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Analysis of the Levels of Biomarkers of Systemic Inflammation, Surfactant Protein D and Melatonin in COPD Patients with Varying Degrees of Airflow Obstruction.

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ABSTRACT

For decades chronic obstructive pulmonary disease has been a major medical and social problem. Recent studies have demonstrated that certain drugs such as melatonin can open up new possibilities in treatment of the disease. The aim of the study is to analyze the clinical course of the disease, levels of biomarkers of systemic inflammation, surfactant protein D and melatonin in patients with chronic obstructive pulmonary disease (GOLD 2-4). The study included 88 patients who were divided into 3 groups based on the degree of airflow obstruction. The analysis of the relationship between the severity of the disease, the degree of the systemic inflammatory response, and the melatonin and surfactant protein D levels has demonstrated significant differences of the values in question in the patients with different degrees of airflow obstruction. The severe course of chronic obstructive pulmonary disease with more frequent exacerbations and more prominent symptoms is directly related to the lowered melatonin level. The lowered levels of melatonin and surfactant protein D and an imbalance in the system of pro- and anti-inflammatory cytokines are associated with the reduced antioxidant defense mechanisms, maintaining the activity of the systemic inflammation, worsening the immune states of the patients.

eywords: chronic obstructive pulmonary disease, systemic inflammation, cytokines, melatonin, surfactant protein D.

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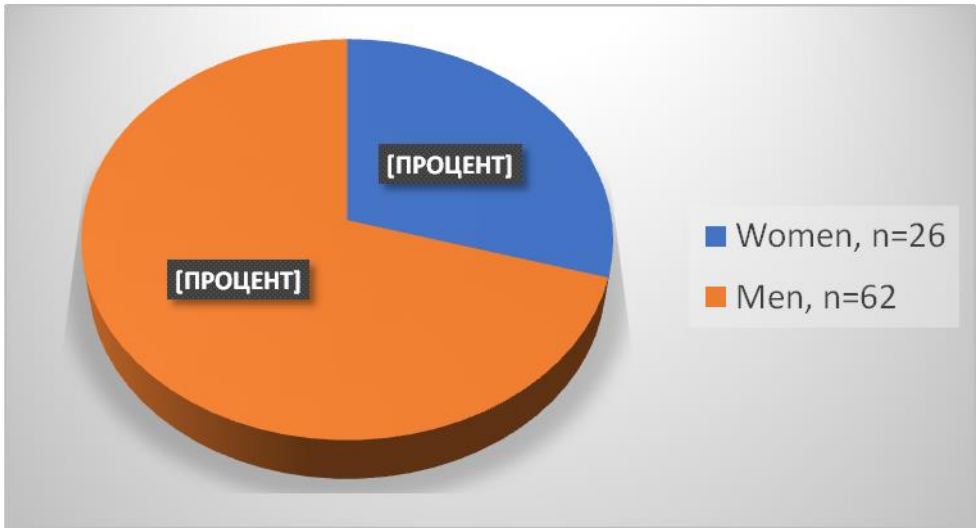
INTRODUCTION

For decades chronic obstructive pulmonary disease (COPD) being one of the most common chronic lung diseases has been a major medical and social problem due to its high prevalence, a tendency towards increasing numbers of severe forms of the pathology, higher disability and mortality rates [1]. Despite the vigorous development of innovative medical technologies in treatment of COPD, we are still faced with insufficient effectiveness and high cost of pharmacological therapy and unsatisfactory quality of life (QOL) of the patients [2]. Recent studies have demonstrated that certain hepatoprotective drugs open up new possibilities in treatment of COPD. For instance, the use of melatonin can be an efficient approach to the treatment of COPD preventing premature aging of the lungs. One of the most important functions of melatonin is its antioxidant activity [3]. Melatonin as an antioxidant works everywhere in the body penetrating all biological barriers [4]. Mechanism of the antioxidant activity of the hormone is based on the ability to bind free radicals and exogenous carcinogens [5]. At the same time, melatonin is able to enhance the production of glutathione through the enzyme activation as well as stimulate superoxide dismutase and catalase. Due to this the balance between antioxidant and pro-oxidant enzymes eventually shifts in favor of antioxidants [6]. At present the imbalance between proinflammatory and anti-inflammatory cytokines closely connected with oxidative stress is considered one of the components of the COPD pathogenesis [1,7]. It is known that an imbalance between oxidative and antioxidative processes is regarded as a universal mechanism of tissue damage [8]. One of the most severe dysfunctions that can be caused by the pro-oxidative-antioxidative imbalance is an impaired immune status that is accompanied by changes in the cytokine profile [9].

The aim of the study is to analyze the clinical course of the disease, clinical, instrumental and laboratory states of the patients, levels of biomarkers of systemic inflammation, surfactant protein D (SP-D) and melatonin in patients with COPD (GOLD 2-4).

MATERIALS AND METHODS

The study included 88 patients (62 men, 26 women) diagnosed with COPD (GOLD 2-4) belonging in the group D, the “frequent exacerbations phenotype”, without acute exacerbation, aged 40-80 years old (the average age being 68.61±0.72 years) (Diagram 1). The study did not include patients below 40 and above 80 years old, ones with acute COPD exacerbations, stage II and more chronic heart failure, bronchial asthma, other severe comorbidities and their complications. The research was carried out within the framework of the grant of the President of the Russian Federation for the state support of young Russian scientists - Candidates of Sciences (МК-2016 Competition).



Based on the COPD stage 3 groups were formed. The first group consisted of 31 patient (22 men, 9 women, the average age being 67.42 ± 1.38 years) with the diagnosis of COPD (GOLD 2). The 2nd group included 29 patients (19 men, 10 women, the average age – 68.83 ± 1.21 years) with the diagnosis of COPD (GOLD 3). The 3rd group included 28 patients (21 men, 7 women, the average age – 69.71 ± 1.09 years) with the diagnosis of COPD (GOLD 4).

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For the objectivization of clinical symptoms of COPD we used the Visual Analogue Scale (VAS) for the qualitative assessment of the severity of chronic cough, sputum viscosity, and dyspnea, and the modified Medical Research Council scale (mMRC) for the evaluation of the severity of dyspnea. To measure health impairment and assess QOL of the COPD patients we used St George's Respiratory Questionnaire (SGRQ). The function of external respiration was studied using the basic method - forced spirometry, which included the measurement of lung volumes and capacities and the expiratory flows. The assessment of exercise tolerance and objectivization of the functional status of the patients was carried out using a 6-minute walk test which corresponds to the submaximal load (6MWD). The levels of proinflammatory cytokines - interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP), tumor necrosis factor (TNF) - and anti-inflammatory cytokines – interleukin-4 (IL-4), interleukin-10 (IL-10) - were determined with the help of the solid-phase sandwich enzyme immunoassay. The melatonin concentration was determined with the help of solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using reagent kits from IBL International GmbH (Germany), and the level of the SP-D, a specific marker of the inflammatory process in COPD, was determined by solid-phase sandwich ELISA using reagent kits from BioVender (Czech Republic). All the laboratory values analyzed were converted to the ones accepted in the International System of Units (SI).

RESULTS AND DISCUSSION

The analysis of the relationship between the severity of the disease, the degree of the systemic inflammatory response, and the melatonin and SP-D levels has demonstrated significant differences of the values in question in the patients with different degrees of airflow obstruction (GOLD 2-4). The activity of the systemic inflammatory response was also directly connected to the severity of COPD.

For instance, there was a direct correlation between the levels of proinflammatory cytokines (IL-6, IL-8, CRP) and the severity of airflow obstruction. As for anti-inflammatory cytokines such as IL-4 and IL-10, their serum levels decreased significantly with an increase in bronchial obstruction (Table 1). In addition, the severity of airflow obstruction was inversely related to the level of SP-D, a specific marker of the inflammatory process in COPD (Table 2).

Table 1: Pro- and anti-inflammatory cytokines in COPD patients with varying degrees of airflow obstruction (GOLD 2-4)

Parameters, pg/ml	Patients with COPD (GOLD 2), n=31	Patients with COPD (GOLD 3), n=29	Patients with COPD (GOLD 4), n=28
IL-6	11.17±1.09	14.88±0.71*	21.56±1.44*
IL-8	21.96±1.21	26.32±1.42*	30.32±11.60*
CRP	11.08±0.23	18.16±0.55*	24.52±1.22*
TNF	8.64±0.19	13.03±0.24*	19.85±0.52*
IL-4	2.92±0.20	2.16±0.13*	1.92±0.12*
IL-10	25.11±3.02	23.70±2.23*	22.13±0.67*

Note: The differences between the values are statistically significant ($p<0.05$).

Table 2: Surfactant protein D levels in COPD patients with varying degrees of airflow obstruction

Parameters, ng/ml	Patients with COPD (GOLD 2), n=31	Patients with COPD (GOLD 3), n=29	Patients with COPD (GOLD 4), n=28
SP-D	106,34±1,45	73,25±3,12	53,34±2,77
	F=11,96; p=0,0011		
		F=7,45; p=0,0088	
	F=26,25; p=0,0000		

Table 3: Melatonin serum levels in COPD patients with varying degrees of airflow obstruction

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Parameters, pg/ml	Patients with COPD (GOLD 2), n=31	Patients with COPD (GOLD 3), n=29	Patients with COPD (GOLD 4), n=28
Melatonin	23,62±2,14	18,16±1,03	12,32±0,69
	F=5,40; p=0,0242		
		F=21,42; p=0,0000	
	F=24,33; p=0,0000		

Table 4: Correlation of the cytokine profile, SP-D and melatonin in COPD patients (GOLD 2-4)

Parameters	Melatonin serum level	Melatonin urine level	SP-D
IL-6	-0,11	**-0,23**	**-0,21**
IL-8	0,03	-0,16	0,01
CRP	0,13	-0,06	**-0,29**
TNF	-0,02	-0,12	0,05
IL-4	**0,29**	-0,06	-0,01
IL-10	-0,01	-0,15	**0,20**
SP-D	-0,05	**0,23**	

Table 5: Correlation of the parameters in COPD patients with varying degrees of airflow obstruction (GOLD 2-4)

Parameters	IL-6	IL-8	CRP	TNF	IL-4	IL-10	Melatonin serum level	Melatonin urine level	SP-D
Severity of COPD (GOLD 2-4)	**0,26**	**0,23**	**0,23**	**0,22**	**-0,25**	0,09	-0,11	**-0,66**	-0,11
Number of acute COPD exacerbations	**0,21**	**0,25**	**0,24**	**0,21**	**-0,20**	-0,10	-0,17	**-0,21**	**-0,28**
Number of visits to the general practitioner (GP)	-0,15	-0,06	0,09	0,05	-0,02	-0,04	0,30	-0,17	-0,09
Chronic cough (VAS)	**0,39**	**0,25**	**0,23**	**0,38**	**-0,20**	-0,14	0,30	-0,15	-0,11
Thick sputum (VAS)	**0,32**	**0,24**	**0,24**	**0,39**	**-0,27**	**-0,20**	0,09	0,05	**-0,27**
Dyspnea(VAS)	-0,06	-0,06	-0,05	-0,05	**-0,25**	-0,06	-0,03	-0,06	-0,10
Dyspnea (mMRC)	0,30	0,21	0,32	0,22	-0,28	-0,21	-0,15	-0,06	-0,22
FEV_1	-0,14	-0,15	**0,46**	-0,02	0,09	0,03	0,11	0,17	-0,08
FVC	-0,11	0,18	**-0,29**	-0,14	0,02	-0,02	-0,06	0,19	-0,16
FEV_1/FVC ratio	-0,02	**-0,26**	**0,49**	**0,22**	0,09	0,05	0,01	0,02	0,04
An increase in FEV_1	**-0,30**	**-0,29**	**-0,24**	**-0,23**	**0,20**	**0,22**	-0,05	-0,05	**-0,21**
6MWD	-0,14	-0,10	0,02	-0,19	0,15	0,11	0,09	0,05	0,30
Symptoms (SGRQ)	**0,43**	**0,45**	**0,35**	**0,40**	**-0,42**	**0,40**	-0,06	-0,07	**-0,31**
Activity (SGRQ)	**0,33**	**0,29**	**0,34**	**0,27**	**-0,20**	**-0,31**	0,30	**-0,15**	**-0,20**
Impacts (SGRQ)	**0,32**	**0,40**	**0,31**	**0,49**	**-0,31**	**-0,25**	**-0,27**	**-0,31**	**-0,21**
Total score (SGRQ)	**0,31**	**0,29**	**0,24**	**0,30**	**-0,27**	**-0,30**	**-0,21**	**-0,28**	**-0,24**

What's more, the melatonin serum level was also inversely related to the severity of COPD (Table 3).

The dependence and the degree of correlation between the melatonin concentration in the blood serum and urine, the SP-D serum level, and the levels of pro- and anti-inflammatory cytokines are shown in the table 4.

The melatonin serum level was directly related to the degree of the influence of COPD on patients' daily life, social functioning and QOL of the patients according to SGRQ, and the IL-4 serum level. In turn, there is a negative relationship between the SP-D level and the number of acute COPD exacerbations, sputum viscosity according to VAS, the degree of reversibility of bronchial obstruction (response of FEV1 to inhaled bronchodilators), the influence of COPD on QOL of the patients, and proinflammatory cytokines serum levels (IL-6, CRP). The correlation analysis allows us to consider a decrease in the SP-D concentration to be a

diagnostic marker of the intensification of the inflammatory process in the lungs and use it to predict the course of COPD.

The correlation analysis data obtained confirmed the intermediate results of the study (Table 5): the intensification of the activity of the chronic systemic inflammation with an increase in the proinflammatory cytokines levels and a decrease in the anti-inflammatory cytokines levels is directly linked to the more severe course of COPD, more frequent acute exacerbations, more prominent symptoms and their negative effect on patients' life, more severe airflow obstruction, and worse QOL of the patients; as for the SP-D that is responsible for the immune system modulation and reducing the inflammation, the serum level tends to decrease. The severe course of COPD with more frequent exacerbations and more prominent symptoms affecting QOL of the patients is also directly related to the lowered melatonin level.

CONCLUSIONS

The levels of the blood serum and urinary melatonin are linked to the states of antioxidant and immune systems of the patients and the intensity of the systemic inflammatory response. The lowered levels of melatonin and SP-D and an imbalance in the system of pro- and anti-inflammatory cytokines are associated with the reduced antioxidant defense mechanisms, maintaining the activity of the systemic inflammation, worsening the immune states of the COPD patients, which lead to an increase in the number of acute exacerbations and the number of visits to the general practitioner, and more severe clinical symptoms of COPD.

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