

Mechanisms Of Local And Systemic Inflammation In Chronic Obstructive Pulmonary Disease With Comorbid Hypertension And Obesity

Ganna Stupnytska, Oleksandr Fediv

Abstract: Today chronic obstructive pulmonary disease (COPD) is considered not only as a disease with changes in the bronchial tree and lungs, but also as a systemic inflammatory syndrome with systemic effects and the presence of comorbidities. The objectives of this study were to evaluate the lipid peroxidation, antioxidant system and proteolysis intensity in the blood and exhaled breath condensate (EBC), as well as local balance between pro- and anti-inflammatory cytokines in COPD patients' with hypertension and obesity. The study included 25 healthy subjects and 50 patients with COPD without hypertension and with comorbid hypertension and obesity (36 patients). General tripsin-like proteinase (TLP) activity was determined by Kunits method, neutral proteolytic activity of citrate blood plasma and EBC – by azoalbumin, azocasein and azocol lysis. Superoxyddismutase (SOD) and catalase (Ct) activity, malone dialdehyde (MDA) level, HS-group content in the blood and EBC were also examined. The concentration of cytokines (TNF α , IL-1 β , TGF- β 1, IFN γ) were marked with ELISA method. Patients with COPD and comorbid hypertension and obesity showed more elevation of TNF α , IL-1 β , TGF- β 1, in EBC, local and systemic higher MDA index, decrease of Ct and SOD activity in EBC and increased Ct activity in blood, activation of neutral proteolytic systems and acid TLP, reduced collagenolysis intensity. The course of COPD with hypertension and obesity was characterized by more intensification of inflammatory process on the level of the bronchial tree and with more systemic inflammatory response.

Index Terms: chronic obstructive pulmonary disease, hypertension, obesity, inflammation, exhaled breath condensate, blood.

1 INTRODUCTION

CARDIOVASCULAR diseases, chronic respiratory diseases, and diabetes are the most frequent chronic degenerative disorders, particularly in the elderly; more than half of all elderly people have at least three chronic medical conditions, and a significant proportion have five or more, that are often unrecognized and untreated [1]. Cigarette smoking, the most important and best-established risk factor for chronic obstructive pulmonary disease (COPD), and there is also a major risk factor for all other chronic diseases and cancer, not only because it damages the lung directly, but also because it may simultaneously cause systemic effects affecting all organs. The most common comorbidities of COPD that are possibly related to the systemic effects of smoking are congestive heart failure, arrhythmias, hypertension, peripheral and coronary artery diseases, diabetes and metabolic syndrome, osteoporosis, cancer (particularly lung cancer), pulmonary vascular abnormalities, psychiatric disorders, cachexia, skeletal muscle abnormalities, and infections [2]. COPD is a leading cause of morbidity and mortality worldwide and results in a economic and social burden that is both substantial and increasing [3]. Patients with COPD, particularly when the disease is severe and during exacerbations, have evidence of systemic inflammation, measured either as increased circulating cytokines, chemokines and acute phase proteins, or as abnormalities in circulating cells [4]. Systemic inflammation is not only present in patients with COPD, but is also a common feature in various other chronic diseases. Elevated levels of inflammatory markers such as CRP and IL-6 were observed in patients with stable coronary artery disease, peripheral arterial disease, and diabetes, compared with individuals without disease. These conditions have to be taken into account when the causative role of COPD in systemic inflammation is investigated because these diseases often occur together. Systemic inflammation is potentially a common pathway leading to these chronic diseases and might explain the high prevalence of multiple chronic diseases in the same

patient [5]. Thus, pathogenesis of COPD, hypertension and obesity has general factors, which intensification is able to influence greatly on the clinical course of COPD. The aim of this work is: to study the changes of lipid peroxide oxidation (LPO), antioxidant system (AOS), proteolysis intensity in blood and exhaled breath condensate (EBC), as well as local balance between pro- and anti-inflammatory cytokines in COPD patients with hypertension and obesity.

2 MATERIALS AND METHODS

135 individuals were examined: 25 healthy subjects (non-smoker) and 86 patients with COPD exacerbation without (I group) and with comorbid hypertension and obesity (II group). Demographic and clinical characteristics are presented in table 1. Diagnostics and treatment of COPD and hypertension were conducted according to the national recommendations. Body mass index (BMI) was determined by the formula: $BMI = m/h^2$, where the m-weight (kg), and h-height (m). Evaluation of body weight and degree of obesity was conducted by the WHO classification (1997): normal weight - BMI 19-24,9 kg/m², overweight - BMI 25-29,9 kg/m², and obesity degree - BMI 30-34,9 kg/m², second degree - BMI 35-39,9 kg/m², third degree - BMI ≥ 40 kg/m². All the patients received complex of clinical-functional, laboratory and special examinations. At the beginning of therapy, from the patients in the morning on empty stomach was taken EBC before taking medicines [6]. The parameters of pulmonary function test in the patients, that were examined, were detected by means of computer spirographic apparatus "BTL-08" ("SpiroPro", UK). General tripsin-like proteinase activity was determined by Kunits method, neutral proteolytic activity of citrate blood plasma and EBC – by azoalbumin, azocasein and azocol lysis ("Simko Ltd", Ukraine). Superoxyddismutase (SOD) and catalase (Ct) activity, malone dialdehyde (MDA) level, HS-group content in the blood and EBC. Cytokines content in EBC were conducted in immune-enzymatic analyzer "Uniplan-M" (Russia) by means of "ProCon IL-1 β " reagents to determine interleukin 1 β (IL-1 β)

(Russia), and "ProCon TNF α " (OOO "Protein contour", Russia) to determine tumor necrotizing factor (TNF α). Transforming growth factor β 1 (TGF- β 1) content in EBC was detected by immune-enzymatic analysis method with the use of "TGF- β 1 ELISA" reagents by "DRG Instruments GmbH" Company (USA), interferon γ (IFN γ) concentration – by means of "IFN γ ELISA KIT" reagents by "DIACLONE Research" (France).

Table 1: Demographic and clinical characteristics

Parameters	COPD II-III stages without hypertension and obesity	COPD II-III stages with hypertension and obesity
N (mean)	50	36
Age (mean years)	66,7 \pm 1,3	69,7 \pm 1,2
Male (%)	68,4	70,1
BMI	24,67 \pm 0,87	35,85 \pm 1,79
Smoked for 10 years or more (%)	56,6	61,2
Pack-years (mean)	38,2	44,4
Dyspnea (%)	76,1	82,1
Wheeze (%)	69,1	74,3
Cough (%)	75,1	79,2
FEV1/FVC ratio	61,35 \pm 0,92	59,66 \pm 1,16
FEV1, % pred.	44,81 \pm 1,11	43,77 \pm 1,26
Systolic blood pressure (mean)	129,42 \pm 1,08	157,12 \pm 1,31
Diastolic blood pressure (mean)	78,20 \pm 1,05	97,85 \pm 0,94

Significance of the statistical tests was defined as a p value of < 0.05. The calculations were performed using standard commercial statistical and calculation software packages (STATISTICA, Excel).

3 RESULTS AND ANALYSIS

The study of indices of cytokine content in EBC in COPD patients without comorbidities showed that the content of proinflammatory cytokines IL-1 β and TNF- α as well as TGF- β 1 and IFN- γ increased. In patients with comorbid hypertension and obesity the level of IL-1 β (16,7%) and TNF- α (20,2%) and especially TGF- β 1 (27,6%) in EBC were higher than in patients with COPD without hypertension and obesity (table 2). Inflammation is known to be accompanied by accumulation of phagocytic cells and activation of their oxygen metabolism in loco morbi. The recent results in increased production of active oxygen forms, local intensification of lipid peroxide oxidation processes and bioantioxidant exhaustion [7]. Thus, intensity of lipoperoxidation and the state of some components of antiradical defense system on the level of bronchial tree were necessary to examine. Intensity of lipoperoxidation processes in COPD patients of I and II groups was studied by the content of MDA in EBC. MDA level in EBC as compared with the healthy subjects was 2,1 and 2,6 times higher (table 2). Antiradical defense systems were estimated by the activity of catalase and SOD in EBC. Ct activity in the I and II groups was 2,4 and 3,6 times lower, although its activity in COPD patients with hypertension and obesity was 33,3% lower than that of those without comorbidities. COD activity in EBC of the I group of patients was 44,1% lower, while in patients with hypertension and obesity – 60,3% lower. But in the II group patients COD activity was found to be 29,0% lower as compared with COPD patients without comorbidities. The

number of HS-group in EBC was found to be higher in 1,8 (in I group of patients) and 1,9 (II group of patients) times compared with the healthy subjects. Thus, COPD exacerbation was accompanied by local intensification of free radical oxidation, which is proved by increased MDA content with simultaneous inhibition of antiradical activity in EBC, which is evidently indicated by decrease of catalase activity in EBC. COPD patients with hypertension and obesity showed more pronounced disbalance between pro- and antioxidant systems on the level of the bronchial-alveolar apparatus, which is indicated by reliably of higher MDA index in EBC, decrease of catalase and SOD activity. To give more accurate definition of correlation between the local and systemic signs of inflammatory process in the bronchi, it would be advisable to analyze the direction and intensity of inflammatory process in EBC and blood according to healthy subjects (table 3). While comparing the changes of intensity of lipoperoxidation and parameters of antioxidant defense in the blood and EBC, in COPD patients with and without hypertension and obesity, our attention was attracted to practically equal (double) increase of MDA level and the number of HS-groups both in the blood and EBC. At the same time, the system of catalase enzymatic antiradical defense underwent different changes, characterized by increased activity of catalase in the blood with its decrease in EBC (table 1,2). As intensification of lipid peroxidation processes are usually accompanied by proteolytic activation influencing greatly upon the intensity of chronic inflammation, it should be advisable to examine the changes of lysis of low-, high-molecular proteins and collagen, as well as determine activity of tripsin-like proteinases in EBC and blood. Intensity of EBC-induced low-molecular proteinolysis in COPD patients of the I and II groups was 50,6 % and 57,1% higher as compared with the healthy subjects, while in patients with hypertension and obesity this index was on 13,1% higher than in patients without comorbidities. Proteolytic break down of high molecular proteins in patients without hypertension and obesity was 32,1% higher than in healthy subjects, and in COPD patients with hypertension and obesity – 43,9%. Although azocasein lysis in EBC was 17,4% higher (table 2).

Table 2: Parameters of cytokine profile, lipid peroxide oxidation and antioxidant system, intensity of proteolysis in exhaled breath condensate in COPD patients with and without hypertension and obesity

Variable	Healthy subjects n=25	COPD II-III stages (group 1) n = 50	COPD II-III stages with hypertension and obesity (group 2) n=36
IL-1 β , pg/ml	36,09 \pm 1,5 6	154,69 \pm 5,20 p<0,001	185,60 \pm 8,06 p<0,001 p ₁ <0,01 222,44 \pm 11,2 9
TNF- α , pg/ml	38,61 \pm 2,2 7	177,55 \pm 6,67 p<0,001	p<0,001 p ₁ <0,01 109,13 \pm 7,54
TGF- β ₁ , pg/ml	18,43 \pm 1,5 1	78,97 \pm 3,46 p<0,001	p<0,001 p ₁ <0,001
IFN- γ	32,77 \pm 2,9	158,01 \pm 4,08	151,79 \pm 6,35

pg/ml	5	p<0,001	p<0,001 p ₁ >0,05
Malone dialdehyde, nmol/1 mg of protein	0,62±0,04	1,29±0,03 p<0,001	1,61±0,04 p<0,001 p ₁ <0,001
Catalase, mkmol H ₂ O ₂ / min per 1 mg of protein	9,23±0,68	3,84±0,17 p<0,001	2,56±0,18 p<0,001 p ₁ <0,001
SH-group, mkmol/1 ml	0,14±0,02	0,39±0,03 p<0,001	0,35±0,03 p<0,001 p ₁ >0,05
Superoxyd-dismutase, un/1 mg of protein per 1 min	16,23±0,79	9,07±0,34 p<0,001	6,44±0,27 p<0,001 p ₁ <0,001
Low-molecular proteinolysis, mkmol of azoalbumin/1ml per hour	1,18±0,04	2,39±0,03 p<0,001	2,75±0,03 p<0,001 p ₁ <0,001
High-molecular proteinolysis, mkmol of azocasein/1ml per hour	1,48±0,05	2,18±0,05 p<0,001	2,64±0,06 p<0,001 p ₁ <0,001
Collagenolysis, mkmol of azocol/1ml per hour	0,25±0,01	0,14±0,01 p<0,001	0,13±0,01 p<0,001 p ₁ >0,05
Proteinase activity by Kunits, mkg of tripsin equivalents/1ml per hour	0,53±0,02	0,85±0,02 p<0,001	1,00±0,02 p<0,001 p ₁ <0,001

Notes: *p* – degree of reliability of indices concerning the control; *p*₁ – degree of reliability of indices between the I and II groups, *n* – number of observations.

At the same time collagenolytic activity in EBC appeared to be 44,0% lower in patients without comorbidities and 48,0% lower - with hypertension and obesity, than that of the control group. Lysis intensification of low molecular and high molecular proteins were accompanied by 37,6% increased activity of tripsin-like proteinases in EBC of patients without hypertension and obesity, and in combination with COPD, hypertension and obesity – 47,0% higher, although it was 15,0% higher in the II group of patients as compared with the I group. While comparing changes of proteolytic activity of EBC and blood in COPD patients of the I and II groups, the increasing level (concerning the healthy subjects) of intensity of EBC-induced lysis of low molecular proteins, was higher than in the blood. The intensity of azocasein lysis and activity of tripsin-like proteinases increased practically in the same way. At the same time collagenolysis intensity in the blood did not change in both groups of patients (table 3).

Table 3: Parameters of lipid peroxide oxidation and antioxidant system, intensity of proteolysis in blood in COPD patients with and without hypertension and obesity

Variable	Healthy subjects n=25	COPD II-III stages (group 1) n = 50	COPD II-III stages with hypertension and obesity (group 2) n=36
Malone dialdehyde, nmol/1 mg of protein	8,03±0,46	17,23±0,52 p<0,001	16,37±0,43 p<0,001 p ₁ >0,05
Catalase, mkmol H ₂ O ₂ / min per 1 mg of protein	35,45±2,71	93,16±3,49 p<0,001	110,71±3,97 p<0,001 p ₁ <0,01
SH-group, mkmol/1 ml	1,79±0,06	3,26±0,12 p<0,001	3,33±0,11 p<0,001 p ₁ >0,05
Low-molecular proteinolysis, mkmol of azoalbumin/1ml per hour	3,44±0,10	5,41±0,14 p<0,001	5,63±0,11 p<0,001 p ₁ >0,05
High-molecular proteinolysis, mkmol of azocasein/1ml per hour	3,21±0,10	5,13±0,08 p<0,001	5,28±0,10 p<0,001 p ₁ >0,05
Collagenolysis, mkmol of azocol/1ml per hour	0,38±0,03	0,52±0,03 p<0,01	0,73±0,04 p<0,001 p ₁ <0,001
Proteinase activity by Kunits, mkg of tripsin equivalents/1ml per hour	0,74±0,02	1,16±0,03 p<0,001	1,34±0,03 p<0,001 p ₁ <0,001

Notes: *p* – degree of reliability of indices concerning the control; *p*₁ – degree of reliability of indices between the I and II groups, *n* – number of observations.

Thus, COPD patients demonstrate increased proteolytic low molecular protein break down both in EBC and the blood. But more pronounced increase of azoalbumin lysis and more substantial inhibition of collagenolytic activity in EBC, especially in combination with COPD, hypertension and obesity are indicative of the fact that local inflammatory reaction on the level of the bronchoaveolar apparatus are more intensive than its general signs in the blood. The results are indicative of local activation of the neutral proteolytic systems with simultaneous inhibition of collagenolysis and activation of acidic tripsin-like proteinases resulting in damage of the mucus of the respiratory passages, proliferation of the connective tissue elements and inhibition of regenerative ability of the ciliary epithelium with the development of ciliary dysfunction, as one of the leading mechanisms of bronchial obstruction in COPD patients. It should be noted, that in COPD with hypertension and obesity, local activation of the

proteolytic systems was more pronounced, but collagenolytic activity did not differ in the I and II groups of patients.

4 DISCUSSION AND CONCLUSION

By the results of our research, COPD patients without hypertension and obesity showed an increasing content of MDA, both in the blood and EBC, which is indicative of local (on the level of bronchoalveolar apparatus) and systemic activation of lipid peroxidation processes [8]. Intensity of LPO processes was more pronounced in case of combination of COPD, hypertension and obesity. Increased level of HS-groups in EBC can be stimulated by the influence of oxygen active forms and lipoperoxides on the protein molecules, and increased protein concentration in EBC of COPD patients, can be connected with increased permeability of the lung capillary filter, presence of inflammatory exudates in the alveoli and pathologic content of the tracheal-bronchial tree (purulent or mucous-purulent sputum) [8, 9]. LPO is known to be performed through the system of antioxidant defense – antioxygen, antiradical and antiperoxide mechanisms. The disorder of the balance between the processes of lipoperoxidation and antioxidant system results in avalanche-like reaction of overoxidation, leading to death of the cells [10]. Literary data indicates, that intensification of lipoperoxidation is accompanied by inhibition of antiradical provision [11]. According to the data obtained, the changes of the antiradical system were found in considerable inhibition of antiradical enzymes activity, on the level of the bronchial tree, especially in patients with hypertension and obesity, which was characterized by reduction of catalase and superoxydedismutase activity. At the same time, we observed increasing activity of catalase in the blood, which can be of an inducible character, i.e. is the result of antiradical response to the intensification of active oxygen forms intensification. Along with increased formation of lipoperoxides, we observed in COPD patients with without hypertension and obesity, proteolysis activation both in the blood plasma and EBC taken place on the background of collagenolysis inhibition. It should be noted, that COPD patients with hypertension and obesity had higher proteolytic blood activity, especially on the level of the bronchial-alveolar apparatus, than in COPD patients without hypertension and obesity. Disintegration of the connective tissue exchange is connected with proteolytic activation, elastase in particular. Disorders on the level of the connective tissue in case of lung pathology are various, and appear under the influence of different factors, forming pathology of the bronchial-alveolar apparatus, other systems and organs. Increased amount of proteases and antiproteases deficiency, result in lesion of elastic connective tissue frame of the lungs, and disorders of elastin and collagen metabolism, promote the development of fibrosis and lung emphysema [8]. These processes are not limited only by the lungs, increasing the level of changes of ventilation-perfusion correlations, hemodynamics of the lungs, intensify hypoxia, transform metabolic lung function with corresponding changes in other organs, the cardio-vascular system in particular [2,12]. Proinflammatory cytokines are also responsible for generalization of chronic inflammation, and through the induction of the nuclear factor NF κ B they launch the chain of protein synthesis of an acute phase [13]. In recent years pathogenetic mechanisms of cytokines status disorders in COPD patients are discussed, as well as the role of this disbalance in the support of chronic inflammation [4,5,13]. Our examination of the content of pro- and anti-inflammatory cytokines in EBC revealed, that patients with and without hypertension and obesity showed pronounced increase of IL-1 β , FNP- α , IFN- γ , and especially TGF- β 1. Pronounced increase of a TGF- β 1 in patients COPD and comorbidities can intensify fibrous changes in the

bronchial tree leading to progressing of bronchial obstruction and hypertension. The literary data proves that increased TGF- β 1 secretion is associated with such consequences of excessive proliferation of the connective tissue as nephrosclerosis, liver cirrhosis, atherosclerosis, lung fibrosis, sclerodermitis and rheumatoid arthritis [4]. Excessive activation of LPO and proteolysis is reflected on the structure and function of the cellular membranes, including into pathogenetic chains of stress reactions, atherosclerosis, ischemic heart disease, and arterial hypertension. Lipoperoxide peroxidation results in hypercoagulation, changes of structures and membrane properties, disorders of enzyme membrane-binding functions, destruction of membranes and death of cells [8,14]. That's why, COPD patients with hypertension and obesity should be administered to medical preparations possessing antioxidant [11] and anti-inflammatory properties as well as ability to renew the structure of the cellular membranes. Experience of drug administration in clinical and experimental therapy proved that liposomal preparations possess such properties. The course of COPD on the background of comorbid hypertension and obesity is accompanied by more substantial intensification, than in case of hypertension absence, of inflammatory process on the level of the bronchial tree, which is proved by pronounced elevation of pro- and anti-inflammatory cytokines in EBC (especially TGF- β 1), local intensification of free radical lipid oxidation with simultaneous inhibition of antiradical defense factors, activation of neutral proteolytic systems and acid tripsin-like proteinases, reduced collagenolysis intensity. COPD patients with comorbid hypertension and obesity are characterized with more pronounced systemic inflammatory response, which is proved by more substantial increase of malone dialdehyde and HS-groups in the blood, on the background of changes of different directions from the system of antioxidant defense (increase of catalase activity), increase of caseinolytic and albuminolytic activity, and increase of tripsin-like proteinase activity, on the background of stable intensity of collagenolysis; more pronounced activity of neutral and acid proteolytic systems in the blood. Thus, the course of COPD with hypertension and obesity characterized more substantial intensification of inflammatory process on the level of the bronchial tree and with more systemic inflammatory response.

REFERENCES

- [1] Luppi F, Franco F, Beghe B: Treatment of Chronic Obstructive Pulmonary Disease and Its Comorbidities. Proc Am Thorac Soc 2008, 5, 848–856.
- [2] Bolton CE, Cockcroft JR: Lung function and cardiovascular risk: a stiff challenge? Am J Hypertension 2010, 23, 584–588.
- [3] Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of chronic pulmonary disease, 2011. <http://www.goldcopd.org/Guidelines/guidelines-resources.html>.
- [4] Barnes PJ, Celli BR: Systemic manifestations and comorbidities of COPD. Eur Respir J 2009, 33, 1165–1185.
- [5] Nussbaumer-Ochsner Y, Rabe KF: Systemic manifestations of COPD. Chest 2011, 139, 165-173.
- [6] Grob NM, Aytakin M, Dweik RA: Biomarkers in exhaled breath condensate: review of collection, processing and

analysis. *J Breath Res* 2008, 2, 1-33.

- [7] Bartoli MI, Novelli F, Costa F: Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. *Mediators of inflammation* 2011, 1-7.
- [8] Rahman I. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. *Cell Biochem Biophys* 2005, 43, 167–188.
- [9] Borrill ZL, Roy K, Singh D: Exhaled breath condensate biomarkers in COPD. *Eur Respir J* 2008, 32, 472-486.
- [10] Louheinen N, Myllamiemi M: Airway biomarkers of the oxidant burden in asthma and chronic obstructive pulmonary disease. *International J of COPD* 2008, 3(4), 585-603.
- [11] Ochs-Balcom HM, Grant BJB, Muti P: Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. *Eur J Clin Nutr* 2006; 60: 991–999.
- [12] MacCallum PK: Markers of hemostasis and systemic inflammation in heart disease and atherosclerosis in smokers. *Proc Am Thorac Soc* 2005, 2(1), 34-43.
- [13] Magnussen H, Watz H: Systemic inflammation in chronic obstructive pulmonary disease and asthma: relation with comorbidities. *Proc Am Thorac Soc* 2009, 6, P.648-651.
- [14] Ashitani J, Mukae H, Arimura Y, Matsukura S: Elevated plasma procoagulant and fibrinolytic markers in patients with chronic obstructive pulmonary disease. *Intern Med* 2002, 41(3), 181-185.