ORIGINAL ARTICLE

PREVALENCE OF HUMAN ADENOVIRUS IN PAEDIATRIC PATIENTS PRESENTING WITH ACUTE RESPIRATORY SYMPTOMS AT DIFFERENT HOSPITALS OF PAKISTAN

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Background: Human Adenovirus (HAdV) is one of the most common causes of infection in children. HAdV commonly affects respiratory system, however can also involve other parts of the body like nervous system, eyes and urinary tract. The virus usually causes a mild infection of the lower and upper respiratory tract. Objective of the study was to find the prevalence of HAdV in paediatric patients presenting with Influenza like symptoms and severe acute respiratory illness across Pakistan. **Methods:** This cross-sectional study was conducted at the National Institute of Health, Islamabad. Respiratory swabs were collected from 389 children with age less than five years from 14 hospitals in different regions of Pakistan from October 1, 2017 to September 30, 2018. Patients' demographics, signs and symptoms were recorded through a predesigned proforma while Real-time polymerase chain reaction (RT-PCR) was performed for respiratory samples. **Results**: Out of all 389 samples, HAdV was found in 25 (6.4%) cases. The proportion of HAdV obtained was greater in females 18 (4.6%) than male 7 (1.8%). The influenza-like illness in children attending outpatient department had a higher prevalence of HAdV 13 (3.3%) compared to admitted children 12 (3.1%). Similarly, patients from one to 6 months of age had higher positive outcome than older children. Majority of positive patients were from Islamabad (2.0%) followed by Gilgit (1.8%), Azad Jammu Kashmir (1.0%), Multan (0.5%), and Karachi (0.5%). The most frequent signs and symptoms were cough, fever, sore throat, nasal congestion and shortness of breath. **Conclusion:** The present study concludes that HAdV infection is common in Pakistan especially in female patients aged 1–6 months. It's crucial to improve the diagnosis of HAdV infections in our country to prevent complications associated with the virus. Furthermore, genetic analysis may help find different genotypes of HAdV circulating in Pakistan.

Keywords: Human Adenovirus; Severe acute respiratory illness; Paediatric patients; Real time PCR

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INTRODUCTION

Human Adenovirus (HAdV) is a class of DNA viruses that affects multiple organs including airway, lungs and eyes.[1] HAdV is a non-enveloped double-stranded virus of the Mast-adenovirus genus and belongs to the family Adenoviridae.[2] It has more than 70 genotypes identified through various bioinformatics tools.[3,4] The virus infects lower and upper respiratory tract, conjunctivae, gastrointestinal tract, meninges, kidneys, pancreas, liver and urinary bladder. HAdV causes infections in younger children due to a lack of humoral immunity.[1] The infection spreads through faecal-oral route, inoculation of the conjunctiva and inhalation of aerosolized virus containing droplets.[5]

Epidemics of the HAdV usually occur due public swimming pools, orphanages, children's homes, schools, training centers, day-care facilities, psychiatric, neonatal nurseries, and hospitals.[6] Similarly, those children having congenital immunodeficiency syndromes, solid organ transplant recipients and hematopoietic stem cell transplant recipients are at higher risk of developing the disease.[7,8] HAdV can be detected by various techniques including shell vial cultures, conventional vial cultures, direct/indirect immunofluorescence, and most commonly polymerase chain reaction (PCR).[9,10]

Respiratory HAdV infections are usually treated with live oral vaccines which have been proved effective in most of the cases.[1]

Acute respiratory infections are the main cause of increased mortality and morbidity throughout the world. A study conducted in Changzhou China, on children below 14 years of age showed HAdV in 4.9% patients where the most prevalent serotype HAdV2.[11] Another study conducted in Kuwait from 2013 to 2016 showed a 3.5% HAdV prevalence with highest in March and November which highlights the importance of seasonal variations of the infection.[12] A Korean study showed alarmingly high prevalence of 16.9% of HAdV in patients presented with respiratory symptoms in children less than 5 years of age.[13]

Till date, more than 50 HAdV serotypes have been categorized within a total of seven species of the virus (A-G). Researchers have also reported different genotypes of HAdV e.g., HAdV-3, HAdV-2, and HAdV-7. Species, genotypes or serotypes identification is important for treatment purpose as they have similar symptoms.[14–16]

Studies conducted at the tertiary care hospitals of Islamabad on children of age 2 years showed the prevalence of HAdV is around 8% and the frequency was higher during December and January in the admitted patients.[17,18] Children under the age of 5 years are frequently affected by the virus. Currently, limited data is available on HAdV prevalence in some parts of Pakistan. A complete and latest epidemiological data on HAdV infection may help improve our understanding of HAdV infections. The present study may help improve our knowledge about the burden of HAdV among respiratory infections in children using molecular detection of RT-PCR.

MATERIAL AND METHODS

This cross-sectional study was conducted on children less than 5 years of age across Pakistan. Samples were collected from patients with clinical presentation of acute respiratory infections from 1st Oct 2017 to 30th September 2018. A total of 389 samples were collected and processed at Virology department, National Institute of Health (NIH), Islamabad. Nasopharyngeal and throat swabs were collected from both Outdoor and Indoor patients in different hospitals of Islamabad and sentinel sites like District Head Quarter Hospital (Gilgit), Civil hospital (Karachi), Mayo hospital (Lahore), Nishtar Hospital (Multan), Abbas Institute of Medical Sciences (Azad Jammu & Kashmir) and Bolan Medical College (Quetta). Before the collection of samples, informed consent was taken from patients. Patients' demographics and clinical findings were collect using a predesigned proforma. Samples were transported to department of Virology, NIH in insulated carriers with ice pack. The project was approved by the Institutional Ethical Board and sampling was done after informed consent.

Two criteria were used for sample selection; Influenza-like illness (ILI) defined as fever higher than 38 °C with coughs less than 10 days whereas Severe Acute Respiratory Illness (SARI) defined as fever higher than 38 °C and cough started in last 10 days with issues like shortness of breath, tachypnoea, expiratory crackle which needed admission to hospital. Children with more than 5 years were excluded from the study. Samples were tested for HAdV following biosafety precautions as coherent by the Center for Disease Control and Prevention (CDC), Atlanta, Georgia, USA.[19]

Samples were vortexed, followed by centrifugation for 10 minutes on 15000 rpm at 4°C. One milliliter supernatant was added to micro-centrifuge tubes inside Class-II biosafety cabinet and samples were stored at -80 °C. *QIAamp* Viral RNA Minikit (Qiagen GmbH, Germany) was used to extract DNA and sample lysis was carried out by AVL buffer. 140µl of the sample was added to a tube containing 560µl of AVL buffer. This was subjected to pulse vortexing for 15seconds and incubated at 30 ºC for 15minutes. After incubation, 560 µl of 100% ethanol was added and vortexed for 15sec.

QIAamp mini spin column was transferred to sterile 2ml collection tube and filled with 630µl of sample solution. Centrifugation was carried out at 8000 rpm for 10 minutes. 500 µlAW1 buffers was used for washing the spin column at 8000 rpm; followed by adding 500 µl of AW2 buffer centrifuged at 14000 rpm for 3 minutes. 60µl elution buffer was added and left at room temperature for 1–2 minutes. The column was centrifuged at 8000 rpm for 1 minute. Extracted DNA was used to carry out qRT-PCR reactions. DNA was stored at -70 ºC till further analysis. qRT-PCR was used for viral diagnostics and the conserved region was targeted for HAdV detection.

Primer sequences (5'–3') used:

Forward primer:

[GCCCCAGTGGTCTTACATGCACATC]

Reverse primer:

[GCCACGGTGGGGTTTCTAAACTT]

Probe:

[TGCACCAGACCCGGGCTCAGGTACTCCGA].[20]

PCR master mix was prepared according to the manufacturer's instructions given in the kit manual with thermal profile shown in table-1.

Results were interpreted from automatically generated files utilizing SDS v1.4 software given with the apparatus. Cycle threshold (CT) values for positive samples and negative controls were studied in amplification plots generated by the software. Each run was done with negative and positive control. If reaction curves cross the positive threshold line in 40 cycles,

samples were positive. Statistical analysis was done using Microsoft Excel 2010.

RESULTS

Samples collected from various hospitals of different parts of the country are shown (Figure-1). The highest number of samples was from Islamabad (48.8%) followed by Gilgit (9.0%), Azad Jammu Kashmir (7.7%), Multan (7.2%), Karachi (6.4%), Lahore (5.9%), Rawalpindi (5.9%), and Quetta (5.1%). Most of the sample was collected from the major cities of the country however; samples from some areas like Swabi, Rahim Yar Khan, Gojra, Faisalabad, Wah Cantt 1.0% and Mohmand were less as shown in the figure-1. The total number of children with influenza like illness were n= 214 (55%) whereas SARI were n=175(45%). The female children having ILI were n=95 (24.4%) while SARI were n=74(19.0%). Male children having ILI were n=119 (30.6%) while SARI were n=101 (26.0%). Clinical follow up of children till 2 weeks for appearance of signs and symptoms is shown in table-2.

The study population was followed for signs/symptoms such as fever, cough, nasal congestion, sore throat, wheezing, chest pain, and shortness of breath. Children with ILI had fever and cough (100 %) followed by sore throat (99%), SOB (93%), Nasal congestion (91%), and wheezing (82%). Children with SARI presented with cough (99%) fever (97%), sore throat (96%), Nasal congestion (93%), and wheezing (84%). Younger children and males were more symptomatic as compared older children and females as shown table-3.

The overall HAdV distribution on the basis of positive PCR was n=25 (6.4%) in the study population. These results showed that females had higher positive results of HAdV n=18 (4.6%) while males were n=7 (1.8%). The ILI children had a bit higher prevalence of HAdV n=13 (3.3%) compared to SARI children n=12 (3.1%). Similarly, infants from 1 to 6 months had higher positive results n=12 (3.1%) as compared to other age groups as shown in table-4.

The prevalence of HAdV from various cities of Pakistan was analyzed. Highest prevalence (2.01%) was recorded from Islamabad, followed by Gilgit (1.8%) and then Azad Jammu Kashmir (1.0%). From Mohmand, Multan, and Karachi equal 0.5% prevalence of HAdV was recorded. However, the total sample collected from these cities varied (Islamabad 48.8%, Gilgit 9.0%, Azad Jammu Kashmir 7.7%, Multan 7.2%, Karachi 6.43%, and Mohmand 1.3%) as shown in figure 2.

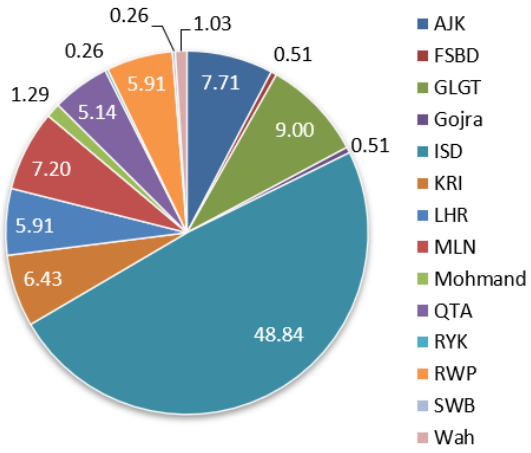

Figure-1: Study samples (%) collected from different cities of Pakistan

*AJK: Azad Jammu & and Kashmir; FSBD: Faisalabad; GLGT: Gilgit Baltistan; Gojra: Gujranwala; ISD: Islamabad; KRI: Karachi; LHR: Lahore; MLN: Multan; Mohmand: Mohmand Agency; QTA: Quetta; RYK: Rahim Yar Khan, RWP: Rawalpindi; SWB; Swabi; Wah: Wah Cantt

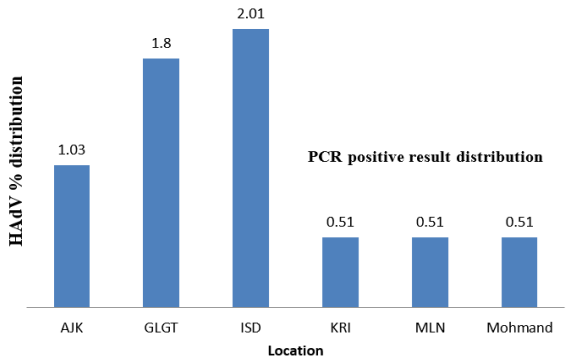

Figure-2: HAdV % distribution in various cities of Pakistan

*AJK: Azad Jammu & and Kashmir; GLGT: Gilgit Baltistan; ISD: Islamabad. *KRI: Karachi; MLN: Multan; Mohmand: Mohmand Agency

Table-1: Thermal profile used for RT-PCR

Step	Stage	Repeats	Thermal Profile	
			Temp	Time
Reverse transcription	1	1	50 °C	30 minutes
RT inactivation/initial denaturation	2	1	95 °C	10 minutes
Amplification	3	45	95 °C	15 sec
			45 °C	31 sec

Table-2: Follow up of children for sign and symptoms as per type of illness and gender

Type of Illness		ILI			SARI			Grand Total n (%)
	Gender	Female n (%)	Male n (%)	Total	Female n (%)	Male n (%)	Total	
Follow up/ Symptoms appearance	Day 1	2 (0.5)	4 (1.0)	6 (1.5)	0 (0)	0.3 (1)	1 (0.3)	3 (0.7)
	Day 2	22 (5.6)	31 (8.0)	53 (13.6)	12 (3.1)	12 (3.1)	24 (6.2)	73 (18.8)
	Day 3	33 (8.5)	45 (11.6)	78 (20.0)	27 (6.9)	39 (10.0)	66 (17.0)	144 (37.0)
	Day 4	19 (4.9)	23 (5.9)	42 (10.8)	20 (5.1)	29 (7.4)	49 (12.6)	91 (23.4)
	Day 5	5 (1.3)	1 (0.3)	6 (1.5)	5 (1.3)	7 (1.8)	12 (3.1)	18 (4.6)
	Day 6	2 (0.5)	1 (0.3)	3 (0.8)	1 (0.3)	3 (0.8)	4 (1.0)	7 (1.8)
	Day 7	12 (3.1)	13 (3.3)	25 (6.4)	8 (2.1)	10 (2.6)	18 (4.6)	43 (11.0)
	Day 8	0 (0)	1 (0.3)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (0.3)
	2 Weeks	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	1 (0.3)	1 (0.3)
Grand Total		95 (24.4)	119 (30.7)	214 (55.0)	74 (19.0)	101 (26.0)	175 (45.0)	389 (100)

*ILI-Influenza like illness, SARI- Severe Acute Respiratory infection. *n= total number of patients, (%)= percent

Table-3: Signs/symptoms (%) among children with regard to type of illness, age, and gender

Parameters		Fever	Cough	Nasal congestion	Sore throat	Wheeze	Chest Pain	SOB	Total
Type of illness	ILI	100.0	100.0	91.1	99.1	82.7	74.3	93.9	91.6
	SARI	97.7	99.4	93.7	96.0	84.6	46.3	71.4	84.2
Age	1-6 months	98.6	100.0	91.2	98.1	87.0	63.3	88.4	89.5
	7-12 months	97.8	100.0	91.3	97.8	69.6	60.9	84.8	86.0
	1< 2 years	100.0	100.0	92.5	95.0	82.5	50.0	70.0	84.3
	2< 3 years	100.0	100.0	94.2	98.1	82.7	59.6	82.7	88.2
	3<5 years	100.0	97.2	97.2	97.2	83.3	69.4	72.2	88.1
Gender	Male	99.1	100.0	91.4	98.2	81.4	60.0	82.7	87.5
	Female	98.8	99.4	93.5	97.0	86.4	63.9	85.2	89.2
Total		99.0	99.7	92.3	97.7	83.5	61.7	83.8	88.2

*ILI-Influenza like Illness, SARI- Severe Acute Respiratory Infection. *SOB= Shortness of breath

Table-4: Distribution of HAdV as per clinical presentation, age and gender

Element	Types of illness	Total number of HAdV cases (=n)	Percentage
Clinical presentation	ILI	13	3.4
	SARI	12	3.0
Age	1-6months	12	3.1
	7-12 months	4	1.0
	1< 2 years	3	0.8
	2< 3 years	2	0.5
	3<5 years	4	1.0
Gender	Female	18	4.6
	Male	7	1.8
Total		25	6.4

*ILI-Influenza like Illness, *SARI- Severe Acute Respiratory Infection. *n-Number of Patients

DISCUSSION

Acute respiratory tract infections are a major cause of mortality and morbidity throughout the world. The HAdV is one of a major cause of acute respiratory infections in children.[21] In Pakistan, due to limited health care resources, HAdV screening is not done in acute respiratory infections and patients are treated empirically based on their clinical presentation. If proper screening is done, HAdV associate morbidity and mortality can be minimized up to some extent.

In the present study, sampling was done from 14 cities of Pakistan where most of the samples (48.84%) were from Islamabad. However, population of Islamabad region is representative of the whole country. Another possible reason could be the seasonal increase in respiratory disease in Islamabad due high pollen count however that is not a contributing factor in HAdV. In this study, we determined sign and symptoms of patients in which most common was found dry cough, high grade fever, sore throat, and nasal congestion with proportion of 99%, 98%, 97% and 92% respectively. These results were supported by a study conducted in Islamabad, Pakistan where the prevalent clinical presentation was dry cough (100%) and fever (70%).[17]

Most of the studies conducted on HAdV prevalence showing the same trend of clinical presentation of the disease.[11,22] HAdV was detected in samples from most of the cities including Islamabad, Gilgit, Azad Jammu Kashmir, Mohmand, Multan, and Karachi. The overall prevalence of the HAdV was 6.4. Our finding of this study is supported by

studies from Guangzhou and Xining city of China where the prevalence HAdV was 4.9% and 8.2% respectively.[11,22] Another study reported from China shows 8.5% positivity ratio of HAdV, however their sample size was large.[23] Similarly, a study on HAdV conducted in two hospitals of Islamabad Pakistan reported 8.4% showed a higher prevalence than our study but their sample size was small and the representative population was from a single city.[17] Some studies conducted in Saudi Arabia reported the HAdV prevalence of 15% and 17% respectively which is higher than our findings in the present study. These findings may due to the age differences of the study population in both studies however some genetic and environmental differences may also have a potential role.[24,25]

In the present study, the HAdV infection was more in female children than males (1.8%), however studies from Chunguz China and Korean University Hospital showed a higher prevalence of 4.6% and 5.7% in males respectively.[11,13] The higher female HAdV prevalence in our study may be due gender preferences and care for male children in our society.[26]

The present study also showed that prevalence of HAdV was higher in babies 1-6 months as compared to older children. However, a study from China shows contrasting results with a low prevalence in children less than one year.[27] This contrast in the age group may be due the higher prevalence of malnutrition in mothers during perinatal period giving birth to immune-deficient babies.[28]

CONCLUSION

The present study concludes that HAdV, a highly pathogenic virus is one of the causative agents of upper and lower respiratory infections in children. Children with respiratory diseases are commonly not screened for HAdV in our hospitals. HAdV is more prevalent in children less than 6 months of age with a higher prevalence in females. Gentic studies of HAdV are needed to know which genotypes serotypes are circulating in our population. HAdV testing may also help in treatment planning and will help prevent antibiotics misuse. Furthermore, female children must get full attention and maternal nutritional status should be considered during perinatal period to avoid immuno-compromised status arising from nutrition deficiency. Population based education programs must be implemented for clean and healthy habits and vaccination of children to overcome neonatal infections.

Limitations:

The study duration was short and only few cities were included with a smaller sample size. Genotypes and serotypes of HAdV were not studied due to budget constraints. Similarly seasonal pattern of HAdV was not considered due to short period of the study.

AUTHORS' CONTRIBUTION

SA: Data collection, project design, manuscript writing. AM: Project design supervision, manuscript writing. IU: Data analysis, manuscript writing, critical appraisal and correspondence. NB: Data collection and analysis. INK: Critical review, supervision. AA: Critical review, final correction. AM: Manuscript writing, references. MUK**:** Critical appraisal, final proof reading

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