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Research paper

Molecular Detection and Genotyping of Human Adenovirus in Patients with Keratoconjunctivitis in Iraq

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Abstract

The objective of this study was to detect and genotype human adenovirus strains in patients suffering from keratoconjunctivitis in Iraq. Additionally, it aimed to understand the distribution and prevalence of different adenovirus types associated with the disease in this population.

Seventy conjunctival swabs and whole blood samples were collected from patients with suspected HAdV infection. Viral DNA extracted and Conventional PCR was used to amplify the hexon gene, and the PCR products were sequenced. Phylogenetic analysis was conducted using NCBI-BLAST and MEGA X to compare local isolates with global strains.

of the 70 samples, 64 (91.4%) were positive for HAdV, with viral loads ranging from 1.32×10^3 to 6.62×10^8 copies/mL. Genotyping revealed three predominant genotypes: HAdV-8 (50%), HAdV-54 (40%), and HAdV-19 (10%). Phylogenetic analysis showed high similarity with global strains, with sequence identities ranging from 99.04% to 99.62%. Males and individuals aged 20–40 years were more frequently affected, with higher infection rates observed in urban areas. No significant correlation was found between viral load and demographic factors or clinical severity.

This study highlights the predominance of HAdV-8, HAdV-54, and HAdV-19 in Iraqi patients with keratoconjunctivitis. The findings underscore the need for continuous surveillance and molecular characterization of HAdV strains to inform public health interventions and prevent outbreaks.

Keywords: *Human Adenovirus; Keratoconjunctivitis; Real-time PCR; Genotyping; Phylogenetic Analysis; Iraq.*

1. Introduction

Epidemic keratoconjunctivitis (EKC) is a severe ocular infection caused primarily by Human Adenovirus (HAdV), particularly species D types 8, 19, 37, 53, 54, and 56 [1,2]. HAdV is highly contagious, with transmission occurring through direct contact with infected individuals or contaminated surfaces. The virus can remain viable on instruments and surfaces for extended periods, contributing to outbreaks in clinical settings [3].

Adenoviral conjunctivitis diagnosis can be discerned through various methods including, conjunctiva swab to determine the adenoviral infective antigen [4]. Early diagnosis and distinguishing among the various causes for conjunctivitis is important for appropriate treatment going forward [5]. Microsporidial keratitis, once associated with immunocompromised persons with HIV/AIDS primarily, is now considered an emerging ocular pathogen and infection in immunocompetent persons, often associated with ocular trauma or use of soil and contaminated water, reminding us that corneal infections are constantly evolving [6]. The non-specific and indiscriminately acute keratoconjunctivitis symptoms lend themselves to geo-cloning of a causative agent: if left untreated, the condition could ultimately result in a corneal transplant or blindness [7]. Infectious keratitis is an urgent ophthalmic condition warranting immediate evaluation and management to avert the progression and possible serious corneal complications or perforation plus endophthalmitis [8]. The limited experience among some of the practitioner ophthalmologists will result in misidentification of clinical signs among the various types of microbial keratitis, plus the challenge of limited diagnostic equipment and practitioners in some hospitals and primary care units [9]. In areas like Burkina Faso, where applying basic standards to practice such as observation and reassurance may not be enough with conjunctivitis patients, should some result in a diagnosis of a treatable systemic infection related to conjunctivitis (which could ultimately be life threatening), a need for thorough evaluation is unquestionable [10].

HAdV infections are a significant cause of morbidity worldwide, affecting individuals of all ages. Symptoms of EKC include eye redness, pain, photophobia, and corneal involvement, which can persist for months [12]. Despite the self-limiting nature of the disease, severe cases can lead to long-term visual impairment, particularly in immunocompromised individuals [13].

Molecular techniques, such as Real-time PCR and DNA sequencing, have revolutionized the diagnosis and genotyping of HAdV. These methods provide rapid, sensitive, and specific detection of viral nucleic acids, enabling the identification of novel genotypes and genetic

variations [14], [15]. Genomic sequence analysis is particularly valuable for understanding the epidemiology of HAdV and tracking the emergence of new strains [16].
In Iraq, limited data are available on the molecular epidemiology of HAdV infections. This study aimed to detect HAdV in Iraqi patients with keratoconjunctivitis, quantify viral loads, and identify circulating genotypes using molecular methods. The findings will contribute to a better understanding of the genetic diversity of HAdV in the region and inform public health strategies to prevent and control outbreaks.

2. Materials and Methods

1) Ethical Considerations:
This study was approved by the Institutional Ethics Committee of Al-Garaawi Private Hospital under the approval number 2540, issued on January 20, 2025. All procedures adhered to the ethical guidelines outlined in the Declaration of Helsinki. Blood collection from healthy controls was conducted with full ethical oversight to minimize risks. Informed consent was obtained from all participants after providing detailed information about the study's purpose, procedures, potential risks and benefits, and the right to withdraw at any stage.
2) Type of Sampling and Reasons for Selection:
A total of seventy conjunctival swabs and whole blood samples were collected from patients with suspected Human Adenovirus (HAdV) infection attending ophthalmology clinics in Iraq, including Ibn Al-Haitham Teaching Eye Hospital, Al-Hilla Teaching Hospital, Al-Imam Al-Sadiq Hospital, and Al Garaawi Ophthalmology Specialist Center. Control samples were collected from 70 healthy individuals. This sampling approach was chosen to compare infected individuals with healthy controls to assess viral presence and genetic variations. All control individuals underwent a clinical examination to rule out signs of conjunctivitis or other ocular infections. Additionally, conjunctival swabs from the control group were tested for HAdV using real-time PCR to confirm the absence of viral DNA.
All control samples were clinically screened to ensure that ocular symptoms were absent in the patients sampled. Conjunctival swabs taken from the control individuals were also retested with the same real-time PCR applied to the patient samples. All control samples were found to be negative for HAdV DNA, thereby verifying their role as true negative controls in our study.
3) Patient Consent Statement:
Written informed consent was obtained from all patients and control individuals before sample collection, ensuring voluntary participation and adherence to ethical standards.
4) Inclusion Criteria:
Patients included in the study met the following criteria:
- Clinical suspicion of HAdV infection based on ophthalmological assessment.
- No prior antiviral treatment before sample collection.
- Willingness to provide conjunctival and blood samples for analysis.

5) Exclusion Criteria:
Patients were excluded from the study if they met any of the following conditions:
- Presence of other confirmed viral or bacterial eye infections.
- Recent history of immunosuppressive therapy.
- Incomplete consent documentation.

6) Sample Collection and Processing:
Conjunctival swabs and whole blood samples were collected from patients and control individuals. Conjunctival swabs were placed in viral transport medium (VTM) and stored at -80°C until DNA extraction.
7) DNA Extraction:
Viral DNA was extracted using the Accuprep® Genomic DNA Extraction Kit following the manufacturer's instructions. Each 200 µL sample was combined with 20 µL of proteinase K and 200 µL of binding buffer, incubated at 60°C for 10 minutes, followed by isopropanol addition. The lysate was transferred to a GD Binding filter column, washed with buffers W1 and W2, and eluted with 50 µL of elution buffer. DNA quality was assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) by measuring the A260/A280 ratio.
A volume of 100 µL of conjunctival swab suspension in VTM was used for extraction of DNA for each patient. The fixed volume across all samples provided for consistency and reliable determination of yield and viral load quantification.
8) Conventional PCR and Gel Electrophoresis:
A 560 bp fragment of the hexon gene was amplified using specific primers; however, the primer sequences were presented in the results section rather than in the methods. The reaction mixture consisted of 5 µL of DNA template, 1.5 µL of forward and reverse primers (10 pmol each), and 12 µL of PCR water. PCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. Despite using a commercial DNA extraction kit, the study does not specify the initial quantity of conjunctival swab material used, which is essential for evaluating DNA recovery efficiency. Additionally, key PCR optimization parameters, such as annealing temperature validation and $MgCl_2$ concentration, were not detailed. PCR products were analyzed via agarose gel electrophoresis, but the agarose concentration was not specified, which could impact DNA band resolution. The amplified fragments were sequenced by Macrogen, South Korea.
PCR optimization was performed to secure reliable amplification of the hexon gene:
9) Concentration of $MgCl_2$:
The mixture contained 1.5 mM of $MgCl_2$, which was about half of the maximum concentration, chosen to achieve a proper balance between amplification efficiency and amplification specificity.
10) Annealing Temperature Testing:
A temp gradient (54° C - 62° C) was done using the positive control DNA. The 58° C temperature provided the clearest and specific bands so that temperature was determined to be optimum.
11) Agarose Gel Concentration:
The PCR product was analyzed using 1.5% agarose gel, this concentration was established to give better resolution on the target fragment of 560 bp.
12) Negative and Positive Controls:
All PCR runs consisted of a no-template control (NTC) and a simple positive control, this ensured 1) that no contamination happened to total reaction batch and 2) the success of the reaction.

13) Real-Time PCR Analysis:
Real-time PCR was conducted using primers and probes targeting the HAdV hexon gene. The reaction mixture contained 5 µL of DNA template, 1 µL each of forward and reverse primers (20 pmol), 2 µL of a TaqMan probe (25 pmol), and 11 µL of DEPC-treated water. The amplification was performed in a MiniOpticon real-time PCR thermocycler with an initial pre-denaturation step at 95°C for 5 minutes, followed by 50 cycles of denaturation at 95°C for 20 seconds and annealing/extension at 60°C for 30 seconds. However, the study lacks details on assay validation, including sensitivity, specificity, limit of detection, and limit of quantification, which are essential for assessing the accuracy and reliability of the real-time PCR results. Additionally, the primer sequences were reported in the results section rather than in the methods, making it difficult to evaluate the primer design and specificity. Viral load was quantified based on threshold cycle (Ct) values, but further information on standard curve generation and controls used for quantification was not provided.
14) Analytical Sensitivity (Limit of detection)
Analytical sensitivity was evaluated by using 10-fold serial dilutions of quantified HAdV DNA standard (10^7 to 10^1 copies/mL). The assay was able to detect as low as 1×10^3 copies/mL consistently, which establishes the limit of detection (LOD).
15) Analytical Specificity
The primer/probe combination of the hexon gene was evaluated using DNA from other ocular pathogens that are commonly found (HSV-1, HSV-2, CMV, Staphylococcus aureus, and Pseudomonas aeruginosa). There was no cross-reactivity found which suggests that it has reasonable analytical specificity for HAdV.
16) Standard Curve and Efficiency
A standard curve was established from the serial dilutions and yielded:
Correlation coefficient (R^2): 0.996
Amplification efficiency: 97%
This indicates a very linear relationship and reaction efficiency, which is ideal across the dynamic range evaluated.
17) Reproducibility
Intra-assay variability and inter-assay variability were determined by testing three different standard concentrations in triplicate in three runs. The coefficients of variation (CV) were less than 5% for all concentrations, indicating a high level of precision for the assay.
Negative and Positive Controls
18) Each run included:
A control without a template (NTC) to ensure that no contamination occurred.
A control containing HAdV DNA that established amplification.
19) Sequence Analysis and Phylogenetics:
DNA sequences were aligned using ClustalW in MEGA X. Phylogenetic trees were constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Sequence identities were confirmed using NCBI-BLAST.

Table 1: PCR Primer for Hexon PCR.

Primer	Sequence		Size
Hexon PCR	Forward	CTGTACTCGAACGTGGCCTT	560bp
	Reverse	TTGGCTGGGATGGGGTAAAG	

Table 2: PCR Thermocycler Settings by Using Conventional PCR Thermocycler System

PCR Step	Temperature	Time	No. of Cycles
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	30 sec.	
Annealing	58°C	30 sec.	30 Cycle
Extension	72°C	45 sec.	
Final Extension	72°C	7 min.	1
Hold	4°C	Forever	-

Table 3: Real-Time PCR Primer for Human Adenovirus Hexon Primer and Human Adenovirus Hexon Probe

Primer	Sequence		Amplicon
Human Adenovirus hexon primer	Forward	AGCACGTACTTTGACATCCG	140 bp
	Reverse	ACACCAGCTCCTGTTTTTGC	
Human Adenovirus hexon probe	FAM-GCGCCCCCAATCCCAGTCAG-BHQ1		

Table 4: Real-Time PCR Thermocycler Settings for Human Adenovirus Hexon Primer and Human Adenovirus Hexon Probe

Step	Condition	No. of Cycles
Pre-Denaturation	95 °C 5 min	1
Denaturation	95 °C 20 sec	50
Annealing / Extension	60 °C 30 sec	
Detection (Scan)		

3. Statistical Analysis

Statistical analyses were performed using SPSS version 24 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean, standard deviation, and interquartile range (IQR), were used to summarize continuous variables, while categorical variables were expressed as frequencies and percentages. The Shapiro-Wilk test was applied to assess the normality of continuous data. Comparisons between patients and control groups were conducted using the independent t-test or Mann-Whitney U test for continuous variables, depending on data distribution, and the chi-square test or Fisher's exact test for categorical variables.
Spearman's correlation test was employed to examine associations between viral load and demographic factors, including age, gender, BMI, and the presence of chronic illnesses. A P-value < 0.05 was considered statistically significant. All statistical tests were two-tailed.

4. Results and Discussion

1) Demographic Characteristics

The demographic characteristics of patients and control subjects are summarized in Table (3-1). The study included 70 patients with suspected HAdV infection and 70 apparently healthy control subjects. The mean age of the patient group was 33.56 ± 19.77 years, while the mean age of the control group was 33.54 ± 19.97 years, showing no significant difference between the two groups (P = 0.761).

When the participants were categorized into age intervals (<20 years, 20–40 years, and >40 years), there was no significant difference in the distribution of patients and control subjects across these age groups (P = 0.761). This suggests that all age groups are equally susceptible to HAdV infection, with no particular age group showing a higher predisposition.

With respect to gender, the study included 42 male patients (60.0%) and 28 female patients (40.0%), resulting in a male-to-female ratio of 1.5:1. To maintain comparability, the control group was structured with a similar gender ratio, consisting of 42 healthy males (60.0%) and 28 healthy females (40.0%). The higher proportion of male patients may reflect increased exposure to risk factors, such as occupational or environmental conditions, or differences in healthcare-seeking behavior between genders.

Samples were collected from four hospitals across different regions of Iraq. A breakdown of genotypes by hospital showed that HAdV-8 was the predominant strain across all locations, while HAdV-54 was detected more frequently in patients from Al-Garaawi Private Hospital. This may suggest possible localized transmission patterns, though further investigation is required to confirm regional differences.

Table 5: Correlation of Viral Load to Demographic Characteristics and Chronic Illnesses

Characteristic	R	P
Age	0.076	0.531 NS
Gender	0.061	0.618 NS
Governorate	0.081	0.503 NS
Residency	0.064	0.599 NS
BMI	0.019	0.874 NS
Chronic Disease	-0.003	0.983 NS
Recurrent Infection	-0.184	0.126 NS

r: Correlation coefficient according to spearman correlation; NS: non-significant at P > 0.05.

2) Viral Detection and Quantification

Real-time PCR detected HAdV in 64 samples (91.4%), with viral loads ranging from 1.32×10^3 to 6.62×10^8 copies/mL. The median viral load was 1.14×10^7 copies/mL (IQR: 3.59×10^{15}). The wide range of viral loads observed in this study may reflect differences in the stage of infection, host immune responses, or viral replication efficiency. Notably, no significant correlation was found between viral load and demographic factors such as age, gender, or residency (P > 0.05). This suggests that viral load alone may not be a reliable predictor of disease severity or clinical outcomes, as previously reported by Uemura et al. [17]. Although no significant correlation was found between viral load and demographic factors (P > 0.05), it is possible that other factors—such as the stage of infection at the time of sampling or specific clinical symptoms—may influence viral load. Patients with early-stage infections may exhibit higher viral loads due to active replication, while those in later stages may have lower viral loads due to immune response clearance. Future studies should consider longitudinal sampling to assess viral dynamics over time.

Table 6: Real-Time PCR Detection of Human Adenovirus in Patients Infected with Keratoconjunctivitis

Characteristic	Value
Positive Cases, No. (%)	64 (91.4%)
Negative Cases, No. (%)	6 (8.6%)
Viral Load Median (IQR), Copy/ ml	1.14 × 107 (3.59 × 1015)
Range	$1.32 \times 10^3 – 6.62 \times 10^8$

3) IQR: inter-quartile range

Correlations of viral load to demographic characteristics and chronic illnesses are demonstrated in table (3-6). These correlations were assessed according to Spearman correlation test. Viral load was not significantly correlated to any of the demographic characteristic of patients enrolled or their possession of chronic medical illness (P > 0.05).

To improve interpretability, effect sizes and 95% confidence intervals (CI) were calculated for Spearman's correlation coefficients:

- Age and Viral Load:
- Spearman's r = 0.076; 95% CI: –0.16 to 0.30; P = 0.531
- Gender and Viral Load:
- Spearman's r = 0.061; 95% CI: –0.17 to 0.28; P = 0.618
- BMI and Viral Load:
- Spearman's r = 0.019; 95% CI: –0.21 to 0.24; P = 0.874
- Chronic Disease Status and Viral Load:
- Spearman's r = –0.003; 95% CI: –0.23 to 0.22; P = 0.983
- Recurrent Infections and Viral Load:
- Spearman's r = –0.184; 95% CI: –0.39 to 0.04; P = 0.126

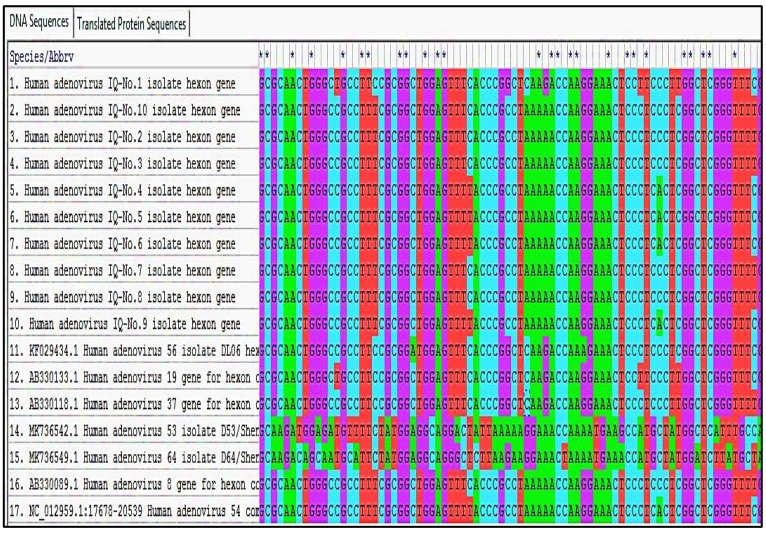

Fig. 1: Multiple Sequence Alignment Analysis of Hexon Gene in Local Human Adenovirus IQ- Isolates and NCBI-Genbank Human Adenovirus Isolates. The Multiple Alignment Analysis Was Constructed Using Clustalw Alignment Tool in (MEGA X). That Showed the Nucleotide Alignment Similarity as (*) And Substitution Mutations in Hexon Gene.

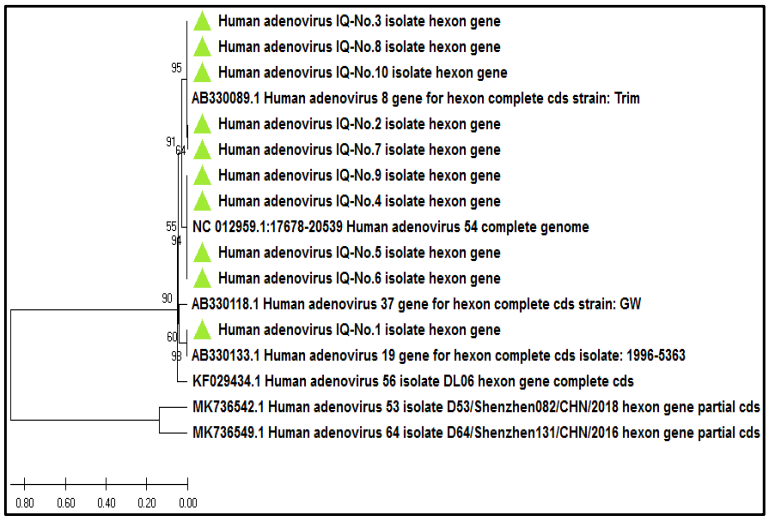

Fig. 2: Phylogenetic Tree Analysis Based on Hexon Gene Partial Sequence in Local Human Adenovirus Isolates That Used for Genetic Analysis. The Phylogenetic Tree Was Constructed Using Unweighted Pair Group Method with Arithmetic Mean (UPGMA Tree) in (MEGA X). The Local Human Adenovirus Isolate No.1 Was Showed Closed Related to NCBI-BLAST Human Adenovirus Type (19) Isolate (AB330133.1). Local Human Adenovirus Isolate No.2, No.3, No.7, No.8 and No.10 Were Showed Closed Related to NCBI-BLAST Human Adenovirus Type (8) Isolate (AB330089.1). Local Human Adenovirus Isolate No.4, No.5, No.6 and No.9 Were Showed Closed Related to NCBI-BLAST Human Adenovirus Type (54) Isolate (NC012959.1) at Total Genetic Changes (0.03-0.01%).

Table 7: NCBI-BLAST Homology Sequence Identity (%) between Local Human Adenovirus Isolates and NCBI-BLAST Submitted Human Adenovirus Genotypes Isolates

Human Isolate	Adenovirus	GenBank Accession Number	Identical Adenovirus Isolate	Human Genotype	GenBank Accession
Human Adenovirus No. 1	Blank	MN242691	Human Adenovirus 19	AB330133.1	99.40%
Human Adenovirus No. 2	Blank	MN242693	Human Adenovirus 8	AB330089.1	99.44%
Human Adenovirus No. 3	Blank	MN242694	Human Adenovirus 54	AB330089.1	99.04%
Human Adenovirus No. 4	Blank	MN242695	Human Adenovirus 54	NC012959.1	99.43%
Human Adenovirus No. 5	Blank	MN242696	Human Adenovirus 8	NC012959.1	99.42%
Human Adenovirus No. 6	Blank	MN242697	Human Adenovirus 8	NC012959.1	99.43%
Human Adenovirus No. 7	Blank	MN242698	Human Adenovirus 54	AB330089.1	99.62%
Human Adenovirus No. 8	Blank	MN242699	Human Adenovirus 19	AB330089.1	99.43%

4) Genotyping and Phylogenetic Analysis

Genotyping of 10 randomly selected positive samples revealed three predominant genotypes: HAdV-8 (50%), HAdV-54 (40%), and HAdV-19 (10%). Phylogenetic analysis showed high similarity with global strains, with sequence identities ranging from 99.04% to 99.62%. The local isolates were closely related to NCBI-BLAST reference strains AB330089.1 (HAdV-8), NC012959.1 (HAdV-54), and AB330133.1 (HAdV-19).

Due to resource constraints and cost associated with sequencing, the analysis was restricted to 10 of the 64 HAdV-positive samples. The samples were selected to facilitate a balanced representation of patients of different ages, genders, and clinical sites to assess initial genotype diversity. Although the number of genotypes identified is limited, genotypes HAdV-8, HAdV-54, and HAdV-19 are consistent with global literature trends (reported by Sun, et al., 2021) and provide a baseline dataset should future surveillance studies in Iraq be conducted.

The prevalence of ocular infections was high, with conjunctivitis being the most common condition [18-20]. This study provides valuable insights into the molecular epidemiology of Human Adenovirus (HAdV) infections in Iraq, identifying HAdV-8, HAdV-54, and HAdV-19 as the predominant genotypes associated with keratoconjunctivitis. The high prevalence of HAdV-8 (50%) aligns with global trends, as this genotype is a well-documented cause of epidemic keratoconjunctivitis (EKC) [21,22]. However, the emergence of HAdV-54 (40%) is noteworthy, as this genotype has been reported more frequently in East Asian countries, particularly Japan, rather than in the Middle East [17]. The presence of HAdV-19 (10%), which has been associated with both ocular and respiratory infections, suggests genetic diversity among circulating strains [2].

5) Comparison with Regional and Global Studies

The findings of this study should be evaluated in the context of other regional and global reports. Data on HAdV infections in Iraq are limited, making direct comparisons challenging. However, studies from Iran and Saudi Arabia have also identified HAdV-8 as a dominant genotype in ocular infections, supporting the notion of its widespread circulation in the Middle East [18,19]. In contrast, HAdV-54 has been more frequently reported in East Asia, particularly in Japan and South Korea, suggesting potential regional variation in genotype distribution [17,23]. The absence of previous genotyping studies in Iraq highlights the need for longitudinal surveillance to track viral evolution and transmission patterns.

6) Genetic Comparisons and Phylogenetic Insights

Phylogenetic analysis revealed that the local Iraqi HAdV strains exhibited high sequence similarity (99.04–99.62% identity) to NCBI-BLAST reference strains from other countries. Specifically, local HAdV-8 isolates clustered closely with Japanese and Iranian strains, suggesting possible regional transmission links [17]. The HAdV-54 isolates, although genetically similar to global strains, have been less commonly reported in Middle Eastern populations, raising questions about their potential introduction routes and evolutionary origins. Further comparative genomic studies incorporating whole-genome sequencing would be beneficial in identifying unique mutations and understanding the evolutionary dynamics of these strains [16].

The phylogenetic clustering of local HAdV-54 isolates with strains isolated previously in East Asia, specifically Japan and South Korea, raises important questions about regional transmission. The fact that HAdV-54 was detected in patients in Iraq may indicate a recent introduction (i.e., through international travel, mobility, or local undocumented circulation). Although we did not have available a travel history for the patients with the most similar isolates, there is a close genetic similarity that suggests a relatively recent common ancestor (i.e., a short period since introduction or local divergence from a common origin).

The small value of sequence divergence (99.04 to 99.62% identity) also suggested that this is likely a strain (or strains) from a common ancestor that has not acquired many mutations since its introduction. These findings highlight the need for more extensive genomic data about adenoviral strains to monitor their evolution. Whole-genome sequencing and metadata with respect to patient mobility would be useful to identify the possible sources of introduction (local or international) and assess if the detected strains were isolated events or part of an emergent event.

7) Implications for Public Health and Surveillance

The high detection rate (91.4%) of HAdV in conjunctivitis cases underscores the need for improved diagnostic capabilities and surveillance programs in Iraq. Routine molecular testing, such as real-time PCR and sequencing, should be integrated into ophthalmology clinics and public health laboratories to facilitate early detection and strain monitoring [3], [14]. Given the emergence of HAdV-54, regional collaboration with neighboring countries is essential to understand its epidemiological trends and prevent potential outbreaks [17]. Additionally, hygiene protocols in healthcare settings, including strict disinfection of ophthalmic instruments and hand hygiene compliance, should be reinforced to reduce nosocomial transmission [21].

8) Study Limitations and Future Directions

While this study provides important insights, it has certain limitations that must be acknowledged. The sample size was relatively small, which may affect the generalizability of the findings. The lack of longitudinal data limits the ability to assess viral persistence, reinfection rates, and immune response variability among patients. Additionally, while phylogenetic analysis confirmed the genetic relatedness of local and international HAdV strains, whole-genome sequencing could provide a more comprehensive understanding of genetic variations and adaptive mutations in these strains [15], [16].

9) Future studies should focus on:

Expanding sample size and including longitudinal patient follow-ups.

Conducting whole-genome sequencing to identify mutations and recombination events.

Strengthening national and regional HAdV surveillance networks to track genotype shifts.

Evaluating potential vaccine candidates targeting predominant HAdV strains.

5. Conclusion

This study highlights the predominance of HAdV-8, HAdV-54, and HAdV-19 in Iraqi keratoconjunctivitis cases, revealing close genetic relationships with global strains. The findings underscore the need for continuous molecular surveillance, improved infection control measures, and regional collaboration to mitigate the public health impact of adenoviral eye infections. Future research should focus on expanding genetic analyses, tracking viral evolution, and developing targeted intervention strategies to control the spread of HAdV in Iraq and beyond.

1) Outcomes of the Study:

The study aimed to detect and characterize HAdV infections in ophthalmology patients using molecular techniques. The outcomes included determining the prevalence of HAdV, viral load quantification, and phylogenetic classification of identified strains

2) Rationale of the Study:

This study was conducted to enhance the understanding of HAdV infections in ophthalmology patients by utilizing molecular diagnostic techniques. Identifying prevalent strains and their genetic characteristics can aid in improving clinical diagnosis and treatment approaches.

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Authors Contributions

Ziyad Kamel Al-Jenabi was responsible for the conceptualization, design, data collection, and laboratory analysis of the study. Adil Abdulhamza Mohammed Akraa contributed to data analysis, interpretation of results, and manuscript drafting. Both authors participated in the critical revision of the manuscript and approved the final version for publication.
All authors have read and agreed to the published version of the manuscript.

Conflict of Interests

The authors declare no conflict of interest.

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