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First Record of HAdV-D20 Among Keratoconjunctivitis Patients in Iraq

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Abstract

Background: Human Adenovirus species D (HAdV-D) was common human viral pathogen especially in eye infection, consists of several types of which HAdV-D8, -D19 and –D37 were common in eye infection. This study includes detection of HAdV-D types implicated in conjunctivitis based on L2 (Penton protein) gene similarity.

Methods: Conjunctival swabs were collected from Keratoconjunctivitis patients as eye infection related to adenovirus. Viral nucleic acids were extracted and specific primer pairs for HAdV-D L2 gene (encoding for penton base protein) was used to amplify the target gene and only positive samples were sent to sequencing.

Results: The results revealed that only 6 samples give positive results for L2 gene amplification and then sent for sequencing for L2 (penton protein) gene-based typing. The results show that 4 local isolates (S1, S2, S3, S6) were similar to HAdV-D8 and 2 local isolates (S4, S5) were similar to HAdV-D20. Also the results display that the HAdV-37, prominent HAdV-D type of human eye infection, may be variant of HAdV-D20 due to that six variation were seen in S4and S5 local isolates nucleotide sequence in relation to HAdV-D37: T>C at position 14364, A>C at position 14411, T>C at position 14427, C>A at position 14448, G>A at position 14540 and T>C at position 14617, leading to only 2 amino acid change in resulted penton protein: T (Threonine) instead of K (Lysine) at position 204 and N (Asparagine) instead of D (Aspartic acid) at position 247.

Conclusions: The current study concludes the possibility of implication of HAdV-D20 in eye infections especially conjunctivitis.

Keywords: HAdV-D8, HAdV-D20, HAdV-D37, Conjunctivitis, Iraq.

Introduction

Conjunctivitis is an inflammation of the conjunctiva resulted from an allergic reaction or infection (viral or bacterial). Children are furthermost disposed to viral infections and viral conjunctivitis may be acquired by airborne transmission, unintended contact with virus, and may be via swimming pools (1,2). Adenoviruses-associated conjunctivitis is most common infection worldwide (3). Human adenoviruses (HAdVs) have been categorized in *Mastadenovirus* genus, containing 7 known species of the HAdV, i.e., HAdV-A to HAdV-G (4,5). Human adenovirus D, include the following types: 8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, 53, 54, 56 (6). HAdV-D8, -D37, -D42, -D48, -D53, -D56 and -D64 compile 63.7% of HAdV conjunctivitis in Beijing, 2011–2013 (7).

Penton base is one of the outer surfaces of viral major capsid proteins (penton-hexon-fiber) contributed to antigenicity and utilized in descriptions and categorization of new recombinant strains of the HAdV (8,9). Phylogenetic analysis also can be achieved

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using penton base for type discrimination and assigning (10). The penton base play a vital role in adenovirus cell entry via loops of RGD that extend from penton base bind to α-vβ3 or α-vβ5 integrins. So, the tissue tropism and infection selectivity may mainly depend upon penton base variant and so the HAdV-D type-specificity will determine the type of infection (11,12). The objective of current study for investigating Human adenovirus species D type based on Penton base (L2) gene sequence variation.

Materials and Methods

One hundred patients referred to AL-Hilla teaching hospital /Babylon, Al-Imam AL-Hussein medical city hospital/Kerbela, and AL-Hakeem teaching hospital/AL-Najef from December 2018 to June 2019 were recruited for this study. All were examined by experienced ophthalmologists and diagnosed with kerato-conjunctivitis. Conjunctival swabs were collected, were inserted to viral transport media, and stored at -80 ºC.

the number of cells increases logarithmically.

Ethical Approval

Informed consent was obtained from all adult participants or parents or legal guardians of minors.

Polymerase chain reaction (PCR)

Viral nucleic acid extraction has been accomplished with the use of the (FavorPrep Viral Nucleic Acid Extraction Kit II) (Cat. No.: FAVNK 002 (50 Preps) according to instructions of manufacturer (Favorgen/Tiwan). Viral nucleic acids were electrophoresed by 0.7% agarose gel electrophoresis and visualized by Gel Documentation system QUANTUM-ST5 (Vilber/France) to check the extracted nucleic acid (13). Conventional PCR for L2 gene (Penton protein gene) was accomplished using Forward: TTCGCAAGAAGCAACCTTT and Reverse: TCTTGCATGAGGTCCGG (14).

Sanger Sequencing and analysis

Trimming of L2 (Penton protein) gene sequences was performed by FinchTV and then submitted to NCBI-BLASTN to see the identity of sequences with reference sequences within NCBI data (15). All trimmed and confirmed sequences then Aligned with most frequent types of HAdV species: HAdV-D8 (AB448767.1), HAdV-D19 (JQ326209), HAdV-D20 (JN226749.1), and HAdV-D45 (JN226764.1).

Results

Results of PCR revealed that only 6/60 samples were belonged to HAdV species D. The results of Multiple alignment of 6 local isolates of HAdV-D (S1-S6) revealed that: S1, S2, S3 and S6 have same sequence (except G instead A in S3) while S4 and S5 have same sequence but differ from those of S1, S2, S3, S6 (Fig. 1). Results of BLASTn for L1 (penton protein) partial sequence of S1 (as representative of first group) revealed that S1, S2, S3, and S6 belong to HAdV-D8 (AB448767.1) with only two variations in S1, S2 and S6 G>A at position 14334 and C>T at 14681 (Table 1). Concern S4 and S5 the results of BLASTn revealed that they are belong to HAdV-D20 (JN226749.1) (Table 2). Phylogenetic tree of S3 with HAdV-D8, -D19, -D20 and –D37 revealed that zero differences between S3 and HAdV-D8 (AB448767.1) while faraway from HAdV-D37, -D20 and –D19 subsequently (from nearest to far). Phylogenetic tree of S4 with HAdV-D8, -D19, -D20 and –D37 revealed that zero differences between S4 and HAdV-D20 (JN226749.1) while faraway from HAdV-D37, -D19 and –D8 subsequently (from nearest to far) (Fig. 2). All six HAdV-D isolates were submitted to GenBank with following accession no. (OL840385, OL840386, OL840387, OL840388, OM069720 and OM069721).

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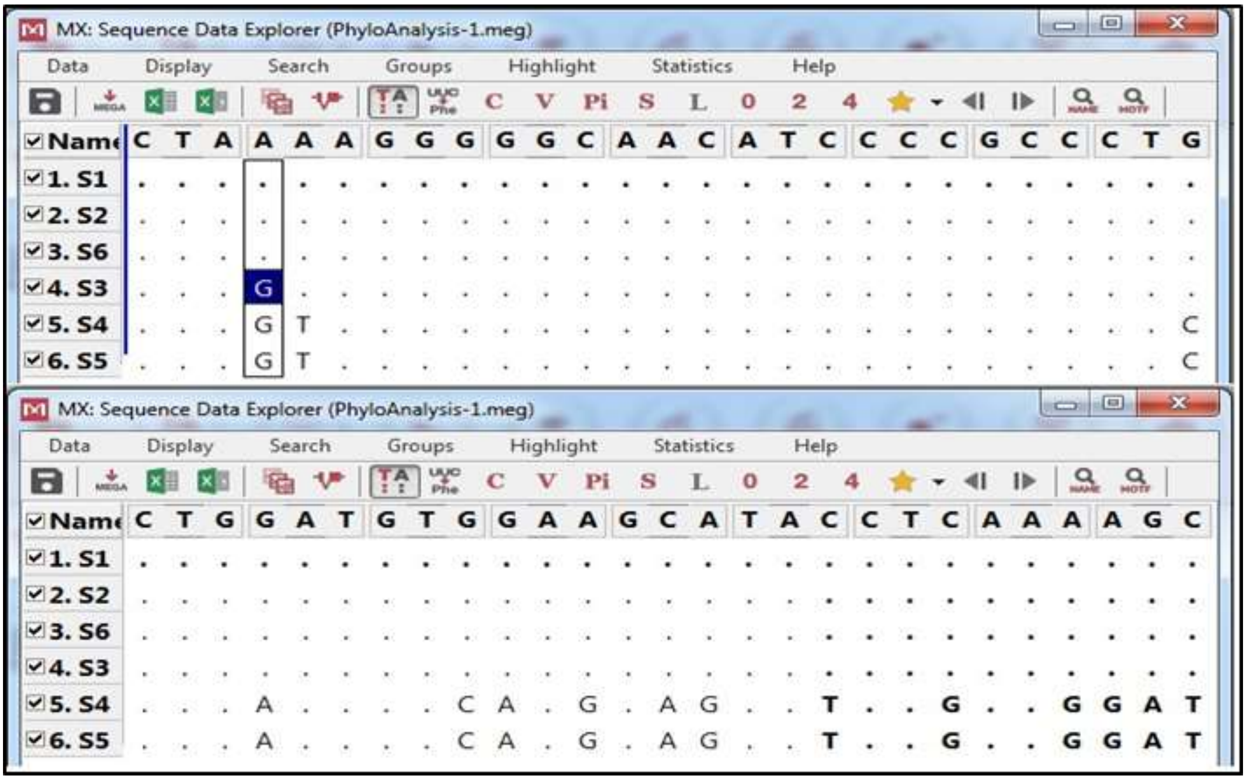

☑ Name	C	T	A	A	A	A	G	G	G	G	G	C	A	A	C	A	T	C	C	C	C	G	C	C	C	T	G
☑ 1. S1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 2. S2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 3. S6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 4. S3	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 5. S4	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
☑ 6. S5	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C

☑ Name	C	T	G	G	A	T	G	T	G	G	A	A	G	C	A	T	A	C	C	T	C	A	A	A	A	G	C
☑ 1. S1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 2. S2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 3. S6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 4. S3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
☑ 5. S4	.	.	.	A	.	.	.	.	C	A	.	G	.	A	G	.	.	T	.	.	G	.	.	G	G	A	T
☑ 6. S5	.	.	.	A	.	.	.	.	C	A	.	G	.	A	G	.	.	T	.	.	G	.	.	G	G	A	T

Fig. 1. Multiple Alignment of S1-S6 local isolates of HAdV-D.

Table 1. Alignment of S1 local isolates with HAdV-D8 (AB448767.1).

Score	Expect	Identities	Gaps	Strand
665 bits (360)	0.0	364/366(99%)	0/366(0%)	Plus/Plus

Query 1 CTAAAAGGGGGCAACATCCCCGCCCTGCTGGATGTGGAAGCATACCTCAAAAGCAAGAAT 60
Subject 14331 CTAGAAGGGGGCAACATCCCCGCCCTGCTGGATGTGGAAGCATACCTCAAAAGCAAGAAT 14390

Query 61 GATCGGGAGGAAGCCACCCAGAATGCAAACAGAGTTGCTGCAAATGGAGGTGGTGAAATT 120
Subject 14391 GATCGGGAGGAAGCCACCCAGAATGCAAACAGAGTTGCTGCAAATGGAGGTGGTGAAATT 14450

Query 121 AGGGGAGATACTTTTCTTACCACCGAACAGCTAAGAGCCGCTGACAAGGAGCTGGTTATT 180
Subject 14451 AGGGGAGATACTTTTCTTACCACCGAACAGCTAAGAGCCGCTGACAAGGAGCTGGTTATT 14510

Query 181 AAGCCCATTAAGGAAGATGCTAGCAAGAGAAGCTATAATGTCATAGGGGACACCCATGAC 240
Subject 14511 AAGCCCATTAAGGAAGATGCTAGCAAGAGAAGCTATAATGTCATAGGGGACACCCATGAC 14570

Query 241 ACCCTGTACCGCAGCTGGTACCTGTCCTATACCTACGGGGACCCCGAGAAGGGGGTACAG 300
Subject 14571 ACCCTGTACCGCAGCTGGTACCTGTCCTATACCTACGGGGACCCCGAGAAGGGGGTACAG 14630

Query 301 TCGTGGACGCTGCTCACCACCCCGGACGTCACCTGCGGCGCGGAGCAAGTTTACTGGTCG 360
Subject 14631 TCGTGGACGCTGCTCACCACCCCGGACGTCACCTGCGGCGCGGAGCAAGTCTACTGGTCG 14690

Query 361 CTGCCG 366
Subject 14691 CTGCCG 14696

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Table 2. Alignment of S4 local isolates with HAdV-D20 (JN226749.1).

Score	Expect	Identities	Gaps	Strand
654 bits (354)	0.0	354/354(100%)	0/354(0%)	Plus/Plus

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Query 1        CTAGTAGGGGGCAACATCCCCGCCCTCCTGAATGTCAAGGAGTATCTGAAGGATAAGGAA 60
Subject 14333  CTAGTAGGGGGCAACATCCCCGCCCTCCTGAATGTCAAGGAGTATCTGAAGGATAAGGAA 14392

Query 61       GAAGCTGGCACAGCAGATGCAAATACCATTAAGGCTCAGAATGATGCAGTCCCAAGAGGA 120
Subject 14393  GAAGCTGGCACAGCAGATGCAAATACCATTAAGGCTCAGAATGATGCAGTCCCAAGAGGA 14452

Query 121      GATAACTATGCATCAGCGGCAGAAGCCAAAGCAGCAGGAAAAGAAATTGAGTTGAAGGCC 180
Subject 14453  GATAACTATGCATCAGCGGCAGAAGCCAAAGCAGCAGGAAAAGAAATTGAGTTGAAGGCC 14512

Query 181      ATTTTGAAAGATGATTCAAACAGAAGCTACAATGTGATCGAGGGAACCACAGACACCCTG 240
Subject 14513  ATTTTGAAAGATGATTCAAACAGAAGCTACAATGTGATCGAGGGAACCACAGACACCCTG 14572

Query 241      TACCGCAGTTGGTACCTGTCCTATACCTACGGGGACCCCGAGAAGGGGGTGCAGTCGTGG 300
Subject 14573  TACCGCAGTTGGTACCTGTCCTATACCTACGGGGACCCCGAGAAGGGGGTGCAGTCGTGG 14632

Query 301      ACGCTGCTCACCACCCCGGACGTCACCTGCGGCGCGGAGCAAGTCTACTGGTCG 354
Subject 14633  ACGCTGCTCACCACCCCGGACGTCACCTGCGGCGCGGAGCAAGTCTACTGGTCG 14686
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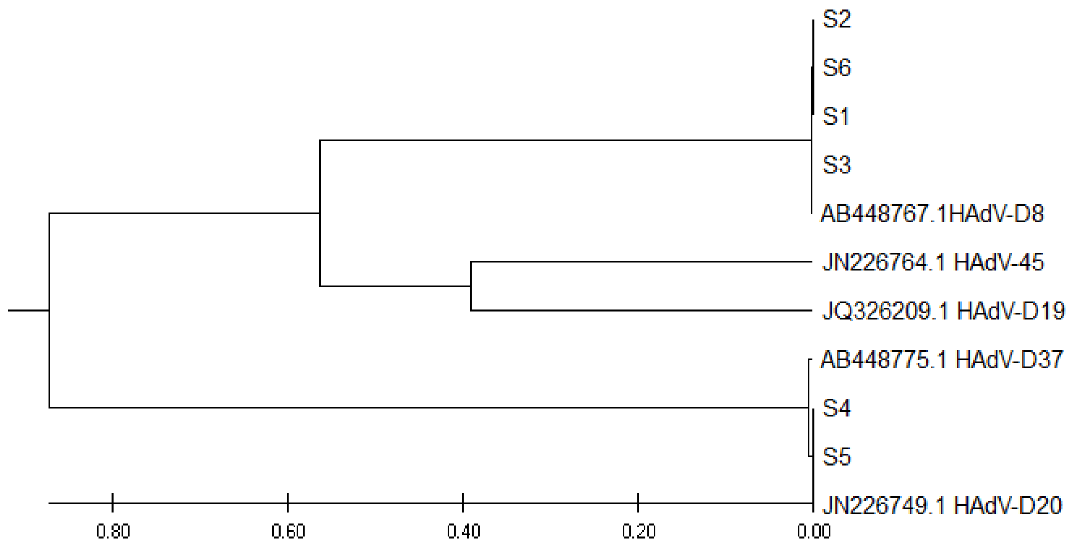

Fig. 2. Phylogenetic tree UPGMA method of all six isolates with HAdV-D8 (AB448767.1), -D19 (JQ326209.1), -D20 (JN226749.1) and -D37 (AB448775.1) and –D45 (JN226764.1).

Discussion

Our results revealed that, the six local isolates of HAdV group belong to two groups: S1, S2, S3 and S6 highly related to HAdV-D8, S4, and S5 highly related to HAdV-D20. Viral Keratoconjunctivitis is mainly resulted from the HAdV especially group B, D and E (16,17). HAdV- D8 is one of the main causative agents of the epidemic kerato-conjunctivitis, often related to the military, community, industrial, and nosocomial outbreaks (18). Among HAdV group D, HAdV-19, HAdV-8, and HAdV-37 cause more serious conjunctivitis in comparison with others (19). Nguyen et al. (20) found that 5

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different types of the HAdV that are related to the conjunctivitis in Hanoi, including HAdV-4 (2.20%), HAdV-3 (4.30%), HAdV-37 (2.20%), HAdV-8 (89.10%), and one of the recombinant types between the HAdV-8 and HAdV-3 (2.20%) types.

The main corneal pathogens, all within the species D, include HAdV-D8, 53, 37, 56, 54, 64 (previously typed as 19a), 85 and82 (the latter 2 have emerged lately) (2,21). Three studies from Japan stated that, some types of HAdV-D are associated with ocular infections, of which HAdV-8, -37, -53, -54, -56 and -64 are predominant (22,23) and Hashimoto et al., (2018) (24). It seems that this is the first study who report implication of HAdV-D20 in conjunctivitis.

Concern implication of HAdV-D20 in Human infection we did not find any research whose document it accepts one study examine virus-spread ability in cell line which find that the HAdV-D20 can propagate successfully in HEK293 cells (Human embryonic kidney 293 cells) (25). Also, HAdV-D20 was showed to be associated with HIV/AIDS and was detected in the samples of stool from the patients who have AIDS with diarrhea, pneumonia, or both (26).

The current study concludes the possibility of implication of HAdV-D20 in eye infections especially conjunctivitis.

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All authors declare that the is no conflict of interest.

References

1. Sow AS, Kane H, Ka AM, Hanne FT, Ndiaye JM, Diagne JP, et al. Senegalese experience with acute viral conjunctivitis. J Fr Ophtalmol. 2017;40(4):297-302.

2. Lee CS, Lee AY, Akileswaran L, Stroman D, Najafi-Tagol K, Kleiboeker S, et al. Determinants of outcomes of adenoviral keratoconjunctivitis. Ophthalmology. 2018;125(9):1344-1353.

3. Keen M, Thompson M. Treatment of acute conjunctivitis in the United States and evidence of antibiotic overuse: isolated issue or a systematic problem?. Ophthalmology. 2017;124(8):1096-1098.

4. Huang GH, Xu WB. Recent advance in new types of human adenovirus. Bing Du Xue Bao. 2013;29(3):342-8.

5. Buckwalter SP, Teo R, Espy MJ, Sloan LM, Smith TF, Pritt BS. Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. J Clin Microbiol. 2012;50(3):766-71.

6. Berman JJ. Taxonomic guide to infectious diseases: understanding the biologic classes of pathogenic organisms. Academic Press;2019;31.

7. Li J, Lu X, Jiang B, Du Y, Yang Y, Qian H, et al. Adenovirus-associated acute conjunctivitis in Beijing, China, 2011–2013. BMC infectious diseases. 2018;18(1):1-8.

8. Nemerow GR, Stewart PL, Reddy VS. Structure of human adenovirus. Current opinion in virology. 2012;2(2):115-121.

9. Ma J, Duffy MR, Deng L, Dakin RS, Uil T, Custers J, et al. Manipulating adenovirus hexon hypervariable loops dictates immune neutralisation and coagulation factor X-dependent cell interaction *in vitro* and *in vivo*. PLoS Pathog. 2015;11(2):e1004673.

10. Otto WR, Lamson DM, Gonzalez G, Weinberg GA, Pecora ND, Fisher BT, et al. Fatal Neonatal Sepsis Associated with Human Adenovirus Type 56 Infection: Genomic Analysis of Three Recent Cases Detected in the United States. Viruses. 2021;13(6):1105.

11. Short JJ, Pereboev AV, Kawakami Y, Vasu C, Holterman MJ, Curiel DT. Adenovirus serotype 3 utilizes CD80 (B7. 1) and CD86 (B7. 2) as cellular attachment receptors. Virology. 2004;322(2):349-59.

12. Sirena D, Lilienfeld B, Eisenhut M, Kälin S, Boucke K, Beerli RR, et al. The human

[DOI: 10.52547/rbmb.11.1.176]

membrane cofactor CD46 is a receptor for species B adenovirus serotype 3. J Virol. 2004;78(9):4454-62.

13. Jarrar YB, Ghishan M. The nudix hydrolase 15 (NUDT15) gene variants among Jordanian Arab population. Asian Pacific journal of cancer prevention. 2019;20(3):801-808.

14. Madisch I, Hofmayer S, Moritz C, Grintzalis A, Hainmueller J, Pring-Akerblom P, et al. Phylogenetic analysis and structural predictions of human adenovirus penton proteins as a basis for tissue-specific adenovirus vector design. J Virol. 2007;81(15):8270-81.

15. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. NCBI BLAST: a better web interface. Nucleic Acids Res. 2008;36(Web Server issue):W5-9.

16. Aoki K, Ishiko H, Konno T, Shimada Y, Hayashi A, Kaneko H, et al. Epidemic keratoconjunctivitis due to the novel hexon-chimeric-intermediate 22, 37/H8 human adenovirus. J Clin Microbiol. 2008;46(10):3259-69.

17. Meyer-Rüsenberg B, Loderstädt U, Richard G, Kaulfers PM, Gesser C. Epidemic keratoconjunctivitis: the current situation and recommendations for prevention and treatment. Dtsch Arztebl Int. 2011;108(27):475-80.

18. Kaneko H, Iida T, Ishiko H, Ohguchi T, Ariga T, Tagawa Y, et al. Analysis of the complete genome sequence of epidemic keratoconjunctivitis-related human adenovirus type 8, 19, 37 and a novel serotype. J Gen Virol. 2009;90(Pt 6):1471-1476.

19. Kaneko H, Suzutani T, Aoki K, Kitaichi N, Ishida S, Ishiko H, et al. Epidemiological and virological features of epidemic keratoconjunctivitis due to new human adenovirus type 54 in Japan. Br J Ophthalmol. 2011;95(1):32-6.

20. Nguyen TT, Le TA, Nguyen VH, Nguyen TU, Nguyen PT, Tran TT, et al. Molecular typing of conjunctivitis-causing adenoviruses in Hanoi, Vietnam from 2017 to 2019 and complete genome analysis of the most prevalent type (HAdV-8). J Med Virol. 2020;92(12):3100-10.

21. Jonas RA, Ung L, Rajaiya J, Chodosh J. Mystery eye: Human adenovirus and the enigma of epidemic keratoconjunctivitis. Prog Retin Eye Res. 2020;76:100826.

22. Nakamura M, Hirano E, Kowada K, Ishiguro F, Yamagishi Z, Adhikary AK, et al. Surveillance of adenovirus D in patients with epidemic keratoconjunctivitis from Fukui Prefecture, Japan, 1995–2010. Journal of Medical Virology. 2012;84(1):81-6.

23. Hiroi S, Morikawa S, Takahashi K, Komano J, Kase T. Molecular epidemiology of human adenoviruses D associated with epidemic keratoconjunctivitis in Osaka, Japan, 2001–2010. Jpn J Infect Dis. 2013;66(5):436-8.

24. Hashimoto S, Gonzalez G, Harada S, Oosako H, Hanaoka N, Hinokuma R, et al. Recombinant type Human mastadenovirus D85 associated with epidemic keratoconjunctivitis since 2015 in Japan. J Med Virol. 2018;90(5):881-889.

25. Uchino J, Curiel DT, Ugai H. Species D human adenovirus type 9 exhibits better virus-spread ability for antitumor efficacy among alternative serotypes. PloS one. 2014;9(2):e87342.

26. Hierholzer JC. Adenoviruses in the immunocompromised host. Clin Microbiol Rev. 1992;5(3):262-74.

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