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Letter to the Editor

Molecular characterization of adenovirus from an ongoingmulti-centric keratoconjunctivitis study in India

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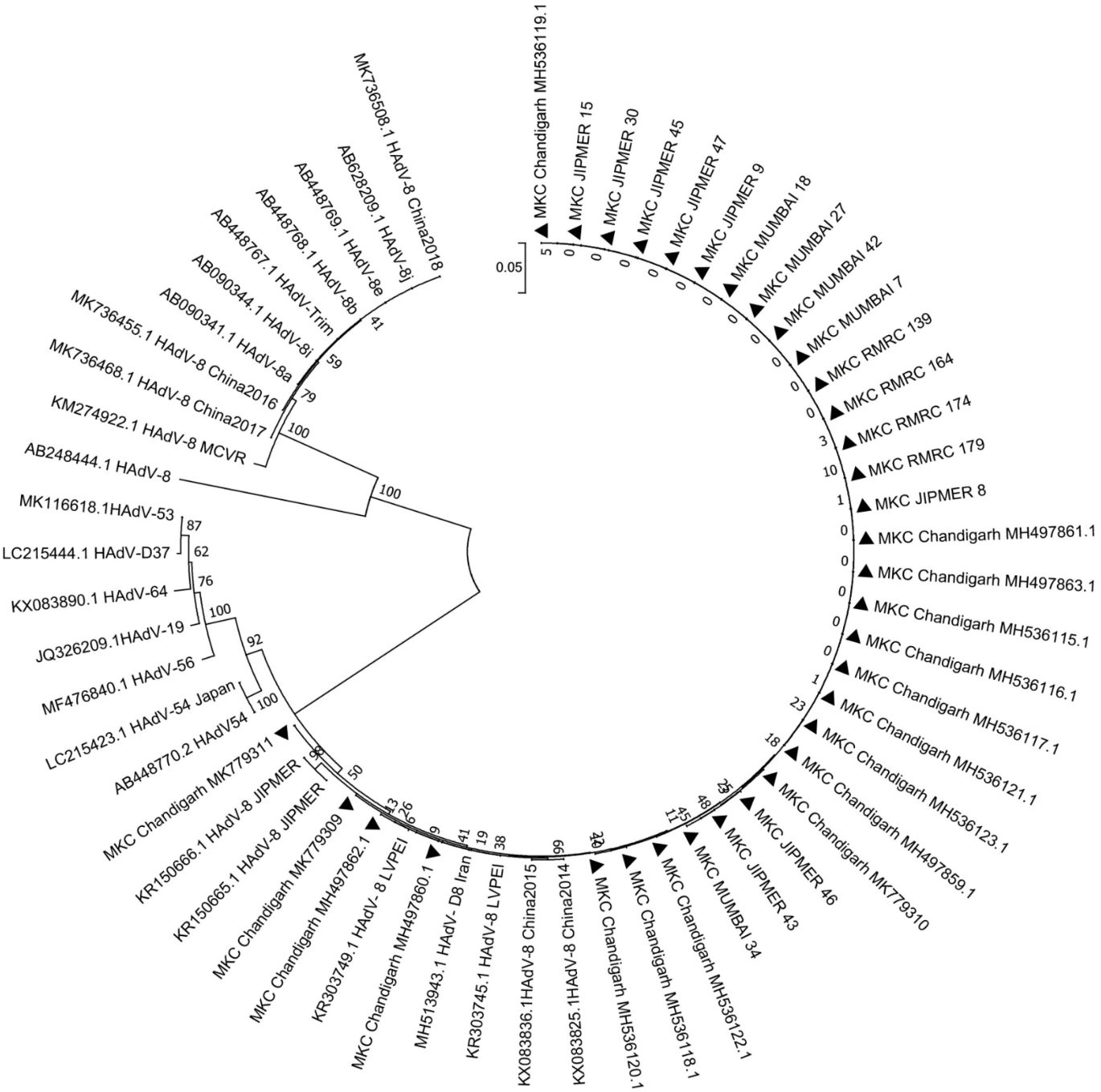
Dear Editor,

Epidemic keratoconjunctivitis (EKC) is a highly contagious form of viral eye disease, which usually causes epidemics in schools, camps, clinics and among population in close quarters. Human Adenoviruses (HAdV) are the known major causative agent of EKC, representing 15% to 70% of conjunctivitis worldwide [1] and in India it has been reported to be 65.2% [2]. EKC usually presents as severe hyperemia, diffuse infiltration, lacrimation, follicular conjunctivitis, pseudomembrane formation with potential permanent symblepharon formation or punctual occlusion, and regional lymphadenopathy, such as mild swelling of the preauricular nodes [3,4]. Multiple subepithelial corneal infiltrates (MSIs) occurs following corneal infilteration in less than 50% of EKC cases and thought to be a by-product of inflammatory response [5]. In few cases the spotted opacities found under corneal epithelium stays persistently resulting in visual decline, glare sensation, photophobia, and irregular astigmatism [4,5]. Rarely patients do present with flu-like symptoms also, which includes myalgia and fever [4]. The incubation period has been shown to vary from between 2 days to 2 weeks, and the patients become contagious after the onset of symptoms for up to 2 weeks thereafter. Bilateral presentations are common with HAdV infections owing to their high infective nature but usually the symptoms are more intense in the firstly infected eye [6]. Although, EKC does not progress to blindness, ocular infections with the above-mentioned clinical features eventually result in significant morbidity and economic damage.

HAdV belongs to the genus *Mastadenovirus* of the *Adenoviridae* family. There are about 54 types divided among seven species A (HAdV-A) through G (HAdV-G). They are non-enveloped, double-stranded DNA virus and are highly resistant to environmental conditions. This makes them remain infectious for

extended periods, thereby facilitating transmission via hand-to-hand, through ophthalmic examination tools or even eye drops [7]. For about fifty years HAdV-8, HAdV-37, HAdV-64 have been considered as the major contributors in EKC epidemics [8], but recent surveillance data have shown emergence of recombinant HAdVs which includes HAdV-53 [9], HAdV-54 [10,11], and HAdV-56 to be involved as well. Hence it is necessary to have continuous surveillance for monitoring the circulating HAdVs, to establish comprehensive criteria in EKC diagnosis, development of treatment strategies and preventive measures against outbreaks.

Figure 1. Molecular Phylogenetic analysis of partial Hexon region of HAdV samples by Neighbor-Joining method.



Phylogenetic analyses of partial hexon region for serotype determination. The evolutionary distances were computed using the p-distance method. The analysis involved a total of 59 nucleotide sequence, of which 25 are reference sequences. Current study samples are indicated by ▲. The numbers in the nodes are support values for the major branches (bootstrap; 1000 replicates).

The Report

This work was carried out as a part of a collaborative project which aimed to characterize the agents causing keratoconjunctivitis throughout India. It includes 5 centres: JIPMER (Co-ordinating centre), PGIMER – Chandigarh (North), NEIGRIHMS - Shillong (North-east), TNMC &BYL NAIR Hospital – Mumbai (West), RMRC – Port Blair (South). This study was approved by institute ethics committee (JIP/IEC/20 17 /0279). A total of 692 conjunctival swabs were collected from keratoconjunctivitis patients with a mean age of 34 ± 17 years during the period of September 2017 to March 2019, with percentage male: female ratio being 59:41. A total of 255 samples (36.8%) were positive for Adenovirus by real time PCR (targeting the hexon region) and representative samples from RMRC-Port Blair (n = 4), PGIMER- Chandigarh (n = 17), JIPMER (n = 8), and TNMC &BYL NAIR Hospital – Mumbai (n = 5) were included in the current study. DNA was extracted from these samples using QIAamp DNA Mini kit (Hilden, Germany) and was subjected to multiplex PCR targeting the partial hexon region (Set A – 3, 4, and 8 serotypes and Set B- 7, 19, 37 serotypes) for identification of the serotype using Adenovirus genotyping PCR kit from HELINI Biomolecules (Chennai, India). Amplicons (serotype e3-150 bp; serotype 4-230 bp; serotype 7-250 bp; serotype 8-550 bp; serotype19-103 bp and serotype 37-322 bp) were detected by agarose gel electrophoresis. We found all the 34 samples to be positive for serotype 8 (550bp) which were subjected to sequencing in ABI 3500. The obtained sequences were edited and BLAST search was conducted to confirm the identity of the sequences. The present study samples revealed 97-100% nucleotide identity among themselves. The phylogenetic tree was constructed with a total of 59 sequences including 34 from the current work, and the remaining belonging to HAdV-8, 19, 37, 64 and novel recombinant genotypes (HAdV53, HAdV54, HAdV56) which are known common causative agents of EKC, from GENBANK in MEGA v.7 [12]. The phylogenetic tree was drawn by using Neighbor-Joining method [13] with bootstrap analysis of 1000 replicates. Analysis of the phylogenetic tree revealed the current isolates to be placed closer to strains from China (KX083836, KX083825), Hyderabad- India, (KR303745, KR303749), Iran (MH513943), previous reported strain from our centre - India (KR150666, KR150665) with a nucleotide identity of ~98% and also with the newly described HAdV-54 (AB448770) (nucleotide identity 94%) (Figure 1). At the aminoacid level we found our sequences aligned at 17678 to 20539 bp corresponding to the prototype strain NC_012959, covering 717th to 879th amino acid of the C terminal end of Adenoviral Hexon- Major Coat protein region. A total of 6 known variations were observed: I769V, L820V, F822L, T830A, M837L, E854Q.One centre NEIGRIHMS – Shillong did not have any HAdV positivity during this study period.

In India, many outbreaks of conjunctivitis have been reported earlier in different parts of the country with HAdV-8 being the commonest [2,14,15]. Reports from the 2014 outbreak in Pune and Chandigarh showed an overall positivity for HAdV to be 60% (14/23) and 65% (15/23); of which HAdV-8 positivity was 78% and 65% accordingly. Our previous published study, also conducted during the same period from Pondicherry revealed 53% (23/43) positivity for HAdV of which 100% belonged to serotype 8. When comparing with these reports the overall positivity for HAdV in our current multi-centric study is less (36.8% (255/692)), however it is similar to results published recently from China [16]. The probable reason for this could be attributed to the larger number of samples included in the present report which could reflect the true contribution of HAdV towards keratoconjunctivitis in India. In phylogenetic analysis we observed that the present samples which were serotyped to be HAdV-8 were actually closer towards the newly reported serotype 54 (AB448770 and LC215423). HAdV-54 was first reported in 2000 during a nosocomial outbreak in Japan [11]. Earlier it was mistyped as HAdV-8 as it has cross reactivity in serological assays. On comparing the entire genome level we find they are almost >95% similar, with differences at the penton base and hexon regions. These data suggest that it could be the product of some ancestral recombination event.

Conclusion

From these reports stating high similarity between the serotypes 8 and 54, we can conclude that the current strains in this study as well as from our previous study could be closer towards HAdV-54rather than HAdV-8. However, this needs to be substantiated with further studies like whole genome analysis using next generation sequencing.

HAdVhas gained significant importance especially in case of epidemics because of their resistant nature of towards sterilizing agents and resulting significant morbidity. Hence continuous surveillance is warranted which aids in early detection of aetiological agent thereby preventing spread of disease as some of serotypes of adenoviruses can cause a severe form of conjunctivitis.

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