Enteric and non-enteric adenoviruses in children with acute gastroenteritis in Western India

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Abstract

Human adenoviruses (HAdVs) are among the most important etiological agents of acute gastroenteritis (AGE). HAdVs found in 5.2% of children admitted for AGE between 2013 and 2016 in seven different hospitals across four cities of Western India. The dominance of subgroup-F (52.4%) strains was observed, followed by the occurrence of non-enteric adenoviruses of the subgroup A (17.4%), C (11.4%), B (8.2%), and D (3.2%). The subgroup-F strains were predominant in Ahmadabad (78.5%), Mumbai (61.5%), and Surat (57.1%) cities, followed by subgroup A strains. In Pune city, subgroup B and C strains were detected in 62.5% of AGE patients, with none of the subgroup A strains. Clinically, patients infected with enteric and non-enteric HAdV strains were indistinguishable from each other. However, a high viral load was observed in subgroup-F specimens as compared to non-subgroup-F. The study highlights the need for viral load estimation to ascertain the role of enteric and non-enteric HAdV strains associated with diarrheal etiology.

1. Introduction

Acute gastroenteritis (AGE) is a major cause of morbidity and mortality and is still a significant public health problem for infants and young children worldwide. Human Adenoviruses (HAdVs) are members of the genus*Mastadenovirus* in the family *Adenoviridae* and are known to cause a wide spectrum of acute and chronic diseases, including gastroenteritis, conjunctivitis, hepatitis, myocarditis, and pneumonia. HAdV is a linear, double-stranded DNA virus with a genome size of 26–45 kb and classified into 7 subgroups (A to G) and more than 100 genotypes [*https://talk.ictvonline.org/taxonomy/*]. HAdVs of subgroup F consisting of serotypes HAdVF-40 and HAdVF-41, also known as enteric adenoviruses, are the most common cause of gastroenteritis in children and adults (Akihara et al., 2005; Shimizu et al., 2007; Lee et al., 2012). The association of other subgroups of HAdV with gastrointestinal tract infections has also been reported over the period (Hierholzer 1992; Li et al., 2005; Banyai et al.,2009; Knipe et al., 2013; Afrad et al., 2018). In the present study, on the background of scanty data on HAdVs circulating in India, clinical, epidemiological, and virological investigations were conducted on AGE patients hospitalized in four cities of India between 2013 and 2016.

2. Materials and methods

2.1 Study site

The Indian Council of Medical Research (ICMR)-National Institute of Virology (ICMR-NIV) in Pune, Maharashtra state is a part of the Indian Rotavirus Surveillance Network supported by the Indian Council of Medical Research (ICMR), New Delhi since 2005. The National Rotavirus Surveillance Network (NRSN) is a multi-centric project conducted at four referral and seven regional centers across India. Each center has peripheral in-patient facilities that collect the clinical data and specimens and send them to the corresponding referral and regional laboratories for testing and characterization. The criteria for recruitment,

stool sample collection, laboratory detection, and genotype characterization protocols used were common at all sites. ICMR-NIV, Pune is one of the referral centers that represents the West Zone of India. The stool specimens collected from the West Zone consisting of four cities and seven hospitals, namely Pune (KEM Hospital, Bharati Hospital and Shaishav Clinic), Mumbai (Lokmanya Tilak Municipal GH & Medical College), Ahmadabad (BJ Medical College & Civil Hospital), and Surat (Surat Municipal Institution of Medical Education & Research) during 2013–2016 were included in the present study. These hospitals are tertiary-level health care centers, providing both general and specialized medical care.

2.2 Enrolment criteria and clinical assessment

A case of acute gastroenteritis was defined as the passage of [?]3 watery stools in a day with or without associated symptoms such as vomiting, fever, and abdominal pain. All patients were examined for fever, number of episodes and duration of vomiting and diarrhea, extent of dehydration and treatment for the assessment of disease severity score (Ruuska and Vesikari, 1990). According to the scores obtained, the disease condition of each patient was categorized as mild (scores 0–5), moderate (scores 6–10), severe (scores 11–15) and very severe (scores 16–20).

2.3 Sample collection and selection

Thirty one hundred and ninety eight faecal specimens were collected from sporadic cases of acute gastroenteritis in patients < 5 years of age. These patients were admitted to four sites, namely Pune (Maharashtra state, n = 1007), Mumbai (Maharashtra state, n = 654), Ahmadabad (Gujarat state, n = 441), and Surat (Gujarat state, n = 1096) during the period of 2013-16. The data on RVA testing and genotyping analysis has been published earlier (Girish Kumar et al. 2020).

From a 3198 faecal specimens stored at -70°C, it was decided to test representative samples for HAdV. Assuming a 50 ±/- 5% prevalence rate of the unknown agents, the required sample size for each region was calculated to be 384. However, due to cost constraints, only 300 samples were selected at random by the Simple Random Sampling method from each region (Pune, Mumbai, Surat, and Ahmadabad). To confirm that the random sample from each region was properly representative of all samples available from that region, the expected frequencies for each parameter (age group, gender, year/month of the collection, vesikari score, and Rotavirus A (RVA) positivity) in the random sample were calculated by using corresponding percentages in the entire data. Then the observed frequencies in the random sample were compared with expected frequencies by using the chi-square test. The p-value >0.05 for each comparison showed that the random sample was properly representative of the entire set of samples in each region.

2.4 Viral DNA extraction, Polymerase Chain Reaction and nucleotide sequencing

Viral DNA was extracted from a 30% stool suspension by using the TRIzol method (Invitrogen, Waltham, MA) according to the manufacturer's instructions. PCR assay with Platinum® Taq DNA Polymerase kit (Invitrogen, USA) was used for amplification, using hexon gene specific primers for AdV detection (Allard et al., 1990) and penton gene specific primers for genotyping (Fujimoto et al., 2012). The nucleotide sequences were determined by using the Big Dye Terminator cycle sequencing kit v3.1 (Applied Biosytems, Foster City, CA, USA) and an ABI 3130XL genetic analyzer. The specificity of the nucleotide sequences obtained was confirmed using BLAST (*www.ncbi.nlm.nih.gov/blast*) and phylogenetic analysis was carried out using the neighbor joining method with 1000 bootstrap replicates (Tamura et al., 2013). The nucleotide sequences of the strains examined in the study have been deposited in the Gen Bank (Accession numbers OP699436-OP699483; OP939983- OP939984; OP939989- OP939995) for partial penton and hexon gene (Accession numbers OP939985-OP939988).

2.5 qPCR assay for estimation of viral load

Amplicons of HAdV-2, hexon gene (nt 18856–19137) were used for the preparation of the standard positive control using primers, probes, and methodology as described earlier (Heim et.al 2003, Koul et.al 2018, Potdar et.al 2020). A standard curve generated by testing ten-fold serial dilutions of standard positive control and

plotting the log dilution of standard DNA against Ct value to determine the sensitivity of the assays. The qPCR assay was performed on an ABI 7500 machine (Applied Biosystems Inc, USA).

2.6 Statistical analysis

All statistical analyses were carried out in PASW 18. Proportions were compared by Chi-square test with Yate's correction wherever applicable. For comparison of the mean values of several groups, univariate ANOVA was performed with Tukey's post-hoc test for multiple pair-wise comparisons. P-value less than 0.05 was considered as statistically significant.

3. Results

3.1 Detection of HAdV in acute gastroenteritis patients

Out of 1200 specimens tested for HAdV, 33 were eliminated from the study because they had < 3 episodes of diarrhea per 24 hr. period (n =7) or > 5 days of diarrhea (n =26). Out of these 33 specimens, two and nine were HAdV and RVA positive, respectively, and 21 had a severe or very severe disease outcome. Among the remaining 1167 faecal specimens, 61 (5.2%) showed the presence of HAdV and were distributed among patients between 2 - 48 months (median-12.5 months) of age, with 85.2% below 2 years of age (Table 1), and 31 were females. A significantly lower proportion (1.2%) of HAdV positive cases were observed in the 37–60 age group as compared to the age groups of 13–18 (7%, p = 0.0461) and 25–36 (8%, p = 0.0351) months (Table 1). A similar comparison for RVA (n = 360) showed significantly higher percent positivity in the 7–12 (39.1%) and 13–18 months (37%) age groups as compared to other groups ($p<0.05$ for each). The mean age of only HAdV (n = 54; 15.4 +- 9.3 months) and RVA (n = 353, 13.8 +- 9.0 months) positive patients, as well as patients with dual infections (n = 7, 12.0 +- 8.4 months) and patients negative for both viral agents (n = 753, 16.7 +- 13.5 months) was compared with each other. The mean age of only RVA positive patients was significantly lower as compared to the patients negative for both RVA and HAdV. No significant difference in the mean age of HAdV and RVA positive patients was observed.

Sixty-one HAdV positive cases were distributed throughout the year, with an overall detection rate of 6.5% (12/184) in the year 2013, 5.7% (24/419) in 2014, 4.5% (15/349) in 2015 and 4.2% (10/215) in 2016. Fecal specimens collected from Pune, Mumbai, Ahmadabad and Surat showed 5.5 (16/292), 4.3 (13/300), 5.7 (16/278), and 5.4 (16/297) percent HAdV positives respectively.

The seasonal distribution of the HAdV positive AGE cases showed occurrence of the virus throughout the year with higher percent positive cases during March to August months (Table 2). RVA positive cases were significantly high, (53.6%) in December – February and in September – November (38.7%) months as compared to those of the March-May and June- August months ($p < 0.05$ for each) (Table 2).

3.2. Genotyping analysis

Among 61 HAdV PCR-positive specimens, 57 were genotyped using penton gene-specific primers. Four non-typable specimens (Ahmadabad n=2; Surat n=2) were confirmed to be HAdV after nucleotide sequencing of the partial hexon gene. Analysis of the nucleotide sequence data of the HAdV strains was carried out using the BLAST and phylogenetic analysis. The phylogenetic analysis of the study strains depicts the grouping of the study strains in different subgroups and corresponding genotypes with high bootstrap support (Figure 1). Among 61 HAdV positive specimens, 52.4% (n=32) belong to HAdV-F and genotyped as HAdV-40 (n=14) and HAdV-41(n=18). The HAdV-40 and HAdV-41 strains of the study clustered with corresponding reference strains (MK883611; MH465394) and showed 100 percent nucleotide identity (PNI) values. Eleven strains (17.4%) grouped with subgroup HAdV-A, and genotyped as HAdV-12 (n=4), HAdV-18 (n=2), and HAdV-31 (n=5) and showed 98-100, 100, 99.5, and 99.3-100 PNI values with corresponding prototype strains. Subgroup HAdV-B with genotype HAdV-7 observed in five (8.2%) specimens with 99.4 - 100 PNI values with MNO11575.1 prototype strain. Seven study strains (11.4%) were classified as subgroup HAdV-C and genotyped as HAdV-2 (n=5) and HAdV-5 (n=2) with 94.9- 100 and 97.8- 100 PNI values with corresponding prototype strains namely JX173081 (Egypt) and KF429754 (USA) respectively. Subgroup HAdV-D observed

in two specimens with PNI value of 98.6 with HAdV-23 (Prototype KF268327,), and 98.7 with HAdV-69 (Prototype KJ626292) strain.

The distribution of the different HAdV subgroups and genotypes in different study years and cities is shown in Table 3. The subgroup F strains were detected in all four cities with 11, 8, 8 and 5 strains in Ahmadabad, Mumbai, Surat and Pune receptively. It should be noted that the HAdV-7 strains of subgroup B were found only in Pune city with absence of subgroup A in Pune and subgroup C strains in Surat. Two strains of subgroup D identified in the study were isolated from AGE patients of Pune and Surat city.

Analysis of the distribution of the HAdV-F strains showed 30.0 (95% C.I: 1.60, 58.40), 65.2 (95% C.I: 45.75, 84.68), 60.0 (95% C.I: 35.21, 84.79) and 55.6 (95% C.I: 23.09, 88.02) percent in the year 2013, 2014, 2015 and 2016. Similar analysis for cities namely Pune, Mumbai, Ahmadabad and Surat, showed 31.2 (95% C.I 8.54, 53.96), 61.5 (95% C.I 35.09,87.99), 78.6 (95% C.I 57.08,100.00) and 57.1 (95% C.I 31.22,83.07) percent patients with HAdV-F infections respectively. The difference in percent positivity among different years as well as cities is not statistically significant with exception of the significantly low proportion of HAdV-F cases in Pune compared to Ahmadabad ($p = 0.0097$). HAdV-F strains were identified consecutively between 2013-2016, 2014–2016 and 2013-2015 in Ahmadabad, Surat and Mumbai respectively and in Pune only in 2013 and 2015.

Comparison of the mean age of the patients infected with different subgroups (A, B, C, D and F), and between HAdV-F (genotype 40 and 41) (n=32; 16.0+- 9.172) and non HAdV-F genotypes (n=29; 13.97 +- 9.443) showed no significant difference ($p = 0.732$; p=0.397).

3.3. Clinical severity of disease

Among patients positive for sole HAdV (n= 54) and sole RVA (n=353), 62.9, 79.6, 70.4 and 52.7, 88.7, 79.5 percent showed clinical history of high body temperature, vomiting, and severe/very severe disease outcome respectively. Statistically significant difference was not observed for these parameters among sole HAdV and sole RVA patients as well as patients with mixed infections (n=7) ($p > 0.05$ for each). Comparison of the clinical features, of the AGE patients during the study period is displayed in Table no.4. The mean values of the number of days, number of episodes per 24 hr. of diarrhea and vomiting, body temperature as well as severity score of the patients infected with RVA, HAdV, mixed infection, and negative for both viral agents were compared. The significantly high mean values of diarrheal episodes were observed only in RVA patients as compared to the group of patients negative for both HAdV and RVA (p= 0.006).

A total of 15 and 30 out of 61 HAdV positive and 97 and 180 out of 360 RVA positive patients showed $>$ 5% and $<$ 5% degree of dehydration respectively. The comparative analysis of the HAdV and RVA positive patients for degree of dehydration (<5%, >5% and none) showed no significant difference ($p > 0.05$ for each). Similarly 3 and 19 patients with oral treatment (n=50) and 58 and 341 patients with intravenous treatment (n=1117) were positive for HAdV and RVA, respectively. Comparison of type of treatment used among these groups also showed no significant difference ($p > 0.05$).

3.4 Comparison of the clinical characteristics of the AGE patients infected with HAdV-F and non HAdV-F (Subgroup A, B, C, D, untypeable penton) adenoviruses

Mean Vesikari score of the patients infected with different subgroups A (n=11, 11.82 +- 2.56), B (n=5, 10.20 +- 1.30) C (n=7, 11.71 +- 2.360) and D (n= 2, 12.0 +- 1.41) and F (n=32; 12.2+- 2.35)) as well as comparison of HAdV-F versus non-HAdV-F strains (n=29; 11.48 +- 2.41) showed no significant difference (Table 5, $p >0.05$). There was no significant difference in the mean number of days, mean number of episodes per 24 hr. of diarrhea and vomiting as well as the body temperature of the patients among these groups (Table 5).

The AGE patients infected with only HAdV-F (n=28, cases with co-infection of RVA or NoV were omitted from the analysis) and only non-HAdV-F strains (n=21) showed 18 (64.2%) and 13 (61.9%) patients with high body temperature and 25 (89.3%) and 15 (71.4%) with a clinical history of vomiting respectively. These proportions were not significantly different from each other (p=0.864; $p = 0.110$ respectively). Similar

comparison for <5% and >5% degree of dehydration (HAdV-F: 78.6% (22/28), non HAdV-F: 79.2% (16/21); p = 0.843), and severe and very severe disease outcome (HAdV-F: 82% (23/28), non HAdV-F: 57% (12/21); p = 0.055) showed no significant difference. The number of days and number of episodes in 24 hrs duration of diarrhea ranged between 1-5 days (Median 3) and 4-25 episodes (Median 10) for HAdV-F and 1-4 days (Median 3) and 4-20 episodes (Median 7) for non-HAdV-F patients. Analysis of the vomiting days and episodes indicated 0-4 days (median 2), and 0-20 episodes (median 4) in HAdV-F and 0-4 days (median 1) and 0-8 episodes (median 3) in non-HAdV-F patients.

Each group of patients, with only HAdV-A (n=8), HAdV-B (n=5), HAdV-C (n=5), HAdV-D(n=1), and penton untypeable (n=2) adenovirus infection was compared for clinical characteristics with HAdV-F (n=28) strains. No significant difference for the percentage of patients with high body temperature, 5% or more dehydration and severe disease outcome was observed with a single exception. In the case of subgroup B infections significantly low number of cases with severe disease outcome (40.0%) were observed as compared to subgroup F infections (82.1%) (p = 0.043).

Comparison of the clinical characteristics of the patients infected with HAdV-40 (n=13) and HAdV-41(n=16) with each other showed no significant difference. The vomiting episodes per 24 hr. ranged between 0-20 (Median 4) for HAdV-40 patients and 0-5 (Median 3) for HAdV-41 patients.

Mixed infection of HAdV (Subgroup F, n=3; Subgroup A, n=1; Subgroup D, n=1, non-typable , n=2) and RVA (G1P[8], n=5; G2P[4], n=1; G9P[4], n=1) was observed in a total of 7 AGE patients aged between 14 - 29 months of age. These seven cases were distributed in Ahmadabad (n=3), Surat (n=2), Pune (n=1), and Mumbai (n=1), and four of them with severe disease outcomes were infected with HAdV-31 (Subgroup A), HAdV-69 (Subgroup D) and non typable penton strains.

3.5 Detection of Viral load

Using standard DNA, the qPCR assay detection limit was estimated to be 9.1 copies per reaction and the slope and correlation coefficient of the standard curve were -2*557 and 0*981 respectively (Figure 3). HAdV genome was detected by qPCR in all 61 PCR positive samples with Ct values ranging from 12 to 39.9 (median Ct value: 28.8) (Figure 3). Fifty-five specimens with Ct values < 35 found to contain DNA copies between 3.3 X 10^2 and 5.7 X 10^{10}. Among them, 46 specimens were negative for RVA and NoV and of which 71.7% were with severe and very severe disease outcome. Analysis of the mean Ct values and viral load in specimens with different subgroups showed no significant difference (Table 5). However, the viral load in patients infected with HAdV-F strains was significantly high (n=32; 5.24+- 2.38) as compared to non HAdV-F strains (n=29; 4.11+- 1.61, p=0.036).

Among HAdV positive patients, 9, 28, 18 and 6 specimens showed Ct values between 10-20, 20-30, 30-35 and 35-40 respectively (Table 6). The distribution of patients with moderate, severe and very severe disease outcome in different groups of Ct values was not significantly different from each other (p=0.571, Table 6). Comparative analysis of the mean +- SD values obtained for disease severity score among these groups was without any significant difference. Analysis of moderate, severe and very severe disease outcome in different groups of Ct values in HAdV-F and non HAdV-F strains showed no significant difference (p= 0.299, Table 6)

Six specimens with Ct values >35 showed DNA copies between 2.7X10^2 and 0.69 per reaction and belong to genotype HAdVF-40, HAdVF-41, HAdVA-31, HAdVA-12, HAdVC-5 and untypeable hexon strain. Out of these six specimens, five belonged to patients with severe disease outcome displaying dual infection with Norovirus in two and RVA in one.

4. Discussion

The overall HAdV detection rate among AGE cases was 5.2% in four cities of Western India and ranged between 4.3% (Mumbai) and 5.7% (Ahmadabad). Previously, 4.9 - 11.8% detection rate has been reported from Western, Northern, and Eastern parts of India (Verma et al., 2009; Borkakoty et al., 2016; Akhil et al., 2016, Gupta et al., 2017; Banerjee et al., 2017; Gopalkrishna et.al, 2021). Globally, the prevalence of

HAdV among AGE patients was reported to be between 3.3 - 16.2% (Tran et al., 2010; Sanaei Dashti et al., 2016; Mukhtar et al., 2016; Colak et al., 2017; Afrad et al., 2018). Exceptionally, high HAdV detection rates (26.6- 42.5%) were reported in fecal specimens of diarrheal children <14 years of age with known or unknown HIV status from rural and urban settings in Kenya and Africa (Magwalivha et.al 2010).

Similar to earlier reports, majority of the (85.2%) HAdV infections were detected in children below two years of age (Verma et.al, 2009; Gopalkrishna et.al, 2021) and significantly high in children aged between 7 and 18 months (Table 1). The patients with HAdV infection were seen throughout the year with a maximum number of cases during warmer months in line with earlier reports (*Borkakoty* et.al 2016; Afrad et.al 2018; Gopalkrishna et.al, 2021). The severity of the clinical disease among patients with sole HAdV infection was not significantly different as compared to those with sole RVA infection or mixed infection.

The HAdV-F, the known causative agent of gastroenteritis, is reported to be responsible for 25 to 52% of gastroenteritis cases (Filho et.al 2007, Verma et.al 2009, Qiu et.al 2018; Kumthip et.al 2019; Gopalkrishna et.al, 2021). The study strains showed the dominance of the HAdV-F (52.5%) followed by an occurrence of non-enteric adenoviruses of the HAdV-A (17.4%), HAdV-C (11.4%), HAdV-B (8.2%) and HAdV-D (3.2%). The HAdV-F strains were detected in all four cities with their predominance in Ahmadabad (78.5%), Mumbai (61.5%), and Surat (57.1%) cities consecutively for 3-4 years along with second most dominance of HAdV-A strains. The role of HAdV-18 and HAdV-31 strains in sporadic and HAdV-12 in sporadic and outbreak cases of AGE has been reported (Verma et.al 2009; Portes et.al 2016; Afrad et al., 2018; Qiu et.al 2018; Kumthip et.al 2019 ; Gopalkrishna et.al, 2021). Identification of the common epitope of the HAdV-A and F strains on protein VI was suggested as a sign of potential determinants of the tropism and their close evolutionary relationship. However, it is not shared by the HAdV B, C, D, or E strains (Brown et.al 1996). It is noteworthy that during the phylogenetic analysis of the study strains using the penton region, clustering of HAdV-A and F strains together with high bootstrap support was observed (Figure 2).

Multiple studies reported considerable change in the relative incidence of HAdV-40 and HAdV-41 infections over the period in specimens collected from the same geographical region (de Jong et.al 1993; Dey et.al 2011; Banerjee et.al 2017). Among HAdV-F (n=32), 56.2% of strains belonged to the HAdV-41 genotype, and of which only 16.6% were of Pune city. In AGE patients admitted during 2005 - 2007 and 2017 - 2019 in Pune city, 80% and 84.6% of the HAdV-F strains were reported to be HAdV-41 (Verma et.al 2009, Gopalkrishna et.al, 2021). The dominance of the HAdV-B (n=5) and C (n=5) (10/16, 62.5%) strains in Pune city followed by HAdV-F (29.4%) was observed with the absence of HAdV-A strains. The absence of HAdV-A strains was in line with earlier studies on the samples collected between 2005 and 2007 (Verma et.al, 2009) and 2017-2019 (Gopalkrishna et.al, 2021) reporting a very rare occurrence of HAdVA-31 in Pune city. The immune selection pressure in favor of one genotype over the other is known for the shift in dominant genotypes over the period and thus, emphasizes the need for long-term monitoring.

The clinical presentation of the patients infected with only HAdV-F and non-HAdV-F strains were indistinguishable from each other with exception of the less severe patients in the HAdV-B group. No difference in viral load of the AGE patients and control groups or between HAdV41 positive patients and healthy controls was reported suspecting the diarrheal etiological role of HAdV-41 (Qiu et.al 2018). However, a high viral load was observed in HAdV-F-positive study specimens as compared to non-HAdV-F. The absence of correlation of the Ct values with the severity of the disease observed in the study is in line with an earlier report (Bergallo et.al, 2019).

The fecal specimens of HAdVA-12 positive AGE patients < 25 months of age were demonstrated with high viral loads (mean 1.9 X 10^7 DNA copies/g stool) using qRT-PCR assay (Portel et.al 2016). Among HAdVA-12 positive fecal specimens (n=4) of the study, three showed viral load between 2.4 X 10^3 and 1.4 X 10^5. The viral load in the HAdV-18 (n=2) positive specimen with dual infection of Norovirus was 8.4 X 10^2, while in the remaining sample it was 7.8 X 10^7. Among five HAdV-31 positive specimens, three showed viral load between 1.2 X 10^3 and 7.1 X 10^6. The remaining two specimens with dual infection with RVA and Norovirus showed a viral load of 7.2 X 10^1 and 1.2 X 10^6 respectively. Overall, eight out of 11 subgroup A strains showed viral load between 10^3 to 10^7 which suggests the need for viral load studies for further confirmation.

All of the HAdV-B, majority of the HAdV-C, and single HAdV-D subgroup strains identified in the study belong to Pune city. To date, several studies reported the presence of non-enteric HAdV strains among AGE patients along with a recent study documenting HAdV-C as the leading agent followed by HAdV-F and B (Hierholzer 1992; Li et.al 2005; Filho et.al 2007; Banyai, et al. 2009; Lee et.al 2012; Knipe et.al 2013; Sriwanna et.al 2013; Afrad et al., 2018; Kumthip et.al 2019). Failure to isolate the majority of the HAdV-C strains from fecal specimens of the patient and detection of the HAdV-F along with other serotypes in healthy individuals was speculated to be due to viral persistence (Fox et al., 1977; Garnett et al., 2002; Roy et.al 2011; Qiu et.al 2018). Therefore, HAdV strains responsible for non-AGE diseases, past infections, or as a part of normal human virome in the gut due to persistent infection needs to be differentiated from the causative HAdV strains of AGE. To rule out the possibility of non-AGE diseases clinical history of the HAdV-positive patients of the study was analyzed. Two patients with upper respiratory tract infection were observed, of which one was positive for HAdV-41 (Ct value- 28.5), and the other for HAdV-18 (Ct. value – 19.3) genotype.

Further, it should be noted that all HAdV-B positive specimens (viral load 327 - 6.2X10^{7}copies/run) were collected in winter (October-December months), and the majority of HAdV-C strains (viral load 8.4X10^2 – 4.5 X10^6) in summer season (March-Aug months). Taking into consideration the summer season pattern of the HAdV AGE cases, further studies in context to seasonality, subgroup/genotype, and viral load are highly essential to ascertain the etiology. High detection rates and viral load were reported in the summer season (2.19x10^3 to 6.72x10^5 gc/g of feces) among asymptomatic adult humans (Vetter et.al, 2015). Hence, further studies, on fecal viral load between symptomatic and asymptomatic children might be useful to identify the role of enteric and non-enteric HAdV strains identified in diarrheal patients.

The difference in the genome-wide composition or the susceptibility among diarrhea and healthy populations suspected to be the reason behind the selective pathogenicity of the HAdV41 strains (Qiu et.al 2018). Further, full genome studies on HAdV strains will be necessary to address this issue. Recently, a serotonin-dependent cross talk between HAdV-41, and human Enterochromaffin cells and Enteric Glia Cells suspected to be the mechanism behind enteric adenovirus diarrhea (Westerberg et.al 2018). Similar studies on non-enteric HAdV strains will help to know their exact role in diarrheal patients.

In Maharashtra and Gujrat states of Western India, universal immunization program for RVA was introduced in August 2019. Recently, a significant proportion of HAdV infections were documented in rotavirus-vaccinated children (Msanga et al., 2020). On this background, the present study on faecal specimens collected between 2013 and 2016 (pre vaccination era) from AGE patients hospitalized in four cities will be of major importance for future comparative analysis to know the exact impact of RVA vaccination in children in Western India. Overall, the existence and co-circulation of multiple HAdV genotypes in AGE patients in the region further demands the necessity of surveillance studies in different parts of India to understand the etiological role and disease burden of adenoviruses in sporadic and outbreak cases of gastroenteritis.

Competing interests: The authors declared that there is no conflict of interest.

Authors' Contributions:

MJ: key role in supervision, interpretation and writing of the manuscript, VS and NC: experimental work and recorded demographic and clinical data, AW: statistical and phylogenetic analysis, VP, VV, ML: guidance and co-ordination of real time PCR for estimation of the viral load of the specimens, VGK: design and overall execution of the study. All authors approved the final manuscript.

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