

## FREE RADICAL OXIDATION CHARACTERISTICS IN BIOLOGICAL MEDIA IN OFTEN ILL CHILDREN AND CHILDREN WITH ASTHMA

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It was performed a comparative study of the free radical oxidation parameters in various biological media in children with diseases of the respiratory system – asthma and frequent acute respiratory diseases. The study involved 215 children, who were divided into 3 groups: 58 healthy children (group 1 – control), 74 children (group 2) – frequently ill children, 83 children (group 3) – children with asthma. The changes in the indicators of free radical oxidation intranasal lavage and exhaled breath condensate groups often ill children and children with asthma are manifestations of systemic disorders in the body of patients surveyed. Similar shifts are identified and in terms of the peripheral blood of these patients: in frequently ill children and children with asthma observed a significant increase in the concentrations of diene conjugates and malondialdehyde relative to the control level, while the value of the activity of antioxidant enzymes superoxide dismutase and catalase were significantly reduced relative the corresponding values in the control group of children. The identified biochemical changes confirm that a better balance of pro- and antioxidant systems of the body should be a prerequisite for complex treatment and preventive measures undertaken for children with common infectious diseases in the developing allergic background.

**Keywords:** free radical oxidation, bronchial asthma, often ill children, upper respiratory tract

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It is generally known that in the body of patient with the lung disease and disease of upper respiratory system, the changes develop on the cell and molecular levels as a result of pathological process which are virtually lack in normal [2–4]. These changes can be examined with the help of laboratory analysis of biological samples, for example, fluids are getting from bronchoalveolar lavage, nose irrigation, blood test and lung biopsy.

It is known at the present time that the changes of process of Free Radical Oxidation (FRO) and the functional disorder of Antioxidant System (AOS) make a valuable contribution to development of some diseases which are concomitant with the changes of cytochemical descriptions of upper respiratory tract. In particular, the active forms of oxygen play important role in the pathogenesis of bronchial asthma (BA) [1, 3, 12]. The courses of all pathological conditions that concomitant with concentration increase of active forms of oxygen are characterized by oxidation stress, including acute respiratory disease of ailing children (AC) [4, 9].

At the present time a lot of works devoted to study the role of active forms of oxygen in different pathological processes concerning survey and experimental descriptions, this is indicative of recognition basic meaning of active forms of oxygen in pathogenesis of different diseases [5, 14, 15]. The main mechanism of progress of oxidizing medium connected with free radical damage of plasmatic, mitochondrial and nuclear membrane, nuclear and mitochondrial genome, blood lipoprotein that lead to damage the vessels and histohematogenous barriers [8]. At the same time the report about pathological role of FRO processes and condition disorder of AOS in pathology of lungs and upper respiratory tract is not systematized. This is relevant in full to the assess-

ment of description of these processes for those children with different pathological respiratory system, in particular, those children who suffer from bronchial asthma and ailing children who have periodically acute respiratory disease. There is no such kind of information in accessible literature.

**The aim of work** is comparative inquiry of characteristics of Free Radical Oxidation in different biological environments of children with respiratory system disease.

### Materials and methods of research

There was checkup of 215 children on the basis of allergology department, Osh Regional Children Clinical Hospital and they were divided into three groups:

- 58 healthy children (1<sup>st</sup> group-control);
- 74 children (2<sup>nd</sup> group) ailing children;
- 83 children (3<sup>rd</sup> group) suffer from bronchial asthma.

There is distribution of patients according to age, gender and average lasting of disease in the following Table 1.

There was organized condensate collection of expired air by G.V. Belov's method (2005) [2] and also collection of rhinal lavage by general method. Concentration of some matters and indicators were tested in these biological environments: levels of total lipids, hydroperoxides, diene conjugates, oxidation index.

As well there was evaluation of estimate activity of Free Radical Oxidation processes and Antioxidant System patients that had been examined. At the same time there was tested concentration of diene conjugates, malonic dialdehyde, activity of catalase and superoxide dismutase (SD). Identification of malonic dialdehyde in blood was tested by fluorimetric method which is based on that thiobarbituric acid interact with low-molecular dialdehyde (mainly with malonic) in acid medium making pink color [7].

Identification of diene conjugates in blood plasma was implemented by UV absorption of heptanoic and isopropanolamic extracts which is based on measuring intensity absorption approximately 232–234 nm that specified with conjugative dialdehyde structures (beforehand extracted from plasma) which is appeared in forming hydroperoxides polyunsaturated fatty acids [7].

Table 1

Distribution of patients according to age, gender and average period of disease

Characteristics		1 <sup>st</sup> group (control group) <i>n</i> = 58	2 <sup>nd</sup> group (ailing children) <i>n</i> = 74	3 <sup>rd</sup> group (children suffer from bronchial asthma) <i>n</i> = 83
Age		13,9 ± 2,1	13,7 ± 1,8	12,8 ± 2,6
Gender	Female	30 (51,7%)	39 (52,8%)	45 (54,2%)
	Male	28 (48,3%)	35 (41,3%)	38 (45,8%)
Period of disease (year)		–	9,9 ± 2,1	5,7 ± 3,5

The activity of catalase was determined using spectrophotometric method which was based on identification of speed decomposition of hydrogen peroxide mm/m (wavelength 230 nm). There was added ethanol for stabilization hemolysate and decomposition of complex catalase H<sub>2</sub>O<sub>2</sub> [7].

Identification of superoxide dismutase activity was implemented by the method that suggested V.A. Kostjuk and others (1990) which was based on oxidation reaction of quercetin [6].

Statistical analysis of data was arranged with the help of program package Statistica 8.0. To determine the statistical important differences of characteristics, in those groups where the patients were examined, U-criterion Manna Whitnye (distribution of parameters value fundamentally differ from normal as shown Kolgomorov-Smirnov's test). The results were evaluated as statistical important in  $p < 0,05$  value.

#### Results of research and their discussion

The analysis of FRO of endonasal lavages showed that concentration of total lipids at the end was  $0,492 \pm 0,023$  mg/ml, and the levels of this parameter were significantly low  $0,426 \pm 0,021$  and  $0,412 \pm 0,031$  mg/ml in groups of Ailing children and Children suffer from bronchial asthma (Fig. 1).

Level of hydrogen peroxide content in endonasal lavages of examined children in control group was  $0,242 \pm 0,016$  mg/ml, the

results in group of Ailing Children was a bit higher  $0,267 \pm 0,016$  mg/ml, its meaning significantly did not exceed like that during the control. Children suffer from bronchial asthma had different result and it was higher in 1,5 size than in control, significantly exceeding this level and also exceeding adequate meaning level in second group (Ailing Children).

Concentration comparison of diene conjugates showed that the level of this result was  $0,045 \pm 0,011$  mg/ml in the group of ailing children rather like the level in control  $0,037 \pm 0,004$  mg/ml. The meaning of this result was maximum  $0,051 \pm 0,003$  mg/ml in group of children suffer from bronchial asthma significantly exceeding the control level (Fig. 2).

Evaluation results of oxidation index brought out significant differences in group of examined children. Like this, if the level was  $0,483 \pm 0,019$  in control group, the meaning of this result was significantly higher ( $p < 0,05$ ) –  $0,628 \pm 0,021$  in the group of Ailing Children. The level of oxidation stress was maximum  $0,849 \pm 0,034$  in the group of children suffer from bronchial asthma and significantly exceeded ( $p < 0,05$ ) the same levels of first and second groups (Fig. 3).

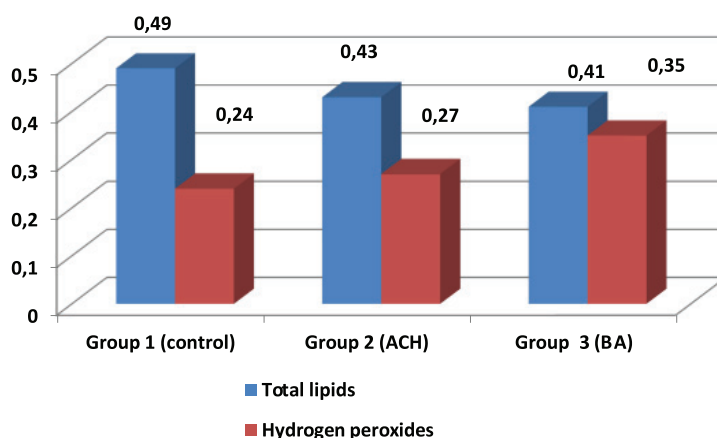


Fig. 1. Concentration of total lipids and hydrogen peroxides in rhinal lavages of examined children

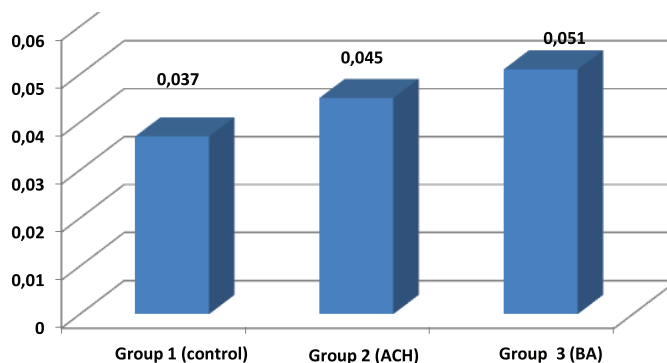


Fig. 2. The levels of diene conjugates in rhinal lavages of examined children

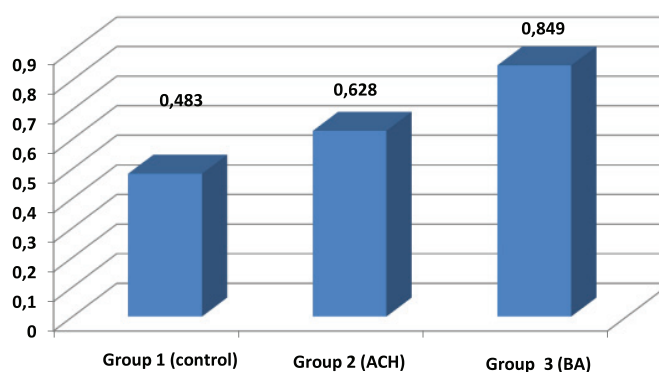


Fig. 3. Oxidation index of rhinal lavages in examined children

The process investigations of Free Radical Oxidation in condensate of expired air testified that the concentration of total lipids was  $0,106 \pm 0,007$  mg/ml in control group. The level of these parameters in the group of Ailing children and Children suffer from bronchial asthma were a bit lower  $0,093 \pm 0,0005$  and  $0,091 \pm 0,008$  mg/ml but significantly intergroup differences were no found (Fig. 4).

The level content of hydrogen peroxide in condensate of expired air (CEA) in control group was  $0,054 \pm 0,006$  mg/ml, the meaning of this parameter in the group of Ailing Children was significantly higher ( $p < 0,05$ ) –  $0,072 \pm 0,007$  mg/ml and this result in the group of children suffer from bronchial asthma was more higher  $0,079 \pm 0,005$  mg/ml significantly exceeded ( $p < 0,05$ ) the level of control group.

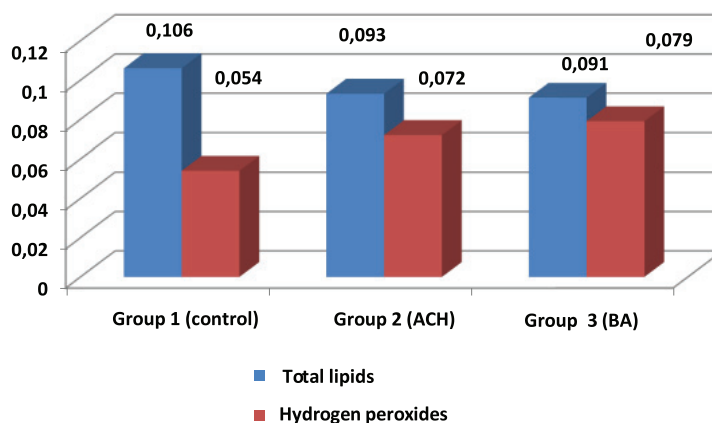


Fig. 4. Concentration of total lipids in condensate of expired air in examined children

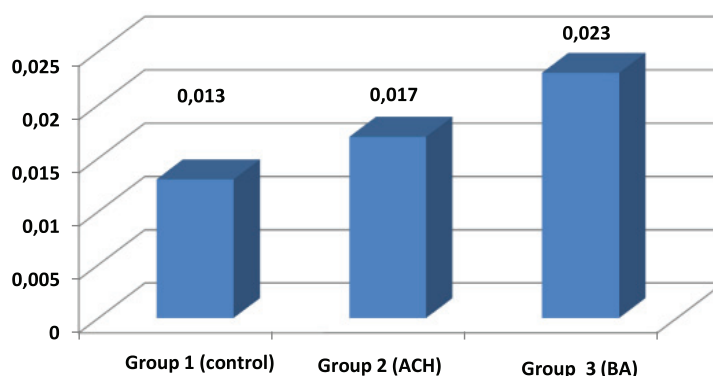


Fig. 5. The levels of diene conjugates in condensate of expired air in examined children

The level comparison of diene conjugates showed that the level of this result in the group of Ailing Children was  $0,017 \pm 0,004$  mg/ml considerably having no differed from the result in control –  $0,013 \pm 0,002$  mg/ml. The maximum meaning of this result was  $0,023 \pm 0,003$  mg/ml in the group of children suffer from bronchial asthma which significantly exceeded ( $p < 0,05$ ) the same levels in control group (Fig. 5).

The meaning comparison of the result of Oxidation index showed significant differences in the groups of examined children. The level in the control group was  $0,485 \pm 0,018$ , the meaning of this result in the group of Ailing Children was significantly higher ( $p < 0,05$ ) –  $0,781 \pm 0,021$ . The level of Oxidation stress in the group of children suffer from bronchial asthma was maximum  $0,856 \pm 0,033$ , that significantly exceeded ( $p < 0,05$ ) the same levels in the first and second groups (Fig. 6).

Probably, it says that brought out changes of results of Free Radical Oxidation in endonasal lavages and in condensate of expired air are become apparent of systemic disturbance in the body of examined patients from the group of Ailing Children and Children suffer from the bronchial asthma. Analogous changes certainly should be uncovered in peripheric blood of this contingent of patients which were showed in our further investigations.

The comparative evaluation characteristics of FRO and Antioxidant System of peripheric blood from examined children showed some changes as in the Fig. 2. Evidently the concentration of diene conjugates and malonic dialdehyde of ailing children and children suffer from bronchial asthma are significantly higher ( $p < 0,05$ ) relatively to control level at the same time the meaning of enzyme activity of antioxidant system of superoxide dismutase and catalase were significantly reduced relatively the same meanings in control group.

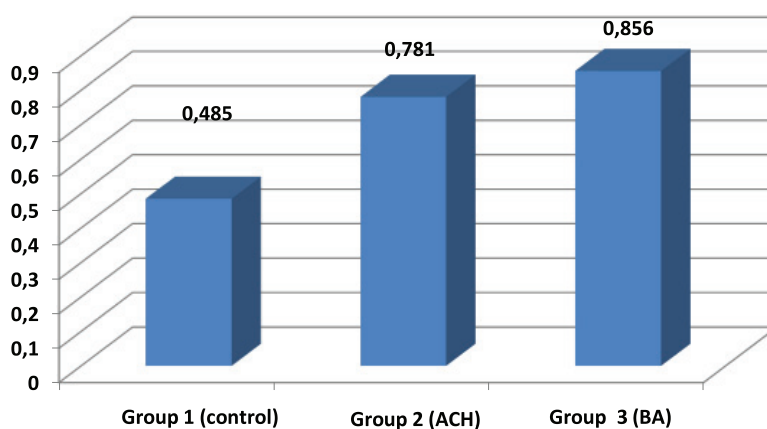


Fig. 6. Oxidation Index Condensate of expired air in examined patients

Table 2

Characteristics	Groups of children		
	Group 1 (control) (n = 58)	Group 2 (Ailing children) (n = 74)	Group 3 (Children suffer from bronchial asthma) (n = 83)
Diene conjugates (units of relative density/mg of gen. lipids)	0,212 ± 0,029	0,374 ± 0,52*	0,414 ± 0,77*
Malonic dialdehyde (units)	2,08 ± 0,35	3,67 ± 0,36*	3,85 ± 0,21*
Superoxide dismutase (units/mg Hb)	1,72 ± 0,41	0,62 ± 0,19*	0,55 ± 0,08*
Catalase (CAT) (ME/g Hb)	28,5 ± 3,2	13,8 ± 3,9*	17,3 ± 2,8*

Note: the difference of authentic (in  $p < 0,05$ ) relatively appropriate meaning of indicators of control group.

### Conclusion

Thus, at the present time described role of the processes of FRO in several homeostatic processes, including – the basis of synthesis of many biologically active compounds – leukotrienes, purine deoxyribonucleotides, uric acid, high energy compounds – the changes of concentrations in tissues may contribute to a number of pathological state [11, 13]. The active forms of oxygen (AFO) and free radicals are formed mainly in the sequential accession electrons to oxygen in the process of lipid peroxidation (LPO). LPO is one of oxygen utilization pathways in the cell, membranes. LPO is a defensive reaction that facilitates the updating of phospholipid membranes [9, 10, 14]. It is obvious that these changes are manifestations of the processes occurring at the molecular and cellular levels in the mucosa of the upper respiratory tract and pathologies under consideration underlying the pathogenesis of nosology. One of these ways is the pathogenesis changes of free radical oxidation and violations of antioxidant protection.

The investigation has shown that ailing children and children suffer from bronchial asthma observed that increased activity of free radical oxidation, in particular increasing the concentration of malonic dialdehyde and diene conjugates in blood plasma and in the KBB. At the same time it showed a reduction in the activity of the antioxidant system in these patients, which manifested weakening enzyme systems – superoksiddissmutazy (SOD) and catalase. The identified biochemical changes confirm that the view expressed by many authors the optimize the balance of pro- and antioxidant systems of the body and should be an important mechanism and a necessary condition for complex treatment and preventive measures undertaken for children with frequent infections, developing on an allergic background.

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