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Detection and Molecular Characterization of Human Adenovirus Infections among Hospitalized Children with Acute Diarrhea in Pakistan

Zainab Khattak Nayab Mahmood[2], Muhammad Masroor Alam[2], Sohail Zahoor Zaidi[2], Muhammad Usman[2], Ribqa Akhtar[2], Nighat Mushtaq[2], Muhammad Salman[2], Aamer Ali Shah[1*]

[1]*Department of Microbiology, Quaid-i-Azam University, Islamabad-45320, Pakistan*

[2]*Department of Virology, National Institute of Health, ChakShahzad, Park Road, Islamabad-45500, Pakistan*

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Abstract

Viral gastroenteritis afflicts an enormous number of persons all over the world. It establishes a main hazard particularly to susceptible people for example children, aged people, and immune compromised individuals. *Rotavirus* and *adenovirus* are the two important agents associated with hospitalization for diarrhea especially in children. Pakistan is among one of the Asian countries with higher diarrhea related child's death. The aim of the study is the assessment of the molecular epidemiological study of HAdV in children with gastroenteritis in Pakistan. During January 2017 to December 2018, 1,086 stool samples were collected from children under 5 years of age from five hospitals; Benazir Bhutto Hospital, Rawalpindi (BBH), Mayo Hospital, Lahore (MHL), Children's Hospital, Lahore (CHL), Kharadar General Hospital (KGH), Karachi and National Institute of Child Health (NICH), Karachi, Pakistan. A demographic and clinical study was performed for determination of the relationship among viral infection and clinical consequences of patients. Atotal 198/1,086 (18.23%) stool samples were determined to be positive for HAdV by PCR. Among them 26.76% (n=53) were found to be co-infected with *rotavirus*. There was a significant relation of diarrhea related clinical parameters with HAdV. This study provides vital data on HAdV in Pakistani children. The presence of higher disease load of HAdV in early age children of our country produced additional emphasis on the prioritization of the analysis of HAdVs particularly in the neonates and early-age infants. Briefly, this study demonstrates that HAdV is the main cause of gastroenteritis in Pakistani children.

* **Corresponding Author:** Aamer Ali Shah ✉ alishah@qau.edu.pk

Introduction

Viral gastroenteritis afflicts an enormous number of persons all over the world and is a severe viral infection. It establishes a main hazard particularly to susceptible people for example children, aged people, and immune compromised individuals(La Rosa *et al.,* 2015). Globally, about 3 to 5million children are affected by gastroenteritis annually that depicts for 1.5 to 2.5 million mortalities per year or 12% of total mortalities between children <5 years of age. In children, viruses are the more significant etiologic agents that are liable for around 70% of the episodes of acute gastroenteritis. Universally, for producing this infections, *rotavirus* is quiet the most common viral cause which is followed by *adenovirus* types 40 and 41, *astrovirus*, and *calicivirus*. Enteric *human adenoviruses* (HAdVs) cause acute diarrhea periodically, in addition to in outbreaks. In developed countries, the rate of enteric *adenovirus* 40 and 41 differs from 1–8% while in developing countries it differs from 2–31% with high prevalence rate in immune compromised individuals (Dashti *et al.,* 2016).

In 1953, Rowe and colleagues while studying the growth of *polioviruses* in adenoidal tissue first isolated *adenoviruses*(Ghebremedhin, 2014). HAdVs are members of the genus *Mastadenovirus* in the *Adenoviridae* family. HAdVs are non-enveloped with linear double-stranded DNA genome. They are categorized into seven species namely HAdV-A to G(Radke and Cook, 2018)having 85 genotypes (Yousefi *et al.,* 2018)which are determined mostly through the hexon hyper variable regions (HVRs) that organize most of the surface of the virion (Yu *et al.,* 2017). Clinical symptoms of the infection is determined by means of the tissue tropism of HAdV species. Species B, D and E of HAdV can infect conjunctiva while the upper and lower respiratory tract sand the gastrointestinal tract can be infected by species B, C and E and species F, G of HAdV. Species A–Gof the HAdV rotate universally and can be responsible for causing restricted, sporadic outbreaks of infection(Radke and Cook, 2018). However, the clinical period of the infection is usually mild and self-preventive but these infections may cause epidemics with severe development that infrequently directing to a fatal consequence also in immune competent persons(La Rosa *et al.,* 2015).

In unindustrialized countries, the association of enteric *adenoviruses* with prolonged diarrhea contributed to child dehydration and malnutrition(Vergatat 2007;Chow *et al.,*2010)that most principally disseminate through the fecal-oral route(Santosham 2002;Chow *et al.,*2010). Typically, periodic diarrhea take place subsequent to an incubation period of 8 to 10 days, having low-grade fever, vomiting, abdominal pains, and dehydration(Santosham 2002;Wilhelmi *et al.,* 2003).

Presently, very limited data is available from developing countries like Pakistan on viral causes of acute gastroenteritis(Alam *et al.,* 2015)except few reports that mainly focused on *rotavirus* in the latest years(Alam *et al.,* 2013). Agboatwalla *et al.* (1995) reported from Karachi, Pakistan that the prevalence of enteric *adenoviruses* was 10% in 1992 in children with gastroenteritis. During this study Enzyme Linked Immunosorbent Assay (ELISA), technique was used for the detection of enteric *adenoviruses* and *rotaviruses*(Agboatwalla *et al.,* 1995). To the best of our information, this is the first comprehensive epidemiological study that focused on the prevalence of HAdV in hospitalized children presented with acute gastroenteritis in Pakistan. The present study intended to assess the epidemiology of prevalent HAdV associated with acute gastroenteritis in hospitalized children in Pakistan.

Materials and methods

Ethical Approval and Sampling

The National Institute of Health's (NIH) Internal Review Board Committee approved this study(Alam *et al.,* 2015). During January 2017 to December2018, 1,086 stool samples were collected from children under 5 years of age from five hospitals; Benazir Bhutto Hospital, Rawalpindi (BBH), Mayo Hospital, Lahore (MHL), Children's Hospital, Lahore (CHL), Kharadar General Hospital (KGH), Karachi and

National Institute of Child Health (NICH), Karachi. In the upper Punjab and northern area of Pakistan, BBH, Rawalpindi is a main teaching hospital. MHL is one of the oldest and largest hospital in Lahore, Pakistan while CHL is the largest public children's hospital situated in Lahore, Pakistan. KGH has been contributing valued health services to the poor, socio-economical, and educationally deprived individuals of 4 million people of low-lying areas of Karachi, Pakistan. NICH is the first children hospital of the country and currently in the province of Sindh, Pakistan; it is one of the major and the only children hospital. In Pakistan for *rotavirus* surveillance program, World Health Organization (WHO) selected these sentinel sites due tohuge patient outcome from both rural and urban regions. For all cases, clinical and demographic information were gathered. For this study, cases were included according to the WHO standard case definition for gastroenteritis: a child reporting with three or more loose stools or any vomiting during last24 hr(Alam *et al.,* 2015).

Inoculation of Stool Samples into Cell Lines

Prior to inoculating the stool samples into cell lines, a pre-treatment is essential in order to eliminate the fungal, bacterial and other probable cytotoxic contaminations. Stool specimens were subjected to chloroform pre-treatment. Briefly, about 1 gram of each sample was mixed with 1000 µl of Phosphate Buffer Saline (PBS) and 1ml of chloroform. The solution was shaken vigorously for 20 minutes and centrifuged (10 min, 100 ×g, +4°C). The supernatant was used for propagation in HEp-2c (Human epithelial carcinoma) cell line, which were maintained in minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). Cultures were observed for a maximum of 10 days for a cytopathic effect (CPE). After a CPE was observed, the isolates were maintained at -20°C.

Polymerase Chain Reaction (PCR) for Virus Detection

DNA Extraction and Hexon Gene Amplification

DNA was isolated using nucleospin DNA extraction kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to manufacturer's protocols. *Human adenovirus* was confirmed by polymerase chain reaction (PCR) following protocol as described by Qurashi *et al.,* 2012, targeting loop 1 and loop 2 region of hexon gene of *human adenovirus* with the help of primers specific for the region. Primers HSAd1 (5' CTG ATG TAC TAC AAC AGC ACT GGC AAC ATG GG 3') and HSAd2 (5' GCG TTG CGG TGG TGG TTA AAT GGG TTT ACG TTG TCC AT 3') were used for the amplification of the L2 region of the hexon gene that produced amplicon of a size 605 and 629 bp depends on the virus type. Nested PCR was used for the amplification of L1 region of the hex on gene using primers described by Lu and Erdman 2006. The external primers were AdhexF1 (5'TIC TTT GAC ATI CGI GGI GTI CTI GA 3') and AdhexR1 (5'CTG TCI ACI GCC TGR TTC CAC A 3'). The internal amplification was then performed using 1µl of the amp icons obtained from the above reaction using internal primers AdhexF2 (5'GGY CCY AGY TTY AAR CCC TAY TC 3') and AdhexR2 (5'GGT TCT GTC ICC CAG AGA RTC IAG CA 3') to generate an amp icon of 820 bp(Qurashi *et al.,* 2012).

PCR Conditions

The PCR mixture contained 10X Taq PCR buffer, (pH 9, and Applied Bio systems), 25mM $MgCl_2$ (Applied Bio systems), 10mM concentration of each deoxynucleoside triphosphate (Promega, Ref U1515), 10 uM of each primer, and DNA polymerase (AmpliTaq @ DNA, 250 U, Cat: N808016). 10 µl of Nucleospin-extracted template was added in a final volume of 50 µl. All amplifications(PROFLEX Thermo cycler, Applied Bio systems, Warrington, UK) starts for loop 1 with a single denaturation cycle of 94˚C for 2 min and ended with a final step of 72˚C for 5 min. Cycling was 94˚C (1 min), 45˚C (1 min) and 72˚C (1 min) for L1. All for 35 cycles for L2 amplification starts with denaturation cycle of 95˚C for 10 min and ended with a final step of 72˚C for 5 min. Cycling was 95˚C (1 min), 51˚C (1 min), and 72˚C (1 min) for L2. All for 40 cycles. The visualization of the PCR products were performed on a 2% agarose gel stained with SYBR safe dye below

trans illuminator (Qurashi *et al.*,2012).

Statistical analysis

The descriptive statistical analysis such as percentages, mean, median and range were carried out for age, gender, duration of symptoms and treatment. The t-test was performed for the assessment of different clinical characteristics between HAdV positive cases and HAdV-*rotavirus* co-infected cases. The p-value < 0.05 (two sided) were considered statistically significant(Alam *et al.,* 2013). All of the statistical analyses were performed by using Graph Pad Quick Calcs (Brumback *et al.,* 2018).

Results

General findings

A total of 496 out of 1086(45.67 %) stool samples were culture positive (Fig. 1), which were then confirmed by L1 PCR (nested-PCR) and L2 PCR (single-round PCR) (Fig. 2.). According to screening results total 198/1,086(18.23%) stool samples were determined to be positive for HAdV by PCR in our laboratory. 100% of all HAdV cases reported through this time period were subsisted after Oral Rehydration Therapy (ORT) and/or intravenous rehydration therapy.

Demographical Features of HAdV Positive Cases

Among the 198/1,086 (18.23%) positive cases for HAdV presented with gastroenteritis, the mean age for these cases was11.033±8.539 months (*P*-value= <0.0001) with median 9 months; range 1 to 60 months. HAdV infections were more frequently found in males (57.07%) as compared to females (42.92%) correspondingly. Among positive cases, the highest proportion of children infected with HAdV were found among 1 to12 months of age (72.22%) followed by those between 13 and 24 months (23.23%) of age, then from 25 to 36 months (3.03%) of age, subsequent from 37 to 48 months (1.01%) ofage and then between 49 to 60 months of age, only 1 patient was positive (Fig.3.).HAdV infection was observed throughout the year with the peak months in April and May (start summer) (Fig.4.).

The HAdV positive rate per year was 25% (153/612) in 2017 and 9.49% (45/474) in 2018. HAdV positive rate per hospital was 13.90% (31/223) in BBH, 18.84% (26/138) in MHL, 35.43% (45/127) in CHL, 12.08% (22/182) in KGH and 17.78% (74/416) in NICH.

Clinical Features of HAdV Positive Cases

The mean duration of HAdV symptoms were recorded as 3.10 ± 1.47 (*P*-value = <0.0001). Among the HAdV positive cases, the mean of vomiting and diarrhea episodes per 24 hours was found to be 3.77 ± 2.32 (*P*-value = <0.0001) and 5.77 ± 1.86 (*P*-value = <0.0001) respectively. The symptoms of vomiting persisted for 2.77 ± 1.86 (*P*-value = 0.0018) whereas diarrhea continued for 3.06 ± 1.41days (*P*-value = <0.0001) (Table 1).

Table 1. Demographic Details of Samples Positive for Human Adenovirus during January2017 to December 2018.

Characteristics	Mean ± S.D	Range	*P*-value
Age (months)	13.29±12.86	1-60	<0.0001
Temperature (˚C)	37.70±0.452	37-38.5˚C	<0.0001
Duration of Symptoms (days)	3.10±1.47	1-5	<0.0001
Vomiting Episodes per 24 hours	3.77±2.32	1-7	<0.0001
Vomiting Duration (days)	2.77±1.86	1-5	0.0018
Diarrhea episodes per 24 hours	5.77 ± 1.86	1-9	<0.0001
Diarrhea Duration (days)	3.06± 1.41	1-5	<0.0001

Co-Infection of Adenovirus with rotavirus

Stool samples were also confirmed for *rotavirus*, which is worldwide the most frequently, stated viral cause for diarrhea. As a total, *rotavirus* was detected in23.11% (n=251) of tested samples. Out of the 198samples positive for HAdV, 26.76% (n=53) were

found to be co-infected with *rotavirus*. For the co-infected cases, the mean temperature was recorded as 37.451 ± 0.624 (*P*-value= <0.0001). The range of vomiting and diarrhea episodes per 24 hrin HAdV–*rotavirus* co-infection cases was recorded as 3.26 ± 2.52 (P-value= <0.0001) and 5.91 ± 1.40 (*P*-value=<0.0001) whereas the range of vomiting and diarrhea duration in HAdV–*rotavirus* co-infection cases was recorded as2.66 ± 2.11 (*P*-value = <0.0001) and 3.72 ± 1.32 (*P*-value = <0.0001) (Table 2).

Table 2. Demographic Details of Co-infected Human Adenovirus with Rotavirus Samples during January 2017 to December 2018.

Characteristics	Mean ± S.D	Range	*P*-value
Age (months)	8.575± 6.060	1-60	<0.0001
Temperature (˚C)	37.451± 0.624	36-39˚C	<0.0001
Duration of Symptoms (days)	3.55± 1.31	1-6	<0.0001
Vomiting Episodes per 24 hours	3.26± 2.52	1-9	<0.0001
Vomiting Duration (days)	2.66± 2.11	1-6	<0.0001
Diarrhea episodes per 24 hours	5.91± 1.40	1-9	<0.0001
Diarrhea Duration (days)	3.72± 1.32	1-6	<0.0001

In *adenovirus* patients, there was a significant association among *adenovirus* infection and clinical parameters, which includes diarrhea, fever and vomiting. The severity of disease in mono-positive and co-infected cases was not considerably different. Similar to *rotavirus* infections, these clinical parameters were the main predictor variable in *rotavirus-adenovirus* co-infections.

Discussion

Worldwide the principal causes of preventable child mortalities are remains to be pneumonia and diarrhea. They attributed about0.7 and 1.3 million deaths correspondingly in children aged less than5 years of age. In five countries comprising Pakistan, 50% of these mortalities in young children are concerted. During 2011, in Pakistan around 352,000 less than 5 years mortalities were stated among which about 30% were attributed to diarrhea and pneumonia. Child deathratioin Pakistan is above 100 deaths/ 100,000 live births having about 74,000 deaths/ annum of which 23,000 deaths were described for *rotavirus* infection. In spite of these miserable data, there is no official nationwide surveillance system present for monitoring of the diarrheal disease load and related threats in Pakistan. Therefore, for addressing the UN Millennium Development Goal 4 (MDG-4) on infant mortality reduction, there must be presence of an improved population-based information on the numbers and etiologies of newborn and infantile death. For influential control of diarrheal infections, awide-ranging laboratory built surveillance and monitoring is therefore necessary(Alam *et al.,* 2015).

In various countries in the past decade, *rotavirus* and *adenovirus* are significant sources of viral diarrhea, which have directed to noticeable mortalities in children(Bernstein, 2009). Nevertheless, in few developed countries in the past, other viruses for example *nor viruses* and *astroviruses* were described for the several documentedg astroenteritid is viral infections (Tran *et al.,* 2010). Anyhow, diarrhea is a costly illness that effect the government budget greatly so studying the contributory agents comprising viruses are quintessential for the health policy decision creators(Motamedifar *et al.,* 2013). For surveillance goals, precise determination of gastroenteritis pathogens is vital with the purpose of understanding which organisms are most predominant in particular regions and to propose precise prevention actions, vaccination policies and empiric management schedules (Sarkar *et al.,* 2014;Varela *et al.,* 2015). In the framework of epidemic studies, quick pathogen detection is also regularly essential for rapidly implementation of effective preventive strategies. For guiding the treatment of single patients principally for those

having serious or continued disease or in immune compromised hosts, laboratory detection can also be supportive. From this perspective, viral screening particularly for children below 5 years turns out to be essential (Mokomane *et al.,* 2018).

In this study, 198 (18.23%) positive *adenovirus* cases were detected out of 1,086children, which is related with the report from Nigeria (18%)(Babalola *et al.*,2015). Our finding is higher than those reported for Brazil (12.47%)(Reis *et al.,* 2016), China (4.7%)(Lu *et al.*,2017), and India (9.30%)(Dey *et al.,* 2011).

Some other reports are also showed with lower prevalence rate of HAdV then ours like New Zealand (3%) (McAuliffe *et al.*,2013), Thailand (9.3%) (Chansaenroj*et al.,* 2017), turkey (8.6%)(Biçer *et al.,* 2011)and Venezuela (11.5%)(Alcalá *et al.,* 2018).

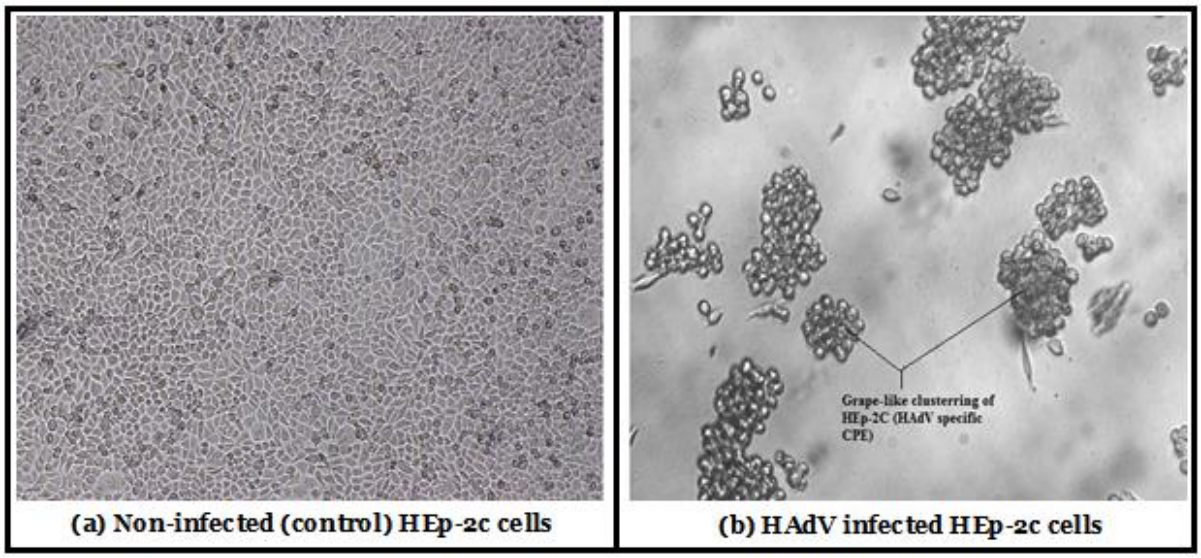

Fig. 1. Human Adenovirus Isolation on Hep-2C cells. (a) Non-infected (Control) (b) Infected with HAdV.

As Pakistan is located in Asia, so in comparison with other Asian countries that revealed the prevalence rate of HAdV as Bangladesh (10.7%)(Afrad *et al.,* 2018), China (9.8%)(Liu *et al.,* 2014), India (6.1% and 7.5%)(Chitambar *et al.,* 2012), Korea (5.5%)(Lee *et al.*,2012) and Thailand (5.84%) (Sriwanna *et al.,* 2013)which is lower than prevalence rate of HAdV in our study. Similarly, our results are also higher from other European countries like France (0.7%)(Cardine *et al.*,2002), Germany (5.5%)(Mayindou *et al.,* 2016), Italy (8.73%)(La Rosa *et al.,* 2006), Portugal (12.4%)(Ribeiro *et al.,* 2015), Turkey (3.3%)(Öztaş *et al.,* 2016) and UK(15%)(Cunliffe *et al.,* 2010). With Middle East countries like Iran (5.18%)(Dashti *et al.*,2016), Iraq (3%) (Jaff *et al.,* 2016)and Qatar (6.25%)(Al-Thani *et al.,* 2013), the prevalence rates for HAdV are lower as compared to the present study. In case of the African countries, the comparable prevalence rate of HAdV is lower than our findings. These rates are from Sudan (16.2%)(Elhag *et al.*,2013), Tanzania (3.5%)(Moyo *et al.,* 2014) and Nigeria (5.1%)(Arowolo *et al.,* 2019). There are some other reports, which showed the increased rate of detection of HAdV then our study. These reports are from different countries likeIran (20.30%)(Sharifi-Rad *et al.,* 2015), Italy (23.1%)(Biscaro *et al.*,2018), Gabon (19.6%)(Lekana-Douki *et al.*,2015), China (28.94%)(Qiu *et al.,* 2018), Brazil (43%)(Costa *et al.,* 2017), Albania (23.2%) (La Rosa *et al.,* 2015)and Burkina Faso (31.2%)(Ouédraogo *et al.*,2016). These increased rates highlighted the role of HAdV in gastroenteritis cases.

In this study, we noted a peak in frequency of *adenovirus* in April and May. This peak coincided with the report from Albania (La Rosa *et al.,* 2015)where *adenovirus* peak occur in April and May as well. Few other studies assured this pattern of seasonality for the virus(Pereira *et al.,* 2007), though former data described HAdV to be isolated during

whole year with no seasonal pattern or any peak in incidence of *adenovirus* over the year(Carraturo *et al.,* 2008;Wong *et al.,* 2008). HAdV-*rotavirus* co-infection rate in this study was 26.76% with frequent co-infection in January by the rate of 30.18% of all co-infections. In gastroenteritis incidences, co-presence of over one agent is not uncommon(Motamedifar *et al.,* 2013).

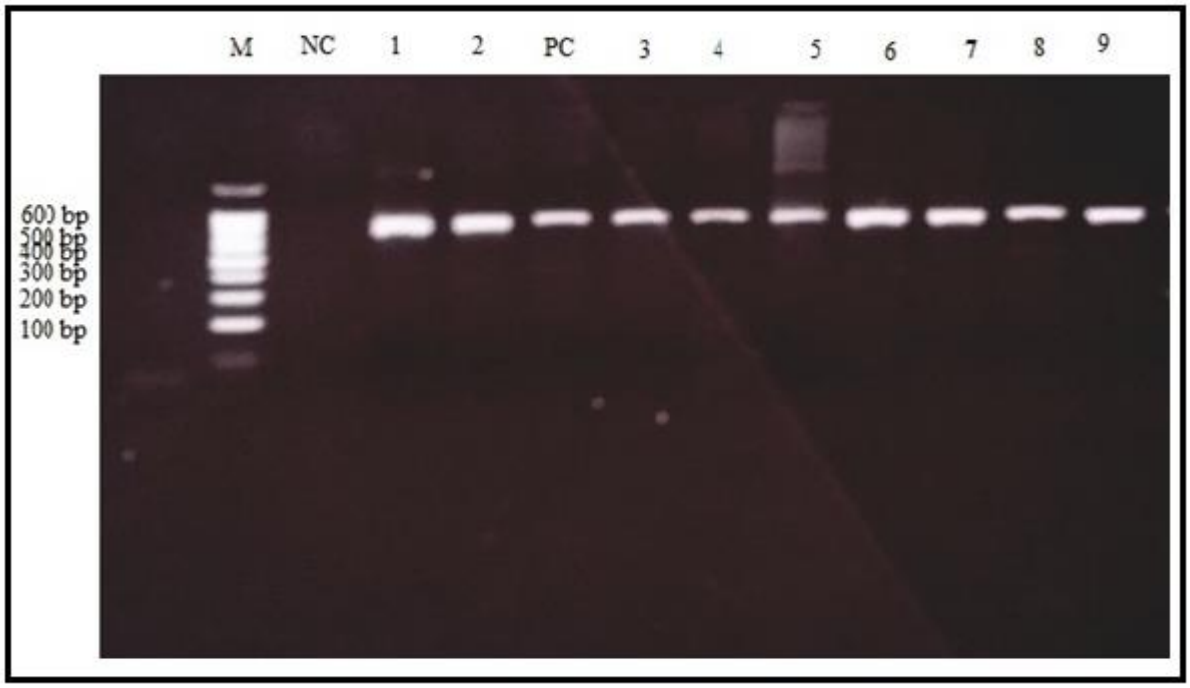

Fig. 2. SYBR safe dye stainedagarose gel showing PCR amplification products using a nested PCR primer pairs of adenovirus (Al-Qurashi*et al.,* 2012). Lane 1-2, 3-9: positive samples (600 bp), NC: negative control, PC: positive control (AdB7 DNA). M: molecular marker (100 bp DNA ladder, Life technologies).

In this study, the rate of viral co-infection was higher in male cases compared to females with no statistical difference present among them. Here in our report there were significant relationships among co-infection and the prevalence of clinical symptoms detected in children.

The co-infection rate in this study is somehow consistent with report from Albania(La Rosa *et al.,*2015), which showed the rate as 27.2%. Moreover, children with co-infection possessed quite serious clinical manifestation with greater possibility of becoming seriously dehydrated, irrespective of age and living style(Valentini*et al.,*2013). According to our results, children with co-infection did not that much differ from those with mono-infection with regard to clinical parameters. Different co-infection rates was reported in various studies (Aminu *et al.*, 2007; Japhet *et al.,*2012, Motamedifar *et al.,* 2013, Babalola *et al.,*2015).

Human adenovirus (HAdV) is one of the pathogens accountable in industrialized and unindustrialized countries for gastroenteritis between infant and children(Vetter *et al.,* 2015).In our study, peak incidence of HAdV infections has been detected in children between 1 to 12 months of age. The greater ratio of HAdV infection produce frequently in ≤ 2 years of children(Biçer *et al.,* 2011). In our findings, the rate for ≤ 2 year children is 95% which is higher than reported data for Iran (53%)(Motamedifar *et al.,* 2013), Turkey (73.7%)(Biçer *et al.,*2011), Taipei (76.6%) (Lin *et al.,*2000)and Iraq (78 %)(Jaff *et al.,* 2016).This elevated level in ≤ 2 years old children proposes that the younger group is quite simply infected by *adenovirus* as compared to the elder group. The reason behind this may be by the fact that young children grows more susceptible to infections then elder with improved fortified with the growing innate and adaptive immune systems(Simon *et al.,* 2015).

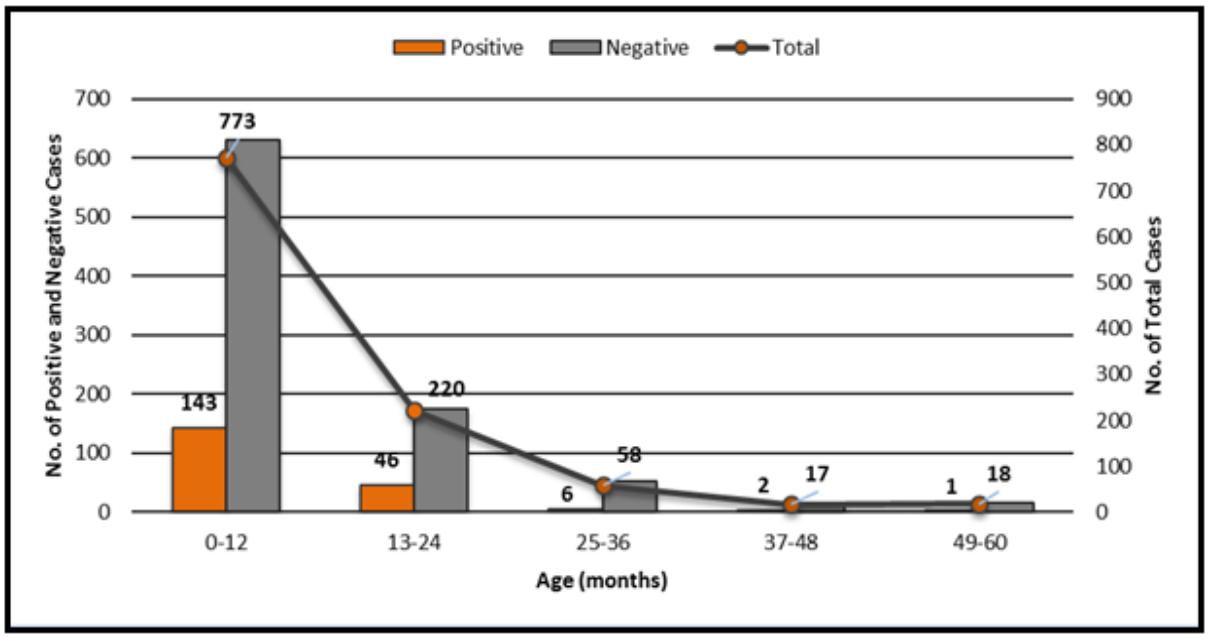

Fig. 3. Age-wise distribution of Human Adenovirus positive cases. The Y-axis indicates the number of cases while X-axis represents five age groups with a difference of 12 months. Total number of samples analyzed is given on secondary axis. Values on top of bars indicate number of samples positive for Adenovirus and values along the line on secondary axis indicate total number of tested samples.

There is a limited report regarding the prevalence of enteric *adenoviruses* from Pakistan. Agboatwalla *et al.,* (1995) reported from Karachi, Pakistan that the prevalence of enteric *adenoviruses* was 10% in 1992 in children with Gastroenteritis(Agboatwalla *et al.,*1995). During this study ELISA technique was used for the detection of enteric *adenoviruses* and *rotaviruses*.

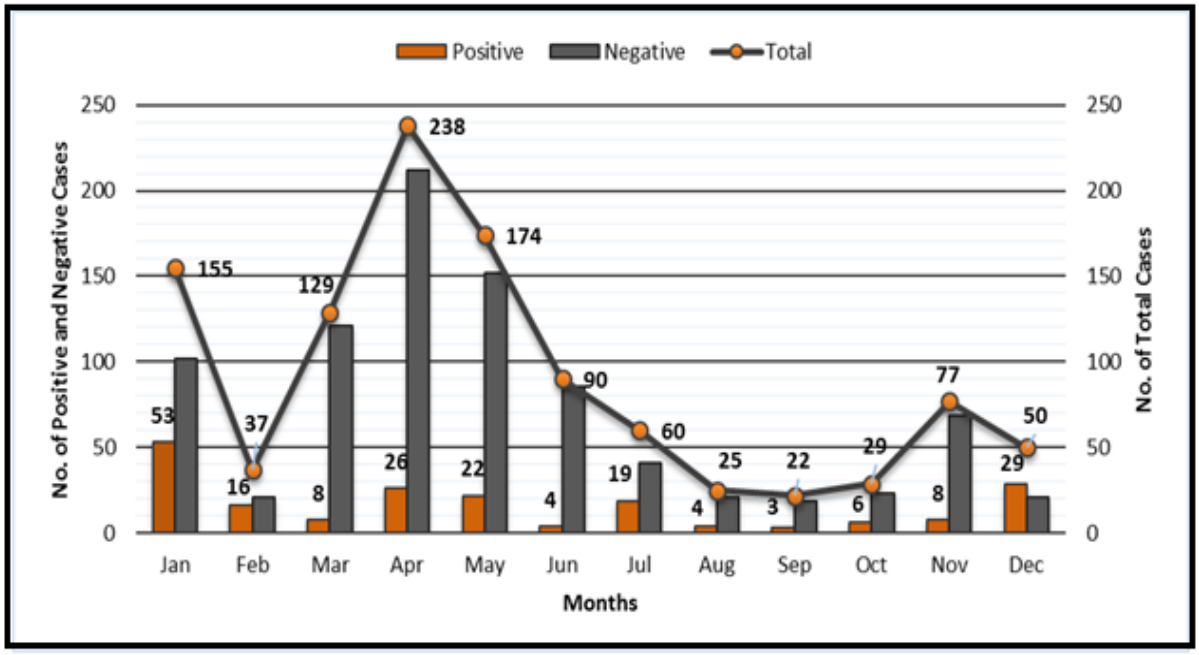

Fig. 4. Month-wise distribution of Human Adenovirus cases with Y-axis indicating the number of positive and negative cases. The total number of samples tested under this study is shown through secondary axis. X-axis indicates the months from January to December, 2017-2018. Values on the top of black bars indicate number of Adenovirus positive samples. The total number of samples are indicated along line given on the secondary axis.

Another study conducted by Ahmed *et al.,* (2016) from Peshawar, Pakistan that showed the prevalence of HAdV as 38.94%(Ahmad *et al.,* 2016). This study was conducted to determine the quality of water by finding the prevalence of different enteric viruses (*rotavirus, adenovirus, hepatitis A virus* and *enterovirus*) in water samples collected from different regions of Peshawar. No other study is conducted for screening of HAdV from diarrheal samples from young children in Pakistan.

One of the limitations of this study is that not all of the samples collected were analyzed because of the financial restraints. However, in receiving at the sample selected, a symbolic sample including all age groups, geographical distribution, seasonality, and different years of study were deliberated. Furthermore, this study was unable to collect all of the climatic variables, which may be significant for perceiving the local epidemiology of the viruses. It further roofer supplementary data about the effect of immunization for controlling this common disease in the country. The findings in our study highlight the importance of the use of broad range assays for the detection of all HAdV species, in studies on the viral etiology of gastroenteritis.

Conclusion

This study provides vital data on HAdV in Pakistani children. In conclusion, the prevalence of *rotavirus* in study population is 23.11% and *adenovirus* is 18.23%, which indicates that HAdV infections are quite common in our population after *rotavirus*. Pakistan is among one of the Asian countries with higher diarrhea related deaths. Regardless of these dismal reports, screenings of the viruses are not completed in our country hospitals because of the lacking appropriate laboratory services attributable to limited assets. Besides, absence of awareness between the clinicians leads to the undiagnosed or misdiagnosed viral infections, which are commonly cured, with needless antibiotic schedule. The presence of higher disease load in early age children of our country produced additional emphasis on the prioritization of the analysis of HAdVs particularly in the neonates and early-age infants. In diarrheal infection, a precise considering of the possible role of HAdV is probably to add to infection prevent policies. Briefly, this study demonstrates that HAdV is the main cause of gastroenteritis and is more predominantamong1 to 24 months old children with males more liable to effect as compared to females.

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