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Case report

Identification of coxsackievirus A-24 GIV C5 strain as the cause of acute hemorrhagic conjunctivitis outbreak in Hyderabad, India in 2022

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ABSTRACT

Viral infection is frequently the cause for acute hemorrhagic conjunctivitis (AHC) epidemics. AHC can result from adenoviruses, with enterovirus 70 and coxsackievirus A24 being the primary agents. AHC was initially identified in Ghana in 1969, caused by enterovirus 70 and leading to a global pandemic. Since 2000, outbreaks of AHC linked to coxsackievirus A24 variant have been documented in Spain, Pakistan, Singapore, India, Korea, and China. A sudden surge of conjunctivitis cases reported in October 2022 in and out of the Hyderabad region. This infection presented with usual symptoms of redness of the eyes, discharge, pain in the eyes and crusting. Occular swab samples from 110 patients were collected in order to identify and characterize the virus that was causing the epidemic. We examined adenovirus, enterovirus, COVID-19 and Herpes Simplex Virus by using commercially kits available at the hospital. Conserved regions in the enteroviral 5′-UTR and VP2 gene were analyzed further for characterization of serotype at the National apex laboratory. None of them was found positive except Enterovirus in 16.36 % (18/110) of the patients. From enterovirus-positive samples, the coxsackievirus A24 was observed in all 18 positive samples. These clinical isolates constitute a new lineage cluster associated with genotype IV-C5, according to additional sequencing of the full-length VP2 genes and subsequent phylogenetic analysis. In conclusion, the current outbreak of acute haemorrhagic conjunctivitis in Hyderabad, India was traced to the coxsackievirus A24 strain GIV C5.

1. Introduction

One of the most prevalent ocular conditions seen in ophthalmic emergency departments is conjunctivitis, also referred to as "red eye" [1]. On a global scale, AHC epidemics have repeatedly been documented. According to several studies [2,3], the main cause of AHC outbreaks is coxsackievirus A24 (CVA24). CVA24 was first isolated from the AHC outbreak in Singapore in 1970 as an antigenic variation of CVA24 [4]. The CVA24 related acute hemorrhagic conjunctivitis (AHC) epidemic that followed had primarily affected

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Southeast Asia and India [5]. The outbreak of AHC in Singapore in 1985, however, broken this confinement, and the CVA24 epidemic that resulted soon expanded across Asia, America, and Africa. Following the 1970s, the world experienced three distinctively different phases of the dynamic process of the AHC triggered by CVA24. The 1980s saw a spike in the number of cases, which led to a period of rapid expansion; the 1990s saw a period of relative quiet, with only sporadic outbreaks; and the 2000s saw a period of re-emergence and extremely quick spread [6].

Acute hemorrhagic conjunctivitis (AHC) outbreaks usually occur in India during the hot and humid months of August and September. Previous reports from India have documented epidemics related to Enterovirus 70 (EV-70) and Coxsackievirus A24 (CVA-24) [7–12]. Additionally, neurological symptoms have been associated with EV-70 infections during the pandemic [13].

New diagnostic techniques and innovative therapy drugs have been developed recently in the study of infectious conjunctivitis. Through direct examination or detection of their antigens and antibodies, individual viral strains have been identified in the laboratory using serological assays, ELISA, electron microscopy and polymerase chain reaction (PCR), Q-PCR techniques.

Between October 22nd and 23rd, 2022, there was a notable increase in conjunctivitis cases reported at an Eye Hospital in Hyderabad. Within just two days, over 100 cases of conjunctivitis were documented at this hospital, leading to concerns about a rise in conjunctivitis cases across Hyderabad. The objective was to identify the causative agent and outline the clinical features of an outbreak among patients receiving treatment at the ophthalmology department of Hospital in Hyderabad city, India.

2. Case/case series presentation

Case history: A sudden surge of conjunctivitis cases from 22nd-23rd October 2022 reported at one of the eye hospital in Hyderabad. Over 100 conjunctivitis cases were reported at Hyderabad's Eye Hospital within two days, prompting reports of an increase in conjunctivitis cases in Hyderabad. The index case in this study was a student who came back from his home after the completion of Dusshera vacations and infected other students. As the contacts had already been exposed, isolating the case after the onset of clinical symptoms was ineffective at the time of the study. Among these samples, 110 samples were further sent to Virus Research & Diagnostic Laboratory (VRDL) at Hyderabad, for screening of viral etiological agents. This infection presented with usual symptoms of redness of the eyes, discharge, pain in the eyes, fever and crusting.

Description of patient(s): Out of 110 cases 5 were having fever (Females-3; Males-2), 78 were with redness of the eye (F-26; M – 52), 59 were showed Discharge (F-24; M – 35) and 12 were presented with pain (F-1; M – 11) [Fig. 1]. Thirteen cases (F-3; M – 10) were having the epidemiological history of presence of similar case in the same house where as 10 cases were reported the presence of similar cases in the village/locality. No case has history of travel in last 10 days. The age range of patients for whom samples were analyzed was from 11 months to 74 years (mean-50 years). Forty two percent of these patients were female.

Diagnostic assessments: At the time of sample collection, all patients exhibited clinical symptoms of AHC. Samples were collected from individuals across all age groups, including infants and the elderly. Sterilized cotton swabs were employed to gently clean the affected eyes, and these swabs were then transported to the laboratory in 1 ml of viral transport medium.

Viral genome was extracted using viral RNA mini (Qiagen, Cat. No-52906) according to the manufacturer's instructions. All the samples were further screened for Adenovirus (Huwel RT-PCR Kit Cat No.QT-ADV-25), COVID-19 (Qline RT-PCR Kit Cat No.P211204), Herpes Simplex Virus (Dia-pro HSV 1 + 2 IgM Kit Cat No.0122/M) and Enterovirus (Realstar Altona RT-PCR Kit Cat No. 037794) by using commercially kits available at hospital.

All the samples were found negative for adenovirus, SARS-CoV-2 and herpes Simplex virus except for enterovirus. Pan-enterovirus qRT-PCR and further genotyping were done at the National apex laboratory, at Pune. All the samples viral genome extraction was performed using an automated MagMAX viral/pathogen Nucleic Acid Extraction kits II (MVP II) (Applied Biosystem, Cat. No. A48383) according to the manufacturer's instructions. Out of 110 tested specimens 18 sample tested positive for pan-enterovirus 5′ untranslated region (5′-UTR) SSIII one-step qRT-PCR (Invitrogen Cat No.12574026) [14] were subjected to nested reverse transcription polymerase chain reaction (RT-PCR) using primers targeted to VP2 [Table-1]. There is no clinical differentiation between enterovirus positive and negative cases.

All of these positive samples underwent further VP2 region-based typing analysis. CVA-24 was observed in each of the 18 samples through subsequent enterovirus species-specific PCR testing. Full-length VP2 gene amplification was used to further confirm CVA- 24

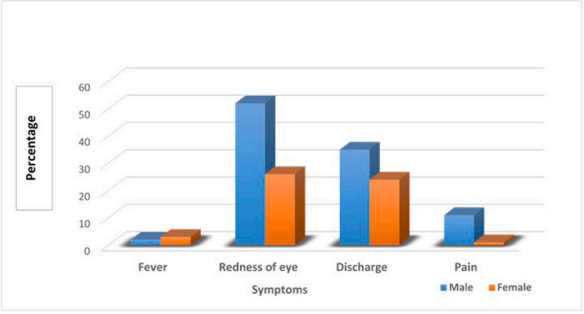

Fig. 1. Symptoms of Conjunctivitis cases present in the study subjects.

Table 1
Primers sequences for detection and characterization of Enteroviruses from AHC cases.

Sr. No.	Primer 5′-3′	Sequence	Location	Reference
1	EV Primer F	GGCCCCTGAATGCGGCTAATCC	449–470	5′ NCR qRT PCR Machado et al 2020
2	EV Primer R	GCGATTGTCACCATWAGCAGYCA	599–577	
3	Probe	FAM-CCGACTACTTTGGGWGTCCGTGT-BHQ1	537–559	
4	AM11	GARGCITGYGGITAYAGYGA	962–981	VP2 RT PCR
5	AM12	GARGARTGYGGITAYAGYGA	962–981	Nasari et al., 2007
6	AM21	GGITGGTGGTGGAARYTICC	1178–1197	
7	AM22	GGITGGTAYTGGAARTTICC	1178–1197	
8	AM31	TTDATDATYTGRTGIGG	1545–1529	
9	AM32	TTDATCCAYTGRTGIGG	1545–1529	

positive sample results. The VP2 genomic sequences underwent phylogenetic analysis, along with comparisons to other clinical isolates and reference strains for which sequences were available in the GeneBank database (Fig. 2) and found that the current AHC outbreak was due to the coxsackievirus A24 strain GIV C5.

Nucleotide sequences were analyzed using SeqScapev2.5 and nucleotide BLAST and phylogenetic analysis was done RIVM automated genotyping tool for enteroviruses (www.rivm.nl/mpf/typingtool/enterovirus) and Genome Detective Virus Tool for enterovirus V2.52 (www.genomedetective.com) [Vilsker et al., 2019]. Phylogenetic trees were generated using Clustal W alignments of nucleotide sequences. The evolutionary history was inferred by using Maximum Likelihood method based on the Kimura 2-parameter model was implemented in MEGA V. 6 (http://www.megasoftware.net/) with bootstrap resampling values of 1000 replicates. The genome sequences generated in this study submitted to NCBI. The annotated genome assembly has been deposited in NCBI GenBank under accession no. (OQ909106-OQ909113).

2.1. Therapeutic interventions

The treatment of all the cases were followed up till their complete recovery. Cases were managed with analgesics, cold compresses, and artificial tears. The rate of clinical accuracy in diagnosing viral conjunctivitis is less than 50 % compared with laboratory confirmation. So at the time of outbreak the patients were prescribed Ciprofloxacin eye drops (Cipro) and Moxifloxacin eye drops (Moxiflox) to avoid possible bacterial eye infections [15,16]. They also received Chloromycetin Applicaps, for various bacterial infections. Additionally, they were given paracetamol for pain relief and fever reduction, as well as cetirizine for allergy symptoms such

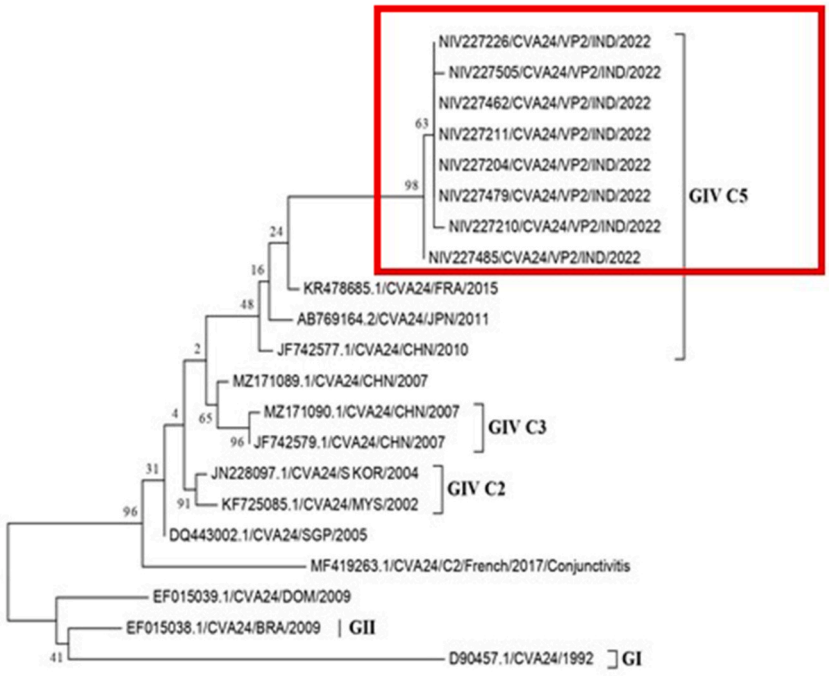

Fig. 2. Phylogenetic analysis of VP2 region of the human CoxsackievirusA-24 (CA24) from an outbreak of acute hemorrhagic conjunctivitis that occurred in Hyderabad, Andhra Pradesh state of India, 2022. Phylogenetic trees were generated using Clustal W nucleotide sequences alignments. The evolutionary history was inferred by using Maximum Likelihood method based on the Kimura 2-parameter model was implemented in MEGA V. 6 (http://www.megasoftware.net/) with bootstrap resampling values of 1000 replicates.

as itching, sneezing, and a runny nose.

2.2. Expected outcome and the actual outcome

This study highlights the investigations done because of the sudden surge in the cases of acute hemorrhagic conjunctivitis (AHC) in the particular area of Hyderabad city of India. Since 1970, CVA24-related AHC outbreak/epidemic that followed had primarily affected Southeast Asia and India. In our study, we identified Coxsackievirus A24 (CVA-24) as the causative agent behind the conjunctivitis outbreak. Modern diagnostic tools like rapid kits and Q-PCR assays are now available for accurately detecting these viral infections. Our research utilized an EV screening and a CVA-24 specific genotyping PCR, which pointed to Enterovirus (EV) as the main cause of the Acute Hemorrhagic Conjunctivitis (AHC) outbreak.

While we obtained molecular serotyping results for some samples, we concluded that CVA-24 was the specific subtype of EV since only this virus was detected, with no positive results for any other pathogen. Preventing such outbreaks in the future requires education and awareness about early symptom recognition, following personal hygiene measures, and implementing immediate isolation protocols.

It noted that the lack of public awareness of the "preventable" occurrence of conjunctivitis endangers the health of patients and imposes a significant economic burden on society. Despite the fact that 80 % of infectious conjunctivitis is caused by viral conjunctivitis, misdiagnosis of viral conjunctivitis as bacterial conjunctivitis, self-medication, and overuse of drugs exacerbate the problem of antibiotic resistance (AMR). To stop the spread of infection throughout the population, the original cluster must be identified and its transmission limited.

3. Discussion

Since the 1970s, CVA24 has been mostly mentioned in studies on the pandemic of AHC, a highly contagious eye illness for which there are now no vaccinations or antiviral medications available.

Our routine disease surveillance system frequently overlooks conjunctivitis outbreaks until they attract the attention of the local media. In order to stop a widespread outbreak in the population, it is essential to identify the conjunctivitis cluster and prevent its spread. We looked at an epidemic of acute conjunctivitis in a hostel/residential facility in a school. Congregational environments like hostels, barracks, religious gatherings, and social events encourage the spread of disease by droplets [17–20].

The majority of AHC outbreaks recorded globally during the past 20 years have involved CV A24 [21–23]. Due to a significant drop in immunity within seven years after infection, it has been hypothesized that widespread transmission of CV A24 is due to a loss of herd immunity. In India, CVA24 is a frequent AHC cause that has been identified in a number of AHC epidemics [24,25].In this investigation into the current epidemic, all 18 strains were sequenced, aligned and compared to strains from other nations, including Asia, where previous outbreaks were reported. It's interesting to note that all current strains were found to be grouped together in one lineage (genotype IV C5) with strains from several other Asian nations, including Korea (JN228097), China (JF742579, MZ171090), Japan (AB769164). Sequence similarities between Indian samples and strains reported from other Asian nations of Clade-1 were 94.4 % and 98.4 %, respectively. With 98.60 % nucleotide identity, our Indian strains were closely linked to the Japan and France strains. As per RIVM and genome detection phylogenetic analysis tools all the study strains were aligned with Picornaviridie Enterovirus C (CV-A24).

Our study has a few significant flaws. Just 16 % of samples tested positive because of delays in collection and testing. In our study, we found CVA-24 is the causative agent for the conjunctivitis. Rapid diagnostics kits and Q-PCR based assays are available nowadays for the identification of these viral infections. Our research demonstrated that EV was the AHC outbreak's primary cause using an EV screening CVA-24 specific genotyping PCR. Although molecular serotyping results were obtained for some of the specimens, we could infer that CVA-24 was the causative serotype of EV, because only that agent was identified and no positive results for any alternative agent were obtained. Such outbreaks in the future in similar settings would be avoided by education and knowledge regarding early symptom recognition, observance of personal hygiene precautions, and prompt isolation.

4. Conclusion

This study highlights the investigations done because of the sudden surge in the cases of acute hemorrhagic conjunctivitis (AHC) in the particular area of Hyderabad city of India. Since 1970, CVA24 related AHC outbreak/epidemic that followed had primarily affected Southeast Asia and India. We performed molecular identification of enteroviruses from AHC cases and phylogenetic analysis, as well as a determination of clinical relevance. Molecular characterization was done for circulating CV-A24 strain and it was found out the current outbreak of acute haemorrhagic conjunctivitis in Hyderabad, India was traced to the coxsackievirus A24 strain GIV C5.

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Data availability statement

The genome sequences generated in this study submitted to NCBI. The genome assembly with annotation deposited in the NCBI

GenBank under accession numbers (OQ909106- OQ909113). The authors confirm all supporting data, code and protocols provided within the article.

CRediT authorship contribution statement

Nutan A. Chavan: Writing – review & editing, Investigation, Data curation. **Vannavada Sudha Rani:** Investigation, Conceptualization. **Pooja Shinde:** Methodology, Formal analysis. **Manohar Shinde:** Methodology, Investigation. **Sanka Pavani:** Resources, Conceptualization. **Mote Srinath:** Resources, Conceptualization. **Syeda Fakiha Mehreen:** Resources, Investigation. **Palkonda Shashikala Reddy:** Resources, Data curation. **Mallika Lavania:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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