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DIAGNOSIS OF HERPES VIRUS INFECTION

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ABSTRACT

Today, it is generally accepted that the herpes simplex virus can affect various organs, and its significance in carcinogenesis and secondary infertility has been proven. Increasingly, there are reports about the role of the virus in the pathology of the liver, brain, prostate, diseases of other systems.

Keywords: infectious diseases, general genetics, immunity, herpesvirus infection, immunotherapy.

INTRODUCTION

Today we can say that the incidence of HSV is extremely high and, according to WHO statistics, it ranks second among human viral infections, second only to influenza. The same situation, according to the WHO, is also observed for the causes of death from viral infections - 35% of the influenza virus and 15.8% of HSV [1]. According to modern estimates, the infection rate of the population with HSV is approaching 100%. At the age of 15 years, antibodies to HSV-1 are detected in 75% of patients and

HSV-2 - in 11%. In adults, these figures are 99% and 73%, respectively. At the same time, approximately 30% have antibodies to both viral serotypes.

HSV infection leads to lifelong persistence of the virus, with the possibility of virus reactivation and cross-contamination with another HSV serotype. HSV is characterized by neurotropism. Through sensitive nerves, it penetrates into the nerve ganglia, where the infection acquires a latent course.

Diagnosis of HSV has a number of features and problems. The predominance of chronic and asymptomatic forms of the course of the disease, as well as the possibility of atypical manifestations, calls into question the diagnosis by external signs. Approximately 20% of patients with HSV-2 have no symptoms at all, and 60% of individuals have signs that cannot be diagnosed and are not accepted by the doctor and the patients themselves for herpes (atypical manifestations) [1]. Both of these groups are at risk of infecting their partners. In these cases, the determination of seroconversion is especially important, because. Treatment of herpesvirus infections is different from other infections.

Another problem is the detection of HSV-2. Specialists in most clinics do not type HSV, and this is a serious omission, since HSV-1 and HSV-2 differ in the frequency of virus reactivation (the frequency of rashes caused by HSV-1 is 3 times lower than that caused by HSV-2), as well as by the probability of transmission partner infection (higher in HSV-2) [5]. Diagnosis of HSV-2 has its own characteristics. The antigens used to diagnose HSV-1 and HSV-2 have a high homology (up to 50%) among themselves. Therefore, the existing tests for the diagnosis of HSV-2 are mostly imperfect and have low specificity [2].

In some cases, staging of the infection is particularly important for diagnosis. Primary infection, as a rule, is not accompanied by clinical manifestations, but its diagnosis in some cases is

extremely important. Specific IgM cannot be used as a reliable marker for diagnosing acute and, especially, primary infection, since IgM to HSV can be formed both during primary infection and during reinfection and during virus reactivation, but at the same time they can be produced in sufficient for diagnosis in only 30% of people. The only way to immediately and reliably diagnose a primary infection is to determine the avidity index of specific antibodies. Another diagnostic method that allows differentiating the stages of infection is the change in antibody titer in the study of paired samples.

The aim of our work was to create a reliable test for the determination of specific immunoglobulins to HSV-1,2, and test systems for the differential diagnosis of genital herpes, allowing to determine the avidity and titer of anti-herpes antibodies.

Materials and methods. We used recombinant proteins produced by NPO Diagnostic Systems: HSV-1 gD (266-394aa), HSV-2 gD (266-394aa), HSV-2 gG (525-578aa) (cat. AHSV 101, AHSV 102 , AHSV103).

We studied 650 blood serum samples from normal donors, containing and not containing antibodies to HSV, 11 blood serum samples containing HSV DNA (PCR positive), 13 samples from patients with clinical manifestations of genital herpes from the collection of the Nizhny Novgorod NIKVI of the Ministry of Health of the Russian Federation.

Tests for the detection of antibodies to HSV produced by DSL (USA) and Sentinel (Italy) were used as reference systems.

Results and discussion. Recombinant proteins reproducing the main linear and conformational immunoreactive determinants of the herpes virus were used to create two enzyme immunoassay test systems: "DS-ELISA-ANTI-HSV-1,2" - to detect class G antibodies to HSV types 1 and 2 and test for the differential diagnosis of HSV-2 - "DS-IFA-ANTI-HSV-2".

As a result of the studies, it was shown that for a reliable diagnosis of HSV-2 by enzyme immunoassay, it is not enough to use only one strong immunogenic epitope of herpesvirus (a type-specific fragment of glycoprotein G) as an immunosorbent. The presence of additional epitopes in another viral protein, gD, makes it possible to achieve maximum test sensitivity.

To prevent cross-reactivity, which is possible due to 50% homology between the amino acid sequences of the HSV-1 and HSV-2 gD fragments [4], we applied a new technique that provides a high specificity of the test. A special reagent with specific reactivity to anti-gD antibodies of only type 1 herpes virus was introduced into the block solution for sera. The complex formed between the special reagent and antibodies to HSV-1 is removed when washing. Thus, in the studied samples, only antibodies to HSV-2 are detected, and a high specificity of diagnostics is ensured.

The diagnostic potential of the test was evaluated using 11 known positive samples (patients with clinical manifestations of genital herpes). Table 1 shows the results of a comparison of test systems for the diagnosis of HSV-2 produced by Diagnostic Systems (Russia, Nizhny Novgorod), DSL (USA) and Sentinel (Italy).

The given data demonstrate the maximum sensitivity of the “DS-ELISA-ANTI-HSV-2” test we created.

The use of the avidity index as a marker of the primary stage of infection is successfully used in the serodiagnosis of infections with atypical dynamics of antibody formation (when the presence of IgM is not a reliable and sufficient sign for differentiating the stages of the disease).

It is known that during primary infection, more than 50% of antibodies that persist in the body have low avidity to the antigenic determinants of the virus, so the resulting antigen-antibody complex is fragile and is destroyed under the influence of denaturing agents [3,6].

The ELISA test system that we have created makes it possible to determine the avidity of specific antibodies in the samples under study.

To determine the avidity index, an additional step is introduced into the standard analysis procedure, during which the antibodies are exposed to 8 M urea, which acts as a denaturing agent. The further reaction proceeds according to the usual scheme. This innovation extends the set-up by only 10 minutes, but allows a clear separation of high and low avidity sera and thus the diagnosis of primary infection.

One of the recommended and widely used methods for monitoring and differentiating the stages of HSV infection is the change in antibody titer.

However, the widespread direct titration (by sequential transfer of a certain amount of a sample, where the maximum dilution of the sample at which a positive result is recorded is taken as the titer of antibodies to HSV) is not economically viable and has a large error due to multiple repetitive operations.

The method developed by us - the use of calibration equations allows you to determine the titer of antibodies to the herpes simplex virus in a single study of the sample diluted 100 times.

Antiherpetic antibody titers were analyzed in 80 blood serum samples with different optical density (OD) using new enzyme immunoassay systems. Experimental data (empirical titration curves) were used to determine the equation relating the value of the ratio of the OD of the sample to the OD of the critical one with the titer of antibodies to HSV

With the help of the Table Curve program, approximation dependencies are obtained, which best characterize the experimental data.

To test the diagnostic reliability of the method, a comparison was made between the calculated and actual antibody titers.

The accuracy of the method was more than 90% with an error of +/- one dilution and 100% with an error of +/- two dilutions. Considering that a 4-fold change in titer is diagnostically significant, the method proposed by us is a convenient and reliable diagnostic tool.

Conclusions. New enzyme-linked immunosorbent assays "DS-ELISA-ANTI-HSV1,2" were created - to determine AT class G to HSV types 1 and 2, and the test system "DS-ELISA-ANTI-HSV-2" for the differential diagnosis of genital herpes with high sensitivity and specificity.

The uniqueness of our new test "DS-IFA-ANTI-HSV-2" lies in the use of two highly informative antigenic epitopes of herpes simplex virus type 2 as an immunosorbent. The introduction of a special blocking method allows for a high level of specificity.

Comparative data demonstrate the high diagnostic potential of new tests that allow differential diagnosis of primary infection, determine the titers of specific antibodies, with a lower cost of analysis of one sample and provide greater accuracy and reliability in the diagnosis of herpesvirus infections.

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