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RESEARCH ARTICLE

Impact of distant and regional metastasis of non-small-cell lung cancer on endotoxicosis development

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ABSTRACT

Background: The complex metabolic disorders and non-specific clinical manifestations that accompany the development of malignant neoplasm are characterized as a syndrome of endogenous intoxication. **Aims and Objectives:** The aim of this study is to establish the patterns of changes in the parameters of endogenous intoxication and lipid peroxidation processes in the saliva of patients with non-small-cell lung cancer (NSCLC) depending on the presence/absence and extent of prevalence of distant and regional metastasis. **Materials and Methods:** A total of 505 people took part in the case-control study: The main group (NSCLC, n = 290) and the control one (healthy, n = 215). All participants were questioned and underwent the biochemical examination of saliva and histological verification of the diagnosis. The parameters of endogenous intoxication and lipid peroxidation were determined spectrophotometrically. **Results:** Dynamic regularities of lipid peroxidation indicators are highly pronounced with metastasis in lymph nodes regardless of histological type of the lung cancer. Hence, alongside regional metastasis, the level of diene conjugates decreases, whereas the level of triene conjugates and Schiff bases increases. Dynamics of albumin concentration is mainly due to regional metastasis, but the presence of distant metastases in the lungs drops albumin concentration greatly. The nature of changes in the malondialdehyde concentration is generally similar with the presence/absence of distant metastases, the maximum accumulation of lipid peroxidation products is observed for stages N_3M_0 and N_3M_1. Distribution coefficient MCM 280/254 nm varies differently: It grows only in case of regional metastasis and reduced in case of distant metastasis for both histological types of the NSCLC. **Conclusion:** The results of the study support the hypothesis of association of endogenous intoxication and lipid peroxidation processes with the development of NSCLC.

KEY WORDS: Saliva; Medium Molecular Weight Toxins; Lipid Peroxidation; Lung Cancer; Oncology

INTRODUCTION

In recent years, the pathogenetic role of oxygen-free radicals and the initiated by them processes of lipid peroxidation in the development of diseases, including oncological, have been widely discussed.[1-3] Oxidative stress manifests itself in the accumulation of damaged DNA, products of protein oxidation, and lipid peroxidation, as well as in reducing the level of antioxidants and related increased susceptibility of lipid membranes and lipoproteins toward prooxidants.[4,5] In particular, in lungs, oxidative stress induces protein modification, macrophage activation, and neutrophil recruitment in central and peripheral airways, accumulation of lipid peroxidation toxic products, hydrogen peroxide, nitrosothiols and nitrates in membranes of the lungs, blood, and inspired air.[6-10] Moreover, oxidative stress can provoke

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hyperplasia of mucous membranes of glands and apoptosis of bronchial epithelial cells.[11] The complex metabolic disorders and non-specific clinical manifestations that accompany the development of malignant neoplasm are characterized as a syndrome of endogenous intoxication.[12-14]

The indicators of endogenous intoxication, as well as the products of lipid peroxidation, are traditionally determined in blood plasma; however, there is a possibility of using saliva as a substrate.[15,16] It should be noted that the study of saliva has advantages over the use of venous or capillary blood, which is due to non-invasive collection and the absence of a risk of infection when producing the biomaterial.[17] Thereat, the saliva adequately reflects the biochemical status and physiological state of a person, which allows using it in clinical laboratory diagnostics.[18,19]

The object of this study is to establish the patterns of changes in the parameters of endogenous intoxication and lipid peroxidation processes in the saliva of patients with lung cancer depending on the presence/absence and extent of prevalence of distant and regional metastasis.

MATERIALS AND METHODS

Study Design

For the case-control study, we have selected the volunteers and divided them into 2 groups: The main group (with the diagnosis of lung cancer) and the control group (conditionally healthy). Inclusion into both groups was made in parallel. The inclusion criteria included age of patients (30-75 years old), the absence of any treatment at the time of the study, including surgical, chemotherapeutic or radiation, the absence of signs of active infection (including purulent processes), and conduction of oral cavity sanitation. The exclusion criterion was the absence of histological verification of the diagnosis.

Patient Recruitment and Sampling

The patients of the main group were examined at the Clinical Oncology Dispensary (Omsk, the Russian Federation). The patients for the control group were recruited under the routine checkups from the city ambulatory-care clinic No. 4 database (Omsk, the Russian Federation). The biochemical studies were carried out in the laboratory of KhimServis LLC (Omsk, the Russian Federation).

All participants had a saliva intake of 2 ml before treatment. Samples of saliva were collected in the morning on an empty stomach by spitting into sterile tubes and centrifuged at 7000 rpm. The patients of the control group underwent photofluorographic examination. The patients of the main group were hospitalized for radical surgery in the volume of lobectomy, bilobectomy, pneumonectomy, combined treatment, or video-assisted thoracoscopic surgery for tumor biopsy. In each case, a histological verification of the diagnosis was made.

The main group included patients with a histologically confirmed diagnosis of a lung cancer. The main group was additionally divided into subgroups according to the following characteristics: Histological type of a tumor adenocarcinoma (AC) and squamous cell carcinoma (SCC) of the lung), tumor, node, and metastasis staging, and presence/absence of distant and regional metastasis. The control group included conditionally healthy patients who had no pulmonary pathology during routine clinical examination. The evaluation of the biochemical parameters of saliva of the patients from the control group was performed without additional subdivision into subgroups.

Test Methods

All participants had a saliva intake of 2 ml before treatment. Samples of saliva were collected in the morning on an empty stomach by spitting into sterile tubes, centrifuged at 7000 rpm. By spectrophotometric methods, we determined the content of substrates for lipid peroxidation processes - diene conjugates, triene conjugates, and Schiff bases by Volchegorskii method[20] and albumin by reaction with green bromocresol.[21] The content of the final lipid peroxidation product (malondialdehyde [MDA] in μmol/l) was determined in the reaction with thiobarbituric acid by Gavrilov et al. method.[22] The MM level was determined by ultraviolet spectrophotometry at wavelengths of 254 and 280 nm.[23] The results were expressed in units that are quantitatively equal to the extinction indicators. In addition, the value of the distribution coefficient (MM 280/254 nm) was estimated as the ratio of extinctions at wavelengths of 280 and 254 nm, respectively.

Ethical Review

The study was carried out in accordance with the Helsinki declaration (adopted in June 1964 in Helsinki, Finland and revised in October 2000 in Edinburgh, Scotland) and approved at a meeting of the Ethics Committee of the Omsk Regional Clinical Hospital "Clinical Oncology Center" on July 21, 2016 (Protocol No. 15).

Statistical Analysis

The statistical analysis was made using Statistica 10.0 (StatSoft, the USA) and R (version 3.2.3) software by a non-parametric method with implementation of the Wilcoxon test in dependent groups and with implementation of Mann-Whitney U-test in independent groups. The sample was described by calculating the median (Me) and interquartile range in the form of the 25th and 75th percentile (lower quartile; upper quartile). The differences were considered to be statistically significant at $P < 0.05$. The statistical

interrelations were studied using the non-parametric correlation analysis by performing the calculation of Spearman correlation coefficients (R).

RESULTS

The study included 290 patients from the Omsk clinical oncology dispensary and 215 practically healthy people selected as a control group. The average age of the patients was 58.9 ± 1.1 years old for the main group and 57.4 ± 1.5 years old for the control group. The main group included 290 patients with lung cancer of different histological types. A detailed description of the group is presented in Table 1.

The assessment of dynamics of the study parameters depending on the metastasis in the lymph nodes (Table 2) is important from the diagnostic and prognostic point of view.

It was shown that, regardless the histological type of a lung cancer, a decrease in the albumin concentration in the direction from N_0M_0 to N_3M_0 (−41.1 and −22.4% for AC and SCC, respectively) was observed. Simultaneously, there is an increase in the content of the final MDA product of lipid peroxidation in the same direction (+27.8 and +26.5% for AC and SCC, respectively). The level of primary products naturally decreases, while the level of triene conjugates and Schiff bases increases uniformly when passing from N_0M_0 to N_3M_0. The level of medium molecular toxins varies differently in the study groups (Table 2). For AC, a uniform growth of both fractions is observed; the maximum values correspond to the lesion of the lymph nodes of lung mediastinal or lung root on the opposite side (N_3). In the case of SCC, we observe a slight increase in the level of intoxication products up to N_2M_0, and then, a sharp decrease (−42.3 and +6.5% relative to N_0M_0 for the fraction 254 and 280 nm, respectively). Nevertheless, despite the revealed differences, an increase in MM 280/254 nm distribution coefficient for both histological types of non-small-cell lung cancer (NSCLC) (+4.0 and 91.9% for AC and SCC, respectively) is observed; thereat, the increase in this parameter is statistically significant for SCC ($P = 0.0180$).

It is interesting to consider the dynamics of lipid peroxidation and endogenous intoxication with simultaneous consideration of both distant and regional metastasis (Table 3). Thus, we have noted a decrease in the albumin concentration in the direction from N_0M_1 to N_3M_1 for both histological forms of a lung cancer (−54.4 and −66.7% for AC and SCC, respectively). The level of endogenous toxins and MM 280/254 nm distribution coefficient decreases in the same direction, whereby in the case of SCC, a decrease in this coefficient against N_0M_1 is more significant (−12.7% for AC and −34.3% for SCC). For lipid peroxidation parameters, the unambiguous regularity of the change is difficult to detect; however, there is a weakly expressed tendency of a decrease in the level of diene conjugates. With due regard to the secondary lipid peroxidation products, the level of triene conjugates and Schiff bases remains approximately constant with a pronounced minimum at N_2M_1 regardless the histological type of a tumor.

Table 1: Description of the study group

Characteristics	ACs, *n* (%), *n*=174	SCCs, *n* (%), *n*=116
Age (years)	61.00 [56.00; 65.00]	59.00 [55.00; 66.50]
Sex		
Male	120 (52)	110 (48)
Female	54 (90)	6 (10)
pT		
T_1	17 (81)	4 (19)
T_2	80 (70)	34 (30)
T_3	15 (29)	36 (71)
T_4	12 (39)	19 (61)
pN		
N_0	76 (63)	44 (37)
N_1	30 (52)	28 (48)
N_2	49 (55)	40 (45)
N_3	19 (83)	4 (17)
pM		
M_0	123 (57)	93 (43)
N_0	70 (63)	42 (37)
N_1	20 (47)	23 (53)
N_2	27 (51)	26 (49)
N_3	6 (75)	2 (25)
M_1	51 (69)	23 (31)
N_0	6 (75)	2 (25)
N_1	10 (67)	5 (33)
N_2	22 (61)	14 (39)
N_3	13 (87)	2 (13)

SCCs: Squamous cell carcinomas, ACs: Adenocarcinomas

The comparison of the data given in Tables 2 and 3 has shown that the dynamics of albumin concentration is mainly associated with the regional metastasis; however, with the presence of distant metastases in the lungs, the decrease in albumin concentration is more pronounced. The nature of MDA concentration change is generally similar in case of the presence/absence of distant metastasis; the maximum accumulation of final lipid peroxidation products is observed for N_3M_0 and N_3M_1 stages. As to intermediate lipid peroxidation products, here, the differences between the histological types of lung cancer are strongly pronounced. Thus, for AC with the presence of only metastases in the lymph nodes, we observed a decrease in the level of primary products and an increase in the level of triene conjugates and Schiff bases, while with the presence of distant metastases, the initial content of these products (N_0M_1, Table 3) corresponds

Table 2: The indicators of lipid peroxidation and endogenous intoxication depending on the presence/absence of regional metastasis

Indicator	HT	N_0M_0	N_1M_0	N_2M_0	N_3M_0
Albumin, mmol/l	AC	0.365 [0.218; 0.552]	0.324 [0.227; 0.425]	0.358 [0.149; 0.772]	0.215 [0.119; 0.535]
	SCC	0.339 [0.238; 0.514]	0.348 [0.198; 0.578]	0.301 [0.140; 0.424]	0.263 [0.111; 0.416]
Diene conjugates	AC	3.90 [3.58; 4.11]	3.87 [2.71; 3.99]	3.82 [3.13; 4.04]	3.79 [2.42; 4.09]
	SCC	3.89 [3.41; 4.09]	3.82 [2.93; 4.00]	3.79 [2.82; 4.05]	3.77 [3.03; 4.21]
Triene conjugates	AC	0.881 [0.787; 1.049]	0.951 [0.894; 1.267]	1.000 [0.936; 1.302]	1.124 [0.957; 1.224]
		-	P_1=0.0387	P_1=0.0034	P_1=0.0230
	SCC	0.932 [0.785; 1.142]	1.028 [0.850; 1.228]	1.090 [0.843; 1.365]	0.943 [0.826; 1.060]
Schiff bases	AC	0.525 [0.488; 0.607]	0.577 [0.533; 0.656]	0.622 [0.502; 0.681]	0.671 [0.634; 0.729]
		-	P_1=0.0490	P_1=0.0037	P_1=0.0083
	SCC	0.557 [0.494; 0.638]	0.614 [0.534; 0.796]	0.621 [0.526; 0.710]	0.596 [0.523; 0.669]
MDA, nmol/ml	AC	7.09 [5.90; 9.49]	7.22 [5.43; 10.00]	7.31 [5.64; 9.49]	9.06 [7.35; 9.32]
	SCC	7.74 [5.38; 10.00]	6.71 [5.56; 8.89]	7.78 [6.15; 9.49]	9.79 [7.26; 12.31]
MM 254 nm	AC	0.297 [0.210; 0.451]	0.240 [0.187; 0.363]	0.298 [0.197; 0.584]	0.336 [0.231; 0.389]
	SCC	0.227 [0.154; 0.314]	0.285 [0.193; 0.370]	0.272 [0.125; 0.406]	0.131 [0.109; 0.153]
MM 280 nm	AC	0.252 [0.202; 0.408]	0.233 [0.167; 0.327]	0.269 [0.153; 0.354]	0.346 [0.202; 0.372]
	SCC	0.201 [0.150; 0.288]	0.257 [0.191; 0.322]	0.265 [0.147; 0.436]	0.214 [0.193; 0.235]
MM 280/254 nm	AC	0.929 [0.829; 1.018]	0.895 [0.825; 1.047]	0.926 [0.790; 0.990]	0.966 [0.874; 1.061]
	SCC	0.861 [0.720; 1.024]	0.898 [0.832; 0.978]	1.017 [0.883; 1.133]	1.653 [1.536; 1.771]
		-	-	P_1=0.0162	P_1=0.0180

HT: Histological type, AC: Adenocarcinoma, SCC: Squamous cell carcinoma, MDA: Malondialdehyde. *P* - Statistically significant differences are compared with the control group values

Table 3: The assessment of the degree of intoxication in case of the presence of both distant and regional metastasis

Indicator	HT	N_0M_1	N_1M_1	N_2M_1	N_3M_1
Albumin, mmol/l	AC	0.563 [0.153; 0.756]	0.315 [0.198; 0.440]	0.286 [0.238; 0.393]	0.257 [0.178; 0.381]
	SCC	0.458 [0.178; 0.737]	0.211 [0.198; 0.330]	0.370 [0.257; 0.490]	0.153 [0.079; 0.226]
Diene conjugates	AC	3.75 [2.43; 3.78]	3.72 [2.52; 4.05]	3.93 [3.64; 4.16]	3.54 [2.16; 4.29]
	SCC	3.60 [2.67; 3.92]	2.41 [1.98; 3.89]	3.01 [2.14; 3.87]	2.88 [2.78; 2.99]
Triene conjugates	AC	1.024 [0.955; 1.333]	1.156 [0.913; 1.311]	0.862 [0.825; 1.020]	1.028 [0.882; 1.239]
	SCC	1.069 [0.954; 1.184]	1.180 [1.062; 1.231]	0.930 [0.779; 1.290]	1.095 [1.032; 1.157]
Schiff bases	AC	0.633 [0.501; 0.769]	0.649 [0.570; 0.708]	0.545 [0.505; 0.593]	0.577 [0.506; 0.719]
	SCC	0.531 [0.499; 0.563]	0.606 [0.567; 0.670]	0.539 [0.476; 0.677]	0.587 [0.552; 0.663]
MDA, nmol/ml	AC	6.28 [5.81; 6.75]	7.31 [5.81; 8.89]	6.75 [6.07; 8.46]	7.52 [4.87; 9.32]
	SCC	9.79 [7.52; 12.05]	6.32 [5.56; 8.21]	6.28 [5.81; 8.38]	7.30 [6.21; 8.00]
MM 254 nm	AC	0.229 [0.202; 0.364]	0.268 [0.199; 0.384]	0.242 [0.126; 0.339]	0.175 [0.123; 0.417]
	SCC	0.277 [0.072; 0.481]	0.189 [0.171; 0.210]	0.271 [0.141; 0.387]	0.186 [0.183; 0.189]
MM 280 nm	AC	0.224 [0.214; 0.298]	0.264 [0.177; 0.334]	0.214 [0.111; 0.319]	0.154 [0.099; 0.277]
	SCC	0.248 [0.147; 0.348]	0.163 [0.150; 0.167]	0.204 [0.120; 0.366]	0.170 [0.127; 0.212]
MM 280/254 nm	AC	0.985 [0.896; 1.149]	0.930 [0.848; 1.004]	0.900 [0.772; 1.041]	0.860 [0.566; 0.896]
	SCC	1.383 [0.723; 2.042]	0.977 [0.862; 1.108]	0.902 [0.851; 1.052]	0.908 [0.694; 1.122]

HT: Histological type, AC: Adenocarcinoma, SCC: Squamous cell carcinoma, MDA: Malondialdehyde

to the N_3M_0 stage (Table 2), and with the progression of the disease, it remains almost constant. For SCC, the content dynamics for lipid peroxidation products are similar, and the contribution of distant metastasis is insignificant. The same thing is typical for medium molecular toxins: Only with the presence of regional metastasis, the increase of both fractions for AC is observed, while with the inclusion of distant metastasis in the same direction, the level of intoxication, vice versa, reduces. For SCC, in both cases, the level of endotoxins generally decreases. The MM 280/254 nm distribution coefficient varies in different directions: It increases only in case of regional metastasis and decreases in case of a distant

metastasis for both histological types of the NSCLC. The absolute values of the distribution coefficient for N_3M_0 and N_0M_1 stages are close to each other (Tables 2 and 3).

DISCUSSION

During the performed studies, we have elicited the fact of an increase in the level of MM, which is an indirect evidence of the excessive generation of active oxygen metabolites: Superoxide radicals and hydrogen peroxide.[24] Hydroxyl radicals are capable to damage the phosphoglyceride membrane structures of cell membranes and its organelles. The object of active oxygen metabolites effect is the arachidonic acid that contains four double bonds separated by CH_2-groups. When exposed to hydroxyl radicals, these double bonds become conjugated and form diene conjugates which a later transformation into lipid hydroperoxides.

It was shown that the level of diene conjugates decreases in case of lung cancer in comparison with the control group. The insufficient level of primary lipid peroxidation products can result from the stability of tumor tissue to initiators of peroxide stress and modification of functioning of enzymatic systems that regulate the lipid peroxidation.[25,26] The level of secondary products against the background of lung cancer increases; the growth of these indicators in the dynamics of the disease was noted. The calculation of the Spearman correlation coefficient has shown that there is a negative correlation between the level of diene and triene conjugates ($R = -0.3665$; $R = -0.5532$, $P = 0.001$) and a positive correlation between the content of triene conjugates and Schiff bases ($R = 0.7283$ and $R = 0.7555$ for AC and SCC, respectively, $P = 0.001$). The increase of Schiff bases level is an adaptive process aimed at removing more toxic metabolites (diene conjugates and MDA) from the cells. Basing on the assumption that the primary products of MM formation are the acyl hydroperoxides and fragments of damaged cell membranes, we can observe a shift in equilibrium toward the accumulation of lipid peroxidation products, while the processes of endogenous proteolysis against the lung cancer slow down.

It is known that the active oxygen forms and products of their reactions with other biomolecules, and in particular lipid peroxides, affect the conformation of albumin, and consequently, its binding properties.[27,28] An increase in albumin concentration may be due to changes in the volume of transport of various metabolites, and primarily, fatty acids which is an important link in the restructuring of energy metabolism with the growth of a malignant tumor.[12,29]

One of the factors that determine the survival rates is the frequency of lymphogenous metastasis. It is known that the metabolism of lung tumor cells when metastasizing NSCLC into lymph nodes is characterized by an increase in the intensity of aerobic processes. The most pronounced increase in the activity of a number of metabolic enzymes in cells of both healthy and tumor lung tissues is revealed among patients with AC.[30]

The level of primary lipid peroxidation products is maximal at N_0 notwithstanding the histological type of tumor, and against the background of regional metastasis, it decreases. The level of secondary products naturally increases in the direction from N_0 to N_3 for both triene conjugates and Schiff bases. It is quite interesting that the maximal growth of these indicators is observed with lesions of peribronchial and/or lymph nodes of the lung root (N_1); then, the level of secondary lipid peroxidation products remains practically constant until the damage of bifurcation or mediastinal lymph nodes (N_2). When moving to the lesion of mediastinal lymph nodes or the lung root on the opposite side (N_3), the further growth of lipid peroxidation parameters is observed.

Generally, against the background of SCC, we have noted a higher level of triene conjugates and Schiff bases and higher values of MM 280/254 nm distribution coefficient. When analyzing the indicators of proliferative activity among patients with different morphological types of the tumor, it was found that the percentage of dividing cells among patients with AC is much lower than among patients with SCC.[31,32] This may indicate a high aggressiveness of the tumor. This fact is confirmed by the higher level of secondary lipid peroxidation products in case of SCC.

The presence of distant metastases in lungs makes a significant contribution into the change in the indicators of endogenous intoxication and lipid peroxidation. However, statistically significant differences between the levels of lipid peroxidation products against the background of the presence of distant metastasis were revealed only for AC. Thus, an increase in the level of triene conjugates is observed when moving from the stage N_0M_0 to N_0M_1 ($P = 0.0431$), while in the transition from the stages N_2M_0 to N_2M_1, the level of triene conjugates decreases ($P = 0.0219$), and Schiff bases, according to statistics, significantly increase ($P = 0.0416$). For SCC, we did not identify such dependencies.

It was shown that the values obtained for N_3M_0 and N_0M_1 stages are nearly similar and close to each other (Tables 2 and 3). An exception is in the dynamics of albumin concentration which is the same both in the presence and in the absence of distant metastasis. During the process of metabolic transformations associated with the intensification of free radical reactions, the processes of lipid peroxidation are intensified and the release of toxic lipid peroxides into the blood increases. Albumin molecules are able to form complex associates with toxins in the form of which they are eliminated to the organs of detoxification. However, with a significant formation of endogenous toxins, the transport and binding capacities of albumin molecules decrease.[12] It is known that a decrease in albumin concentration

is associated with a worse response to treatment and lower survival rate of patients with lung cancer.[33,34]

CONCLUSIONS

Thus, against the background of lung cancer, we observe the development of oxidative stress, which is manifested in the increase in the level of lipid peroxidation and endogenous intoxication products. Nature of changes of the investigated parameters is ambiguous and depends more on the extent of regional metastases than the presence of distant metastases in the lungs.

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