

## METABOLIC STATUS OF ERYTHROCYTES AT PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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### INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the major medical problems throughout the world. It is predicted that in 2020 COPD will become the third leading cause of death worldwide [1, 2]. COPD is considered such as systemic disease, but the mechanisms of its development are not clear.

One of the mechanisms of COPD development has associated with red blood cells (RBCs) disorders. Oxidative stress and reduced antioxidant resources in RBCs of COPD patients may contribute to the systemic effects of COPD [3]. It is considered that oxidative damage of RBCs induced an impairment of rheological properties of erythrocytes by means of increasing of lipid peroxidation, fragmentation of membrane proteins, decrease of membrane – binding enzyme activities and disorder of barrier function of membrane [4,5,6]. The RBCs of COPD patients were altered with respect to control erythrocytes. It was revealed important shape changes and increase in membrane rigidity. The data seem to suggest that changes in erythrocyte shape and rheological properties play a key role in RBCs dysfunction in the progression of COPD [6]. Recently it was found the conversion of hemoglobin in erythrocytes of COPD patients. The results obtained showed increment of the concentration of methhemoglobin, sulfhemoglobin, fetal hemoglobin and glycosylated hemoglobin in RBCs of COPD patients [7].

The aim of the present work was to apply biochemical analysis to detect metabolic perturbations in erythrocytes of patients with different clinical forms and severity of COPD.

### ETHICS

This investigation was approved by the ethics committee of Karaganda State Medical University. All patients and healthy subjects had received the full information on probable inconveniences and complications at the blood sampling before giving their written informed consent.

### PATIENTS AND PROCEDURES

Control subjects were healthy volunteers without any medication. All patients were on hospitalization and inspection. The syndrome of bronchial obstruction has been revealed at 100 percent of patients at receipt in a hospital. Respiratory insufficiency (RI) was established in reason of the syndrome of short wind in a rest condition and at the insignificant physical loading representing walking on 100 meters by slow rate (speed of movement is not higher than 5 steps in one minute). RI of I degrees is diagnosed at 25% of patients and RI of II degrees — at 75 % of patients. Verification of the diagnosis was carried out on the basis of the complex of the standard criteria. At 100 percent of patients the habit to smoking tobacco is revealed. The index of smoking person has made > 200 at 67% of patients of 1<sup>st</sup> group and at 73% of the patients including in 2<sup>nd</sup> group. In 45% of patients are marked professional harm (the experience of underground work on collieries over 10 years, work on woodworking enterprises, cement works), atmospheric pollution (inhabitants of Kazakhstan industrial cities Temirtau, Karaganda, Balkhash have made 79% surveyed).

Basic clinical displays of COPD were cough with sputum and a short wind. Cough was marked during all

day, less often only at night. The quantity of sputum was small, outside of aggravations its character was mucous. At 54% of patients elimination of sputum was occurring after long cough. All patients carried out cytologic research of sputum which found out presence of alveolar epithelial cells, elastic fibres, siderophages. It was found out a plenty of the leukocytes submitted basically neutrophiles at 54% of patients of 1 group and at 61% of patients of 2 groups during an aggravation. It was interpreted as the evidence of pyo-inflammatory process in mucous of bronchial tree.

Patients were divided into 4 groups. 29 patients with COPD, moderate severity mixed form (emphysematous and bronchial), exacerbation, respiratory insufficiency of grade 2 were included in first group. 21 patients with COPD, severity mixed form (emphysematous and bronchial), exacerbation, respiratory insufficiency of grade 2 were included in second group. 35 patients with COPD, moderate severity bronchial form, exacerbation, respiratory insufficiency of grade 2 were included in 3-hd group. 23 patients with COPD, severity bronchial form, exacerbation, respiratory insufficiency of grade 2 were included in 4<sup>th</sup> group. The fifth group consisted of 32 healthy subjects.

Venous blood (3.0 ml) was drawn from patients and healthy ones into a heparinized syringe; blood was centrifuged (2000 g, 10 min, 4° C). The plasma and buffy coat were removed. The erythrocytes were washed (3×) in ice-cold phosphate buffered saline with following centrifugation (2000 g, 10 min, 4° C).

## METHODS

The count of erythrocytes and hemoglobin were detected by using Mindray BC-3200 Hematology Analyzer. The concentration of malondialdehyde (MDA) was measured using thiobarbituric acid [8]. The concentration of MDA was given in mmol/g. The protein reactive carbonyl derivatives protocol of Levine R., et al. [9]. The protein reactive carbonyl derivatives content values were given in nmol/g hemoglobin. Sorption property of RBCs was measured using methylene blue (MB+), unit - %. High erythrocyte membrane permeability was associated with increased methylene blue sorption, which correlated with erythrocyte damage [10]. Determination of glycosylated hemoglobin (HbA1c) was performed using sets of Hemoglobin A1C company BioSystems, unit - % HbA1c.

**Statistical analysis.** Comparisons between patients and controls ones were performed using non-parametric Mann-Whitney U-test (for independent variables) and Wilcoxon matched pairs test.

## RESULTS

The results obtained demonstrated the significant increasing of MDA and protein reactive carbonyl

derivates in RBCs of COPD patients ( $p < 0.001$ ). It was found that MDA accumulation in erythrocytes did not depend on severity and clinical form of COPD. The concentration of protein reactive carbonyl derivatives was higher in erythrocytes of patients with COPD of severity bronchial form in comparison with patients suffering of COPD of moderate severity bronchial form (Table 1).

An increasing of the glycated hemoglobin concentrations (above 6%) was fixed in erythrocytes at 9% of the 1 group patients and at 25% of the second group patients. An increasing of the glycated hemoglobin concentrations were fixed in erythrocytes of 29% of the 3 group patients and at 37 % of the 4<sup>th</sup> group patients.

Analysis of MB+ sorption on RBCs from 1-4 group patients showed the significant differences in respect of control ones. Sorption properties of RBCs from patients of 1 and 2 groups were higher than control one (by 2 and 1.8 times, respectively,  $p \leq 0,001$ ). Sorption properties of RBCs from patients of 3 and 4 groups were higher than control one by 1.5 times ( $p \leq 0,001$ ). In our opinion it would be better interpret those results not from position of erythrocyte membrane permeability. It is known that MB+ acts as an electron donor in the non-enzymatic reduction of methemoglobin [11, 12, 13]. NADPH-dependent reductase catalyses the reduction of methylene blue to leucomethylene blue [11]. We supposed that high sorption properties of RBCs in patients from 1 and 2 groups might be connected with low reductase activity. The trend to declining of sorption properties of RBCs in patients from 3 and 4 groups might be determined by slight increasing of reductase activity. In any cases it may be at higher risk for developing methemoglobinemia. Our data agree well with studies that have found methemoglobin reductase (CYB5R3) to be underexpressed in erythrocytes at COPD patients [14].

Taken together, our results obtained demonstrated the synchronism of lipid peroxidation and accumulation of oxidative-modified proteins in RBCs of COPD patients. In our opinion the hemoglobin and cytoskeleton proteins are the main intracellular targets for carbonyl stress. Oxidative modification of intracellular proteins induced on different perturbations of erythrocyte metabolic pathways. The oxidative modification in lipid and protein components of RBCs plasma membrane erythrocytes of COPD patients may decrease the membrane fluidity and carrier properties. It also may possibly impair the activity of membrane-binding enzymes.

We believe that would be expedient to continue discussion of our results in a context of studying of mechanisms of progressing COPD.

**Table 1.** The concentration of protein reactive carbonyl derivatives and MDA in erythrocytes of patients with different clinical forms and severity of COPD

Groups	Protein reactive carbonyl derivatives	MDA
	median	median
Control subjects	7,91	0,51
Std.Dev.	1,63	0,025170
Group 1	13,56*	0,92*
Std.Dev.	6,01	0,399549
Group 2	12,15*	0,87*
Std.Dev.	3,50	0,123491
Group 3	14,72*	0,90*
Std.Dev.	5,82	0,303187
Group 4	15,12*	0,81*
Std.Dev.	4,88	0,388738

\* Significant difference compared to control ones ( $p \leq 0,001$ )

## FUTURE DIRECTIONS

The further challenge will be to expand analyzing physical and chemical properties of RBCs in blood of patients at different stages and types of COPD and to estimate their impact in progression of COPD.

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## COMPETING INTERESTS

The authors declare that they have no competing interests. All authors read and approved the final manuscript.