

RESEARCH ARTICLE

Prevalence of Human Adenoviruses in Respiratory Tract-infected Patients in Anbar Governorate (West of Iraq): A Descriptive Cross-sectional Study

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Received: 10th October, 2022; Revised: 30th October, 2022; Accepted: 26th November, 2022; Available Online: 25th December, 2022

ABSTRACT

Background: Human adenoviruses (HAdVs) are a significant worldwide problem that causes viral respiratory tract infections affecting millions worldwide each year, especially in children and immunocompromised adults.

Objective: To know the prevalence of HAdVs strains 3, 4, 7 among respiratory tract infected patients aged between 15–42 years in Anbar province (west of Iraq) using enzyme-linked immunoassay (ELISA) test with polymerase chain reaction (PCR) technique.

Material and methods: A descriptive cross-sectional study was done between 22th of January 2021 - October 11, 2021 to notice the frequency of HAdVs in Anbar governorate children and adults with respiratory infections from the various general hospitals and private clinics. Depending on our questionnaires, blood samples were taken from all those patients for hematological and serological parameters. The ELISA test with PCR has been done depending on the manufacturer's instructions.

Results: A total of 104 respiratory tract Infected patients, 11(10.6%) patients were ELISA IgM positive, 9(8.7%) of ELISA IgM positive patients were positive using PCR technology. The 6(5.8%) patients were ELISA IgG positive for HAdV. Out of 32 mild pneumonia patients, 1(3.1%) was positive for HAdV using IgM ELISA. Out of 46 moderate pneumonia patients, 6(13.0%) were positive for HAdV using IgM ELISA. Out of 26 sever pneumonia patients, 4(15.4%) were positive for HAdV using IgM ELISA. Out of 32 mild pneumonia patients, 1(3.1%) was positive for HAdV using IgM ELISA. Out of 46 moderate pneumonia patients, 6(13.0%) were positive for HAdV using IgM ELISA. Out of 26 sever pneumonia patients, 4(15.4%) were positive for HAdV using IgM ELISA.

Conclusion: The prevalence of HAdVs strain 7 in children with respiratory infection was 4.8%, whereas its prevalence in adults in age groups 18–50 and 51+ years was 2.9% for each group using the IgM ELISA test.

Keywords: Human adenoviruses, Respiratory tract infected patients, Anbar, Iraq.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.4.11

How to cite this article: Abd-Alazeez RA, Khalil MA, Ghazzay HI. Prevalence of Human Adenoviruses in Respiratory Tract-infected Patients in Anbar Governorate (West of Iraq): A Descriptive Cross-sectional Study. International Journal of Drug Delivery Technology. 2022;12(4):1547-1551.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Human adenoviruses (HAdVs) (genus Mastadenovirus, family Adenoviridae) are a significant worldwide problematic that leading to cause viral respiratory tract infections that affect millions of people worldwide each year.^{1,2}

Adenovirus is the greatest reason for upper and lower respiratory tract disease,³ and constitutes 5–8% of all Respiratory tract infections (RTIs) in neonate and children.^{3,4} Other research showed that Adenovirus infection range between 5 to 15% of upper and lower RTIs in hospitalized children for respiratory disease.^{5,6}

Adenovirus (AdV) is considered one of the greatest public viruses causing RTIs in immunocompetent adults and elderly

persons.⁷ HAdV-associated with life-threatening military respiratory disease. An increasing frequency of HAdV conditions with isolated cases familiar in civilian communities in the US and other nations indicates an emerging threat of this virus to public health.⁸

Recent HAdV-7d outbreaks in college and military surroundings have also been recognized.^{9,10}

Adenoviruses (ATVs) are classified in the genus mastadenovirus, family adenoviridae¹⁰ with an icosahedral nucleocapsid containing double-stranded linear DNA.

Presently, 103 diverse HAdV serotypes are assembled into 7 HAdV species A - G according to their biophysical, biochemical, whole-genome sequencing, phylogenomics,

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and genetic characteristics.¹¹ In the anbar governorate, Iraqi country incomplete evidence concerning Adenoviruses (HAdV) prevalence. A current study was done to show the prevalence of HAdV in this area of Iraq.

Patients and Methods

A descriptive cross-sectional study has been achieved throughout a period between 22th of January 2021 to October 11, 2021, for the discovery of human Adenovirus (HAdV) strains 3, 4, and 7 amongst 104 children and adults with upper and lower RTIs who were attended to AL-Ramadi and Fallujah Teaching Hospitals and from other hospitals in Anbar governorate, in ages between 15–56 years with mean age 35.55 \pm SD 24,46 years.

Study Protocol

Upper and lower RTIs patients were interviewed individually before blood sample collection; for each patient, a well-structured questionnaire form was full carefully included: name, phone number, hospital title, clinic name, gender, age, residency, occupation, material status, educational attainment, smoking with disease history. Family consent was applied to every patient and control, and the scientific committee must approve the research of the College of Medicine, Al-Anbar University.

Blood Specimens Collection and Processing

The 5 mL of fresh blood specimens were drawn from upper and lower RTIs patients through using of venipuncture, 2 mL of blood specimens were transferred in a dry tube, then centrifuged within four hours at 2000 rpm for ten minutes and storage at (-20–40°C) with avoid repeated freeze and thaw cycle). Serological test (IgM & IgG antibodies investigations using enzyme-linked immunoassay (ELISA) test), and other hematological & serological parameters has been done depending on Manufacturer's instructions. Further, 3 mL of blood was collected in sterile edetic acid (EDTA) tubes. Plasma has been separated and transferred to sterile Eppendorf tubes and these tubes are stored at -80°C in the laboratory until for molecular assay (PCR technique).

Table 1: Respiratory human adenovirus prevalence in children and adults with respiratory tract infections using ELISA IgM, ELISA IgG and PCR tests

<i>ELISA IgM result</i>	<i>Frequency</i>	<i>Per cent</i>
Positive	11	10.6
Negative	93	89.4
Total	104	100.0
<i>ELISA IgG result</i>	<i>Frequency</i>	<i>Per cent</i>
Positive	6	5.8
Negative	98	94.2
Total	104	100.0
<i>PCR result</i>	<i>Frequency</i>	<i>Per cent</i>
Positive	9	8.7
Negative	95	91.3
Total	104	100.0

Some hematological and serological parameters, ELISA test (for detection of human adenovirus strains 3, 4, 7 IgM and IgG antibodies) and polymerase chain reaction (PCR) (for detection of human adenovirus strains 3, 4, 7), were done according to the manufacturer's instructions

Statistical Analysis

Through using SPSS version 24, Statistical analysis was performed. The results have been presented in tables and graphic as descriptive results (mean, SD, frequency and percentage), 2x2 cross-tabular test has been performed to compare the HAdV prevalence in upper and lower RTIs patients. The independent sample T-test that compares the means of different serological and hematological parameters in upper and lower RTIs patients has been used. A two-tailed p-value has been used in overall analyses and p-value less than 0.05 were used to decide statistical significance difference.

RESULTS

The mean age of children with respiratory infection with the age group 9–17 was 14.33 SD2.934, whereas the mean age of adults with respiratory illness in the age group 18–50 years was 34.19 SD9.503 and for the period group 51+ year was 61.03 SD 8.431.

A total of 104 respiratory tract infected patients, 11(10.6%) patients were ELISA IgM positive, 9(8.7%) of them were positive also using PCR technology, and the other 2 (1.9%) were negative for PCR. 6(5.8%) patients were ELISA IgG positive for human adenoviruses (HAdV). All patients who were negative for HAdV using the ELISA IgM and IgG tests were also HAdV negative using the PCR technique (Table 1).

Out of the total of 104 Children and adults respiratory tract infected patients, 9(8.7%) of them were positive for human adenoviruses serotype 7 using PCR technique (Table 2). In contrast, no human adenoviruses serotype 3,4 were detected using the same technique.

HAdV prevalence among children and adults with respiratory tract Infected patients using IgM ELISA test were distributed in age groups as following 5(4.8%) in age group

Table 2: HAdV prevalence among children and adults with respiratory tract Infected patients using ELISA and PCR test

<i>IgM ELISA Results</i>	<i>PCR Results</i>		<i>Total</i>	<i>P-value</i>
	<i>Negative</i>	<i>Positive</i>		
Negative	93 (89.4%)	0(0.0%)	93(89.4%)	
Positive	2(1.9%)	9(8.7%)	11(10.6%)	0.000
Total	95(91.3%)	9(8.7%)	104(100.0%)	
<i>IgG ELISA Results</i>	<i>PCR Results</i>		<i>Total</i>	<i>P-value</i>
	<i>Negative</i>	<i>Positive</i>		
Negative	89 (85.6%)	9(8.7%)	98 (94.2%)	
Positive	6 (5.8%)	0(0.0%)	6(5.8%)	0.437
Total	95 (91.3%)	9(8.7%)	104(100.0%)	

Table 3: HAdV prevalence among children and adults with respiratory tract Infected patients among different age groups using IgM ELISA, IgG ELISA and PCR test

IgM ELISA Results	Age group			Total	p-Value
	9-17	18-50	51+		
Negative	7 (6.7%)	54 (51.9%)	32 (30.8%)	93(89.4%)	
Positive	5 (4.8%)	3 (2.9%)	3 (2.9%)	11(10.6%)	0.001
Total	12(11.5%)	57(54.8%)	35 (33.7%)	104(100.0%)	

IgG ELISA Results	Age group			Total	p-Value
	9-17	18-50	51+		
Negative	9 (8.7%)	57 (54.8%)	32 (30.8%)	98 (94.2%)	
Positive	3 (2.9%)	0 (0.0%)	3 (2.9%)	6 (5.8%)	0.002
Total	12 (11.5%)	57 (54.8%)	35 (33.7%)	104 (100.0%)	

PCR Results	Age group			Total	p-Value
	9-17	18-50	51+		
Negative	7 (6.7%)	54 (51.9%)	34 (32.7%)	95 (91.3%)	
Positive	5 (4.8%)	3 (2.9%)	1 (1.0%)	9 (8.7%)	0.000
Total	12 (11.5%)	57 (54.8%)	35 (33.7%)	104 (100.0%)	

Table 4: Association human adenovirus with clinical forms of respiratory tract infections using IgM ELISA

Diagnosis	IgM ELISA Results		Total
	Negative	Positive	
Upper respiratory tract infection	8 (100.0%)	0(0.0%)	8 (100.0%)
Bronchopneumonia	26(86.7%)	4(13.3%)	30 (100.0%)
Pneumonia	59(89.4%)	7(10.6%)	66 (100.0%)
Total	93(89.4%)	11(10.6%)	104 (100.0%)

Table 5: Association human adenovirus with clinical forms of respiratory tract infections using IgG ELISA

Diagnosis	IgG ELISA Result		Total
	Negative	Positive	
Upper respiratory tract infection	7 (87.5%)	1 (12.5%)	8 (100.0%)
Bronchopneumonia	30 (100.0%)	0 (0.0%)	30 (100.0%)
Pneumonia	61 (92.4%)	5 (7.6%)	66 (100.0%)
Total	98 (94.2%)	6 (5.8%)	104 (100.0%)

9-17, 3(2.9%) in the age group 18-50, 3(2.9%) in the age group 51 + Table 3.

Using IgG, ELISA were distributed in age groups as 3 (2.9%) in age group 9-17 and 3(2.9%) in the age group 51 + Table 3. HAdV prevalence among children and adults with respiratory tract Infected patients using PCR test was distributed in age groups as following 5(4.8%) in age group 9-17, 3 (2.9%) in the age group 18-50, 1(1.0%) in the age group 51 + Table 3

Of 30 bronchopneumonia patients, 4(13.3%) were positive for HAdV using IgM ELISA. Of 66 patients with pneumonia, 7(10.6%) were positive for HAdV using IgM ELISA, no HAdV was detected in 8 of the upper respiratory tract infected patients (Table 4).

Out of the eight upper respiratory tract infected patients, 1(12.5%) were positive for HAdV using IgG ELISA. Of 66

Table 6: Association human adenovirus with the severity of Pneumonia using IgM ELISA test.

Severity of Pneumonia	IgM ELISA Results		Total
	Negative	Positive	
Mild	31 (96.9%)	1 (3.1%)	32 (100.0%)
Moderate	40(87.0%)	6 (13.0%)	46 (100.0%)
Sever	22(84.6%)	4 (15.4%)	26 (100.0%)
Total	93(89.4%)	11 (10.6%)	104 (100.0%)

patients with pneumonia, 5(7.6%) were positive for HAdV using IgG ELISA (Table 5).

Of 32 mild pneumonia patients, 1(3.1%) were positive for HAdV using IgM ELISA. Of 46 moderate pneumonia patients, 6(13.0%) were positive for HAdV using IgM ELISA. Of 26 sever pneumonia patients, 4(15.4%) were positive for HAdV using IgM ELISA (Table 6).

A significant difference in the means of ALT(U/L), AST(U/L), creatine kinase (U/L), LDH(U/L), neutrophil% and lymphocyte% between positive and negative IgM patients using the IgM ELISA test.

We concluded that the prevalence of human adenovirus in children with respiratory infection was 4.8%. In contrast, the majority of adults in age groups 18-50 and 51+ years was 2.9% for each group using the IgM ELISA test.

DISCUSSION

HAdV prevalence among children with respiratory tract infection in age group 9-17 years was 5(4.8%), whereas the HAdV prevalence among adults with respiratory tract infection in the age group 18-50 years was 3(2.9%). In the age group, 51+ years was also 3 (2.9%) using the IgM ELISA test. These results were consistent with the results reported by Zhao *et al.* (2021),¹² that HAdV ranges between 5 to 15% of patients with upper and lower hospitalized RTIs.

Current results were in agreement with previous results that show HAdV consider one of the most common viruses associated with upper and lower RTIs, and approximately 4 to 10% of upper and lower RTIs children were infected with HAdV, but disagree with the same information that shows HAdV causes these infections to occur more commonly in children under five years of age.²

Our study was consistent with previous studies that found that HAdV has a significant role in pediatric upper and lower RTIs, as its frequency range between 2 to 5% of the overall upper and lower RTIs and 4 to 10% of pneumonia, particularly in pediatric and immune-compromised patients.^{2,5}

Conversely, a minor percentage of infant and adults develop severe upper and lower RTIs duo to adenovirus, which can lead to acute respiratory failure and high mortality rate in children.² Furthermore, some severe upper and lower RTIs duo to adenovirus may develop chronic difficulties, e.g., bronchiolitis obliterans and bronchiectasis cases.¹²

The current study showed that 1(12.5%) was positive for HAdV using IgG ELIZA in 8 upper respiratory tract infected patients. Of 66 patients with pneumonia, 5(7.6%) were positive for HAdV using IgG ELIZA. These results were consistent with the previous study that show HAdV may cause pediatric infections in a significant percent range between 2–7% of upper respiratory tract infection overall and 4–20% of severe pneumonia amongst immunocompetent patients.⁽¹³⁾ It's consistent with a previous study that showed HAdV is responsible for 15 and 5% of upper and lower respiratory illnesses, respectively amongst immune-compromised adults patients.¹¹

The present research was dissimilar to an earlier report that exposed HAdV was identified in 114 of 583 samples (19.6%), including 6.1% single illnesses and 93.9% co-infections in Tunisia in the pediatric population and HAdV-C was recognized as the most predominant genotypes.¹⁴

HAdV has very important roles in pediatric respiratory conditions, with a percent 2–5% of general respiratory conditions & 4 to 10% of public-acquired pneumonia conditions.¹⁵

A total of 104 Children and adults respiratory tract infected patients, 9(8.7%) of them were positive for human adenoviruses serotype 7 using PCR technology. In contrast, no human adenoviruses serotype 3, 4 were detected using the same technique during the current study. These results were consistent with the results reported by Kujawski *et al.* (2021).¹⁷ and Clark *et al.*(2011),¹⁷ who show that HAdV-7 are predominant circulating genotypes linked with upper and lower respiratory conditions but the difference from other results that showed HAdV-4 HAdV-4 are predominant and liked with sever upper and lower respiratory conditions.¹⁴

Amongst HAdV linked with respiratory illnesses in children and adults, HAdV-7 is known to be predominant serotypes and associated with severe and high mortality rate due to fatal respiratory conditions in pediatrics.³ HAdV-7 is widespread worldwide and constitutes about 20%

of overall HAdV respiratory conditions.⁶ A previous study demonstrated that HAdV-7d is frequent in the population.^{9,10} Adenoviruses (AdVs) are associated with gastroenteritis, hemorrhagic cystitis and keratoconjunctivitis.¹⁸ Several reports indicated that adenoviruses were distributed in COVID-19 patients.^{19,20}

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