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LEVEL OF ZINC AND ITS FRACTIONS AS REFLECTION OF A CONDITION OF ANTIOXIDANT SYSTEM AT THE CHRONIC OBSTRUCTIVE LUNG DISEASE

Abstract

The objective of the study: To estimate a possibility of use of zinc and its fractions levels as the indicator reflecting a condition of antioxidant system at patients with COPD or in groups of high risk. **Materials and methods**. Patients with COPD, smokers and non-smoking healthy people participated in a study. Spirometry was carried out for all study subjects. Forced expiratory volume$_1$ (FEV_1) was determined followed by calculation of % FEV_1 from the normal value. Taking into account the importance of an oxidative stress in COPD pathogenesis, the level of activity of the main enzyme of antioxidant defense — superoxide dismutase, levels of zinc and its fractions as main component of the enzyme and also albumin level as main metabolically active zinc transporter were determined. The indicator "proportion of bound zinc fraction" was introduced to define zinc fractions changes. **The results of the study**. It is defined that at smokers with minimal impairment of the respiratory function the change of indicators are similar to that at patients with diagnosed COPD. Statistically significant differences in the level of the studied indicators at non-smoking patients are also revealed. A Spearman correlation analysis showed significant correlation between % FEV_1 from normal value and SOD activity; between SOD activity and total and bound zinc levels. This confirms a hypothesis of the possibility to use zinc and its fractions levels in screening examinations as a parameter that reflects the state of antioxidant system in smokers.

Key words: *antioxidant system, superoxide dismutase, zinc, zinc pools, smoking, screening, spirometry*

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COPD — chronic obstructive pulmonary disease

The new recommendations for the diagnosis and management of patients with chronic obstructive pulmonary disease (COPD) propose a new disease definition in which the GOLD working group focused on certain risk factors, including smoking in particular. Smokers constitute a risk group for COPD and require examinations of pathophysiological changes that are characteristic of this disease [1].

Oxidative stress is one of the components of the COPD pathogenic mechanism. Cigarette smoke contains a variety of substances with the potential to form free radicals. Such substances are also produced by activated inflammatory cells [2, 3]. It is known that peroxidation processes are involved in spasms of smooth muscle cells in the bronchi and condensation of bronchial mucus leading to an aggravated course of the disease [4].

Effects of oxidative stress vary and include: inactivation of antiproteinases, initiation of endothelial dysfunction, remodeling of blood vessels, and fibrosis [5].

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The substances that control peroxidation processes include catalase, superoxide dismutase (SOD), reduced glutathione, glutathione peroxidase, and vitamin E. Their levels drop in case of COPD, and some researchers attribute this to the exhaustion of antioxidant defenses under long-term oxidative stress [6, 7].
The impairment of free-radical oxidation in smokers that can be observed at the preclinical stage is of clinical interest [6–8].
Being one of the components of anti-radical defense, SOD contains zinc [9]. It has been established that zinc deficiency impairs SOD synthesis, thus leading to increased oxidative stress [10].

Therefore, the aim of our study was to determine total zinc and its fractions levels and SOD activity in patients with COPD and in both smoking and non-smoking healthy subjects. The study's objectives included determination of SOD activity level, albumin, total zinc and its fractions levels in study subjects and the establishment of a correlation between the studied parameters.

Materials and methods

The study was conducted at the Federal State Budgetary Institution of Higher Professional Education Voronezh State Medical University named after N. N. Burdenko, Department of Outpatient Treatment and General Practice; Budgetary Healthcare Institution of the Vronezh Region Voronezh City Emergency Clinical Hospital No. 8; OOO Medical Center of Professional Pathology. After completion of the Informed Consent Form, a total of 30 patients with confirmed COPD were enrolled. The patients had no co-morbidities, and 20 of them were males while 10 were females (with a mean age of (55.8 ± 6.78) years). They had been hospitalized between December 2016 and January 2017. A total of 90 healthy subjects who had undergone periodic physical examination also participated in the study: 48 men and 42 women (with a mean age of (43.7 ± 7.17) years). The Study Protocol was approved by the Ethics Committee of the Federal State Budgetary Institution of Higher Professional Education Voronezh State Medical University named after N. N. Budenko.

Spirometry data from patients with verified COPD were obtained using the Diamant spirometer.

Healthy subjects completed a specifically designed questionnaire to determine their smoking status. Then active smokers entered data on the number of cigarettes they smoked per day and smoking duration to calculate the smoking index. Those who did not smoke at the moment answered questions on their smoking history and exposure to secondhand smoke outside, at work or at home. Subjects had to provide information on chronic disorders (those with chronic disorders were excluded from the study in order to verify effects of smoking on the studied parameters). Respiratory function was evaluated using a portable Spirotest USPC-1 spirometer (made in Russia): forced expiratory volume$_1$ (FEV_1) was determined followed by calculation of % FEV_1 from the normal value.
All patients provided blood samples.
Calorimetric measurement of total zinc level using RAL Clima MC-15 instrument and Vital Development Corporation kits. The following was added to 1.0 mL of mono reagent: 0.05 mL of blood serum in the test sample, 0.05 mL of the calibrator in the calibration sample, and 0.05 mL of double distilled water in the blank sample. The samples were then analyzed using photometry at a wavelength of 560 nm. The following equation was used for calculations:

$$C = Atest/Acal \times 30.6\ [\mu mol/L]$$

To measure bound zinc level, proteins were precipitated with trichloracetic acid and then centrifuged. Zinc level was then calculated in accordance with the method described above.
Albumin levels were measured using Vital Development Corporation kits.
SOD activity was determined using Spekol instrument with a chemiluminescent detector, developed by Carl Zeiss Jena. 70 μL of luminol, 70 μL of methionine, 80 μL of riboflavin, and 3 μL of blood serum (3 μL of distilled water for control samples) were added to 2.7 mL of the buffer solution.
The following equation was used for calculations:

$$\% \text{ quenching} = 100 - \text{test value} \times 100/\text{control value}$$

A statistical analysis was conducted using the Kruskal-Wallis H-test in Microsoft Excel 2010 and Statistica 6.0 software, since we had to compare four independent samples. Kruskal-Wallis H-test is a generalized Mann-Whitney test for two or more independent samples. The test does not require a hypothesis of a normal distribution. The null hypothesis H_0 means that only random differences exist between the samples. The alternative hypothesis H_1 means that the differences in the studied parameter that exist between the samples are not random. The differences were considered significant at $p \le 0.05$. The Spearman correlation analysis in conjunction with the rank correlation coefficient (r) was also used; r coefficient was interpreted as follows: very weak correlation ($0 < r \ll 0.3$); weak correlation ($0.3 < r \ll 0.5$); medium correlation ($0.5 < r \ll 0.7$); strong correlation ($0.7 < r \ll 0.9$); very strong correlation ($0.9 < r < 1$).

Results and discussion

After the analysis of questionnaires and spirometry data (Table 1), all subjects were divided into 4 groups: 1 — smokers with diagnosed COPD (N = 35, mean age of (55.8 ± 6.78) years), 2 – smokers with minimal impairment of the respiratory function (FEV_1 is 80–70 % from the normal value) (N = 25, mean age of (45.6 ± 5.79) years), 3 — smokers with preserved respiratory function (N = 30, mean age of (41.8 ± 7.97) years), 4 — passive smokers (N = 30, mean age of (42.4 ± 9.31) years).

Preventive medical examination of five smoking patients with cough revealed % FEV_1 less than 70 % of the normal value. Therefore, these patients were transferred to Group 1 (smokers with COPD).

At the first stage, SOD activity as the main enzyme of antioxidative defense was determined in the study subjects, and albumin levels were determined

Table 1. *Spirometry results of the study subjects*

	Statistics	FEV_1 (l)	% FEV_1 from normal value (%)
Group 1 (smoking patients with COPD)	M±m	1,68±0,61	48,6±10,5
	min-max	0,91-3,20	33,0-69,0
Group 2 (smokers with minimal impairment of the respiratory function)	M±m	2,99±0,45	74,3±3,0
	min-max	2,04-3,59	71,0-79,0
Group 3 (smokers with preserved respiratory function)	M±m	3,01±0,63	84,8±3,6
	min-max	2,10-4,42	80,0-96,0
Group 4 (secondhand smokers)	M±m	2,85±0,57	88,2±3,1
	min-max	82,0-95,0	82,0-95,0

Table 2. *SOD and albumin levels in patients from different groups (μM)*

		Group 1 (smokers with diagnosed COPD)	Group 2 (smokers with minimal impairment of the respiratory function)	Group 3 (smokers with preserved respiratory function)	Group 4 (secondhand smokers)
Superoxide dismutase	M±σ	36,02±2,77	39,85±1,63	43,47±5,06	52,66±2,71*
	min-max	32,0-45,6	36,8-43,7	37,4-55,6	48,9-57,9
Albumin	M±σ	32,57±2,87	37,77±3,10	42,02±4,57	47,88±1,94**
	min-max	32,6-27,6	33,6-43,7	33,1-49,6	42,9-51,9

Note: * — Differences of the SOD level in the studied groups are significant at p=0,001; ** — Differences of the albumin level in the studied groups are significant at p=0,001

as the main transporter of metabolically active zinc fractions that can participate in enzyme synthesis if necessary (Table 2).

To compare the samples, the Kruskal-Wallis analysis of variance was used for several independent groups. We found statistically significant differences in SOD (H = 91.5898 at p = 0.01) and albumin levels (H = 90.812 at p = 0.01) between the groups.

SOD level in Group 1 (smokers with diagnosed COPD) was significantly higher than in other groups due to active pathophysiological changes involving the enzyme. SOD activity levels in smokers (Group 2 — smokers with minimal impairment of the respiratory function, Group 3 – smokers with preserved respiratory function) were not significantly different. Interestingly, SOD activity level in non-smokers from Group 4 (those exposed to secondhand smoke) was significantly lower than in smokers (Groups 2 and 3). These patterns can be interpreted as activation of antioxidant defense involving SOD.

We found the following differences in albumin levels in the blood between the study subjects: albumin levels in healthy smokers from Groups 2 and 3 (smokers with minimal impairment of the respiratory function and smokers with preserved respiratory function) were significantly higher than in Group 1 (smokers with diagnosed COPD). These could be due to the activation of metabolically active zinc transport to meet the need in this trace element for the production of enzymes that mediate pathogenic changes in the respiratory tract.

To confirm the significance of obtained values for pathophysiological reactions during the development of COPD, we performed a Spearman correlation analysis with rank correlation coefficients.

To verify the significance of changes in SOD activity for respiratory tract obstruction accompanied by changes in the respiratory function, we evaluated the relationship between SOD activity and % FEV_1 of the normal value (Figure 1). A strong positive correlation was revealed (r = 0.80 at p = 0.01). The following equation was obtained in the correlation analysis:

$$\% FEV_1 = -11.0021 + 1.9585 \times SOD$$
$$(r = 0.80 \text{ at } p = 0.01),$$

where % FEV_1 is a percentage of forced expiratory volume during the first second from the normal value, SOD is the superoxide dismutase activity

Revealed patterns confirm the role of SOD in pathophysiological changes of the respiratory tract in patients with COPD.

At the next stage, we analyzed zinc level and its fractions. We also determined the proportion of bound zinc fraction to verify changes in zinc pools, namely, the conversion of some unbound zinc to the bound form (which is metabolically active and necessary for producing zinc-containing enzymes) (Table 3).

Statistical analysis of the obtained results revealed significant differences in the studied parameters between the groups (H = 92.322 at p = 0.01 for total zinc level; H = 90.355 at p = 0.01 for the bound

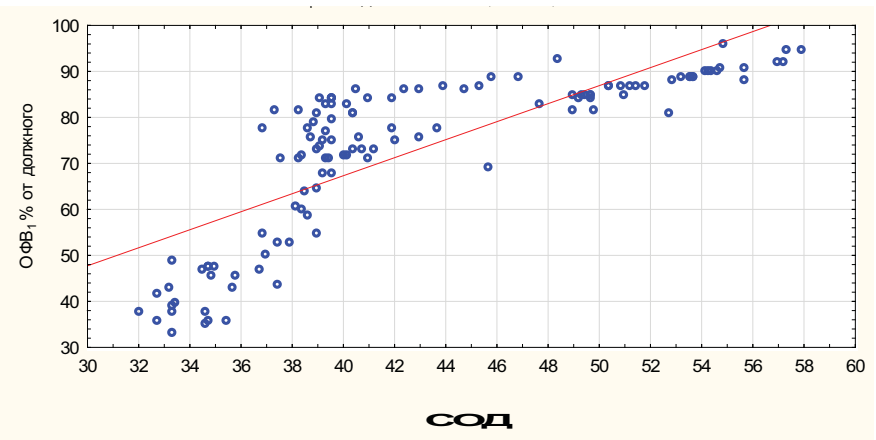

Figure 1. *The scatter plot: % FEV_1 vs SOD*

Table 3. *Total zinc and its fractions levels in study subjects (μM)*

		Group 1 (smokers with diagnosed COPD)	Group 2 (smokers with minimal impairment of the respiratory function)	Group 3 (smokers with preserved respiratory function)	Group 4 (secondhand smokers)
Total zinc	M±σ	11,60±2,62	15,54±1,52	17,50±2,54	22,49±2,17*
	min-max	6,98-18,20	12,30-17,90	13,60-23,40	19,10-27,00
Bound zinc	M±σ	10,73±2,35	14,14±1,37	15,65±2,14	19,74±1,63**
	min-max	6,50-16,63	11,17-16,20	12,28-20,60	17,25-23,27
Unbound zinc	M±σ	0,87±0,28	1,40±0,15	1,86±0,46	2,75±0,56***
	min-max	0,48-1,57	1,11-1,70	1,32-3,23	1,85-3,73
Proportion of bound zinc fraction	M±σ	92,6±0,7	91,0±0,2	89,5±1,2	87,9±1,3****
	min-max	91,4-93,7	90,5-91,4	86,0-90,5	86,2-90,3

Note:* — differences general level of zinc are significant at ρ=0,001; ** — differences related fraction of zinc are significant at ρ=0,001, *** — differences free fraction of zinc are significant at ρ=0,001, **** — differences share of the related fraction of zinc are significant at ρ=0,001

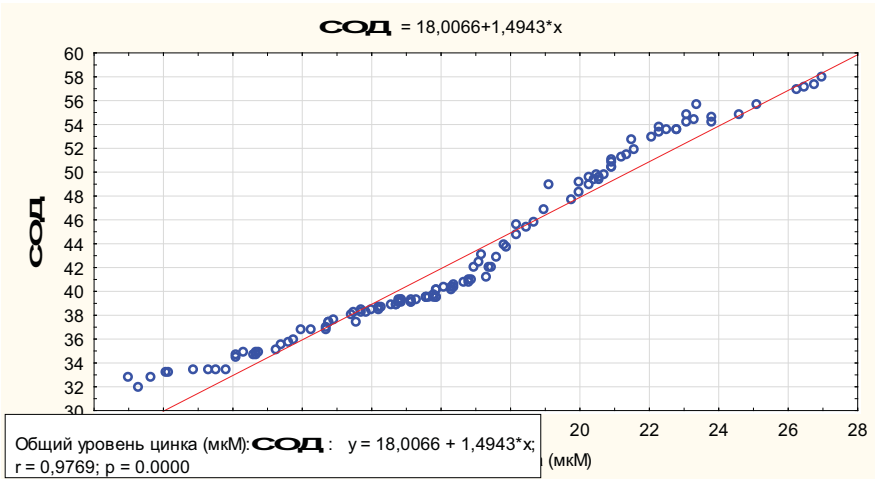

Figure 2. *The scatter plot: total zinc vs SOD*

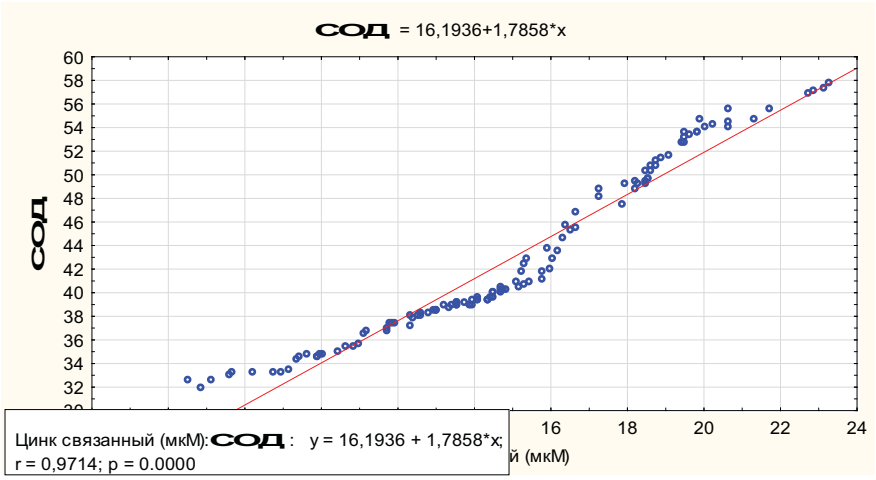

Figure 3. *The scatter plot: bound zinc vs SOD*

fraction; H = 99.748 at ρ = 0.01 for the unbound fraction; H = 104.523 at ρ = 0.01 for the proportion of the bound fraction), with total zinc level decreasing and the proportion of the bound fraction increasing as the % FEV_1 of the normal value decreased (r > 85 % at ρ < 0.05) (Figures 2 and 3).
A Spearman correlation analysis with rank correlation coefficients yielded the following correlation equations:

$$SOD = 18.0066 + 1.4943 \times Zn_{total}$$
$$(r = 0.98 \text{ at } \rho = 0.01),$$

$$SOD = 16.1936 + 1.7858 \times Zn_{bound}$$
$$(r = 0.97 \text{ at } \rho = 0.01)$$

Significant differences in SOD activity, levels of zinc and its pools, and in an additional parameter (the proportion of bound zinc fraction) that were revealed in our study together with strong correlations between the studied parameters confirm that it is possible to use levels of zinc and its fractions as a value reflecting the state of antioxidant system in COPD at-risk groups.

Conclusions

1. The study revealed statistically significant differences in SOD, albumin, total zinc and zinc fractions levels.
2. We obtained correlation equations for the studied parameters and % FEV_1 of the normal value (r > 85 % at ρ < 0.05), which indicated the relevance of the studied parameters for the COPD pathogenic mechanism.
3. Levels of zinc and its fractions can be used in screening examinations as a parameter that reflects the state of antioxidant system in smokers.

Conflict of interests
The authors declare no conflict of interests.

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