



Aspects of Clinical and Laboratory Diagnostics of Enteroviral Infection without CMS Damage

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Abstract: In this study, we analyzed the course of the disease in 79 patients diagnosed with Acute Respiratory Infection (ARI) and catarrhal angina. In order to verify the diagnosis, a polymerase chain reaction (PCR) of blood serum and feces of patients was carried out to identify enteroviruses. According to the data obtained, the PCR diagnostic method was of particular value from the standpoint of early etiological confirmation of the diagnosis of enterovirus infection (EVI), with the aim of the most complete identification of forms without affecting the central nervous infection (CNS). The most frequent forms of ARI of similar enteroviral diseases in the clinic so reminiscent of "small forms" of enterovirus infections were "low fever" or "summer flu", while the typical form of EVI - herpangina - was found with the lowest frequency, but had the most severe course.

Keywords: enterovirus infections without CNS damage, acute respiratory infection, catarrhal angina, polymerase chain reaction, "small forms" of enterovirus infection.

Introduction. At the present stage, there has been a clear tendency for EVI to become more active in the world, and there are no signs of a decrease in its activity. Epidemiological increases in morbidity and outbreaks, constantly recorded in different countries, testify to this [2]. Long-term studies all over the world have shown that the most common manifestation of EVI is enteroviral meningitis, which is widespread both in the form of sporadic diseases and in the form of outbreaks [4]. It should be noted that with the prevalence of forms of EVI occurring without damage to the nervous system, it is not possible to single out the leading form. In most cases (about 85%), EVI is asymptomatic. At the same time, about 12-14% of cases are diagnosed as febrile diseases of mild or moderate severity [6]. The variety of clinical respiratory-like forms in the absence of any specific symptoms complicates the timely registration of EVI. Practitioners are not sufficiently aware of the methods of clinical diagnosis of these diseases; therefore, enterovirus infections are often diagnosed as influenza, pneumonia, etc. As a result, appropriate anti-epidemic measures are not taken in the foci of infection, which contributes to the further spread of infection. This determines the urgency of the problem and the need to study the patterns of regional epidemiology, and the clinical features of enterovirus infections without damage to the central nervous system, as well as the improvement of diagnostic methods.

Purpose of the study: to determine the incidence of enterovirus infection as a causative agent in patients with ARI and catarrhal sore throat with characteristics of the species distribution and features of the clinical and laboratory course of non-polio enteroviruses.

Material and methods: the study included 79 patients of various ages with ARI and catarrhal sore throat, who were inpatient treatment at the Regional Clinical Infectious Diseases Hospital in Samarkand, as well as outpatients in family polyclinics in Karshi. The diagnosis of ARI and catarrhal angina was based on a set of complaints, anamnesis data, clinical picture of the disease, examination of ENT organs and laboratory tests. To confirm the presence of enterovirus infection in patients with ARI and catarrhal angina, a method was used to study blood serum and feces using PCR, followed by culture and neutralization reaction to determine the species distribution of enteroviruses. Isolation of enteroviruses from feces of patients was carried out in the virological department on a nutrient medium "Needle DMEM with L-glutamine". Molecular biological research method (PCR) was carried out in the reference laboratory of the Research Institute of Virology of the Ministry of Health of the Republic of Uzbekistan. The analysis was carried out on the above material using the test system "Ampli-sense Enterovirus" (Russian Federation, Moscow).

The results of the study: showed that the frequency of positive cases of PCR for the detection of genomic RNA particles of enterovirus infection in the blood serum was 11 cases -34.4%, and in fecal samples 11 cases, which was 23.4%. The overall frequency of a positive result for EV in patients with ARI and catarrhal angina was 27.8% of both children and adults. When determining the age factor, it was found that in the sample of patients with a positive result on EVI, children from 1 to 3 years old were 2 ($9.1 \pm 6.1\%$), from 4 to 6 years - 4 children ($18.2 \pm 8, 2\%$), from 7 to 18 years - 9 cases ($40.9 \pm 10.5\%$), and adults - 7 cases ($31.8 \pm 9.9\%$). When determining the sex difference in the group with a positive EV result, it was revealed that the largest number of cases were female 12 ($54.5 \pm 10.6\%$), versus 10 ($45.5 \pm 10.6\%$) male patients. In the epidemiological history, all patients with a positive EV result (100.0%) showed contact with patients with acute viral infection, while parents of sick children and adult patients noted the appearance of symptoms of the disease after being in public places in a crowded population ($p < 0.001$ in relation to the EV group "-"). When analyzing the place of residence, it was revealed that in patients with ARI and a positive result of PCR for EVI, the number of urban residents was 11 cases ($50.0 \pm 10.7\%$) in Karshi, and 4 cases ($18.2 \pm 8, 2\%$) residents of Samarkand. When identifying the seasonality of the disease, it was revealed that patients with ARI and catarrhal angina and a positive result on EV were admitted mainly in the summer-autumn period, while the largest number of patients fell on June - 11 cases ($50.0 \pm 10.7\%$), then July 4 ($18.2 \pm 8.2\%$), August, September, October 2 cases each ($9.1 \pm 6.1\%$) and one in November ($4.5 \pm 4.4\%$). Thus, ARI and catarrhal angina with a positive result for EVI were characterized exclusively by summer-autumn seasonality (22 cases; 100.0 ± 0.0 ; $p < 0.002$).

The next stage of our work was to determine the serotypes of EV in the groups of patients with ARI and catarrhal angina, and a positive PCR result. The results of our work showed that the main pathogens of EVI were the ECHO 7 serotype. The data we obtained were not confirmed by our foreign colleagues, where ECHO 7 would be the causative agent of herpangina or enteroviral fever. To date, only Skachkov MV, 2011 [5], in his research points to ECHO 7 as the causative agent of "small forms" of EVI. This probably indicates the characteristic features of our region in relation to pathogens of "small forms" of EVI, which is confirmed by the studies of domestic scientists [3], where this serotype was found in the population of our region as a permanent carrier, and which could cause EVI in the form of sporadic diseases. The variety of clinical manifestations of EVI primarily concerns the significant incidence of diseases with a clinical picture of acute respiratory viral infection with all its clinical manifestations. In this regard, it was of interest to us to comparatively study the clinical picture and course of the disease in 22 patients admitted with a diagnosis of ARI and catarrhal angina and a

positive PCR result for EVI. It should be noted that when analyzing the timing of admission in the group with a positive result on EV, there was an acute onset with the timing of admission or admission in the first 2 days of the disease, while the main group sought medical help on day 1 of the disease - 18 cases (81.8 ± 8 , 2%), which was significantly higher compared to the comparison group ($p < 0.001$), where the bulk of patients turned on 2 (17 cases $29.8 \pm 6.1\%$; $p > 0.2$) and 3 days from the beginning diseases (17 cases, $29.8 \pm 6.1\%$; $p < 0.05$ in relation to the group with EV "+"), which indicates a gradual, subacute development of the disease in this group. When analyzing the clinical picture of the entire contingent of patients with acute respiratory viral infections and a positive PCR result, it was revealed that a moderately pronounced intoxication syndrome manifested itself in the main contingent of patients (19 cases; $86.4 \pm 7.3\%$; $p < 0.001$), in the rest of the contingent a serious condition was observed (3 cases; $13.6 \pm 7.3\%$), which was the reason for the admission of these patients to the hospital. In patients with a negative result on EV, there was a uniform distribution of patients according to the severity of the disease, there were both mild and severe course of the disease. The temperature reaction in more than half of the patients (12) with a positive PCR result ranged from $38.5.00C$ to $40.50C$, i.e. had a febrile character ($54.5 \pm 10.6\%$; $p < 0.05$), in 1/3 of the patients the temperature was within the subfebrile range ($27.3 \pm 9.5\%$), in the rest of the patients the temperature reaction had normal values (4 cases, $18.2 \pm 8.2\%$). It should be noted that in 1/2 patients, the temperature had a two-wave character ($50.0 \pm 10.7\%$; $p < 0.02$), i.e. on the 3-5th day of illness after a decrease in temperature, its repeated increase was noted. In patients with a negative PCR result, the temperature was predominantly subfebrile (25 cases; $43.9 \pm 6.6\%$), while two-wave fever was observed in only 13 ($22.8 \pm 5.6\%$) patients. Symptoms of intoxication in the form of headache were statistically significantly greater in the group of patients with EV "+" 15 cases ($68.2 \pm 9.9\%$; $p < 0.01$) versus 21 cases in the group with EV "-" ($36.8 \pm 6.4\%$). Loss of appetite or anorexia also predominated in the PCR-positive group, but the difference had no confidence limits. Such general symptoms as severe weakness, lethargy were observed in almost the absolute majority of cases (21) in the group of patients with EV "+" ($95.5 \pm 4.4\%$). The main and leading symptom of any acute respiratory disease is sore throat and cough, as these symptoms were observed in 11 and 7 patients with a positive result on EVI ($50.0 \pm 10.7\%$ and $31.8 \pm 9.9\%$). In the comparison group, these indicators had better results compared with the comparison group in 37 and 38 cases ($64.9 \pm 6.3\%$; $p > 0.2$ and $66.7 \pm 6.2\%$; $p < 0.01$). In the group of patients with a negative result on EV, hyperemia of the mucous membrane of the soft palate, palatine arches, uvula, posterior pharyngeal wall did not significantly differ from the results in the group with a positive result on EV (49 cases; $86.0 \pm 4.6\%$; versus 19 cases ; $86.4 \pm 7.3\%$). Whereas such a sign as the presence of papules and erosions on the palatine arches, uvula, and soft palate was statistically significant as a sign of EVI (7 cases; $31.8 \pm 9.9\%$; $p < 0.05$ versus 3 cases; 5.3 ± 3 , 0%). Symptoms of rhinitis with serous-mucous discharge, as well as pharyngitis, prevailed in the group with a negative PCR result on EVI (38 cases; $66.7 \pm 6.2\%$; $p < 0.01$ and 49 cases; $86.0 \pm 4.6\%$; $p < 0.02$), while in the group with a positive PCR result on EV, there were significantly more signs of regional lymphadenitis (8 cases; $36.4 \pm 10.3\%$; $p < 0.05$) and scleral vascular injection (15 cases ; $68.2 \pm 9.9\%$; $p < 0.001$). A general blood test in patients with a positive PCR result was characterized by the presence of cases of grade 1 - 2 anemia in 8 patients ($36.4 \pm 10.3\%$), while hemoglobin averaged 103.00 ± 2.70 g / l, which was not differed from the indicators of the comparison group (103.50 ± 2.00 g / l, $p > 0.1$). The leukocyte formula showed the presence of cases of leukopenia and lymphocytosis in 11 ($50.0 \pm 10.7\%$) patients, or cases of leukopenia and monocytosis in 8 ($36.4 \pm 10.3\%$) patients, in 3 (13.6 ± 7 , 3%) of patients with leukocyte counts were within normal limits. These indicators differed from those of patients with ARI and catarrhal angina in whom lymphocytosis and monocytosis were observed with a significantly lower frequency (24.6 ± 5.7 and 14.0 ± 4.6 , respectively; $p < 0.05$ and $p < 0.05$ when compared with a group with a positive PCR result). In 19 cases ($86.4 \pm 7.3\%$) ESR was moderately increased, and averaged 13.50 ± 0.57 mm / h. The average level of leukocytes was $4.40 \pm 0.37 * 10^{12}L$, lymphocytes

37.00 ± 1.26, monocytes 6.55 ± 1.00. In the comparison group with a negative PCR result on EVI, a significantly lower level of ESR was observed (10.43 ± 0.47; p < 0.0001), with a lower frequency of observation of cases of increased ESR (24 cases, 42.1 ± 6, 5%; p < 0.001). While the level of leukocytes significantly exceeded the indicators of the comparison group, although it was within the normal range (6.24 ± 0.37 * 10¹²/l; p < 0.0001), in the absence of a difference in the level of lymphocytes (34.77 ± 0.79; p > 0.1) and monocytes (5.14 ± 0.47; p > 0.2). Analyzing the clinical picture of patients with ARI, catarrhal sore throat and a positive PCR result, it can be divided into 3 groups that fit into one of the forms of enterovirus infection according to the classification [1] - herpangina, minor illness (summer flu) and catarrhal (respiratory form). The typical and main form of EVI, herpangina, was diagnosed in 6 patients (27.2%). Characterizing the general course of the disease, it was revealed that the general condition in half of the patients was characterized as a state of moderate severity (50.0 ± 20.4%). At the same time, the rest of the patients had a serious condition, which was accompanied by a febrile temperature reaction in the absolute number of patients (100.0%). The duration of the temperature reaction in patients of this group lasted 5.08 ± 0.30 days, while in all patients the temperature reaction had a second wave, which lasted 2.42 ± 0.20 days. The main complaints of this contingent of patients were moderate sore throat, which was in the absolute number of patients (100.0%), the duration of sore throat was 4.42 ± 0.30 days. Also, the entire category of patients (100.0%) had hyperemia of the pharynx, namely, the mucous membrane of the soft palate, palatine arches, uvula, posterior pharyngeal wall, and with the presence of erosions. It was found that within 24–48 hours, from 5–6 to 20–30 small papules of grayish-white color with a diameter of 1–2 mm appeared, which occurred in groups or separately, in the further papules by an average of 2.47 ± 0.20 day turned into bubbles-erosion, while a corolla of hyperemia was formed around the erosion. Erosions healed on day 4.77 ± 0.38 without mucosal defects. It should be noted that this disease on admission was regarded as acute pharyngitis or catarrhal angina, patients were prescribed an antibiotic in 100% of cases, and interferon was additionally prescribed in 4 patients. Recovering occurred on day 5.92 ± 0.24. In 4 patients (66.7 ± 19.2%), there was a repeated relapse of the disease. The clinical picture of 9 (40.9%) patients fit into the clinic of a minor illness, namely, the main symptom of this form was a short-term increase in temperature (on average 2.89 ± 0.20 days), which was accompanied by vomiting in 8 patients (88.9 ± 10.5%) and abdominal pain in 6 patients (66.7 ± 15.7%), which required the consultation of a surgeon. Almost all patients had a slight hyperemia of the throat (7 patients, 77.8 ± 13.9%) and a single cough was observed. The general condition of patients with "minor illness" was regarded as a state of moderate severity (9 cases, 100.0 ± 0.0). A feature of this sample of patients was the presence of cases of myalgia in 6 patients (66.7 ± 15.7%). Convalescence occurred on average on 4.11 ± 0.18 days. In 7 (31.8%) patients, the clinical picture was similar to the catarrhal form of enterovirus infection. The clinical picture in this sample of patients was primarily characterized by moderate catarrh of the upper respiratory tract. First of all, rhinitis with serous-mucous discharge was typical in the entire sample of patients (100.0%), as well as manifestations of pharyngitis (100.0%), which in 6 (85.7 ± 13.2%) patients was accompanied by symptoms of regional lymphadenitis, i.e. the leading symptom was the presence of nasopharyngitis. Hyperemia and graininess of the throat and dry cough were found in 85.7 ± 13.2% of patients in the study sample. The general febrile reaction had indicators in the range of 37 -37.50C, and the duration of the temperature reaction was 4.62 ± 0.47 days. Recovering occurred on average 6.42 ± 0.42 days from the onset of the disease. Thus, it is likely that the first and early sources of infection are patients with the so-called "minor forms of enteroviral diseases", early diagnosis of which in the initial period of the rise in the incidence of enteroviral infections is difficult due to the similarity of symptoms with acute respiratory viral infections. This, in turn, contributes to the spread of infection due to the lack of preventive measures. Conclusions: the PCR diagnostic method is of particular value in terms of early etiological confirmation of the diagnosis of EVI. At the same time, patients with ARI and catarrhal angina in the summer-autumn season are recommended to carry out PCR diagnostics on EV, in order to most fully

identify all forms of EVI. The identification of the EVI serotype - ECHO 7, confirms the presence of the circulation of this serotype in the environment of our region with the development of sporadic diseases of small forms of EVI. The main forms of ARI of similar enteroviral diseases in a clinic so reminiscent of "small forms" of enterovirus infections were "low fever" or "summer flu", while the typical form of EVI - herpangina - was found with the lowest frequency, but had the most severe course.

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