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ENDOTHELIAL DYSFUNCTION OF VESSELS AT LUNG CANCER

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***Aim*: To evaluate changes in indicators of endothelial function and their relationship with morphological forms of disease, stage of pathological process and tumor markers, by analysis the peripheral blood of lung cancer (LC) patients. *Materials and Methods*: 38 LC patients without metastases (mean age — 57 years) prior chemo- and radiotherapy were included in the study. The duration of the disease manifestation was 18 months. 21% of patients had small cell LC, and the rest — non-small cell LC. The ratio of patients with stages IA, IB, IIA, IIB, IIIA and IIIB LC was 1 : 3 : 3 : 4 : 4 : 4. The enzyme immunoassay, spectrophotometry, and statistical data analysis were used. *Results*: Endothelial dysfunction of vessels was characterized by increased blood levels of vascular endothelial growth factor (VEGF), endothelin-1 (ET1), homocysteine (HCys), cyclic guanosine monophosphate (cGMP), P-selectin (PSel) and nitrites (NO_2) and simultaneously by decreased values of prostacyclin (PgI2). Those were observed in 100; 90; 76; 71; 50; 53 and 79% of LC cases, respectively. Disturbances of vascular endothelial function were associated with patient's age, disease duration, and morphological form and LC stage. Such changes were observed in women with higher prevalence. The studied indices correlated with tumor markers, namely transforming growth factor beta (TGFβ1), fibronectin and osteopontin. *Conclusion*: Indices of vascular endothelial dysfunction in LC can be of diagnostic and prognostic value.**
***Key Words*: lung cancer, blood vessels, endothelium, endothelial dysfunction.**

Lung cancer (LC) occupies the first place in the structure of cancer morbidity in men and it is one of the leading causes of cancer mortality [4, 9]. The incidence of LC in the male population of various countries (including Ukraine) is more than 60 per 100 000, in the female population — 14–30 per 100 000, and in many countries these values increase annually [8, 25]. Every fourth man from newly registered cancer patients and every third of those cancer patients who died are the LC patients, as discussed in [16].

Tumor endothelial cells differ significantly from normal endothelial cells by structural and functional characteristics that finally determine the process of angioneogenesis [22]. Changes of expression pattern of the proteins controlling angiogenesis can serve as prognostic marker of malignant process [26]. They can be used also for monitoring of anticancer treatment efficacy in LC patients [3]. In particular, in case of lung tissue damage, the endothelial system reacts first; drastic increase of the blood level of vasoconstrictor endothelin-1 (ET1) is considered as a marker of activity of destruction process.

Vascular endothelial growth factor (VEGF) plays the leading role among tumor angioneogenesis regulators. It is essential for prognosis of a course of malignant disease [6], for staging and the rate of metastasis of LC [15]. The direct correlation between VEGF levels in blood and tumor size is evidenced [19]. It should be noted that high levels of VEGF in serum are associated with low survival rates in patients with non-small cell LC [2].

Disorders in cell-to-cell adhesion are typical for many malignant tumors [18]. Proteins controlling this process in association with vascular endothelium are involved into LC pathogenesis [13]. The imbalance of E- and P-selectin (ESel, PSel) enhances dissemination of tumor cells [10]. The excess of selectin in culture medium was accompanied by increased adhesion of tumor cells to endothelial cells [7].

Many questions concerning functional state of endothelial blood vessels (FSVE) in LC are still unanswered. For example, it is not clear how the changes of ration between vasoconstrictors and vasodilators, measured in blood and occurs, and its relationship to other tumor markers remains undefined [1, 20].

The aim and the objectives of this study were the assessment of FSVE variations (VEGF, ET1, ESel, PSel, TxA2, HCys, PgI2, NO_2, cGMP) in blood of LC patients, as well as their association with morphological forms of the disease, pathological process stage and the tumor markers (transforming growth factor — TGFβ1, fibronectin — FN, osteopontin — OP).

MATERIALS AND METHODS

38 LC patients without metastases (35–76 years; average 56.6 ± 1.88 years), prior chemo- and/or radiotherapy, were included into the study. 29 (76%) patients were men and 9 (24%) patients— women (who were on average 10 years older; $\chi^2 = 2.23$; $p = 0.032$). The duration of the LC manifestation was 17.9 ± 1.29 months. 21% of patients had small cell LC, and the rest 79% — non-small cell LC. The ratio of IA, IB, IIA, IIB, IIIA and IIIB LC stages was 1 : 3 : 3 : 4 : 4 : 4, respectively. With regard to LC morphological forms and stage there was no differences between genders. The control group

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Abbreviations used: cGMP – cyclic guanosine monophosphate; ET1 – endothelin-1; FN – fibronectin; HCys – homocysteine; LC – lung cancer; NO_2 – nitrites; OP – osteopontin; PgI2 – prostacyclin; PSel – P-selectin; TGFβ1 – transforming growth factor beta; TxA2 –thromboxane; VEGF – vascular endothelial growth factor.

consisted of 30 healthy persons (17 men and 13 women, average age 41.1 ± 6.1 years; $p > 0.05$).

The following methods was used for diagnostic: X-ray, computed or magnetic resonance tomography of the lungs ("Multix-Compact-Siemens", Germany; "Somazom-Emotion-6-Siemens", Germany; "Gygoscan-Intera-Philips", The Netherlands). Enzyme immunoassay (reader "PR2100-Sanofi Diagnostic Pasteur", France) was performed for determination of VEGF, ET1, TxA2, HCys, PgI2, cGMP, ESel, PSel, TGFβ1, FN and OP (kits "R&D-Systems", USA, "DRG", USA, "Amercham", United Kingdom, "IBL", Germany, "Immundiagnostik", Germany, "ProCon", Russia). Blood NO_2 levels were determined by spectrophotometry ("SF46", Russia), using Grace reagent.

Statistical analysis of the obtained results was carried out, using ANOVA/MANOVA dispersion analysis (programs "Microsoft Excel" and "Statistica-StatSoft", USA). Mean values (M), standard deviations (SD) and the errors (m), correlation coefficients (r), criteria of regression (R), dispersion (D), the *t*-Studient (t), the Wilcoxon — Rao (WR), MacNemar — Fisher (χ^2) and the statistical significance (p) were calculated, using abovementioned programs.

RESULTS AND DISCUSSION

Blood levels of VEGF are 90.0 ± 5.5 pg/ml, ET1 — 4.0 ± 0.1 pg/ml, TxA2 — 8.0 ± 1.6 ng/ml, HCys — 9.3 ± 0.6 mmol/l, PgI2 — 72.6 ± 9.0 ng/ml, NO_2 — 5.1 ± 0.1 mmol/l, cGMP — 11.2 ± 0.2 pmol/ml, ESel — 241.6 ± 12.9 ng/ml, PSel — 40.8 ± 1.6 ng/ml in healthy individuals. The increases of VEGF ($t = 16.82$, $p < 0.001$) by 4.7 fold, ET1 ($t = 9.00$, $p < 0.001$) by 1.9 fold, HCys ($t = 7.69$, $p < 0.001$) by 68%, cGMP ($t = 4.89$, $p < 0.001$) by 23%, PSel ($t = 3.52$, $p = 0.001$) by 17% and NO_2 ($t = 2.43$, $p = 0.018$) by 12%, which were accompanied by PgI2 values decrease ($t = 6.73$, $p < 0.001$) by 75%, in LC patients (Fig. 1). These changes (< M ± SD > healthy) were found in 100; 90; 76; 71; 50; 53 and 79% of LC patients, respectively (Fig. 2).

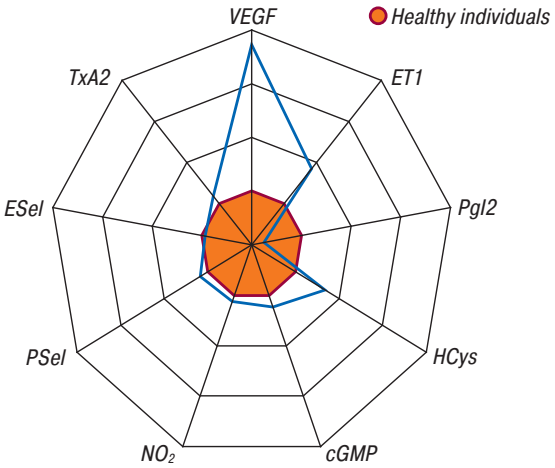

Fig. 1. Variations of FSVE indices in LC patients

Notes: the changes degrees were calculated in % to indices in healthy individuals, each of which was taken as 100%

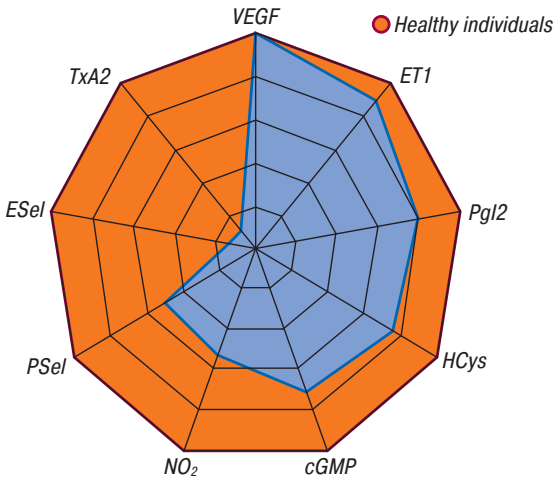

Fig. 2. Deviation rate (%) for each FSVE index in LC patients from those of healthy individuals (100%)

As evidenced by statistical analysis, the LC morphological forms is associated with TxA2 level ($D = 2.08$, $p = 0.047$), and the disease stage affects the blood levels of ET1 ($D = 2.44$, $p = 0.038$) and NO_2 ($D = 3.73$, $p = 0.009$). Regression analysis showed the direct ET1 ($R = +2.39$, $p = 0.024$) and cGMP ($R = +2.09$, $p = 0.046$) patient's age-dependence, HCys ($R+ = 2.12$, $p = 0.044$) disease duration-dependence, and VEGF ($R = +2.53$, $p = 0.017$) and ET1 ($R = +2.19$, $p = 0.037$) process pathological stage-dependence.

According to multifactor Wilcoxon — Rao analysis, only gender of the LC patients influences the integral state of FSVE ($WR = 3.50$, $p = 0.005$). In the women group such indices as VEGF ($t = 2.13$, $p = 0.040$) ET1 ($t = 2.61$, $p = 0.013$) and ESel ($t = 4.54$, $p < 0.001$) were significantly higher (20; 28 and 24%, respectively).

In patients with non-small cell LC the PSel values were 22% higher ($t = 3.39$, $p = 0.002$). Moreover, in these patients the values of NO_2 ($t = 2.86$, $p = 0.008$) and PSel ($t = 5.74$, $p < 0.001$) differed significantly from the levels in healthy individuals, such shifts were not observed in patients with small cell LC.

Concerning PSel, special comments should be made. The partial delay of leukocytes with incomplete "stop" on the endothelial surface (rolling) is going on under the control of the adhesive protein PSel ($CD62^+$). After endothelium stimulation (by thrombin, histamine, reactive oxidized substances, etc.) the PSel is rapidly translocated on the surface of endothelial cell. At LC, the PSel is most probably involved in mediating leukocyte adhesion to activated endothelium upon the cancerose inflammation. The soluble form of PSel in blood of LC patients is the product of proteolysis and, most likely, in this fragment the transmembrane domain is absent. The observed PSel increase in patients with non-small cell LC may be due to excessive accumulation of neutrophils on the endothelial surface, which occurs also in other lung diseases (acute pneumonia, respiratory distress syndrome). Importantly, the malignant cells of LC patients may express the PSel receptors, suggesting that the PSel plays a specific role in the formation of both, tumor and metastases.

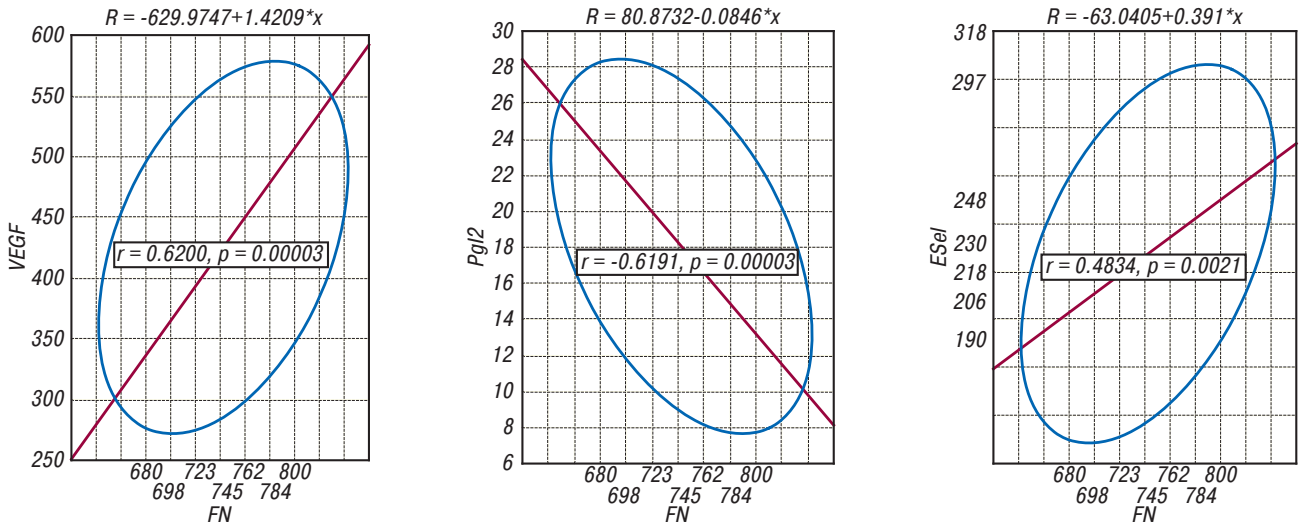

Fig. 3. Histograms of correlation and regression interrelations between FN and certain FSVE indices in LC patients

TGFβ1, FN and OP are considered as LC markers. Their levels in blood of LC patients were 411.9 ± 12.48 ng/ml, 742.7 ± 7.50 g/ml, and 40.4 ± 1.13 ng/ml, respectively. These values are significantly higher ($p < 0.001$) compared with healthy individuals (7.8 times ($t = 25.50$), 10% ($t = 6.13$) and 90% ($t = 12.54$)). It is known that the increased levels of TGF1β cytokine in blood upon tumor growth indicate the activation of mechanisms of immune suppression [12]. Upon tumor progression in LC patients the TGF1β indices are elevated [23]. In the endothelial cells of LC patients TGFβ1 promotes α_2-glycoprotein synthesis that will result in protection of tumor cells from apoptosis [21].

In LC patients FN serves as activator of tumor associated gelatinases (matrix metalloproteinase 2 and 9) [5], besides, concentrations of these matrix proteins are increased both, in blood and in tumor surrounding tissues [17]. Herewith in LC patients the FN level may serve as neoangiogenesis marker, at least partially [24]. In neoangiogenesis process along with VEGF, proinflammatory cytokine OP takes part, which is believed to display unfavorable course of LC [15, 19]. The OP level in blood is associated directly with metastatic disease and with high progression rates in LC patients [11, 14].

In LC patients the specific correlations between studied markers were found (Fig. 3). The FN level correlated directly with indices of VEGF ($r = +0.620$, $p < 0.001$), PgI2 ($r = -0.619$, $p < 0.001$) and ESel ($r = +0.483$, $p = 0.002$). Moreover, the TGFβ1 correlated directly with VEGF ($r = +0.352$, $p = 0.030$) and inversely with PSel ($r = 0.378$, $p = 0.019$). The OP levels correlated positively with HCys ($r = +0.331$, $p = 0.043$). Hence, there are the close interconnections between tumor markers and proteins that determine FSVE in LC patients. The presented data might result in a set of prognosrtic markers.

CONCLUSIONS

LC is characterized by vascular endothelial dysfunction which is manifested in enhanced blood levels of VEGF, ET1, HCys, cGMP, PSel and NO_2 and reduced PgI2 values, and is observed in 100; 90; 76; 71; 50; 53 and 79% LC patients, respectively. In LC patients the FSVE disorders are associated with patient age (ET1, cGMP), disease duration (HCys), morphological form (TxA2) and disease stage (VEGF, ET1, PSel, NO_2) with the higher prevalence in women. Such indices, as VEGF, PgI2, and ESel correlate with tumor markers (TGFβ1, FN, OP), displaying interrelations of these proteins in LC pathogenesis. These changes may be the valuable prognostic markers.

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