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Molecular epidemiology of circulating Human Adenovirus type in acute conjunctivitis epidemic cases in Chennai, India - First report in 2019

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ABSTRACT

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INTRODUCTION

Epidemic keratoconjunctivitis (EKC) presents with watery discharge, hyperaemia, chemosis and ipsilateral lymphadenopathy.[1] Adenoviral conjunctivitis is known to be the most common cause of red eye in the world.[2]Various studies have demonstrated the prevalence of human adenovirus(HAdV) in 70-75% approximately of all conjunctivitis cases worldwide.[3–5] More than 50 different adenoviral serotypes have been identified and divided into six distinct subgroups.[6,7]

Due to the epidemic nature of keratoconjunctivitis, outbreaks have been studied in crowded living conditions and places where people come into contact. Direct contact through ocular and respiratory secretions or by indirect contact with contaminated instruments or solutions leads to viral transmission.[8]Diagnosis of EKC is primarily based on the clinical feature.[8] Laboratory investigations involve viral isolation and staining targeting viral antigens. Polymerase chain reaction (PCR) based identification of the causative agent will aid in rapid identification along with the subtype prevalence in a particular epidemic. This study was conducted to identify the pathogen EKC outbreak in Chennai during October – November 2019.

Methodology

Sample collection: The cases for the study were recruited from two different eye hospitals, Sankara Nethralaya and Dhanvantri eye care, Chennai, India. 20 conjunctival swabs were collected from 11 patients (9 bilateral and 2 unilateral) clinically suspected with viral conjunctivitis. The conjunctival swabs were collected in rayon swab by ophthalmologists after obtaining informed written consent. The samples obtained were transported to Sankara Nethralaya Referral laboratory in viral transport medium in the cold chain.

DNA extraction: DNA was extracted from the samples using QIAGEN DNA extraction kit (Hilden,

Germany) as per the manufacturer's instructions. PCR targeting hexon gene of HAdV were performed using the DNA extracted.

Polymerase chain reaction: Nested PCR was performed using oligonucleotides to amplify 1004 base pair (bp) and 956 bp fragments of DNA coding for adenovirus hexon protein as previously described.[11]

DNA sequencing: PCR positive samples were further subjected for DNA sequencing. PCR amplified products 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 7 and sequenced.

Results and Discussion

The mean age of the patients was found to be 43.9 ± 17.7 years with male: female ratio of 3.6:1. The conjunctivitis was unilateral in 2 patients (18.2%) and bilateral in 9 patients (81.8%). The disease was graded as moderate and severe-based on the presence of chemosis in 8 (72.7%) and 3 (27.2%) patient, respectively. A total of 8 (72.7%) and 3 (27.2%) reported purulent and watery discharge, respectively. Two patients were reported with conjunctival membrane and conjunctival haemorrhage was noted in 5 cases. 6 (54.5%) patients also presented with upper respiratory tract infection.

Out of twenty samples obtained from eleven patients samples collected, nineteen (90.0%) were found to be positive for HAdV by nested PCR. All 19 PCR positive samples that were subjected to sequencing, found to be human adenovirus Type 8(HAdV-8). The sequences were submitted to Genbank. The nBLAST analysis showed a sequence identity of 99%–100% for Type 8. In the present study, positivity of 95% was observed for HAdV among patients with acute conjunctivitis. Prominent investigations from India that have been directed from 1996 to 2015 have demonstrated the positivity of 13.8%–65.2% for HAdV (Type 7a, 3, 4, 37, and 8) among the patients with keratoconjunctivitis.[10–13]Among the different types of HAdV (A-G), B, D and E are all the more usually associated with conjunctivitis. In the present study, we recognized the nearness of HAdV Types 8 having a place with the Group D by utilizing preliminaries targeting on the hexon region as the causative for EKC. From an epidemiological investigation of 76 conjunctivitis flare-ups all around, uncovered that the Genotype 8 is progressively predominant, engaged with up to 44%–100% of pandemic keratoconjunctivitis episodes.[14] The investigations from India have recently detailed the nearness of Type 8 from Pune and Puducherry in 78.6% and 100% of cases, separately, and Type 4 from Chennai and Pune (7.2%).[10,15]

The present study proposes the transcendence of Type 8 strains and their comparable circulating patterns in India. It is important to sequence to recognize any variety in flowing kinds. Just not many nations, for example, the US and Japan, are doing suitable observation of HAdV.[16]The present investigation adds up the information on existing circulating strains of HAdV. Further molecular investigations will help to the distinguishing proof and origin of outbreaks and advancement of control programs.

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