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Improvement of Control the Efficiency of Treatment of Cytomegalovirus Infection in Children

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Abstract: As a result, clinical and epidemiological analysis of primary medical documentation and a comprehensive survey of immunocompromised children (61patients at the age of 3 months-7 years, the entire selection) were diagnosed with chronic reactivation of cytomegalovirus infection (CMV) in combination with other opportunistic infections in 57.4% of cases. Antenatal factors at high risk of fetal infection were 95% of patients. Infection, lymphoproliferative and allergic immunopathological syndromes diagnosed in 77%, 54.1% and 44.3%, respectively. For laboratory monitoring treatment efficacy was estimated dynamics viral load by quantitative amplification method CMV DNA in saliva and urine in a polymerase chain reaction (PCR) in real time. Was ranked at a level of viral load (VL): over-high, high, medium, minimum. Minimum level replicative activity cytomegalovirus (500-1000 copies/ml) has been made in 79.3% of cases.

Key words: Cytomegalovirus infection · PCR diagnostics · Children · Immunotherapy · Rehabilitation

INTRODUCTION

Feature of modern pathology of children is a high prevalence of herpes virus infections of the ability to activate against the background of disorders of the immune protection, exerting by an additional immunosuppressive effect and causing a general sensibilization. Cytomegalovirus (CMV), human beta-herpesvirus type 5 (HHV-5)-the most common opportunistic fetal infection [1] identifies in 70.9-78.9% with severe multi-organ lesions [2] and a wide range of obstetric pathology [3]. There is ranked second to HIV by immunosuppressive activity [4]. Chronic relapsing forms of CMV infection are important clinical marker for syndrome impaired immune protection, achieving 24.6% in early ages children Perm region [5].

The social significance of the problem is determined of growing by the infectivity populations with low economic status, reaching even in British and the United States 80% [6]. Most importantly, if among pregnant women in developed countries, this index is 40%, in developing countries up to 100% [7].

Currently, the laboratory verification of CMV used a set of methods: enzyme-linked immunosorbent assay (ELISA) with the definition of anti-CMV IgM, IgG and avidity index IgG [8] and western blot (WB), rapid culture method (RCM) and the polymerase chain reaction (PCR) to amplify DNA qualitative and quantitative real-time mode [9]. Due to100% specificity and sensitivity of PCR detection of DNA is carried out in the organs and tissues, biological fluids such as blood, saliva and urine, which allows to establish an etiological diagnosis, phase of infection non-invasive method that is most appropriate in pediatric practice.

Informative, fast and exact way to measure viral load could hypothetically be used to assess the intensity of viral replication in the dynamics for the appointment of an adequate basic treatment and determining the nature of the subsequent prevention of activation of latent and chronic forms of CMV reactivation. However, the use of quantitative PCR method based on the ranking of the degree of viral load (VL) to evaluate the effectiveness of therapy, CMV infection in children has not yet been developed. The aim of the study was to improve the

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technology for monitoring the effectiveness of therapy of cytomegalovirus infection in children in an outpatient setting.

MATERIALS AND METHODS

Object were immunocompromised children with CMV infection verified by qualitative PCR of saliva and urine using the test systems "AmpliSens CMV-skrin/monitor-FL", Moscow (61 people, the solid sample) aged 3-6 months (13%), 6-12 months (21%) 1-3 years (43%) of 3-7 years (23 %). Patients were sent to the consultative reception of different clinics in the city of Perm in order to clarify the diagnosis and correct treatment. They were pre- screened for CMV serological method (ELISA determination of IgM, IgG avidity index with the dynamics) and qualitative PCR of blood. PCR of blood were negative and ELISA diagnostic significance indicators.

A clinical and epidemiological analysis of primary medical documentations to assess the risk factors of fetal infections, the underlying disease and comorbidity. All children underwent a pediatric examination of the dynamics of 1-3 months at least 1 year. Laboratory investigation included an analysis of peripheral blood and humoral immunity (immunoglobulins A, M, G, E total), biochemical liver tests (ALT, AST, GGT) and of bacteriological methods of cultivation of oropharyngeal swabs. All children were examined etiologic and pathogenetic symptomatically individual schemes including diagnosis and effectiveness of the control age quantitative PCR in real time based on the ranking of the degree of viral load (VL).

Statistical processing of the materials was carried out using the software package SPSS for Windows v. 13.0. To calculate the relative values for $n < 30$ was used amendment Van der Warden. The conclusion was considered statistically significant at $p < 0,05$.

RESULTS AND DISCUSSION

Clinical and epidemiological analysis of primary medical records of children with verified CMV infection showed that antenatal high risk factors fetal infection had 95 % of patients (58/61). Burdened by the antenatal period was multifactorial combinations of high risk of infection: the threat of termination of pregnancy-55.7 % (34/61), chronic fetoplacental insufficiency-45.9 % (28/61), single and repeated respiratory infections during pregnancy-50.8 % (31/61), exacerbation of chronic extragenital and urogenital infections-39.4 % (24/61), recurrent infection caused by the herpes simplex viruses (HSV)-39.4 % (24/61). Negative serological markers, the results of screening pregnant women for TORCH- complex in the I trimester of pregnancy (57.4%) were: seronegativity to HSV 1,2-9.8 % (6/61) and CMV - 6.6% (4/61), a high antibody response to HSV 1,2 and CMV - 29.5 % (18/61), low IgG avidity index of CMV, HSV- 11.5 % (7/61).

Children without burdened antenatal period (5%) belonged to the group and often chronically ill after the visit to kindergarten as a result of pathological adaptation.

The proportion of children who were hospitalized in the neonatal period in the intensive care unit, department of pathology and preterm infants was 21.3 % (13/61). In all these cases, the diagnosis was registered fetal infections or threat of fetal infections with lesion central nervous system. Indirect hyperbilirubinemia, prolonged more than 21 days, at rates of direct bilirubin more than 10% of the total, with cholestasis syndrome and undulating course that meets the definition of a standard case of fetal infection-associated hepatitis [10], was present in 47.5 % (29/61) patients.

Infectious immunopathological syndrome occurred in 77% (47/61) immunocompromised outpatients and manifested by frequent viral respiratory infections in the first months of life, 57.4 % of children in the study cohort, including not amenable to conventional therapy (31.1%) and Candida and viral and bacterial infections tonsils (13%) and recurrent aphthous stomatitis (6.6%). Long-term low-grade fever was reported in 11.5 % of cases.

Lymphoproliferative (mononucleosis) syndrome, diagnosed more than half of the patients (54.1%, 33/61) was presented polyadenopathy (36.1%) with hepato-and splenomegaly (16.4% and 8.2%, respectively), as well as chronic diseases of upper respiratory tract (18%) in children 3-7 years of age (adenoids; follicular and granulosa pharyngitis).

Allergy syndrome was 44.3 % (27/ 61): recurrent bronchial obstruction (9.8 %) and cutaneous form (34.5 %). Early manifestation of allergic dermatitis was observed in 29.5 % of children and 70% (13 / 18) of them-without genetic determinancy.

Autoimmune syndrome was observed in a few cases in the form of acquired hemolytic anemia, thrombocytopathia and prolonged tolerance to therapy, neutropenia. The prevalence of immune-syndromes is presented in Figure 1.

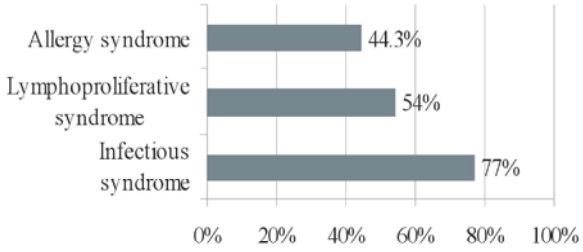

Fig. 1: The prevalence of of immunopathological syndromes in children with CMV infection verified.

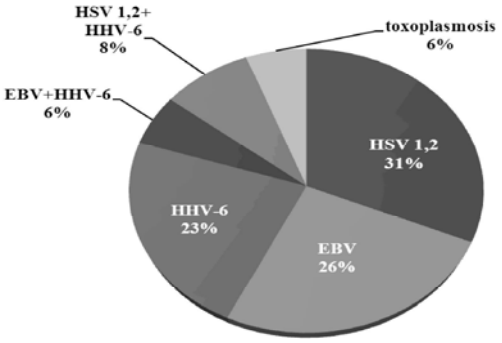

Fig. 2: The etiology of mixed opportunistic infections in immunocompromised children with CMV infection verified.

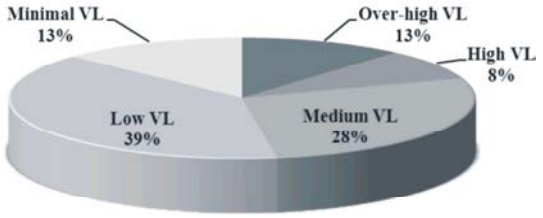

Fig. 3: The degree of viral load detected by quantitative PCR at primary examination immunocompromised outpatients.

Mixed with other forms of CMV infection, mainly herpes infections were detected in 57.4% of cases (35/61) with an infection caused by the herpes simplex viruses (HSV 1,2) - 31.4% (11/35) with infections caused by viruses, Epstein-Barr (EBVI, HHV-4) - 25.7% (9/35) with an infection caused by the human herpes virus type 6 (HHV-6)-22.9% (8/35), as well as two patho-genes: HHV-4 and HHV-6-5.7% (2/35) with HSV1,2 and HHV-6-8.6% (3/35) and in 2 cases-combination with toxoplasmosis (Fig. 2).

Laboratory markers immune deficiency secondary (IDS) were: neutropenia in the dynamics (73.8%), neutropenia combination with lymphocytopenia (19.7%), decreased IgA (68.8%); decrease IgG (45.9%); concomitant hypoimmunoglobulinemia (40.9%) and neutropenia (34.4%).

Bacteriological examination of the oropharynx, in all cases were found expressed disbiotic states with high levels of contamination of the mucous membrane of Enterococcus faecalis (46.7%), Staphylococcus aureus (60 %), Candida albicans (33.3%), requiring medical correction.

On the basis of clinical and epidemiological analysis and laboratory data immunocompromised outpatients was diagnosed with chronic CMV infection, reactivation, mild (52.4%) and moderate (47.6%) on IDS and dysbiosis oropharynx. During the chronic mild and moderate forms of CMV viremia was short-lived and the virus persists and keeps the replicative activity in the tropic tissues. Perhaps that's why, when an adequate serological response (significant titers of IgG; low avidity index; increase titers and avidity index in dynamics), CMV DNA in the blood, in contrast to other medium (saliva, urine), did not show up.

The purpose of the treatment was carried out by clinical and laboratory remission with suppression of viral replication. Special attention was paid to the immunorehabilitation adherence “antigenic protection”: prevention of intercurrent infections (home education and family prevention) mode of the day, sleep and temporary removal from vaccinations to remission of chronic infection.

The positive clinical effect was achieved in all cases, is to improve the condition of the child, reducing the frequency, duration and severity of respiratory infections, exanthematous and subfebrile liquidation process, the normalization of the local condition of the oropharynx.

Laboratory improvement was shown in abolishing neutropenia, lymphocytopenia and thrombocytopenia, increase of hemoglobin, normalization of humoral immunity and blood biochemical parameters.

Quantitative PCR diagnosis showed that the DNA content of CMV in immunocompromised children of different ages at baseline ranged from 15 million to 500 copies in 1 ml of saliva and urine. The degree of viral load (VL) has been ranked by the number of copies of DNA in 1 ml of the medium as follows: over-high VL (6-8) log10, i.e. 100,000,000-1.000.000 copies (11.5 %); high VL (5-6) log10, i.e. 1,000,000-100.000 copies (8.2%); medium VL (4-5) log10, i.e. 100,000-10.000 copies (27.9%), low VL (3-4) log10, i.e. 10,000-1.000 copies (39.3 %), minimum VL < 3 log10, i.e., < 1,000 copies (13.1 %) (Fig. 3).

In the clinical laboratory analysis of children's health study cohort was found that the rate of viral load intensity obtained by quantitative PCR correlated with the severity of chronic viral infection and allowed to evaluate the effectiveness of the therapy. Dynamic reduction in

Table 1: The structure of the distribution of viral load in children with CMV infection before and after treatment

VL	Over-high, abs.(%)	High, abs.(%)	Medium, abs.(%)	Low, abs.(%)	Minimum, abs.(%)	PCR(-), abs.(%)
Before treatment	7 (11.5%)	5 (8.2%)	17 (27.9%)	24 (39.3%)	8 (13.1%)	-
After treatment	-	-	6 (9.8%)	5 (8.2%)	38 (62.3%)	12 (19.7%)

viral load was achieved in 90.2% of cases (55/61) with the persistent absence of CMV DNA (remission during 1 year) in 21.8% (12/55). Minimum CMV replicative activity was achieved in 23 of 29 children with over-high, high and medium VL (79.3%). The increase or save viral load in immunorehabilitation period or shortly after it occurred in 6 cases (9.8%) and was associated with the presence of mixed infections (CMV infection with HSV 1,2, HHV-6) and for mono- CMV infection, with several layers of intercurrent disease (severe acute respiratory viral infection, rotavirus infection, etc.) and complicated teething. The structure of the distribution of VL CMV, according to the rankings, in immunocompromised outpatient study cohort in the dynamics before and after treatment are shown in Table 1.

CONCLUSION

Chronic relapsing form of cytomegalovirus infection has a significant role in the forming of a contingent immunocompromised outpatients with high incidence and severity of infectious, allergic and lymphoproliferative immunopatological syndromes. Negative role during the treatment of herpes virus infection play a mixed identified more than half of the cases. The results of research aimed at the improvement of laboratory methods for monitoring the effectiveness of therapy of cytomegalovirus infection in children, suggest the use of quantitative PCR in real time based on the ranking of the degree of viral load (VL) justified.

Findings:

- A highly specific direct qualitative method for identifying cytomegalovirus DNA by PCR of saliva and urine is the preferred method of noninvasive diagnosis of primary CMV infection in pediatric outpatients.
- The use of quantitative PCR in real time based on the ranking of the degree of viral load can objectively assess the dynamics of the effectiveness of immune therapy and rehabilitation.
- To optimize the therapeutic and diagnostic measures in respect of immunocompromised outpatients is necessary to conduct the PCR diagnosis of herpesvirus mixed opportunistic infections, as well as immunological and biological control.

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