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Minimally Invasive Methods of Diagnosis of Bronchitis Severity in Children.

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ABSTRACT

The development of new minimally invasive differential diagnostic and prognostic criteria of severity of the various forms of bronchitis in children. Total 127 hospitalized children aged 5 to 14 years with various forms of bronchitis were examined. 78 of them suffering from recurrent bronchitis were attributed to group 1; and 49 children with acute bronchitis were attributed to group 2. The control group consisted of 35 conditionally healthy children. A special complex study included the determination of the indicators of oxidative stress using the model of oral neutrophils and the oral colonization resistance indicators. It was revealed that the children had significantly lower colonization index and anti-adhesive saliva activity than the control group, while the lowest value were observed in group 1. Children with acute bronchitis had increase in value of the spontaneous and induced luminol chemiluminescence as compared to control, while in case of recurrent bronchitis these values were below the reference value. Indicators of the induced luminol chemiluminescence in healthy children reached their maximum, in group 2 they tended to decrease, but decreased to minimum values in group 1. The use of minimally invasive diagnostic methods shows promise in pediatric pulmonology. Subject to severe defects of the mucosal protection system, the patients with recurrent bronchitis are extremely "vulnerable" to repeated bacterial infections (diseases). This dictates the need to conduct the targeted preventive and rehabilitation measures in this group.

Keywords: children, bronchites, minimally invasive diagnostics

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INTRODUCTION

Adverse changes in the health status of the population and a high level of depopulation, which occurred in the 90s of XX century, have caused a complex medical and demographic situation and drawn attention to the problems of the health of children and adolescents. In recent years, according to statistics, our country has shown negative dynamics of most health indicators of the younger generation.

The leading diseases in the structure of early childhood, preschool- and school-age morbidity are respiratory diseases [6]. Bronchitis accounts for 30-40% in the structure of respiratory diseases in children of these age group [5]. At the same time, despite the current knowledge on the etiology and pathogenesis of the respiratory tract diseases and the availability of a wide range of medicines, this class of diseases remains significant in both children and adults, due to their high incidence and prevalence [1]. Furthermore, it should be noted that frequent respiratory infections in children can subsequently act as a trigger factor for their chronic bronchopulmonary pathologies, including bronchopulmonary asthma [2, 9]. In turn, the problem of recurrent respiratory disease affects not only the medico-social, but also economic aspects, such as temporary working incapacity of parents due to child care and high-cost treatment of exacerbations.

One of the leading components in the development of infectious diseases is the functional state of the cell membranes and the activity of lipid mediators of inflammation, determining the lipid peroxidation processes in the damaged zone [3]. Their excessive accumulation is a pathogenetic component of most diseases of bronchopulmonary system. The main prerequisite for the implementation of lipid peroxidation is the formation of reactive oxygen species [8]. By consuming oxygen, the neutrophils form an oxygen-dependent bactericidal system for the elimination of the homeostasis-disturbing agents. To evaluate the oxygen-dependent metabolism of neutrophils, the methods of spontaneous (SL CL) and induced (IL CL) luminol chemiluminescence have been applied [4].

Factors of local nonspecific resistance become more important in children of different ages in infectious diseases of bronchopulmonary apparatus [10]. It has been shown that a decrease in their activity is accompanied by an increase in the risk of diseases of both the upper respiratory tract and the bronchi and pulmonary parenchyma. One of the mechanisms of nonspecific resistance is colonization resistance (CR) of the buccal epithelium [7]. CR indicators not only allow for assessment of the adaptation degree of the child body to adverse environmental effects, but also can be used to monitor the effectiveness of the treatment.

Objective of the study was the development of new non-invasive differential diagnostic and prognostic criteria of severity of the various forms of bronchitis in children.

PATIENTS AND RESEARCH METHODS

The study was conducted on clinical bases of the Department of Pediatrics of Kazan State Medical University. The special methods of investigation were applied in a laboratory for the development of fungal allergens of SBFSI "Kazan Research Institute of Microbiologic Epidemiology".

The study involved 127 children suffering from bronchitis, which were divided into 2 groups: the first group consisted of 78 children with recurrent bronchitis, and the second - of 45 children with acute bronchitis. The control group consisted of 35 healthy children. The criteria for inclusion of children in the study group were: age from 5 to 14 years, a verified diagnosis of bronchitis, the absence of exacerbation signs of chronic diseases for at least 6 months prior to the study, and a signed informed consent of the parents. Exclusion criteria were age younger than 5 years and older than 14 years, other forms of acute respiratory disease, presence of exacerbated concomitant diseases, pathological changes of the oral mucosa and teeth, and the parents' disagreement.

Diagnostic algorithm included medical history taking, a pediatric examination, assessment of physical status, for indications - consultation of dentist, otolaryngologist, neurologist and other specialists. The paraclinical methods of examination such as blood tests and urine tests were conducted, and for indications - biochemical blood tests, instrumental examinations (chest X-ray, ultrasound of internal organs, etc.).

Minimally invasive methods were used to determine the indicators of colonization resistance of the oral cavity, as well as the index of buccal epithelial cells colonization (BCI) and anti-adhesive activity of saliva (AASA). The indicators of oxygen-dependent metabolism of neutrophils were also recorded by spontaneous (SL CL) and induced luminol chemiluminescence (IL CLD).

BCI was determined by the method by A.N. Maianskii et al., based on a quantitative calculation of "oral streptococci", adhering *in vivo* to the buccal epithelial cells of a child. The results were expressed in points using the following grading: 0 points - 0 to 10 "oral streptococci" on one buccal epithelial cell, 1 point - 10 to 30, 2 points - 30 to 100, 3 points - 100 to 300, 4 points - more than 300 "oral streptococci" on one buccal epithelial cell. Based on these data, the buccal colonization index was calculated by the formula: $(0 \times n + 1 \times n + 2 \times n + 3 \times n + 4 \times n) / 50$, where n - the number of epithelial cells with different (0 - 4) degree of colonization.

AASA was determined by the method by J. Ofek and E. Beachey in modification by I.V. Maianskii et al. The method consists in assessing the ability of the patient's saliva to prevent the adhesion of bacterial cells to buccal epithelial cells of the donor. As the microbial cell, the culture *C. albicans* was used - a standard strain No.4 from the collection of Kazan Research Institute of Microbiologic Epidemiology. The results were expressed in conventional units (c.u.).

For more in-depth assessment of the homeostatic reserves, the indicators of oxidative stress were studied on a model of oral neutrophils of 73 children who participated in the study. Saliva sampling for the registration of SL CL and IL CL was performed as follows: children rinsed their mouth morning fasting with 10 ml 0.9% NaCl solution for 2 minutes, then the washings were placed in vials and centrifuged at 1500 rpm for 5 minutes. The contents were washed with a solution of 0.9% NaCl. The evaluation was conducted in the number of pulses per minute.

Mathematical analysis involved calculations of the following parameters: arithmetic mean, random and mean square error, index of difference significance by Mann-Whitney test for unrelated samples and the Wilcoxon test for linked samples. The relationship between the studied features was assessed using the Spearman's test.

RESULTS

The analysis of anamnestic data showed that the frequency of recurrent bronchitis in group 1 within 12 months preceding the examination ranged from 3 to 6 times, averaging 4.7 ± 0.9 times. The analysis of the distribution of children of group 1 in accordance with the frequency of bronchitis relapses has shown that almost half of the group were children having 4 or more episodes annually, with short-lived and unstable remission between the bronchitis relapses.

Colonization index in the control group averaged 1.67 ± 0.2 points. Individual analysis of this indicator in conditionally healthy children found its variation from 0.85 to 2.94 points. Since most of the children (68% - 23 persons) had BCI matching (or exceeding) 1.21 points, we took this level as the lower normal limit. Since the value of the colonization index in girls did not differ from that of boys (0.81 ± 0.09 and 0.72 ± 0.054 points, respectively, $p > 0.05$), the subsequent analysis of this indicator was carried out regardless of gender.

Colonization index in children with recurrent bronchitis was significantly lower as compared with the control, and only 5 (7%) patients had their buccal colonization index within normal limits. The average value of the indicator amounted to ___ points ($p < 0.05$) subject to the individual fluctuations from 0.39 to 1.09 points.

BCI in the group 2 averaged 0.74 ± 0.03 points, which was significantly lower than the control values ($p < 0.05$), but significantly higher than its value in group 1 ($p < 0.05$). The results are shown in Table 1 below.

Thus, the majority of children with recurrent bronchitis showed a significant reduction in the colonization by obligate microorganisms ("oral streptococci") of such important biotope as the oral cavity.

Table 1. Average BCI (points) in the study groups

group 1	group 2	Healthy children
0.63±0.04*	0.74±0.03*	1.67±0.2

* p<0.05 as compared with control.

Correlation analysis revealed that the incidence of recurrent episodes of bronchitis in children of group 1 was inversely related to the values of colonization index ($r=-0.54$, $p<0.05$). In other words, the less intense the buccal colonization by "oral streptococci" was, the more bronchitis relapses were recorded.

Analysis of the results of AASA evaluation revealed individual variations of the index from 0.49 to 0.86 c.u., while the average value in the control group was 0.68 ± 0.04 c.u. Given that 76% of children (25 persons) of the control group had indices equal (or exceeding) 0.62 c.u., the level of anti-adhesive activity was taken as a conventional lower normal limit.

Children with acute bronchitis had quite low anti-adhesive saliva activity - 0.35 ± 0.03 c.u. ($p<0.001$). Individual values ranged 0.66-0.11 c.u., and only in 7% (3) of cases the values corresponded to normal.

AASA in children with recurrent bronchitis was significantly lower than in children of group 2 - 0.24 ± 0.06 c.u. ($p<0.05$) and control ($p<0.001$). Variations in individual values among them ranged 0.1-0.51 c.u., and in no case the "normal" AASA values have been recorded. The results are shown in Table 2 below.

Table 2. Average AASA (points) in the study groups

group 1	group 2	Healthy children
$0.24\pm0.06^{**}$	$0.35\pm0.03^{**}$	0.68±0.04

* p<0.05 as compared with control.

* p<0.005 as compared between the groups and with control.

It is important to emphasize that the level of anti-adhesive saliva activity as a basic component of colonization resistance is closely related to the formation of respiratory diseases in children. It is possible that the depletion of anti-adhesion, hyporesistance-forming factors is a marker and a trigger of the bronchitis relapse. This assumption has been confirmed by the results of the correlation analysis, which found an inverse relationship between the bronchitis recurrence rate and the AASA index ($r=-0.61$, $p<0.01$). Consequently, the lower the anti-adhesive saliva activity is, the more often the disease episodes occur.

To deepen the concepts of the state of homeostatic reserves in children with various forms of bronchitis, the indicators of oxidative stress were investigated on the model of oral neutrophils.

Table 3 shows the indicators of both spontaneous and induced luminol chemiluminescence (SL CL and IL CL).

Table 3: Indicators of luminol chemiluminescence in the study groups (pulses per minute).

Indicators	SL CL (pulse/min)	IL CL (pulse/min)
group 1 (n=46)	$104\pm2.9^{**}$	$91.7\pm2.2^*$
group 2 (n=29)	$147\pm2.4^{**}$	$151.2\pm3.1^{**}$
Control (n=19)	10.1 ± 1.9	164.1 ± 3.8

* p<0.05 as compared with control.

** p<0.05 as compared between the groups

*** p<0.05 as compared between the groups and with control.

It was noted that the children of group 2 had significantly higher values of SL CL as compared with control. This indicates an adequate generation of reactive oxygen species and oxygen-dependent metabolism of blood cells in response to the development of an acute infectious process. Conversely, the recurrence of the

inflammatory process in the bronchial tree, apparently, leads to a reduction of neutrophil bactericidal activity as evidenced by significantly lower average values of SL CL in group 1 as compared with group 2 ($p < 0.05$).

In our opinion, the situation is aggravated by insufficient reserves of antimicrobial protection of oral neutrophils in children with recurrent bronchitis, which can clearly be seen in the study of the IL CL indicators. While the group of conditionally healthy children had maximum IL CL values, the development of acute bronchitis promoted their decrease, and the recurrent bronchial inflammation resulted in maximally decreased IL CL values and significantly different not only from the control values, but also from the indicators of group 2 ($p < 0.05$).

The obtained results allow us to state that subject to severe defects of the mucosal protection system, the patients with recurrent bronchitis are extremely "vulnerable" to repeated bacterial infections (diseases). This dictates the need to conduct the targeted monitoring over the health state due to the risk of process chronization.

Thus, this study demonstrates wide opportunities for the non-invasive evaluation of homeostatic reserves in children with bronchitis in order to determine both the treatment tactics and prognosis of an inflammatory process. The simplicity and accessibility of the used methodical approaches allows to apply them as screening methods in the general pediatric practice, including the outpatient stage, for identification of children at risk for the formation of recurrent and chronic bronchopulmonary diseases.

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