biochemical blood plasma parameters is frequently an early sensitive diagnostic criterion in various pathological conditions. Malignant tumor growth is known to cause specific changes in the composition of blood plasma proteins, e.g. reduction in albumin level [4].

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The aim of the investigation was to assess the interaction of tumor marker level, the indices of protein and mineral metabolism, free radical oxidation in blood plasma and the tumor-forming stage in malignant processes in epithelial tissues.

Materials and Methods. Blood plasma of 73 patients, who had had no antitumor treatment before was studied: 46 male (aged 47–74) and 27 female (aged 34–67). Renal cell carcinoma was diagnosed in 16 patients (22%), bladder cancer — in 12 (16%), prostate cancer — in 16 (22%), ovarian cancer — in 14 (19%), laryngeal cancer — in 7 (10%), bowel cancer — in 3 (4%), hysterocarcinoma — in 2 (3%), pancreatic carcinoma — in 2 (3%), gallbladder tumor — in 1 (1%). 18% patients were found to have stage I, 18% — stage II, 46% — stage III, and 18% — stage IV. Blood plasma of 31 virtually healthy people was considered control: 12 male (aged 24–74) and 19 female (aged 25–65).

Blood plasma biochemical parameters were estimated using analyzers "ConeLab 20/20i" (Finland). Tumor markers – prostate specific antigen (PSA), CA 125, CA 19-9, carcino-embryonic antigen (CEA), α-fetoprotein (AFP) were determined by immunochemiluminescence on automatic analyzer Liaison (Italy). Free radical activity was assessed by induced biochemoluminescence [5] on IBM-coupled biochemiluminometer BCM-06, oxidative protein modification (OPM) was estimated by the level of carbonyl derivatives [6]. Element analysis was performed by atomic emission spectrometry with inductively coupled plasma on spectrometer iCAP6300Duo (Thermo Scientific, USA). The findings were statistically processed using software package BIOSTAT.

Results and Discussion. The study showed PSA level (serine proteinase, prostate excretory product) in prostate cancer statistically significantly increases beginning from stage II (Fig. 1).

In 33% of cases at stage III and in 25% of cases at stage IV its level did not exceed 4.5 ng/ml — upper limit of normal. AFP content (glycoprotein, concentration growth of which is observed in hepatocellular carcinoma and testicular and ovarian teratocarcinomas) also increased significantly beginning from stage II. We may assume that the revealed increase of AFP concentration is due to patients' elderly age, since with aging the protein content grows. CEA concentration (having diagnostic value in colorectal carcinoma detection) and CA 19-9 (a marker of pancreatic carcinoma) did not statistically significantly differ from those levels in healthy people.

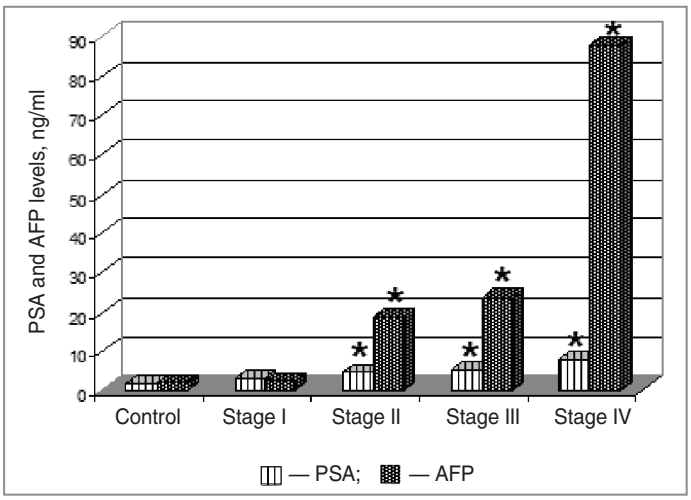

Fig. 1. PSA and AFP levels in blood plasma of patients with prostate cancer: * — differences with control group values are statistically significant, p<0.05

No significant differences in CA 125 level in blood plasma of ovarian cancer patients were found compared with the indices in virtually healthy people (glycoprotein of mucin family, which is a selective marker of ovarian tumors) in stages I and II: 12.93 and 8.33 u/L, respectively. Significant differences were observed starting from stage III (274.79 u/L), and marker levels over upper limit of reference interval were observed in 86% cases. The obtained results support the information on the absence of CA 125 level increase in more than 50% of cases at stage I ovarian cancer [7].

The values of the tested markers in renal and bladder cancer did not statistically significantly exceed those in control group.

In early stages of carcinogenesis in patients with laryngeal cancer there were no significant differences in the levels of oncomarkers with a control group. Only CA 19-9 level at stages III and IV differed from that in the control group, however it did not exceed the upper limit of normal (37 u/ml).

Due to insignificant number of patients with bowel cancer, uterine cancer, pancreatic cancer and bladder cancer, the oncomarker levels were not analyzed by stages, but it can be mentioned that CA 125 content in 50% of cases is increased in hysterocarcinoma (stages III and IV), and CA 19-9 – in 100% of cases in pancreatic cancer (stage IV) and bladder cancer (stage IV).

The study of blood plasma protein homeostasis did not reveal any significant differences in the values of the tested parameters depending on the type of epithelial malignant neoplasms, for this reason we present data obtained in different stages of the disease (Table 1).

There was revealed statistically significant decrease of blood plasma albumin level in all stages of tumor process, though it did not override a lower bound of reference interval (48–65%). Significant increase in blood plasma urea in the absence of such an increase for creatinine proves an idea of the activation of protein catabolism processes in oncological patients [8]. The present increase of α1-globulin fraction levels in epithelial malignant tumors (See Table 1), to which α_1-antitrypsin and acidic α_1-glycoprotein refer to, and being inhibitors of tissue proteases, are likely to have compensatory significance. In carcinogenesis there was also found statistically significant increase of γ-globulins, and in stages I and IV their level exceeds an upper limit of normal (by 10–19%), and in stages II and III it has the same level that is due to stress inflammatory response of the organism on a tumor.

The content of immunoglobulins in blood plasma increases mainly due to IgA that can be conditioned by a specific character of the disease — malignant tumors of epithelial tissues.

Table 1

Blood plasma protein homeostasis values in epithelial malignancies (M±m)

Parameters	Virtually healthy people (control group)	Oncological patients			
		Stage I	Stage II	Stage III	Stage IV
Total protein, g/L	70.59±2.41	73.25±2.56	74.73±3.01	72.39±1.79	72.27±2.13
Albumins,%	59.71±1.36	56.36±1.42*	54.80±2.74*	53.83±4.12*	51.26±3.35*
α1-globulins, %	3.53±0.11	4.22±0.41*	3.69±0.10*	4.27±0.39*	4.77±0.62*
α2-globulins, %	10.08±1.48	8.76±2.21	10.63±1.78	10.09±1.13	9.28±2.67
β- globulins, %	12.13±0.93	10.92±2.14	11.50±1.59	11.75±2.68	10.99±1.74
γ- globulins, %	13.89±1.98	19.07±2.37*	17.31±1.21*	18.82±2.46*	19.29±3.15*
Ig A, g/L	2.89±0.37	3.51±0.22*	3.83±0.49*	4.07±0.76*	3.76±0.33*
g G, g/L	14.17±2.15	12.72±2.13	12.30±1.77	15.33±3.09	13.02±1.79
Ig M, g/L	1.69±0.34	1.25±0.48	1.13±0.53	1.38±0.76	0.92±0.64*
Urea, mM/L	4.81±0.97	7.24±1.11*	6.88±0.97*	8.28±1.59*	7.20±0.85*
Creatinine, μM/L	83.88±8.15	87.92±2.34	95.63±5.14	110.14±12.43	86.42±3.76

* — differences with control group values are statistically significant, p<0.05.

The study of free radical activity of blood plasma (Imax, mV) showed its significant increase in all groups of oncological patients compared to a control group beginning from stage I carcinogenesis — 3 times as much (Fig. 2, *a*). Blastomatous process is accompanied by the increase of superoxide anion radical and other reactive oxygen species (ROS), and a condition of oxidative stress developing in the organism [9]. Superoxide anion radical accumulation at early stages of tumor growth is thought to promote DNA damage, increase in the number of mutations, expression of some genes and neoplastic transformation of cells, the change of physicochemical properties of membranes including a nuclear membrane. There is a hypothesis suggesting the participation of reactive oxygen species, superoxide radical and hydrogen peroxide in particular, in cell proliferation regulation [10, 11].

The activity of free radical oxidation of blood plasma in metastasis increased that manifested in statistically significant dependence between two parameters: the presence of distant metastases and Imax (r=0.394). Matrix metalloproteinases — a family of extracellular endopeptidases able to destroy all type proteins of extracellular matrix — are known to have a significant role in metastasis process [12]. The activity of these enzymes is regulated, among other things, by reactive oxygen species [13].

All patients with epithelial malignant neoplasms were also observed to have significant activation of protein oxidative modifications evidenced by an increased content of carbonyl protein derivatives in plasma starting from stage I of the disease — by 71, 146, 259 and 82% respectively (Fig. 2, *b*).

The analysis of mineral homeostasis as early as at early carcinogenesis stages showed Na level decrease in blood plasma (about 15%), increase of K (by 17%) and P (by 20–58%) compared to indices in virtually healthy people (Table 2). Ca, Cl, Mg content had no significant differences.

Correlation analysis revealed statistically significant interaction of free radical activity with K (r=–0.438) and Na (r=0.488) content in blood plasma. This relationship can be due to the capability of excess ROS concentrations to cause the inhibition of Na^+/K^+ adenosine triphosphatase and, therefore, imbalance of Na and K levels [14].

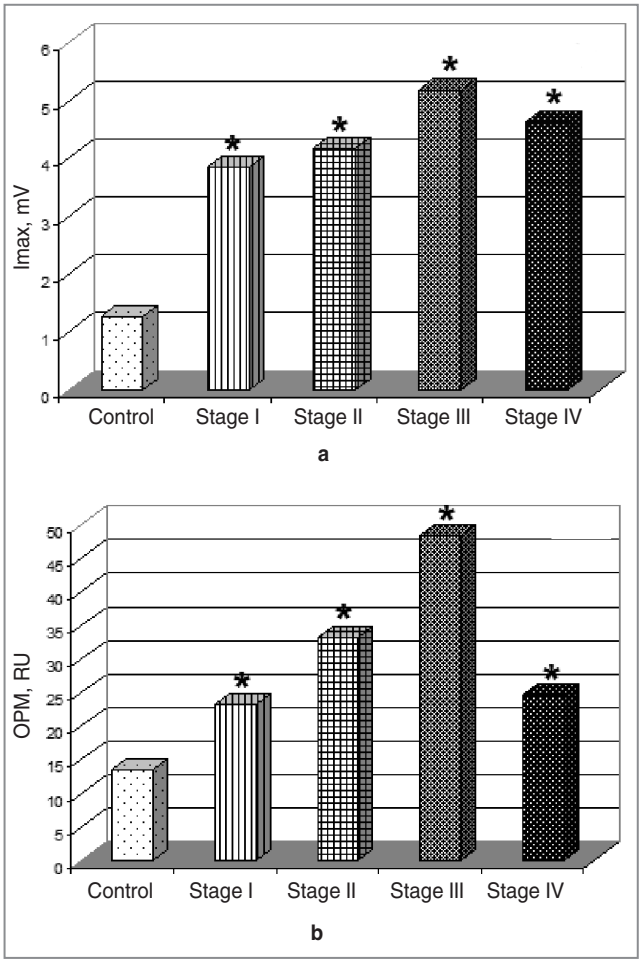

Fig. 2. Free radical activity (a) and protein oxidative modification level (b) of blood plasma of patients with epithelial malignancies; * — differences with control group values are statistically significant, p<0.05

Table 2

The content of macroelements in blood plasma of patients with malignant neoplasms of epithelial tissues (M±m)

Macro-elements	Virtually healthy people (control group)	Oncological patients			
		Stage I	Stage II	Stage III	Stage IV
Na, mmol/L	145.78±2.33	137.70±5.10*	137.72±4.17*	139.43±3.59*	140.76±2.47*
K, mmol/L	3.80±0.22	4.43±0.33*	4.39±0.27*	4.33±0.17*	4.38±0.24*
Ca, mmol/L	2.28±0.35	1.88±0.22	2.06±0.36	2.20±0.28	2.22±0.12
Cl, mmol/L	103.10±2.89	109.67±4.35	104.00±2.76	105.00±4.29	104.67±3.28
Mg, mmol/L	0.81±0.08	0.87±0.17	1.07±0.19	0.86±0.15	0.69±0.10
P, mmol/L	1.07±0.08	1.28±0.09*	1.22±0.07*	1.69±0.15*	1.38±0.07*

* — differences with control group values are statistically significant, p<0.05.

Table 3

The content of microelements in blood plasma of patients with epithelial malignant neoplasms, μg/ml (M±m)

Micro-elements	Virtually healthy people (control group)	Oncological patients			
		Stage I	Stage II	Stage III	Stage IV
Fe	1.24±0.12	0.93±0.13*	0.79±0.21*	0.88±0.19*	0.975±0.10*
Cu	1.36±0.09	0.85±0.23*	0.92±0.30*	1.12±0.28	1.05±0.15*
Zn	0.88±0.13	0.72±0.21	0.83±0.12	0.85±0.16	0.59±0.09*
Li	0.007±0.001	0.001±0.0007*	0.002±0.001*	0.002±0.001*	0.001±0.001*
Ba	0.007±0.002	0.007±0.003	0.009±0.003	0.011±0.003	0.009±0.002
Sr	0.043±0.008	0.074±0.014*	0.082±0.017*	0.061±0.03	0.079±0.002*

* — differences with control group values are statistically significant, p<0.05.

The study of the level of microelements in blood plasma showed concentration decrease of Fe (30–40%), Cu (18–40%), Li (3–7 times), Sr content growth (90%) (Table 3).

Correlation analysis revealed statistically significant dependence between the parameters of free radical activity and the content of such elements as Mg (r=0.381), Zn (r=–0.660), Cu (r=0.273). Similar Zn and Cu interaction is supposed to be due to the participation of these microelements in the action of antioxidant enzymes, e.g. superoxide dismutase. Previously, L.P. Smirnova and I.V. Kondakova [15] found low activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) in tumor cells being in the phase of logarithmic growth.

Lithium data prove the existing information on its role in the decrease of lipid peroxidation processes due to the inhibition of unsaturated fatty acid synthesis [16]. Depending on its concentration, lithium can have a directly opposite effect on cell apoptosis and proliferation [17]. It also can have effect on Na and K level due to membrane potential change in malignant cells.

Conclusion. Antitumor markers do not always make it possible to diagnose a stage and prognosis of oncological diseases. PSA determination in prostate cancer has the most essential findings, but they are not reliable in early stages of the disease. PSA, CEA, AFP, CA 19-9, CA 125 have no diagnostic value in renal and bladder cancer detection. Statistically significant increase of CA 19-9 level is found only in terminal stages of laryngeal cancer, pancreatic carcinoma, gallbladder cancer, and cannot be used for early diagnosis of these diseases. This also holds true for CA 125 in hysterocarcinoma detection. The investigations of blood plasma biochemical parameters enable to conclude about the change of protein and mineral metabolism as early as in the early stage of carcinogenesis. Therefore, an integral study of protein metabolism parameters including the processes of protein oxidative modification and free radical oxidation, as well as the content of macro- and microelements, will considerably improve diagnostic value of tumor marker determination in epithelial malignancy detection.

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