WORLD VIEW

Acute haemorrhagic conjunctivitis outbreak in the city of Fortaleza, northeast Brazil

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Background/aim: Between February and May 2003 an epidemic of acute haemorrhagic conjunctivitis affected more than 200 000 people in all five geographic regions of Brazil (north, south, midwestern, southeast, and northeast). The aim was to identify the aetiological agent and to describe clinical aspects of this outbreak in a group of patients treated at the ophthalmology department of the Hospital Walter Cantídio (OD-HWC) at the Universidade Federal do Ceará, in the city of Fortaleza, capital of the state of Ceará, northeastern Brazil.

Methods: Conjunctival swabs were collected from patients who spontaneously went to the laboratory of virology. Specimens were inoculated in HEp-2 and RD cell lines. The viral isolation was confirmed by performing reverse transcriptase polymerase chain reaction and indirect immunofluorescence assay.

Results: Viral conjunctivitis was diagnosed in 56 patients but only 24 of them allowed the collection of samples. Of 24 conjunctival swabs tested, 11 were positive for a variant of coxsackie virus A24 (CA24v) and one of the isolates reacted with anti-adenovirus monoclonal antibodies.

Conclusion: CA24v was confirmed as the aetiological agent of this outbreak of acute haemorrhagic conjunctivitis in the city of Fortaleza.

Acute viral conjunctivitis (AVC) is a highly contagious infection that results in a significant number of outpatient visits. There are several forms of clinical presentation: pharyngoconjunctival fever, epidemic keratoconjunctivitis, and acute haemorrhagic conjunctivitis (AHC). A variant of coxsackie virus A24 (CA24v), enterovirus 70 (EV70) and, less frequently, the adenovirus are the major aetiological agents of AHC.

The first description of AHC is related to an outbreak caused by EV70 in Ghana in 1969.[1] After the first report of AHC in the western hemisphere in 1981, many epidemics also caused by the EV70 have been observed in many Latin-American countries.[2–6] The identification of CA24v as the agent of an outbreak of AHC in Brazil occurred 17 years after the description of this agent as cause of an outbreak in Singapore, in 1969.[7 8] This agent has prevailed in the majority of the most recent AHC outbreaks in different countries.[9–13]

The diagnosis of AVC is essentially based on clinical features. However, during epidemics, the identification of the agent is justified by the benefits of epidemiological vigilance, the education of doctors and patients, the definition of the pathological process, and the assessment of therapeutic implications. Isolation in tissue culture, direct detection by electron microscopy, detection of viral antigens by immunofluorescence, and amplification of viral nucleic acids are some methods used for identification of the aetiological agent.

In 2003, a national outbreak of AVC, defined chiefly by their haemorrhagic aspect, occurred in Brazil.[14] The aim of this study was to identify the aetiological agent and to describe clinical aspects of this outbreak in a group of patients treated at the ophthalmology department of the Hospital Walter Cantídio (OD-HWC) at the Universidade Federal do Ceará, in the city of Fortaleza, capital of the state of Ceará, northeastern Brazil.

METHODS

Study population

Patients who were treated at the OD-HWC presenting with any of the following conjunctivitis symptoms, foreign body sensation, red eye, tearing, and pruritus, were included in the study. Patients using topic medication, those with a previous history of ocular disease, and contact lens users were excluded. Clinical and epidemiological data were recorded on patient charts. All patients were referred to the virology laboratory of the faculty of medicine at the Universidade Federal do Ceará (VL-FAMED-UFC) for the collection of clinical specimens. Written informed consent was obtained from the population studied. This study was approved by the ethics committes of the Hospital Walter Cantídeo.

Specimen collection

Sterile cotton swabs were used to collect eye discharges and were immediately put into tubes with 2 ml of viral transport medium (1× Hank's balanced salt solution containing 5% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml), pH 7.4).

Viral isolation

Specimens were inoculated in HEp-2 and RD cell lines at 37°C, and examined daily for cytopathic effect (CPE) over a period of 7 days. Viral isolation was confirmed by performing reverse transcriptase polymerase chain reaction (RT-PCR) or indirect immunofluorescence assay (IFA) using the Respiratory Panel 1 viral screening and Identification Kit (Chemicon) based on CPE observed. Normal mouse antibody was used as a negative control in IFA

RT-PCR and nucleotide sequencing

RNA was extracted from 250 μl of virus infected culture supernatant by using Trizol LS (Invitrogen, USA), and the complementary DNA was synthesised with Oligo(dT) (Invitrogen) by using SuperScriptII reverse transcriptase (Invitrogen). Enterovirus group specific RT-PCR was

Abbreviations: AVC, acute viral conjunctivitis; CA24v, coxsackie virus A24; CPE, cytopathic effect; EV70, enterovirus 70; IFA, immunofluorescence assay; RT-PCR, reverse transcriptase polymerase chain reaction

performed by using a primer pair 292 (sequence: 5′-MIGCIGYIGARACNGG-3′) and 222 (sequence: 5′-CCCCIGGIGGIAYRWACAT-3′) that amplifies an approximately 350 bp fragment within the VP1 gene.[15] The PCR products, after being purified from the agarose gels, were submitted to the cycle sequencing reactions. The ABI Big Dye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems) kit was used for this purpose. Sterile water was used as negative control in all reactions. VP1 sequences from our isolates were compared to those available at GenBank by the BLAST software to determine viral identity and serotype (accession nos.AY296249, AY296251, AF545847, AY876178, AY876181, D90457).

RESULTS

AHC was clinically diagnosed in 56 patients from February to May 2003 but only in only 24 of them were eye discharge samples collected for testing. The development of CPE was observed in only 12 samples (50%), all exclusively in HEp-2 cells. Eleven were positive for enterovirus; all were confirmed as CA24v by VP1sequencing. One of the isolates reacted with anti-adenovirus monoclonal antibody.

The patients included in the study ranged in age from 2−66 years. The symptoms lasted for 5–14 days. History of contact with individuals with similar symptoms was reported by 63% of patients. The main symptoms described by patients, in decreasing order of frequency, were foreign body sensation (95%), excessive tearing (86%), ocular pain (70%), pruritus (26.3%), and blurred vision (26%). The key clinical signs observed at ocular examination were conjunctival hyperaemia (100%), watery discharge (99%), bilateral involvement (84,2%), chemosis (61%), subconjunctival haemorrhage (56%), follicular conjunctival reaction (43%), preauricular lymphadenopathy (5%), and punctate epithelial keratitis (3%).

DISCUSSION

During the last few days of February 2003, a growing number of clinically diagnosed cases of viral conjunctivitis were observed in the Brazilian states of Acre, Amazonas, Ceará, Rondônia, Rio de Janeiro, São Paulo, Mato Grosso, Mato Grosso do Sul, Santa Catarina, Rio Grande do Sul, and Paraná. These epidemics peaked in March and ended in May, with a total of 228 227 notified cases, 3737 of them in the state of Ceará.[14] These and other AHC epidemics in Brazil occur during the summer season, a fact that has also been described in other countries.[5 6 8 9 16–18] This viral infection occurs essentially by direct contact with infected secretions and its dissemination is facilitated by conglomerates. In 2003, the ''Carnival,'' Brazil's biggest popular festival, was in the first few days of March, certainly contributing to the fast virus dissemination and the imposing number of cases noted.

From 1981 to 1984 the predominant causative agent of AHC in Brazil was EV70.[4–6]. In Brazil, the first epidemic of AHC caused by CA24v was reported in 1987 and since then no further outbreaks have been registered.[8] CA24v has been more frequent since the 1990s, surpassing the cases relating to EV70. A study analysing the variation of the aetiological agents in viral conjunctivitis outbreaks in Taiwan for 18 years, showed that EV70 rarely appeared after 1984; however, CA24v was the aetiological agent of the four major epidemics of AHC from 1985 to 1994.[13]

The first (1987) and last (2004) outbreaks of ACH in Brazil were restricted to the states of Pará and Rio de Janeiro, respectively. However, the 2003 epidemic began in the south and subsequently spread to the several states of other Brazilian regions.[8 14 18] This outbreak extended to French Guiana where approximately 6000 cases of AHC were observed between April and July and then to Central America, affecting Puerto Rico and the Caribbean Islands from August to October.[12 17] The most recent CA24v related outbreak of AHC in Brazil occurred during April and May of 2004 and was mainly restricted to the city of Rio de Janeiro where more than 60 000 cases were officially reported.[18] Phylogenetic analysis revealed that the CA24v circulating in all these countries in 2003 and 2004 was similar to the genotype which caused outbreaks of AHC in Korea and Malaysia between 2002 and 2003.[11 12 18] The data collected indicate that the dissemination of CA24v in the outbreaks of AHC from 2002 to 2004 spread from Asia to the Caribbean via South America.[10–12 14] However, the first CA24v outbreak of AHC in Brazil followed a different path, moving from Asia to South America via the Caribbean.[8]

In the present study, the CA24v was identified as the aetiological agent responsible for this epidemic although one adenovirus was isolated from one of the inoculated samples. Other studies have shown that adenovirus and CA24v can circulate simultaneously during AHC outbreaks.[11 16] The rate of virus isolation in this study was 50%. According to studies using conjunctival swab specimens and the same cell lineages used in this study rates of CA24v isolation ranged from 30.3% to 70%.[9 11 18] As in this study, HEp-2 cells were only capable of viral isolation of CA24v during the last ACH epidemic in Brazil.[18]

The clinical manifestations shown by patients included in our study do not differ from those reported in other studies.[11 12] The difficulty in collecting samples during medical attendance may have influenced the reduced number of patients submitting samples (24/42.8%). Only a small number of specimens are generally collected during the outbreaks of AHC to characterise their aetiology. Rates of notified cases during AHC outbreaks range from 10 327 to 137 136, while the number of specimens collected for the definition of aetiological agents ranged from 15 to 86.[9 11 12 18]

This report describes the first outbreak of AHC in the city of Fortaleza and although some time has elapsed this is the first study to obtain data for aetiological, clinical, and epidemiological aspects of the major outbreak of AHC in Brazil. The phylogenetic analysis of CA24v isolates from this study and other circulating in Brazil during 2003's ACH epidemic is in progress.

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