

## Original Article

# Epidemiological Investigation of Adenovirus Infection in Individuals Presenting Acute Respiratory Manifestations at The Academic Medical Facilities

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## Abstract

**Background and Aims:** Adenoviruses are dsDNA viruses known for causing respiratory, gastrointestinal, ocular, and genitourinary infections. Epidemiological insights are crucial for public health interventions. This study focuses on the epidemiology of adenoviruses in acute respiratory cases, aiming to enhance preventive and therapeutic strategies.

**Materials and Methods:** This cross-sectional study was performed on collected throat swab samples from individuals under 19 with acute respiratory symptoms. Nested-PCR was used to detect adenovirus presence in 328 samples. DNA extraction, quality assessment, and statistical analyses were conducted to determine prevalence, clinical manifestations, in the population under study.

**Results:** Among the 328 samples analyzed, 72 samples (22%) including 25 male and 47 female tested positive for adenovirus infection using Nested-PCR. Clinical symptoms evaluation revealed distinct manifestations. Fever was presented in 72.6% of cases, cough in 64.6%, and muscular pain in 64%. Gender differences emerged, with 56.3% of infected individuals being females and 34.7% males. Statistical analyses confirmed these associations, providing valuable insights into the prevalence and clinical aspects of adenovirus infections.

**Conclusion:** The identification of distinct clinical manifestations, coupled with gender-specific prevalence, underscores the complexity of adenovirus infections. These findings contribute crucial knowledge to the understanding of adenovirus epidemiology, highlighting the need for targeted interventions and public health strategies to address their substantial burden on affected populations. Further research and ongoing surveillance are imperative for the effective management and mitigation of adenovirus-related respiratory diseases.

**Keywords:** Adenovirus, Acute Respiratory Infections, Epidemiology, Public Health, Prevention

## Introduction

Adenoviruses comprise a diverse group of non-enveloped, double-stranded DNA viruses that span a broad spectrum of serotypes. Renowned for their capacity to induce an array of infections in humans, these

viruses commonly manifest as respiratory, gastrointestinal, ocular, and genitourinary ailments. The intricate genetic variability of adenoviruses has propelled them to the forefront of clinical attention, owing to their substantial impact on public health (1, 2).

Amid the array of health challenges attributed to adenoviruses, respiratory infections emerge as a prominent focal point. Adenoviruses have recurrently been associated with acute respiratory illnesses, including bronchitis, pneumonia, and upper respiratory tract infections.

These infections exhibit varying degrees of severity, particularly in immunocompromised

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individuals and young children, necessitating a deeper comprehension of their prevalence and implications for affected populations. Beyond their respiratory effects, adenoviruses display a remarkable ability to affect the gastrointestinal tract, ocular structures, urinary tract, and more, showcasing their versatile tissue tropism. This adaptability underscores the intricate nature of adenoviral infections and accentuates their noteworthy role as significant contributors to the global burden of infectious diseases (3-5).

Epidemiologic investigations play a pivotal role in unraveling the dynamics of infectious diseases within populations. By methodically dissecting patterns of disease occurrence, transmission dynamics, and risk determinants, these studies furnish indispensable insights for devising public health interventions, preventive strategies, and therapeutic modalities.

In the context of adenoviruses, rigorous epidemiologic inquiries assume a critical stance in formulating efficacious control measures and upholding public health (6-8).

This research undertaking sought to undertake an all-encompassing epidemiologic exploration centered on adenovirus infections in individuals presenting acute respiratory symptoms. Our inquiry transpired within the academic medical facilities of Shiraz University of Medical Sciences.

Through an analysis of the prevalence, serotypes, and clinical ramifications of adenoviral infections in this specific demographic, we aspired to furnish substantial data to the existing knowledge reservoir. Ultimately, this contribution aims to refine preventive and therapeutic stratagems targeting adenovirus-linked respiratory ailments.

## Methods

### Study Population and Sample Collection

The research was conducted in a cross-sectional manner. The study population consisted of individuals under 19 years of age with acute respiratory infections. A throat swab sample was taken from each participant and used in the experiments. Based on the available statistics in the virology laboratory of the Influenza Research Center at the Medical

School of Shiraz University of Medical Sciences, a total of 328 respiratory samples were examined.

Influenza Department staff of the Shiraz Medical School undertook the collection of samples sent from hospitals and associated centers. Historical information was obtained from individuals' files through a questionnaire. This information included their first and last names, gender, age, sampling date, and clinical symptoms such as fever, body aches, and dry cough. After the samples were collected, relevant tests were conducted on them, and the results were recorded.

**DNA Extraction.** Nucleic acid extraction from clinical samples was carried out using commercial kits following the provided instructions from the manufacturer.

Respiratory samples were purified using the Bioneer AccuPrep Genomic DNA Extraction Kit. The extracted DNA was stored in a -70°C freezer until use in reactions.

### Quality assessment

#### DNA Concentration Determination by Optical Absorption Method

To determine the DNA concentration in the extracted sample, a 1:100 dilution of the extracted DNA is prepared. This can be achieved by adding 10 microliters of the DNA sample to 990 microliters of distilled water. After thorough mixing, the optical absorption at wavelengths of 260 and 280 nanometers is measured using a spectrophotometer (the blank for this process is distilled water). The ratio of the absorption at 260 to 280 nanometers indicates the DNA sample's quality.

The absorption at 260 nanometers reflects the DNA concentration, while the absorption at 280 nanometers indicates the protein concentration in the sample. If the 260/280 absorption ratio is approximately 1.8, the DNA is of sufficient quality for PCR amplification. To ensure the accuracy of the extraction, it is advisable to assess DNA quality using an internal control. This control helps confirm the integrity and suitability of the DNA for downstream applications, including PCR.

#### DNA Quality Assessment by PCR

For evaluating the physical condition of the extracted DNA, the quality of the obtained

DNA assessed by amplifying human reference  $\beta$ -globin gene. In the context of PCR process, in the first stage (Initial Denaturation), the temperature is set to 95°C and maintained for 5 minutes, marking the beginning of the amplification process with the DNA template (1 cycle).

During Denaturation, the temperature remains at 95°C for 1 minute. This step is repeated 38 times. Next, in Primer Annealing, the temperature is lowered to 54°C for 1 minute.

Following this stage, in the Extension step, the temperature is set at 72°C for 1 minute, during which the DNA polymerase synthesizes a complementary strand. The third and final stage is the Final Extension, where the temperature is held at 72°C for 5 minutes, ensuring any remaining single-stranded DNA is fully extended (1 cycle).

**Nested-PCR.** After confirming the quality of the extracted DNA, a search operation for the adenovirus genome is performed using Nested-PCR method with the following primers' sequences targeting the hexon gene region shown in Table 1.

**Table 1.** Primer sequences targeting the hexon gene region of adenoviruses.

Primer Name	Forward Sequence	Reverse Sequence
161-AdV	GCCGAGAAGGGCGTG CGCAGGT	TACGCCAACTCGCCC ACGCGC
2-AdV	TGACTTTTGAGGTGG ATCCATG	GGTCTCGATGACGCCG CGGTGC

The reaction components were added to a 0.2 mL microcentrifuge tube in the following order, ensuring all tube materials and steps were performed on ice. Add 0.5  $\mu$ L of 10X buffer, 1  $\mu$ L of dNTP (200  $\mu$ M), 5.1  $\mu$ L of MgCl<sub>2</sub> (50 mM), 2  $\mu$ L of each primer (10 pmol/mL), 5  $\mu$ L of previously extracted DNA, 1 unit of heat-resistant DNA polymerase equivalent to 0.5  $\mu$ M final concentration, and finally add enough sterile deionized water to bring the reaction mixture to a final volume of 50  $\mu$ L. In each experiment, perform negative

control tubes. In the negative control tube, replace DNA with double-deionized water. Centrifuge the materials for 1 second to mix them. Enter the following program steps into the machine: 95°C for 5 minutes (for template denaturation), 95°C for 1 minute, 61°C for 1 minute. Repeat these three steps 35 times, then incubate at 72°C for 1 minute, followed by 72°C for 5 minutes (for final extension).

For result analysis, load 5 to 15  $\mu$ L of the reaction mixture onto a 5.1% agarose gel and visualize using ethidium bromide staining under UV light. After that, another Master mix under the following conditions was prepared: Add 5  $\mu$ L of 10X buffer, 1  $\mu$ L of dNTP (200  $\mu$ M), 5.1  $\mu$ L of MgCl<sub>2</sub> (50 mM), 2  $\mu$ L of each primer (10 pmol/mL), 5  $\mu$ L of the PCR product from the previous stage, 1 unit of heat-resistant DNA polymerase equivalent to 0.5  $\mu$ M final concentration, and add enough sterile deionized water to bring the reaction mixture to a final volume of 50  $\mu$ L. Note that for adding each of the above components in each stage, use a fresh pipette tip. In each experiment, perform negative control tubes.

Centrifuge the materials for 1 second to mix them. Enter the following program steps: 95°C for 5 minutes (for template denaturation), 95°C for 1 minute, 61°C for 1 minute. Repeat these three steps 35 times, then incubate at 72°C for 1 minute, followed by 72°C for 5 minutes (for final extension). After completing the program, remove the samples from the machine and load 5 to 15  $\mu$ L of the reaction mixture onto a 5.1% agarose gel for result analysis. Visualize using safe stain and observe under UV light. The sizes of the PCR products are 161 and 107 base pairs, respectively. In both rounds, the total volume of the main PCR mixture is 50  $\mu$ L for each PCR sample.

**Statistical Analysis.** All statistical analyses were conducted using the statistical software SPSS version 15.

After determining the frequencies, statistical analyses including Pearson's chi-square test, Fisher's exact test, and chi-square test for trend were employed to establish relationships and perform calculations. Furthermore, the validity and significance of all statistical tests were confirmed with a P value less than 0.05.

## Results

**Demographic Data.** In this study, a total of 328 throat swab samples from individuals with respiratory infections, including 150 (45.7%) males and 178 (54.3%) females, were examined. The mean age of individuals with acute respiratory infections was  $37.0 \pm 97.8$  years, ranging from 1 to 19 years. The mean ages of females and males were estimated to be  $60.0 \pm 29.9$  and  $95.0 \pm 60.8$ , respectively. The study population was divided into three age groups: children under 2 years, preschoolers aged 2 to 8 years, and adolescents aged 8 to 19 years. The number of individuals in each group in the collected samples was 145, 143, and 40, respectively.

**Adenoviruses Prevalence:** Among the total samples under investigation, 72 (22%) were identified as patients infected with adenovirus (Table 2).

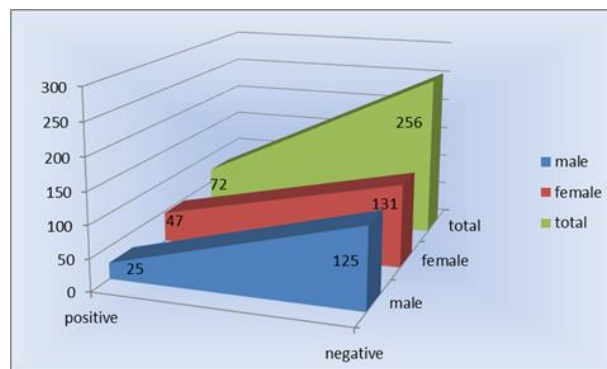
**Table 2.** Prevalence of adenoviruses within the individuals participated in the study.

Adenovirus Status	Percentage (%)	Prevalence Frequency
Positive	22%	72
Negative	78%	256
Total	100%	328

The diagnosis of adenovirus was carried out using the Nested-PCR method, where initially, an amplicon of approximately 176 bp was generated on the agarose gel and the PCR product in the second stage is approximately 107 bp. Among those infected with adenovirus, 47 (56.3%) were females, and 25 (34.7%) were males. The P-value in this study was 0.034, indicating a statistically significant association (Figure 1).

**Clinical Manifestations.** Upon clinical symptom evaluation, it was determined that approximately 238 (72.6%) samples exhibited fever, 212 (64.6%) samples presented with cough, and 210 (64%) individuals experienced muscular pain. Statistically, there was no signifi-

cant correlation observed between disease symptoms and gender ( $p = 0.48$ ).



**Fig. 1.** Prevalence of adenoviruses divided by gender.

**Table 3.** The frequency of clinical symptoms by gender

Clinical Symptoms	Gender	Count	Percentage/total
Fever	Female	128	53.8%
	Male	110	46.2%
Cough	Female	117	55.2%
	Male	95	44.8%
Muscle pain	Female	111	52.8%
	Male	99	47.1%

## Discussion

Viral infections have caused numerous diseases in humans. Acute respiratory infections play a significant role in causing mortality, especially in children. Research in developing countries has demonstrated that annually, approximately 1.5 million deaths occur in children and 2 to 4 million deaths in the elderly due to acute respiratory infections. According to reports from the World Health Organization, around 4 million deaths occur each year as a result of respiratory infections in children under 2 years old. About 20 to 40 percent of hospitalizations in pediatric healthcare facilities are attributed to acute respiratory infections. These diseases can be caused by various

pathogenic factors, with seasonal respiratory infections being a primary reason for hospitalizations and mortality, particularly during the winter season (9-12).

Human adenovirus is a common respiratory-related disease, although different viral serotypes are associated with various conditions including gastrointestinal infections, stomach infections, conjunctivitis, etc. Numerous viral factors contribute to the occurrence of these infections, particularly in pediatric lower respiratory tract infections.

Among the most significant factors are adenoviruses, which often manifest with symptoms such as runny nose, cough, fever, and especially pneumonia (12-15). In the context of adenovirus prevalence, our results indicate that among the female participants (totaling 131), 47 tested positive for adenovirus. This yields a prevalence rate of approximately 35.9% among females in the study.

Among the male participants (totaling 125), 25 tested positive for adenovirus, resulting in a prevalence rate of about 20%. This data suggests a higher prevalence of adenovirus among females compared to males in your research sample.

In the context of its prevalence, several studies have reported its prevalence in different age groups. A systematic review and meta-analysis of human infection with respiratory adenovirus in China from 2009 to 2020, which found that HAdV-3 and HAdV-7 were the most prevalent types and were frequently associated with outbreaks and pneumonia (16).

An investigation was conducted in Wenzhou, China, spanning from 2018 to 2019, to analyze the epidemiological, molecular, and clinical attributes of human adenovirus among children hospitalized with acute respiratory infections. The study revealed that the prevalent types were HAdV-3, HAdV-7, HAdV-2, and HAdV-1, each leading to distinct clinical presentations influenced by the patient's age and the specific type of virus. HAdVs were detected in 1,059 (3.5%) of total samples (17).

In this study, clinical symptoms were examined in relation to gender. The data indicates that among the observed cases, 53.8% presented with fever, with a total count

of 128 individuals. Among these cases, the majority were female (53.8%), while males accounted for 46.2%. Additionally, cough was a prevalent symptom, accounting for 55.2% of the total cases (117 individuals). Among those with cough, a majority were female (55.2%), while 44.8% were male. Furthermore, muscle pain was reported in 52.8% of cases, with a total count of 111 individuals. Among those experiencing muscle pain, 52.8% were female, while 47.1% were male. This data suggests potential gender-specific patterns in the presentation of clinical symptoms within your research sample. Specifically, fever, cough, and muscle pain appear to be more prevalent in females, while males exhibit lower percentages for these symptoms.

Naghipour et al. conducted a research study in Rasht, Iran, involving 50 families, each with a minimum of two children. Over a span of 2 months, the researchers made weekly visits to monitor instances of acute respiratory infections (ARI). They collected swab samples from symptomatic individuals and at specific intervals to track adenovirus presence. The method of Polymerase Chain Reaction (PCR) was used for adenovirus identification, and positively tested samples underwent DNA sequencing. The outcomes revealed that adenovirus contributed to 35% of ARI cases among children (33 out of 94 episodes) and 27% in adults (8 out of 30 episodes). In half of the families (25 out of 50), adenovirus infections were found among their members. Notably, children had higher infection rates compared to adults, particularly when a symptomatic case was present within the same household. Adenovirus shedding persisted for roughly 11 days in children and 7 days in adults. Regarding specific serotypes, adenovirus-7 emerged as the most prevalent, followed by adenovirus-6, -1, -2, and -5.

Interestingly, adenovirus-5 and -7 samples formed two distinct clusters. Importantly, no genetic mutations were observed during the intra-family transmission of the virus (18). In another study by Malekshahi et al., out of the total samples, a significant 45.5% (92 samples) exhibited the presence of respiratory symptoms. Within this group, 5.4% had evidence of

being concurrently infected by two different viruses. Noteworthy findings include the prevalence of respiratory syncytial virus (RSV) as the most frequent culprit at 16.8%. Following closely, adenovirus was detected in 14.4% of cases, while influenza A virus accounted for 4.9%. Parainfluenza virus-3 and parainfluenza virus-1 were identified in 4.4% and 2.9% of cases respectively. Additionally, both influenza B virus and human metapneumovirus (hMPV) were found in 0.49% of cases each (19). The COVID-19 pandemic, driven by the highly contagious nature of SARS-CoV-2, led to the implementation of strict public health measures (20).

These measures aimed to curb the spread of the virus, inadvertently limiting opportunities for other respiratory viruses like RSV to circulate. Preventive measures such as mask-wearing, social distancing, and enhanced hand hygiene not only effectively reduced SARS-CoV-2 transmission but also had a significant impact on the prevalence of other respiratory viruses. By minimizing close contact and respiratory droplet dispersion, these measures played a crucial role in containing a range of respiratory infections. Increased awareness of COVID-19 and improved access to testing prompted individuals with respiratory symptoms to seek prompt medical attention. This proactive approach led to the early identification and isolation of COVID-19 cases, preventing further spread of both SARS-CoV-2 and other respiratory viruses (21, 22). Adeno, RSV, Influenza and other respiratory viruses, follows seasonal patterns.

The emergence of SARS-CoV-2 may have coincided with a naturally low period of RSV activity, potentially contributing to a decrease in RSV cases during the pandemic (21, 23-25). In summary, the heightened prevalence of SARS-CoV-2 during the COVID-19 pandemic influenced the incidence of other respiratory viruses, including Adenoviruses.

Understanding these dynamics is crucial for planning future public health responses to outbreaks of respiratory viruses.

According to our study, among the studied samples, 22% tested positive for adenovirus infection through Nested-PCR. The study

identified fever, cough, and muscular pain as prevalent clinical symptoms. Approximately 72.6% exhibited fever, 64.6% presented with a cough, and 64% experienced muscular pain. The observed prevalence rate of 22% highlights the significance of adenovirus as a causative agent of respiratory infections.

Understanding the clinical manifestations associated with adenovirus infections is essential for accurate diagnosis and appropriate medical interventions.

Further research with larger, more diverse samples could enhance the generalizability of findings. Additionally, conducting longitudinal studies to monitor the dynamics of adenovirus infections over time would provide a more comprehensive understanding of its impact.

## Conclusion

The current study focused on a comprehensive epidemiological exploration of adenovirus infections among individuals with acute respiratory symptoms. The results revealed a prevalence of adenovirus infections in 72 (22%) of the samples, with females (56.3%) and males (34.7%) showing statistically significant differences.

Clinical symptoms included fever, cough, and muscular pain, with varying frequencies across genders. These findings underscore the complex nature of adenovirus infections and emphasize the need for continued research and targeted interventions to mitigate their impact on public health.

In essence, this study contributes insights into the epidemiology of adenovirus infections, particularly in the context of acute respiratory symptoms. Ongoing efforts to understand and manage adenoviral infections, ultimately leading to improved healthcare strategies and outcomes is necessary.

## Acknowledgment

None.

## Conflict of Interest

No conflict of interest is declared.

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