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Microbiological Methods for Determination of Respiratory Tract Microorganisms in Chronic Infection

Fayzullaeyva Zamira Rahmatovna
Tashkent Medical Akademy, Associate Professor of the Department of Microbiology, Virology, Immunology, Ph.D. Uzbekistan

Buriev Abdumalik Yavkochdievich
Karshi State University, Uzbekistan

Abstract: The great social significance of ear, nose and throat diseases is determined by their high incidence in childhood. The prevalence of chronic otorhinolaryngological pathology in children in Uzbekistan ranges from 181.9 to 465.0 per 1000 children, with adenotonsillar pathology occupying a leading position.

Keywords: Staphylococcus aureus, MRSA, MSSA, polymerase chain reaction. Staphylococcus aureus, MRSA, MSSA, polymerase chain reaction.

The problem of children who are often and long-term ill (CHI) remains relevant. Among young children attending kindergartens, the group of children who suffer from frequent and long-term respiratory diseases ranges from 40 to 50% [1.2]. In 40% of cases, by the age of 7-8 years, children with acute respiratory infections develop a chronic pathology, and the risk of chronicity is directly proportional to the increase in the frequency of acute respiratory infections episodes during the year [14]. The work of domestic and foreign researchers has shown the etiological and nosological heterogeneity of recurrent respiratory diseases in children with acute respiratory distress [3.8].

A feature of the modern course of infectious pathology in children is the frequent combination of etiological factors, including viruses, bacteria, fungi and parasitic pathogens. Many infectious agents have the ability for long-term active persistence - representatives of the Herpesviridae family, “atypical” pathogens from the Chlamydiaceae and Mycoplasmataceae families [4.7], bacterial flora of the upper respiratory tract [6.9.14].

Moreover, herpes viruses can cause significant disturbances in the immune status of the macroorganism, thereby forming a vicious circle: chronic active herpes virus infection - secondary immunodeficiency, against which frequent acute respiratory viral infections are observed [10.5.15], recurrent bacterial and parasitic diseases [12.11.13].

Purpose of the work: to present the etiological structure of persistent infections in children with recurrent respiratory diseases and to evaluate the effectiveness of etiopathogenetic therapy.

Materials and research methods:

43 children aged 1 to 17 years who applied to the clinic for frequent long-term and complicated respiratory diseases (otitis, tonsillitis, sinusitis, stenosing laryngotracheitis, bronchitis, pneumonia) underwent a comprehensive clinical, anamnestic and laboratory examination which

included: a clinical blood test, a general urinalysis, determination of the level of alanine aminotransferase and antistreptolysin-O (ASL-O), blood and saliva testing using the polymerase chain reaction to detect DNA of type 4 herpesviruses - Epstein-Barr virus (EBV), Type 5 - cytomegalovirus (CMV) and herpes type 6 (HHV-6) determination of IgM antibodies to the capsid antigen, IgG to the early and nuclear antigen of EBV, IgM and IgG to CMV by enzyme-linked immunosorbent assay (ELISA). The qualitative composition of aerobic and facultative anaerobic micro flora of the nasal and or pharyngeal mucosa was assessed using a semi-quantitative bacteriological (cultural) method.

Children with repeated diseases of the lower respiratory tract (bronchitis (n=34), pneumonia (n=7)), as well as those with a long-term cough (n = 16), were examined by ELISA for IgM and IgG antibodies to Mycoplasma PNUmonia and Chlamydophila PNEUMONIA, as well as using the PCR method on the DNA of these pathogens in the secretions of the upper respiratory tract. Results and its discussion:

The average age of the observed children was 5.8 ± 0.9 years. Children from 1 to 2 years old made up 14.8%, from 3 to 6 years old - 51.0%, from 7 to 11 years old - 18.1% and from 12 to 18 years old - 16.0%. Boys predominated among those who applied (63%). Among children under 7 years of age, 85.6% attended preschool educational institutions. 35.8% of children with acute respiratory illnesses had monthly episodes of respiratory disease, 32.9% had from 6 to 10 episodes with a “light interval”, usually in the summer months.

Frequent acute respiratory infections (ARI) in an uncomplicated form were suffered by 18.9% of children with acute respiratory infections, 8.6% of patients complained of recurrent sore throats, otitis - 16.0%, sinusitis - 7.0%, stenosing laryngotracheitis - 2.5%, bronchitis - 14.0%, pneumonia - 2.9%. In 39.1% of children with acute respiratory syndrome, long-lasting inflammatory phenomena in the ENT organs were observed: symptoms of chronic adenoiditis in 30.5%, chronic tonsillitis in 6.2%, recurrent sinusitis in 3.3% of children.

The most frequent use of antibiotics was observed in groups of children aged 3 to 6 years - 5.4 ± ± 0.2 times per year, and in children aged from 1 to 2 years - 4.9 ± ± 0.3 times, in children with children In younger schoolchildren, antibiotics were used 3.8 ± 0.2 times per year, and in children over 12 years old - 3.3 ± ± 0.2 times per year.

In a bacteriological examination of the nasopharynx and oropharynx, pathogenic and conditionally pathogenic microflora in etiologically significant quantities (>104 CFU/ml) were determined in 47.3% of children with children with acute respiratory syndrome. Growth *Str. Pyogenes* (β-hemolytic streptococcus of group A) was determined in 23 children (9.5%), among them, elevated ASL-O titers were determined in 17 children. Among the entire group of BCH children, elevated ASL-O titers were detected in 32 patients (13%). Thus, streptococcal infection was diagnosed in 15.6% of children, and in 2/3 of cases in association with EBV, CMV or herpes type 6 viral infection. Among opportunistic microorganisms, S. aureus was more often detected in diagnostically significant quantities - 38%, fungi of the genus *Candida* - 16%, less often *Str. pneumoniae* - 6%, *H. influenzae* - 5%, *B. catarrhalis* - 4%, *K. pneumoniae* - 3%, *Ps. aeruginosa* - 2%. Moreover, in 18% of children there was an association of two pathogens, and in 7% - three or more. Mycoplasma and chlamydial infections were detected in 16 and 7 children, respectively, which amounted to 28.1 and 12.3% of the 57 examined children with repeated bronchitis, pneumonia or prolonged coughing.

Laboratory improvement was considered to be cases when the causative agent of the disease was not identified during a repeat study after treatment. Six months after the start of treatment, 86.4% of patients showed clinical improvement, but by a year from the start of treatment, such an effect could be noted in 77.8%. In 30.9% of patients, markers of persistent infections were not determined by 6 months after the start of treatment; by 1 year from the start of treatment, such patients remained in 27.2%.

An analysis of the effectiveness of CBA therapy for children depending on age demonstrated the best results in schoolchildren, among whom clinical improvement was observed in 94.3% within a year from the start of treatment. Moreover, in almost half of the schoolchildren, markers of persistent infections were not detected during repeated examinations. The greatest difficulty was in the treatment of BDD in children aged 3 to 6 years. A stable clinical effect over the course of a year was achieved only in 67.5% of children of this age; the majority (86.2%) retained markers of active herpesvirus infections during repeated studies.

Among children under 3 years of age, therapy was effective in 91.7% of children by 6 months; and by 1 year after the start of treatment, the clinical effect remained in 83.3% of patients, despite the identification of markers of active infections during laboratory monitoring. The therapy was highly effective in eliminating group A β-hemolytic streptococcus, the excretion of which decreased from 9.5 Ґ} 1.8% to 0.8 Ґ} 0.5% ($p < 0.001$) and "atypical" respiratory pathogens diseases (chlamydia, mycoplasma), markers of which were not determined in children 6 and 12 months after treatment.Isolation of opportunistic microflora in diagnostically significant titers during bacteriological examination of smears from the upper respiratory tract decreased by 2.2 times (from 41.6 Ґ} 3.1 to 18.5 Ґ} 2.8%, $p << 0.001$). By 1 year from the start of treatment, the frequency of detection of DNA herpes viruses in the blood decreased by 2.7 times (from 60.1 Ґ} 3.2 to 22.2 Ґ} 2.6%, $p < 0.001$), however, viruses decreased slightly (from 86.8 Ґ} Ґ} 2.1% to 67.9 Ґ} 3.0% of patients, $p < 0.001$). The frequency of use of antibiotics in children after treatment of persistent infections generally decreased by 2.2 times. A particularly significant decrease was noted in the group of schoolchildren - 3.5 times (from 3.3 Ґ} 0.4 times a year to 0.7 Ґ} 0.1, $p < 0.001$).

The presented method for diagnosing persistent infections in children with recurrent respiratory infections made it possible to carry out differentiated etiopathogenetic therapy and immunorehabilitation of this group of patients, as a result of which 78% of children achieved stable normalization of their condition within a year - relief of catarrhal, lymphoproliferative and asthenic syndromes, reduction in incidence to 6 or less episodes of ARI per year in preschool children and 4 or less episodes in schoolchildren.

Conclusions

1. The recurrent course of respiratory diseases in children is in the vast majority of cases associated with persistent infection.
2. The most frequently identified causative agents of persistent infections are representatives of the Herpesviridae family (herpes virus type 6, Epstein-Barr virus and cytomegalovirus), group A β-hemolytic streptococcus, and Staphylococcus aureus.
3. Differentiated etiopathogenetic therapy and immunorehabilitation of children with acute respiratory distress, depending on the diagnosed persistent infection, allows achieving stable normalization of the condition in 78% of cases; reduce the frequency of antibiotic use per year by 2.2 times.

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