JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Letter to the Editor

Outbreak of Adenovirus serotype 8 keratoconjunctivitis in Puducherry, South India: a molecular epidemiological study

Raja Sundaramoorthy[1], Rahul Dhodapkar[1], Subashini Kaliaperumal[2], Belegode Narasimha Harish[1]

[1] Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India
[2] Department of Ophthalmology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India

Key words: Human Adenovirus; keratoconjunctivitis; multiplex polymerase chain reaction.

J Infect Dev Ctries 2016; 10(4):449-452. doi:10.3855/jidc.7225

(Received 04 June 2015 – Accepted 04 August 2015)

Dear Editor,

The present study reports an outbreak of conjunctivitis due to human adenovirus serotype 8 (HAdV-8) for the first time from India. HAdV-8, was detected in 16 samples by Multiplex PCR. Eleven strains were sequenced, analysed by phylogenetic tree with pre-epidemic and global isolates and revealed no variation with pre-epidemic strains.

Acute viral conjunctivitis is the most common cause of infectious conjunctivitis. It can be caused by several viruses such as Human adenoviruses (HAdV), Herpes virus, Enterovirus 70 and Coxsackie virus A24 [1]. Adenovirus is the most common agent of viral conjunctivitis representing 15%-70% of all cases of conjunctivitis worldwide [2]. HAdV (Adenoviridae family) is divided into 7 species (A to G) and 68 serotypes [3]. Serotypes 3, 4, 7, 8, 19 and 37 are responsible for 89% of the cases of viral conjunctivitis worldwide [4]. Adenoviruses are non-enveloped and show resistance to both physical and chemical agents of disinfection. Their stability against such agents make them more infectious and facilitates their transmission in the community through fomites, ophthalmic instruments and even through ocular drops [5].

Information on the monthly incidence of adenoviral conjunctivitis is essential to prevent further infection [6]. In the month of October and November 2014, an outbreak of acute viral conjunctivitis occurred in Puducherry, India, during the rainy season. In this context, the aim of the present study was: to detect adenovirus by (i) viral culture, (ii) immunofluorescence assay (IFA) and (iii) multiplex polymerase chain reaction (PCR). We also conducted a molecular epidemiological study on adenovirus and its serotypes.

The study

An outbreak of acute viral conjunctivitis was observed during routine surveillance in the months of October and November 2014. A total of 42 patients (24 males and 18 females) who presented with viral conjunctivitis in the outpatient department of Ophthalmology at the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) were included in the study. Dacron swabs were used to sample material from the inferior palpebral conjunctiva, transported in viral transport medium (VTM) and processed in the virology laboratory. Samples were vortexed and centrifuged at 10,000 rpm for 30 minutes at 4°C. The supernatant was used for both viral culture and PCR. The pellet was used for IFA. Viral culture was carried out by inoculating 0.5 ml of supernatant into a 24 hours old Hep2 cell line and incubated at 37°C for 30 minutes in a CO_2 incubator in order to achieve enhanced absorption. Maintenance medium (Dulbecco's minimal essential medium supplemented with 2% foetal calf serum) was added and incubated at 37°C. The culture flask was regularly observed for typical cytopathic effects of adenoviruses (grape like clusters). IFA was carried out on all positive cultures. If there were no cytopathic effects at the end of seventh day, blind passage was done and the sample was reported negative after another 7 days. IFA was also carried out for rapid identification of adenovirus directly from samples. The pellet, isolated during

sample processing was spotted on a Teflon coated slide. Immunofluorescence staining was carried out using primary mouse origin anti-adenoviral (Ab) directed against a hexon protein (Catalog No: MAB8051) and secondary anti-mouse FITC labelled Ab of goat origin (Catalog No: CA92590) (Chemicon Millipore, Temecula, California, USA). Staining procedures were carried out with appropriate positive and negative controls, and examined under fluorescence microscope for apple green fluorescence (Supplementary Figures 1 and 2) [7].

In our present work, PCR was carried out to detect adenoviral serotypes. Viral DNA was extracted from the supernatant fluid using a commercial kit (QIAGEN, Hilden, Germany). Adenovirus Universal Semi-nested PCR based on Hexon target region (HELINI Biomolecules, Chennai, India) was carried out to detect adenoviral DNA. The internal controls were run concurrently for each set of experiments along with positive and negative controls. Samples that were positive by the Universal Semi-nested PCR, were subjected to a multiplex PCR for identification of adenoviral serotypes such as 3, 4, 8, 7, 19 and 37 (primer sequences listed in Table 1).

Results and discussion

Epidemic Keratoconjunctivitis is a severe contagious eye disease frequently associated with Human adenovirus Serotype 8 (HAdV-8) [8]. HAdV-8 as an etiological agent of EKC was first reported by Jawetz and Hamma in the year 1959. Till date, HAdV-8 remains an important agent of EKC worldwide and outbreaks have been reported from several studies [9-11]. Results of the present work depict that out of 42 patients, 23 were positive for adenovirus by Universal Semi-nested PCR, IFA or viral culture (Table 2). Multiplex PCR was carried out on 16 PCR-positive samples and 4 PCR negative, viral culture positive samples for serotype detection. All 20 were identified as adenovirus serotype 8. Remaining 3 samples were positive only by IFA and not by other methods. Sequencing was carried out for 11 strains and all were confirmed to belong to serotype 8 (Gen bank accession No. KR150657-667). Additionally, two pre-epidemic strains were sequenced to be serotype 8 (KR084326-27). The sequences were aligned using Clustal-W algorithm. All the sequenced serotypes including the pre-epidemic isolates were aligned for phylogenetic analysis. The analysis revealed that the pre-epidemic and epidemic strains showed no variations in sequence

Table 2. Number of samples positive for Adenovirus by various methods

Methods	No. of Samples positive
PCR, Viral Culture and IFA	9
PCR and IFA	2
PCR and culture	2
Culture and IFA	2
PCR alone	3
Culture alone	2
IFA alone	3

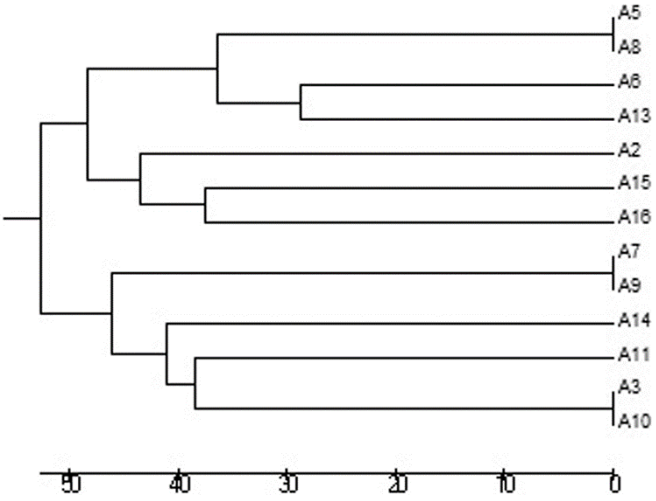
Table 1. Primers used in this study

Serotype	Primers	Product size(base pair)
Universal Semi-nested PCR	F-CTGTGGTCGACTTGCAAGAC R-ACCGCAGAGTTCCACATACT	106
Serotype 3	F- TGCACCTACTATGAGACAAGGG R-GACATGAAGTTGCTGGAGAAGG	150
Serotype 4	F-GGTGGTGGACGAGGTTAACTA R-GACATGAAGTTGCTGGAGAAGG	230
Serotype 7	F-ACATTACTGCAGACAACAAGCCC R-CTCCTCAGCTTCAACATCTCCTTC	250
Serotype 8	F-TTTGTTTACTCGGGCACCATC R-GACATGAAGTTGCTGGAGAAGG	550
Serotype 19	F-CTCTGGTACCAATGCTGCCTA R-GTTACGATCTGCGACTTTGGTATC	103
Serotype 37	F-AGGAACTGGAGCAGAAAAAGATGTTAC R- GTATTGAGGATCGGTACCATTGGG	322

based on the unweighted pair group method with arithmetic mean (UPGMA) method (Figure1). To the best of our knowledge, this is the first study from India, reporting an outbreak of conjunctivitis due to adenovirus serotype 8. Hence, we compared our strains with the global isolates and a phylogenetic tree was constructed (Figure 2).

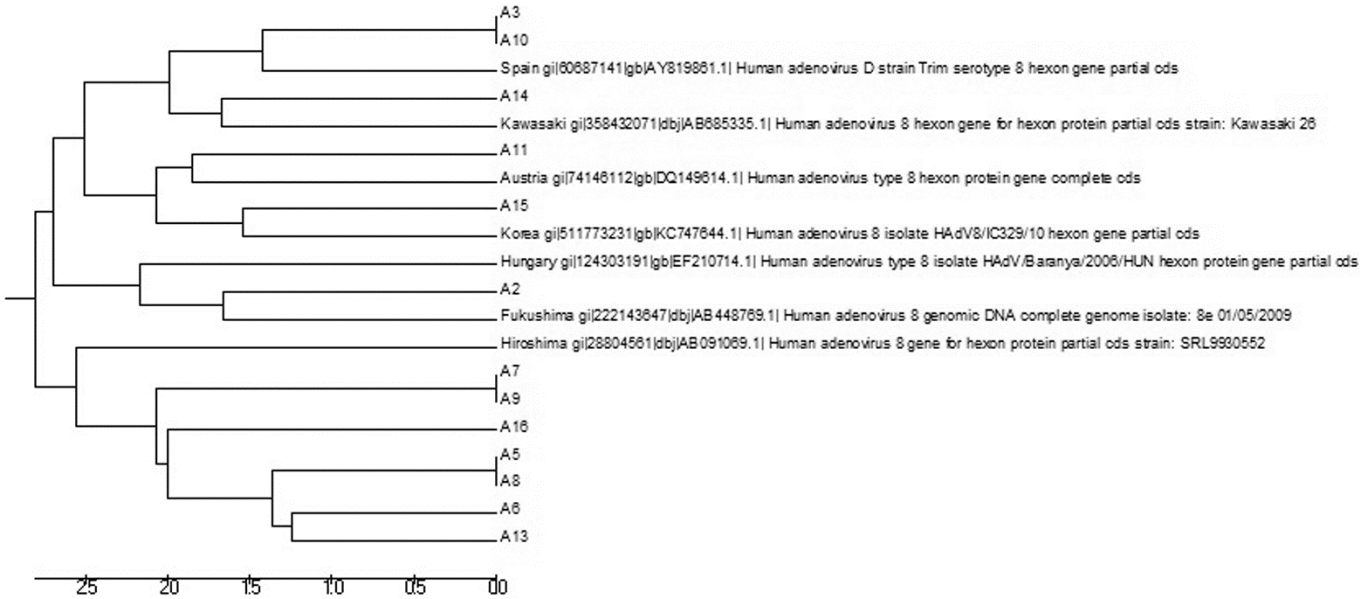
In the present work, phylogenetic tree analysis revealed that our strains belong to two clusters (A5, A6, A7, A8, A9, A13, A16, Hiroshima strain) and (A2, A3, A10, A11, A14, A15, Spain, Kawasaki, Austria, Korea, Hungary, Fukushima strains). In each year, HAdV-8 accounts for the highest numbers of EKC cases worldwide. Previous studies from India reported outbreaks of conjunctivitis due to serotypes 3, 4, 7 [12] and a variant strain closely related to 2 and 6 [13]. Hence this is the first report of viral conjunctivitis due to HAdV-8 from India. Furthermore, we observed that all the patients presented with single eye involvement and within three days of symptoms.

Figure 1. Phylogenetic analysis of pre-epidemic and epidemic strains.



*A2, A3- Pre epidemic strains; A5, A6, A7, A8, A9, A10, A11, A13, A14, A15 A16 – Epidemic strains

Figure 2. Phylogenetic analysis of outbreak strains with global strains.



*A2, A3 - Pre epidemic strains; A5, A6, A7, A8, A9, A10, A11, A13, A14, A15 A16 – Epidemic strains
gi|222143647|dbj|AB448769.1| Human adenovirus 8 genomic DNA, complete genome, isolate: 8e – Fukushima strain
gi|124303191|gb|EF210714.1| Human adenovirus type 8 isolate HAdV/Baranya/2006/HUN hexon protein gene, partial cds – Hungary strain
gi|60687141|gb|AY819861.1| Human adenovirus D strain Trim serotype 8 hexon gene, partial cds- Spain strain
gi|358432071|dbj|AB685335.1| Human adenovirus 8 hexon gene for hexon protein, partial cds, - Kawasaki strain
gi|74146112|gb|DQ149614.1| Human adenovirus type 8 hexon protein gene, complete cds- Austria strain
gi|511773231|gb|KC747644.1| Human adenovirus 8 isolate HAdV8/IC329/10 hexon gene, partial cds – Korea strain
gi|28804561|dbj|AB091069.1| Human adenovirus 8 gene for hexon protein, partial cds, strain: SRL9930552 – Hiroshima strain

Acknowledgements

This work was supported by intramural grant by Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry 605006, India.

References

1. Elnifro EM, Cooper RJ, Klapper PE, Bailey AS, Tullo AB (1999) Diagnosis of viral and chlamydial keratoconjunctivitis: which laboratory test? Br J Ophthalmol 83: 622–627.
2. Engelmann I, Madisch I, Pommer H, Heim A (2006) An outbreak of epidemic keratoconjunctivitis caused by a new intermediate Adenovirus 22/H8 identified by molecular typing. Clin Infect Dis 43: e64–66.
3. Huang G-H, Xu W-B (2013) Recent advance in new types of human adenovirus. Bing Du Xue Bao 29: 342–348.
4. Banik U, Adhikary AK, Suzuki E, Inada T, Okabe N (2005) Multiplex PCR assay for rapid identification of oculopathogenic Adenoviruses by amplification of the fiber and hexon genes. J Clin Microbiol 43: 1064–1068.
5. Madhavan HN (1999) Laboratory investigations on viral and *Chlamydia trachomatis* infections of the eye: Sankara Nethralaya experiences. Indian J Ophthalmol 47: 241–246.
6. Meyer-Rüsenberg B, Loderstädt U, Richard G, Kaulfers PM, Gesser C (2011) Epidemic keratoconjunctivitis. Dtsch Ärztebl Int 108: 475–480.
7. Kasparova EA, Marchenko NR, Loseva IE (1998) Express diagnosis of eye adenovirus infection by fluorescent antibody method. Vestn Oftalmol 114: 24–26.
8. Ford E, Nelson KE, Warren D (1987) Epidemiology of epidemic keratoconjunctivitis. Epidemiol Rev 9: 244–261.
9. Percivalle E, Sarasini A, Torsellini M, Bruschi L, Antoniazzi E, Grazia Revello M, Gerna G (2003) A comparison of methods for detecting adenovirus type 8 keratoconjunctivitis during a nosocomial outbreak in a neonatal intensive care unit. J Clin Virol 28: 257–264.
10. Adhikary AK, Ushijima H, Fujimoto T (2012) Human adenovirus type 8 genome typing. J Med Microbiol 61: 1491–1503.
11. Tabbara KF, Omar N, Hammouda E, Akanuma M, Ohguchi T, Ariga T, Tagawa Y, Kitaichi N, Ishida S, Aoki K, Ishiko H, Ohno S (2010) Molecular epidemiology of adenoviral keratoconjunctivitis in Saudi Arabia. Mol Vis 16: 2132–2136.
12. Dalapathy S, Lily TK, Roy S, Madhavan HN (1998) Development and use of nested polymerase chain reaction (PCR) for the detection of adenovirus from conjunctivitis specimens. J Clin Virol Off Publ Pan Am Soc Clin Virol 11: 77–84.
13. Janani MK, Malathi J, Madhavan HN (2012) Isolation of a variant human adenovirus identified based on phylogenetic analysis during an outbreak of acute keratoconjunctivitis in Chennai. Indian J Med Res 136: 260–264.

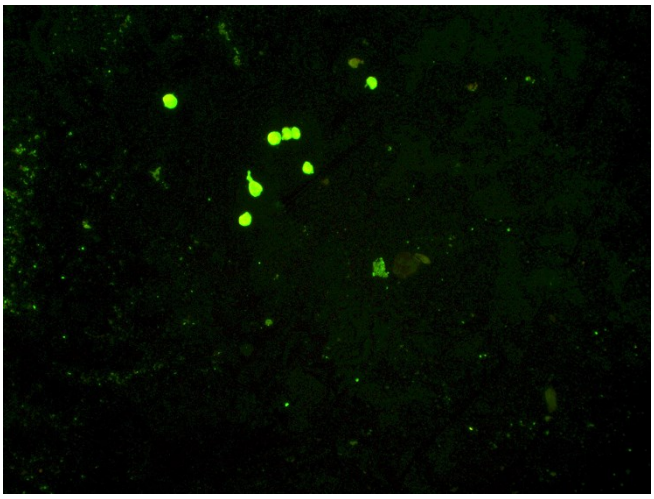
Corresponding author

Rahul Dhodapkar, MD, Associate Professor
Department of Microbiology,
Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER),
Dhanvantri Nagar, Gorimedu, Puducherry, India-605006
Phone: +91-9344560708
Fax: 0413-2272067
Email: rahuldhodapkar@gmail.com

Conflict of interests: No conflict of interests is declared.

Supplementary Items

Supplementary Figure 1. IFA positive – direct sample.



Supplementary Figure 2. Culture IFA positive.