

The Possibilities of Modern Methods of Oxidative Stress and Immune Profile Study in Determining the Predictors of Chronic Inflammatory Process in the Bronchi of Young Beginning Smokers

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The aim of the investigation was to assess the possibilities of the modern methods for oxidative stress and immune profile research in determining the predictors of chronic inflammatory process in the young smokers bronchi.

Materials and Methods. 85 students of the I–III-year from Nizhny Novgorod State Medical Academy were examined, among them 30 students (group 1) were non-smokers and 55 students (group 2) were addicted to nicotine. The pulmonary function test, oxidant–antioxidant system, the levels of soluble differentiating molecules of leucocytes and histocompatibility molecules were studied along with general clinical examination methods. Only students with normal pulmonary function test indices were included in the study.

Results. Antioxidant system activation and decreasing intensity of lipid peroxidation was characteristic of young smokers. Thus, statistically significant increase in erythrocytes superoxide dismutase was revealed in group 2, and the indices of maximum luminescence intensity I_{max} and S light sum were decreased. The smokers showed significant increase in sCD95 apoptosis modulator, growing content of sCD25 and sCD8 immune cells activation markers, growing total fraction of sCD54 adhesion molecules as well as soluble histocompatibility molecules of class I and II.

Conclusion. At the first stages smoking is accompanied by compensatory immune reactions, antioxidant system activation, as well as decreasing lipid peroxidation intensity. Modern methods of researching oxidant–antioxidant system as well as the levels of differentiation and histocompatibility molecules allow studying the subtle mechanisms of smoking impact on the respiratory system at the “pre-obstructive” stage and tracing the appearance of chronic bronchi inflammation predictors.

Key words: tobacco smoking; chronic bronchi inflammation predictors; soluble differentiating leucocyte antigens; lipid peroxidation.

Cigarette smoking is one of the leading mechanisms of exogenous respiratory system impairment resulting in the development of chronic obstructive pulmonary disease (COPD), emphysema, lung cancer [1, 2]. The pathogenic potential of tobacco smoke includes about 4,700 of different toxic substances contributing to systemic damage of the organism and development of pathological processes in the bronchopulmonary system [3–6]. Smoking activates free-radical processes inducing development of chronic inflammation in the respiratory tract over time [1, 4, 7, 8], including microflora involvement [1, 4].

One of the main tools for body balance control is functioning of oxidant–antioxidant system. Bronchoconstriction and vasoconstriction develop through oxidative mechanisms. Oxidants take part in DNA degradation, surfactant activity decrease, hyperpermeability of endothelium and epithelium, cause emphysema and pneumosclerosis [7, 9].

One of the end products of lipid peroxidation (LPO) is malondialdehyde (MDA) whose formation rate enables to estimate the activation of reactions leading to free radicals generation. On the other hand, proteins and antioxidant system enzymes, such as superoxide

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dismutase (SOD), glutathione peroxidase and others, protect the organism cells from the damaging impact of oxidative stress.

Pro-oxidant-antioxidant system imbalance underlies the development of respiratory system diseases (COPD, bronchial asthma, lung fibrosis, lung cancer), atherosclerosis, ischemic heart disease and others [10–12].

Oxidative stress in the lungs induces modification of plasma proteins, macrophagocytes activation and neutrophils recruitment in the central and peripheral airways, accumulation of toxic LPO products in the lung and blood membranes, increase in LPO products content, hydrogen peroxide, nitrosothiols and nitrates in the expired air [13]. Besides, according to the latest data, it provokes hyperplasia of gland mucous membranes and apoptosis of airway epithelium cells [8].

The mechanisms of inflammation development are in many ways connected with the processes taking place in the immune system. The cells participating in the immune response and inflammation development in COPD have diverse membrane antigens characterizing their lineage [14, 15]. Membrane proteins of immune system cells may have soluble forms which can be found in body fluids (blood serum, synovial fluid and others) and tend to preserve their biological potential regulating different stages of immune response [14, 15]. According to the experts in the field, the content of these molecular markers may be regarded as additional criteria of inflammation activation and COPD severity [3].

Three main groups of soluble membrane proteins have been identified within immune system cells [14, 15]:

molecules of the major histocompatibility complex (HLA) class I and II;

cytokine receptors, such as CD25 antigen, interleukin-2 receptor (IL-2 R α);

different antigens participating in immune system cells maturation and performing effector functions: CD95 (Fas/Apo-1) mediates apoptosis, CD50 and CD54 are intercellular adhesion molecules (ICAM), CD38 participates in adhesion, signal transduction and cell activation processes, CD8 is a co-receptor of T-cell receptor and a cytolytic lymphocyte antigen.

One of the 12 most urgent scientific COPD problems specified by the European Respiratory Society is studying the mechanisms of COPD formation at the initial stages and developing methods of early diagnosis of this disease [16]. Garey et al. [5] found neutrophils increased hemotoxic activity combined with other inflammation signs induced by tobacco smoke in the exhaled condensate of the young “apparently healthy” smokers. In the literature there are no clear data on how fast the first COPD signs associated with smoking may occur. The presence of inflammation in the bronchi, immune mechanisms activation as well as destabilization of oxidant and antioxidant balance are likely to be assumed even at the early stages of tobacco addiction. In this

connection, it is interesting to trace the onset of chronic inflammatory process development in young beginning smokers by analysing LPO dynamics, antioxidant system activity and soluble membrane proteins of the immune system cells.

The aim of the investigation was to assess the potential of modern methods used for oxidative stress and immune profile study in diagnosing the predictors of chronic inflammatory process in the bronchi of young beginning smokers.

Materials and Methods. The study was performed at the Department of Internal Diseases Propedeutics of Nizhny Novgorod State Medical Academy. There were examined 85 I–III-year students from the Department of General Medicine and the Department of Pediatrics (on average 19.5±0.7 years of age; 48 young males and 37 females). Group 1 consisted of 30 non-smoking students and group 2 included 55 students addicted to smoking (30 young males and 25 females). To exclude broncho-obstructive syndrome and other respiratory pathology chest X-ray and pulmonary function test (PFT) data were analyzed: forced expiratory volume per 1 s (FEV1), Tiffeneau index, peak expiratory flow rate (PEFR), maximal expiratory flow at 25, 50, 75% of expiration (MEF25, MEF50, MEF75). The students with pathologic abnormalities were not included in the study.

The intensity of free-radical processes was evaluated by using the induced chemiluminescence method with the aid of biochemiluminometer BCL-07 (Russia). The index of maximum luminescence intensity (Imax) indicating the potential capacity of biological tissues for free-radical oxidation was used for LPO evaluation. Increase in S light sum being an integral indicator of non-enzymatic antioxidant activity (AOA) of plasma and being in an inverse relationship with it may indicate high LPO activity and reduced antioxidant capacity. Plasma MDA level, SOD and erythrocyte catalase activity were also measured.

Imax and MDA levels were considered the criteria of LPO intensity. The antioxidant capacity was evaluated taking into account SOD, catalase and S light sum activity.

The immune system status was assessed by the level of sCD95 apoptosis inhibitor (total and oligomeric fractions), immune cell activation markers (sCD38, sCD25, sCD8), sCD54 intercellular adhesion molecules (ICAM-1), total and oligomeric fraction and sCD50 (ICAM-3), as well as HLA-I and HLA-DR soluble histocompatibility molecules.

The level of soluble membrane antigens was measured with the aid of Stat Fax 2100 enzyme immunoassay device (USA) with the wave length of 450 and 630 nm. The resulting data were expressed in standard units (U/ml).

The study of soluble differentiating leucocyte antigens was carried out at the Scientific Research Institute of Molecular Biology and Regional Ecology of Lobachevsky

State University of Nizhni Novgorod. The LPO and AOA study was conducted in Central Scientific Research Laboratory, Nizhny Novgorod State Medical Academy.

The material was statistically processed with Statistica 7.0 and Minitab 16 software. The results were presented as the mean and standard deviation (M±SD). T-test and Mann–Whitney U-test were used depending on the correctness of the chosen distribution of the sign for comparison of unrelated groups. Reliability criterion was considered to be a significance level of $p < 0.05$. Statistical tendency was estimated to be present at $0.05 < p \leq 0.1$.

Results and Discussion. All the students under study had similarly good PFT indices regardless of tobacco addiction (Table 1). Moreover, they did not show clinical signs of inflammatory lesion in the bronchi.

When comparing the indices of LPO and antioxidant enzymes of plasma and hemolysate of erythrocytes our study revealed significant differences between smoking and non-smoking students. Antioxidant system activation was characteristic of young smokers (Table 2, Figure). So, SOD level of erythrocytes in group 2 was statistically significantly higher than in group 1 ($p = 0.0001$). It should be emphasized that high level of antioxidants (SOD and glutathione) in healthy smokers, which depends on smoking intensity, was found by other authors as well [17].

Table 1

The main spirometric parameters (%) in young smokers and non-smoking relatively healthy students (M±SD)

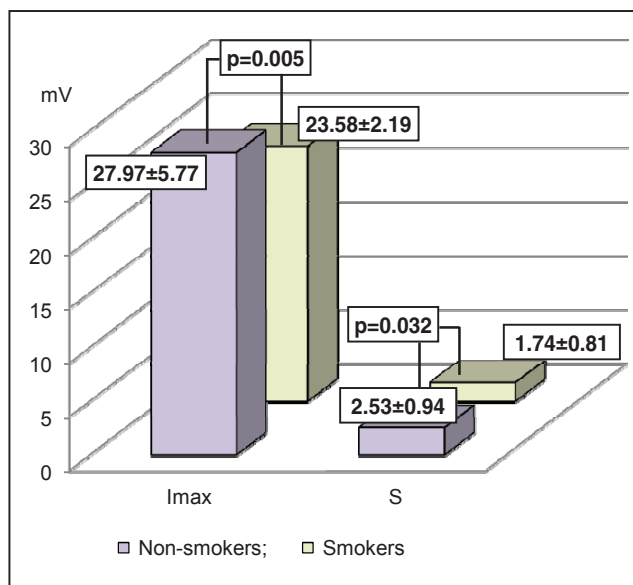
Index	Non-smokers	Smokers	p
FEV1	89.94±13.99	91.85±11.89	0.635
Tiffeneau index	119.0 [119.0; 119.75]	121.0 [118; 121]	0.248
PEFR	92.19±14.86	84.29±13.08	0.076
MEF25	147.13±32.98	149.27±30.09	0.830
MEF50	119.31±23.41	115.50±17.91	0.555
MEF75	101.44±14.87	94.48±14.19	0.135

Note. FEV1: forced expiratory volume per 1 s; PEFR: peak expiratory flow rate; MEF25, MEF50, MEF75: maximal expiratory flow at 25, 50, 75%.

Table 2

Comparative analysis of lipid peroxidation and antioxidant enzyme indices in smoking and non-smoking students (M±SD)

Index	Non-smokers	Smokers	p
SOD (enzymatic activity units/g Hb·min)	280.32±130.98	585.60±259.05	0.0001
Catalase (enzymatic activity units/g Hb·min)	11.09±0.19	13.78±5.53	0.067
MDA (nmol/L)	1.01±0.19	1.09±0.30	0.421
Imax (mV)	27.97±5.77	23.58±2.19	0.005
S (mV)	2.53±0.94	1.74±0.81	0.032



Lipid peroxidation parameters in smoking and non-smoking students

Increase in average values of erythrocyte catalase in group 2 as compared to group 1 was in accordance with the statistical tendency ($p = 0.067$). According to the data provided by the recognized investigators, this index does not alter even in a severe COPD case [18].

On the other hand, in group 2 there was a statistically significant decrease in the index of maximum luminescence intensity Imax indicating the potential capacity of biological tissues for free-radical oxidation ($p = 0.005$) and decrease in S light sum value ($p = 0.032$).

Nowadays there is active research concerning the immune status in many diseases, including COPD. What we do find rather interesting to study is the level of soluble differentiating molecules and histocompatibility molecules in the serum of smoking and non-smoking students.

In our study (Table 3) smoking students were characterised by statistically significant increase in sCD95 apoptosis inhibitor level of both total (sCD95) and oligomeric (sCD95ol) fractions ($p = 0.011$ and $p = 0.010$, respectively). These results are consistent with the obtained earlier experimental data showing that tobacco smoke extract causes intracellular accumulation of the active oxygen forms and induces Fas/Apo-1-associated apoptosis of alveolar epithelium [19].

Besides, in young smokers immune cells activity grew statistically significantly, which was proved by the changes in sCD8 and sCD25 levels (in the smoking group they appeared to be statistically significantly high: $p = 0.029$ and $p = 0.0001$, respectively) as well as the level of sCD54 adhesion molecules total fraction ($p = 0.025$). By contrast, oligomeric fraction values were somewhat increased in non-smokers ($p = 0.05$).

Other authors also found increase in both cellular expression and serous level of soluble adhesion molecules

Table 3

The level of soluble differentiating molecules and histocompatibility molecules in the serum of smoking and non-smoking students (Me [25; 75])

Index (U/ml)	Non-smokers	Smokers	p
sCD95s	372.58 [244.45; 449.20]	533.60 [238.47; 954.57]	0.011
sCD95ol	241.20 [219.17; 287.80]	276.45 [251.33; 358.90]	0.010
sCD8	382.80 [369.55; 389.77]	496.10 [366.73; 1052.33]	0.029
sCD38s	196.20 [184.13; 206.13]	177.15 [107.18; 318.07]	0.523
sCD38ol	254.90 [234.38; 261.25]	249.50 [150.35; 334.05]	0.880
sCD25	405.20 [397.25; 414.58]	526.5 [435.9; 645.8]	0.0001
sCD54s	65.70 [56.0; 70.25]	75.65 [63.95; 95.22]	0.025
sCD54ol	148.65 [130.18; 170.82]	127.40 [97.18; 177.20]	0.05
sCD50	361.50 [333.68; 416.28]	373.60 [317.50; 571.05]	1.0
HLA-I	1041.8 [966.9; 1109.3]	1601.8 [1134.7; 1987.6]	0.003
HLA-DR	104.30 [91.70; 115.93]	266.60 [116.05; 337.70]	0.002

under the influence of active and passive smoking [20]. Recently it has been shown that tobacco smoke extract and the mediators of lavage fluid extracted from the smokers' distal bronchi elevate ICAM-1 expression and adhesion properties of neutrophils, moreover, they induce pro-inflammatory BLT2-receptors triggering tissue damage mechanisms [21]. The published findings of 10-year-long multi-ethnic investigation (MESA Lung Study) involving 1,865 participants also emphasize that in the general population high sICAM-1 level serves as an independent marker of progressing lung emphysema [22].

Earlier, our working group [23] has established, that longtime tobacco smoking and COPD progressing is associated with the low level of soluble adhesion antigens which correlates with a more extensive microbial colonization of the lower respiratory tract. The revealed decrease in the serous content of molecules under study may indicate the adverse effect of antibiotics and systemic glucocorticosteroids on the immune status during hospital treatment of obstructive pulmonary disease aggravation.

In recent years, the interrelation between adhesion molecules, inflammation and oxidative stress have been actively discussed. It has been demonstrated on the cell culture of retinal pigment epithelium that inflammatory TNF- α effects are mediated by induction of the active oxygen forms and increasing ICAM-1 expression [24]. As the experiment on mice showed, antioxidants (α -tocopherol), in their turn, elevate the resistance to bacterial pneumonia modulating neutrophil recruitment to the lungs by impairment of ICAM-1 antigen expression, its ligand and other adhesion molecules [25]. The data obtained by us support the idea of existing interrelation between smoking-induced oxidative stress, adhesion molecules and soluble leucocyte antigens participating in apoptosis regulation and immune cell activation. This has been proved by the works of other authors as well.

So, the analysis of smokers lung tissue samples has shown that T cell inflammation with prevailing CD8⁺ cytolytic lymphocytes is the principal response to antigenic stimuli which is induced by tobacco smoke, and the pathogenesis of emphysema is associated with activation of CD95-dependent apoptosis [26].

The average values of HLA-I and HLA-DR histocompatibility molecules statistically significantly grew in smoking students ($p=0.003$ and $p=0.002$, respectively). The latter may testify of the activation of humoral and macrophage components of immune system response realized by CD4⁺ T-lymphocytes in young beginning smokers. According to the data obtained by other researchers,

histocompatibility molecules class I play a key role in COPD pathogenesis and their level can be regarded as an indicator of this disease severity [27].

Previous investigations [28] show that smoking students of the I–VI courses may already have mild obstructive pulmonary lesions. This research work convincingly demonstrates that these changes are preceded by significant adaptive processes involving many components such as antioxidant process activation and immune shifts. Thus, modern research data on oxidant–antioxidant system as well as the levels of differentiation and histocompatibility molecules may play a key role in understanding the subtle mechanisms of smoking impact on the respiratory system at the “pre-obstructive” stage and in identifying chronic bronchi inflammation predictors.

Conclusion. In young smokers smoking is accompanied by compensatory immune reactions inducing antioxidant system activation and decreasing LPO intensity. Their erythrocyte SOD level rises and the indices of maximum luminescence intensity I_{max} and S light sum reduce significantly.

The first years of tobacco smoking are associated with the activation of protection mechanisms in the organism: increase in sCD95 apoptosis inhibitor level, augmentation of immune cell activity and the level of the total fraction of sCD54 adhesion molecules. Moreover, the values of HLA-I and HLA-DR histocompatibility molecules are observed to grow which may speak of the activation of humoral and macrophage components of the immune response.

The modern research methods of oxidant–antioxidant system as well as the levels of differentiation and histocompatibility molecules allow to study the subtle mechanisms of smoking impact on the respiratory system at the “pre-obstructive” stage and to identify the predictors of chronic bronchi inflammation development.

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Conflicts of Interest. The authors have no conflict of interests to disclose.

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