Optimizing etiological diagnostics and improving the efficiency of treating centralized infectious corneal ulcers

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*The **purpose** of this research was to study the influence of human herpes viruses (HHV) on the development of infectious corneal ulcers. **Material and methods.** 43 patients (21 to 77 years old) with corneal ulcers believed to have been derived from a bacterial infection were examined. Based on anamnesis and ophthalmological examination, 3 groups were identified: the 1st group (26 patients) — with herpetic ulcers (HU), the 2nd group (13 patients) — with secondary bacterial ulcers (SBU), and the 3rd (4 patients) — with primary bacterial ulcers (PBU). Blood (43 samples) and corneal scrapings (29 samples) were examined by nested polymerase chain reaction (PCR) for the presence of DNA (deoxyribonucleic acid) of various HHV, including herpes simplex virus (HSV) 1, 2, Epstein — Barr virus (EBV), HHV-6, and HHV-7. **Results.** HHV was detected in 41.6—53 % of the corneas and in 33.3—50 % of blood, depending on the patient group. Clinical patients with the DNA of HHV evident in their cornea were distinguished by their CU's torpid appearance and in the absence of epithelization following the suppressed purulent process. The addition of antiherpetic drugs resulted in complete epithelialization of the cornea within 3—5 days. **Conclusion.** The frequent representation of HHV-6 and EBV DNA and less frequently detected HSV DNA in the corneal scrapings indicate the possible involvement of HHV in the etiopathogenesis of SBU. The effectiveness of the complex antiherpetic drug therapy supports this conclusion.*

Keywords: Corneal bacterial ulcers, Herpes simplex virus, Epstein — Barr virus, human herpes virus type 6, human herpes virus type 7, PCR.

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Corneal ulcers (CU) are one of the main causes of reduced visual acuity and corneal blindness. CU are evident in 6.3 to 23.2 % of the population in developed countries [1–7].

Amongst inflammatory diseases of the anterior part of the eye, CU are the most difficult to treat and can result in corneal perforation, endophthalmitis, and loss of the eye. Ulcers can be found in any part of the cornea, but more than 70 % of patients develop CU in the central zone, where the infection is more intense, difficult to treat, and scarring in this area always leads to loss of vision [6–9].

Corneal ulcers are organized into two categories – infectious and non-infectious. Infectious CU include herpetic, bacterial, fungal, and parasitic (acanthamoe) CU. Non-infectious diseases include recurrent corneal erosion, marginal corneal ulcer (autoimmune), ulcers from dry eye syndrome, and primary or secondary corneal dystrophy.

According to the Moscow Helmholtz Research Institute of Eye Diseases, there have been significant changes in the etiology of CU among hospitalized patients from 2000 to 2015, suchas, the frequency of herpetic ulcers

(HU) decreased from 66 to 40 %, and bacterial ulcers (BU) increased from 12 to 27 %.

Bacterial ulcers are divided into primary and secondary categories. During this study, the tendency for developing secondary bacterial infection increased, and the ratio of primary to secondary infection changed from 47 and 53 % to 18 and 82 %, respectively [6–8].

We believe that the increase in the number of corneal bacterial infection cases, mostly the secondary type, is caused by untimely diagnosis and, as a consequence, incorrect therapy of herpetic keratitis.

Present data allows us to discuss clinical features of herpes ulcers and bacterial ulcers. In some cases, it helps to establish the etiology of the disease by analyzing biomicroscopy first, immediately followed by etiotropic therapy before the lab results are obtained [1, 4, 6, 7, 9, 10].

However, the clinical etiologic diagnosis of herpetic keratitis and CUis not always possible. This depends on the specialist's qualifications, the epidemiology, individual patient characteristics, and ophthalmic and somatic diseases. Often, this leads to diagnostic errors and inappropriate pharmacotherapy, which can result in a long, intense, and difficult to treat inflammatory process.

A prolonged herpetic keratitis and the irrational use of corticosteroids, antiviral medicine, antibiotics, and anesthetics contribute to theincreased representation of bacterial flora and the development of secondary bacterial ulcers (SBU), which are characterized by a mixed viral-bacterial infection of the cornea [2, 9, 11, 12]. Secondary bacterial infections during ocular herpes are mostly caused by staphylococci, streptococci, and Pseudomonas aeruginosa. The secondary bacterial infection is characterized by its severity, the formation of purulent discharge, and the development of uveitis, which can lead to corneal perforation and endophthalmitis [2, 4, 5, 7].

Despite active medication, the eye can be rescued from the progression SBU only with the help of urgent keratoplasty [4, 9].

According to our data, severe, long-term ulcers occur in 32 %, recurrent ulcers — in 17 %, and early relapses (within a month after recovery) — in 3 % of recorded cases. Complications in the cornea, such as abscess, endophthalmitis, descemetocele, or perforation vary from 5 to 15 % in bacterial ulcers and 2 to 5 % in ocular herpes; according to the literature, these complications can develop from 9 to 37.9 % in bacterial ulcers and from 19 to 32 % in autoimmune ulcers [4, 5, 8].

In such conditions, laboratory etiological diagnostics are very important. Presently, methods such as immunofluorescence (MFA) and microbiological examination (smears and crops) are commonly used to study conjunctival scrapings for indicators of herpes simplex virus (HSV). According to our observations, a follow-up examination of patients who had previously received antiviral and antibacterial therapy significantly reduced their sensitivity to these methods [13].

Because of present ineffective diagnostics and treatment, it is important to develop more accurate and informative methods of laboratory diagnostics that allow clinicians to identify and reliably confirm the etiology of corneal ulcers at early stages of the disease, identify mixed forms of infection, and timely prescribe etiotropic and pathogenetic therapy.

The **PURPOSE** of this research was to optimize etiologic diagnosis and to improve efficiency of treating centralized infectious corneal ulcers.

MATERIAL AND METHODS

43 patients (43 eyes) ranging in age from 21 to 77 years with centralized infectious corneal ulcers were examined. From the moment of hospitalization, the disease was 5–14 days old in 15 patients (34.8 %), 15–30 days in 8 patients (18.6 %), 31–60 days old in 5 patients (11.6 %), and more than 60 days old in 15 patients (34.8 %). All patients underwent a standard clinical examination, which included anamnesis, visometry, and biomicroscopy. As described in previous methodology, the bacteriological examination included the study of smears and cultures from conjunctiva and immunofluorescence (MFA) to detect HSV antigens in the conjunctival scrapings [13].

Blood samples were analyzed using an enzyme immunoassay to determine the presence of IgM-, IgG- antibodies to different antigens of herpes simplex virus type 1 and type 2 (HSV 1, 2), Epstein — Barr virus (EBV), and IgG antibodies to human herpesvirus type 6 (HHV- 6). These findings were used to establish the presence of infection and its stage (primary, chronic, reactivation of chronic).

Blood (43 patients) and scrapings from corneal ulcers (29 people) were also examined with the highly sensitive nested polymerase chain reaction (PCR) for the detection of deoxyribonucleic acid (DNA) of HSV 1 and 2, EBV, HHV-6, and HHV -7. After the patients were placed under epibulbar anesthesia, cells from the surface of the corneal ulcer were gathered using a sterile single-use scalpel and a slit lamp for an ideally controlled procedure. The gathered material was stored at -70 °C until the PCR was prepared.

The results were statistically analyzed using the chi-squared test and Fisher's exact test with the help of the BIOSTADT computer program.

RESULTS AND DISCUSSION

3 groups of patients were identified based on their different medical conditions: 1 group — 26 patients (26 eyes) with herpetic ulcers (HU); Group 2 — 13 people (13 eyes) with secondary bacterial ulcers (SBU), 3rd group — 4 patients (4 eyes) with a corneal primary bacterial ulcers (PBU).

Group 1: HU. 26 (100 %) patients from the 1st group were seropositive to HSV1 and 2, 25 (96 %) to EBV, and 20 (77 %) to HHV type 6. This result reflects the prevalence of these viruses among the Russian Federation population. Serological markers of HSV1 and / or type 2 activity were detected in 21 (82 %) patients. EBV was detected in only 1 patient.

The HSV antigen was detected in the eyelid conjunctival scrapings of only 6 patients out of the examined 26 (23 %). We believe that this is due to analyzing the patients at a later time and collecting the examined material only after starting the antiviral therapy, which significantly reduces the sensitivity of immunofluorescence diagnosis of ocular herpes [13].

Despite the presence of the HSV antigen in the eyelids' conjunctiva, DNA of HSV 1 and 2 was not detected in the analyzed corneal ulcer samples. Many authors emphasize the differences between MFA and PCR results attained for different infections. For example, when examining patients with the flu, N.D. Yushchuk et al. [14] found an antigen in 7.1–17.6 % of their patients' throats and nasal passages, however, they could not detect the DNA of the virus in the studied cells.

Notably, 7 of 13 (53.3 %) patients with HUin corneal cells had genomes of lymphotropic HHV; HHV-6 DNA in 4 (30.8 %), EBV DNA in 2 (15, 4 %), and HHV-7 DNA in 1 (7.6 %) of the patients (Fig.). Recently, more data on the possible role of not only HSV, but also other herpesviruses, primarily HHV-6, has accumulated in the etiopathogenesis of corneal diseases [15–18].

DNA of HSV 1 and 2 was not detected in the blood samples, but the genomes of herpes viruses, characterized by persistence and replication in lymphocytes and blood monocytes, were found in 8 out of 20 (40 %) of the examined; HHV-6 in 4 of 20 (20 %), EBV — in 3 of 20 (15 %), and HHV-7 in combination with HHV-6 — in 1 of 20 (5 %) patients DNA (Fig.).

Group 2: SBU. In general, the herpesvirus infections in Group 2 patients and the detection of HSV 1 and 2 did not differ significantly from group 1 ($p > 0.05$). The HSV antigen was more often detected in the conjunctiva epithelium of patients with SBU compared to patients with HU (5 out of 13–38.4 % and 6 out of 26–23 %, respectively, $p > 0.05$). With PCR analysis, HSVDNA was detected in 2 of 5 antigen-positive patients. From this, we believe that the secondary bacterial flora hinders the elimination of HSV from the mucous membrane of the eye.

HHV-6 DNA was detected in 6 out of 12 (50 %) patients with SBU. 3 instances were detected in the patient's blood, and the other 3 where in the corneal scrapings. At the same time, the HHV-6 gene was not detected in the blood or the cornea (Fig.).

Group 3: There were only 4 patients with PBU. No antigen or genome of HSV was detected in any of the studied biomaterials (blood, eyelid conjunctival scrapings, corneal scrapings).This finding corresponded to a symptomatology that did not fit into the clinical picture of ocular herpes.

The detection of HHV-6 or EBV DNA in the blood or cornea of all patients using PCR should be taken note of. The lack of observations did not allow us to make any conclusions (Fig.). However, it was noticed that it was possible to activate herpesviruses and develop a "secondary" herpetic ulcer when patients were inadequately treated.

Examining patients with different types of CU gave results which confirmed the highly sensitive nature of the PCR technique. However, it should be emphasized that the immunofluorescence method has not lost its value, since MFA and PCR are used to identify different components of the virus and the positive results acquired with

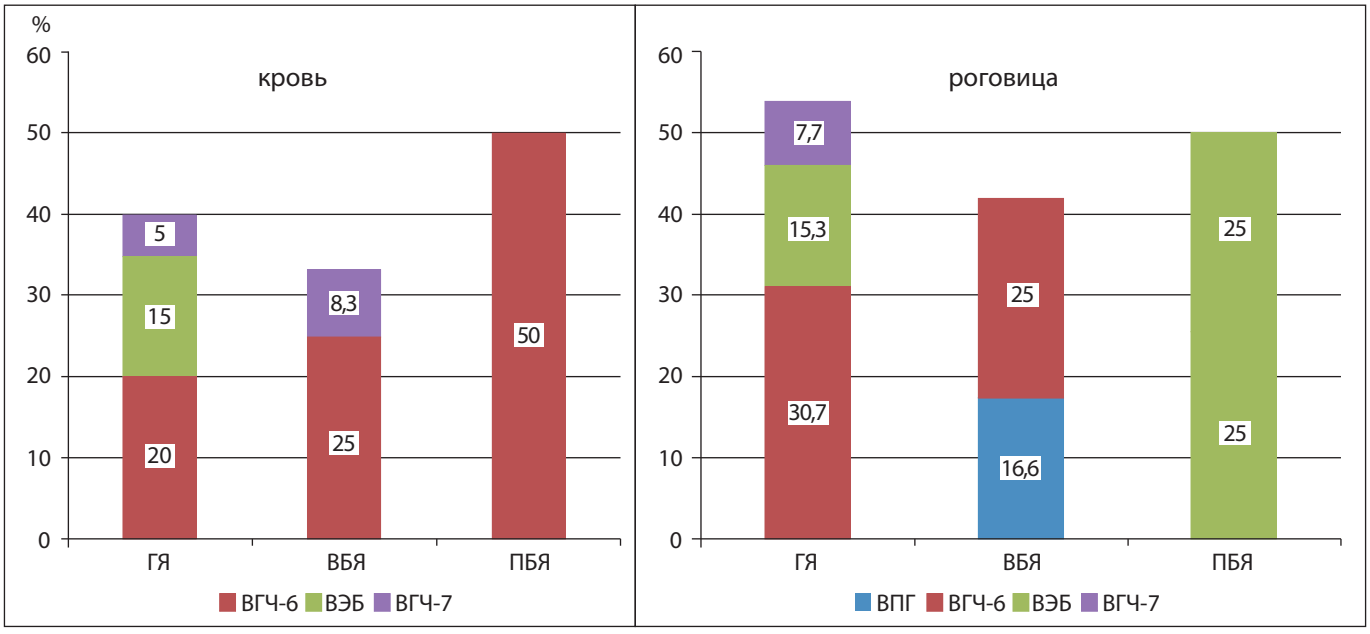

Fig. Frequency of DNA (%) of Herpes simplex virus, Epstein – Barr virus, Human herpesvirus type 6 and 7 detection in biomaterial (blood, corneal scrapings) of patients with corneal ulcer of various etiology. HU — corneal herpetic ulcer, SBU — secondary bacterial corneal ulcer, PBU — the primary bacterial ulcer of the cornea.

MFA were confirmed by only 19 % of the PCR-positive patients.

Laboratory testing becomes increasingly effective when using several methods.

Studying the corneal blood and cells using PCR showed that in addition to HSV, EBV and HHV-6 play a less understood role in the etiopathogenesis of herpetic CU. According to our data, HHV-7 is detected very rarely and mostly in combination with HHV-6. The role of HHV-7 as a co-factor in diseases caused by other HHVs is actively discussed.

HSV, HHV-6 and EBV were also found in the corneal blood and tissue of the patients with bacterial ulcers. This discovery allowed us to treat these ulcers like herpetic ulcers complicated by the secondary bacterial flora (SBU) and to make adjustments to the current therapy.

Etiopathogenetic pharmacotherapy includes specific antibacterial and antiseptic therapy, pathogenetic (metabolic), anti-inflammatory, antiallergic, antihypertensive therapy, and mydriatics [10, 19, 20].

As a result of intensive antibiotic therapy, the CU was cleared of purulent necrotic masses, and further destruction of the corneal stroma completely ceased.

CU took on a torpid appearance in HHV positive patients. After complete suppression of the purulent inflammatory process, the surface area of the ulcer did not decrease, and epithelialization was absent. In connection with this, anti-herpetic preparations were included in the complex therapy: Diphenhydramine + Interferon alfa-2a eye drops 4 times a day, Acyclovir ophthalmic ointment 3 times a day, systemically: inside 200 mg of Aciclovir 5 times a day for 5 days.

The use of antiherpetic drugs led to complete epithelialization of the CU for 3–5 days. With the help of a variety of treatments, antibacterial therapy in combination with antiherpetic drugs, opacity formed on the surface of the place where the resorbed corneal ulcer was located, slightly reducing the visual acuity.

Thus, the proposed methods of diagnosis and treatment made it possible to prevent the prolonged development of corneal bacterial ulcers, intense corneal opacities, and corneal perforation, and to preserve and restore visual functions and avoid the need for surgical treatment (amnioplasty and / or through keratoplasty).

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