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Special Article - Conjunctivitis: Clinical Cases and Images

Outbreak of Epidemic Keratoconjunctivitis Caused by Human Adenovirus Type 2 in Chennai, India in 2014

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Abstract

Human Adenoviruses (HAdV) are the most important and most frequent cause of Epidemic Keratoconjunctivitis (EKC). The present study was undertaken to detect the virus responsible for the recent outbreak of conjunctivitis that occurred during September 2014 –November 2014 at Chennai, India.

Keywords: Conjunctivitis; Adenovirus; PCR-based DNA sequencing

Abbreviation

HAdV: Human Adenoviruses; EKC: Epidemic Keratoconjunctivitis

Introduction

Human Adenovirus (HAdV) is widely known to be an etiological agent causing varied clinical conditions like keratoconjunctivitis, gastroenteritis, upper and lower respiratory tract illness. The prevalence of adenovirus is worldwide and the infections are common during late winter, spring and early summer. Till date more than 50 serotypes of adenovirus has been reported. Among them more than 19 serotypes of adenovirus are associated with keratoconjunctivitis. Adenovirus type 8, 19, 37, 53 and 54 are commonly associated with Epidemic Keratoconjunctivitis (EKC) [1].

Epidemic Keratoconjunctivitis is a highly contagious ophthalmologic infection that causes outbreak in families, schools, health care centers. The acute phase of EKC involves the conjunctiva and the chronic phase involves the cornea presenting with multifocal Sub Epithelial Infiltrates (SEIs). Corneal involvement occurs 7-10 days after the onset of the infection. EKC should be differentially diagnosed from the other types of conjunctivitis as EKC is contagious and can spread to patient to patient rapidly [2].

Adenoviral conjunctivitis is clinically diagnosed based on the ocular signs and symptoms presented by the patient. HAdV can be diagnosed in the laboratory by immunofluorescence assays, isolation of viruses by cell culture techniques and Polymerase Chain Reaction (PCR) [3].

The present study reports on detection, isolation and genotyping of virus responsible for the recent outbreak of conjunctivitis that occurred during September 2014 –November 2014 at Chennai, India.

Materials and Methods

Patients and clinical specimens

Twenty conjunctival swabs were collected from both eyes of 10 patients with acute conjunctivitis who attended the outpatient department during October 2014. The conjunctival material collected in sterile absorbable cotton swab was transported in viral transport medium (Minimum Essential Medium with 3% fetal calf serum and antibiotics). The specimens were collected from the patients between 2 - 4 days after onset of the symptoms of conjunctivitis and were processed within 10 – 20 minutes in the laboratory.

Viral RNA extraction and RT-PCR amplification

The swab was squeezed against the wall the container, discarded and 200 µl of specimen was extracted for viral RNA using QIAGEN Viral RNA extraction kit. The kit reagents were reconstituted before use as per the protocol of the manufacturers' instructions. Reverse transcription/cDNA conversion (RT-PCR) was performed using QuantiTect Reverse Transcription Kit (Fermentas, USA) according to kit protocol.

Isolation of Human Adeno virus

Fifty microlitres of the clinical specimen was inoculated on to a 24 h old monolayer of HEp2 (NCCS, India) cell culture grown over tissue culture plate after aspirating out the growth medium. The inoculated culture was kept in a rocker for 30 min. At the end of 30 min Dulbecco's minimal essential medium supplemented with 1 per cent foetal calf serum was added. The cultures were incubated at 37°C carbondioxide incubator (Thermo Scientifics, USA). The cultures were observed daily under phase contrast microscope (Nikon, Japan) for the presence of cytopathic effect. The isolation of adenovirus was confirmed by subjecting the culture harvest for PCR investigation.

DNA extraction from cell culture harvest

DNA was extracted from the cell culture harvest using QIAGEN DNA extraction kit (Hilden, Germany). The kit reagents were reconstituted before use as per the protocol of the manufacturers' instructions. The viral isolation was further confirmed by PCR targeting Hexon gene of HAdV in the DNA extracted from the virus infected cell culture harvest.

Polymerase chain reaction for detection of Hexon gene: Nested PCR was performed using oligonucleotides to amplify 1004 Base Pair (bp) and 956 bp fragments of DNA coding for adenovirus hexon

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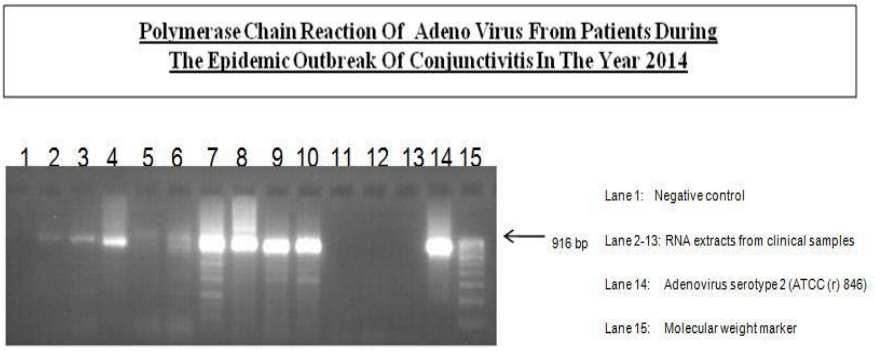

Figure 1: Agarose gel electrophoretogram showing Human Adenovirus PCR results of clinical sample collected from patients during the epidemic outbreak of acute kerato conjunctivitis in the year 2014.

protein. The PCR protocol and primers described earlier was followed [4]. The primers were custom synthesized by Bangalore Genei, India. The forward primer Ad TU 7-5' GCC ACC TTC TTC CCC ATG GC 3' and the reverse primer Ad TU 4'-5' GTA GCG TTG CCG GCC GAG AA 3' were used to amplify a 1004 bp (base pair) product in the first round. For the second round of the nested PCR (nPCR) the forward primer US'-5' TTC CCC ATG GCC CAC AAC AC 3' and the reverse primer UA-5' GCC TCG ATG ACG CCG CGC TG 3' were used to amplify a 956 bp product.

DNA sequencing

Among the PCR positive two were further subjected for DNA –sequencing. PCR amplified products of the two samples were cycle sequenced with both forward and reverse primers. The cycle sequenced products were purified and loaded into ABI 3100 Genetic Analyzer with polymer POP 6 and sequenced.

Results

Twelve samples collected from both eyes of six patients were positive for adenoviral RT-PCR and the 12 samples inoculated Hep-2 cell culture showed cytopathic effect in HEp 2 cell line and were confirmed as HAdV by PCR (Figure 1). The presence of viral RNA implies presence of active transcription. All the specimens positive for culture were collected within 2-3 days after onset of diseases symptoms and none of those with onset of disease beyond 3 days were positive either by PCR or culture.

Two of the 12 isolates harvest subjected for PCR-DNA sequencing confirmed the result on blast analysis. Further DNA sequence had 99.8% homology with Adenovirus [2].

Conclusion

Most of the severe EKC infections are caused by HAdV species B and D (serotypes 8, 11, 19a, 37). Adenovirus type 4, the only member of human adenovirus species E, is one of the major causes of adenoviral conjunctivitis [1].

HAdV-56 is a new recombinant type isolated from Epidemic Keratoconjunctivitis (EKC) patients and has been sporadically isolated in Japan several times.4 HAdV type 2 is commonly associated with Upper respiratory illness and hepatitis [5].

Outbreak of epidemic conjunctivitis is encountered every year in Chennai, India, during the months of rainy season i.e. August-November. The outpatient clinic of Sankara Nethralaya at Chennai, India is generally filled with patients diagnosed to have acute conjunctivitis during this period. Our earlier investigations during some of these epidemics identified Adenovirus serotype 4 in the year 1991, type 3 in 1992-1993 [6].

The identification on the causative agent was possible by application of virus isolation using tissue culture facility and serotyped using anti-adenoviral antiserum types 1, 2, 3, 4, 5, 6, 7a and 14 (NIAID antisera, ATCC, Rockville, MD, USA). Later in the year 1996, viruses from clinical specimens were isolated in HEp-2 cell line), and PCR-RFLP technique confirmed them to be type 7a [3]. The epidemic of the year 1998 continued to December – January 1999 and was due to Coxsackie A24 virus [7]. A variant HAdV was isolated during the Epidemic of Acute Keratoconjunctivitis in 2010 based on Phylogenetic Analysis [8].

The current report is on detection, isolation and genotyping of virus that was responsible for the recent epidemic which commenced in August with the peak reaching in September with a slow decline in the number in October 2010. In this study, HAdV type 2 were isolated from patients attending outpatient department during October 2014 when the peak of the acute conjunctivitis epidemic. Among the twenty clinical samples collected, HAdV was isolated from twelve samples collected from both the eyes of six patients. Among the 6 patients whose Ad isolates were serotyped, all the twelve isolates were serotyped as HAdV type 2 by PCR based DNA sequencing. Though HAdV 2 was commonly associated with respiratory and rarely ocular infection, this is first time the HAdV type 2 was found to be the causative agent of Epidemic Keratoconjunctivits in India. The overall prevalence of isolation of Ads from samples collected in our study was relatively less, but all 10 HAdV type 2 infected patients had clinical manifestations of ocular EKC. Further studies are indicated to elucidate the association of EKC with HAdV type 2 infections, to determine the spectrum of clinical manifestations and natural history of conjunctival HAdV type 2 infection.

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