IJPSR (2019), Volume 10, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 September 2018; received in revised form, 09 December 2018; accepted, 12 December 2018; published 01 June 2019

COMPARISON OF PCR AND CELL CULTURE FOR HUMAN ADENOVIRUS DETECTION IN GASTROENTERITIS AND RESPIRATORY TRACT INFECTED NORTH INDIAN CHILDREN

Rambha Tripathi [1, 2], Shally Awasthi [2], Amreen Zia [1], Swati Saxena [1, 2], Dharmveer Singh [1], Ankita Pandey [1], Piyali Bhattacharya [1] and Tapan N. Dhole [* 1]

Sanjay Gandhi Postgraduate Institute of Medical Sciences [1], Lucknow - 226014, Uttar Pradesh, India.
King George's Medical University [2], Lucknow - 226003, Uttar Pradesh, India.

Keywords:

Human adenovirus, Acute respiratory tract infection, Acute gastroenteritis, Polymerase chain reaction, Cell culture, Sensitivity

Correspondence to Author:
Dr. Tapan N. Dhole

Professor and Head,
Department of Microbiology,
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow - 226014, Uttar Pradesh, India.

E-mail: tndhole@sgpgi.ac.in

ABSTRACT: Acute gastroenteritis (AGE) and acute respiratory tract infection (ARTI) are the most common causes of morbidity and mortality in young children worldwide. This study aimed to analyze the sensitivity and specificity of laboratory diagnostic methods. We compared to cell culture and polymerase chain reaction (PCR) technique for the detection of Human adenovirus (HAdV) in AGE and ARTI children below 6 years of age. We analyzed 1270 samples taken from children, consisting of 537 fecal specimens from AGE and 733 throat swabs from ARTI pediatric patients. Hep-2 cell line was used for the isolation of HAdV. These viruses were identified by targeting partial hexon gene from fecal specimens and throat swabs. The total positivity for HAdV in AGE patients was 5.96% by cell culture and 8.75% by PCR, so increasing the percentage of identification from 5.96% to 8.75% ($p>0.08$). In the case of ARTI patients, 4.64% were positive by cell, while 7.23% by PCR in the increased rate of detection of HAdV infection from 4.64% to 7.23% ($p<0.04$*). Compared to culture as the gold standard, the sensitivity, specificity, and accuracy of PCR was 100%, 97%, and 97% respectively. The sensitivity of PCR was higher than cell culture technique in AGE and ARTI cases. In conclusion, molecular methods were found to be useful for specific and rapid diagnosis of HAdV infections with higher sensitivity compared with the traditional cell culture method.

INTRODUCTION: Human adenoviruses (HAdVs) are ubiquitous DNA viruses, discovered in 1953 [1]. Prevalence of HAdV infections is approximately 4-12% in Acute Gastroenteritis (AGE) and Acute Respiratory Tract Infection (ARTI) [2]. Viral gastroenteritis is an important cause of childhood morbidity and mortality worldwide, especially in developing countries [3]. It is estimated that more than one billion diarrhea episodes occur every year, causing up to 700,000 deaths among children younger than 5 years of age [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(6).2884-91

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(6).2884-91

Among children younger than 2 years of age, 72% of deaths occur due to diarrheal disease [5]. These viruses create a major risk in children, elderly people, and immunocompromised persons [6]. It can cause a broad range of human diseases such as several different clinical syndromes, including gastroenteritis, acute respiratory infections, pneumonia, bronchitis, conjunctivitis, hepatitis, ocular infection, hemorrhagic cystitis, urinary tract infection, and meningoencephalitis [7-8].

HAdV infection is transmitted by inhalation and direct contact with small droplet aerosols or the fecal-oral route [9]. In developing countries, acute respiratory tract infection (ARTI), is one of the major cause of morbidity and mortality among children under the age of five years [10] and it mostly causes, severe lower respiratory infections in children [11].

Acute upper and lower respiratory tract diseases, including pneumonia and bronchitis, have been attributed to HAdVs [12]. The clinical symptoms of infected respiratory patients are fever, cough, nasal congestions, and sore throat. HAdV belongs to the family Adenoviridae and genus Mastadenovirus, and these are non-enveloped, icosahedral, linear, double-stranded DNA viruses that vary in size from 70 to 100 nm [13]. Up to now, based on the antigenic variants of the capsid protein HAdVs are classified into 7 species (A–G) and can be further differentiated into 70 HAdV genotypes [13-14].

Presently, cell culture technique is the "gold standard" for laboratory diagnosis of Human adenovirus infections. On the other hand, it is not suitable as a rapid diagnostic test, because the detection of adenoviruses by cell culture technique is expensive and time-consuming [15].

Usually, culture takes 2–14 or more days to yield results, and therefore, its clinical value is limited. To overcome these limitations, more rapid and modern molecular diagnostic techniques, such as PCR is a sensitive, specific, and rapid technique as compared to traditional cell cultures technique [16].

HAdV was detected by comparing the sensitivity, specificity, and accuracy of the cell culture technique with PCR technique using degenerate primers for the conserved region of partial hexon gene [17]. The diagnosis of HAdV disease has traditionally been performed by cell culture techniques, but this technique has a lower sensitivity than PCR technique. Cell culture lacks the sensitivity for detecting low levels of circulating viruses and may require weeks to deliver definitive results [18]. Molecular diagnostic assays offer advantages in terms of speed, sensitivity, and the ability to quantify viruses [19]. The present study emphasized comparing cell culture and PCR techniques for the diagnosis of HAdV infections in children with gastroenteritis and acute respiratory tract infection in North India.

MATERIALS AND METHODS: The present study is a cross-sectional hospital-based study. A total of 1270 samples (537 AGE +733 ARTI cases), collected from clinically suspected children attending outdoor (OPD) and indoor (IPD) clinics at Sanjay Gandhi Post Graduate Institute of Medical Sciences and pediatric department of King George Medical University, Lucknow, Uttar Pradesh, during March 2012 to April 2015.

Fecal specimens and throat swabs samples collected from children below age 6 years, suffering from AGE and ARTI respectively. Stool specimens were collected and processed according to the WHO (World Health Organization) guidelines [20]. We have collected 10 ml watery stools in a clean, and dry screw capped wide mouth plastic container. Sterile viral transport media (3 ml) was used for the collection of throat swabs. After collection and the addition of the transport medium, samples were immediately sent to a virology laboratory (Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow) for laboratory analysis. All samples were stored at -20 °C until processing.

(a) Processing of Gastroenteritis Sample: Stool suspension (10%) prepared in 0.01 M phosphate buffered saline (PBS) (PH 7.2), followed by vortexing the same at 300 rpm for 20 min. After vortexing, the suspension was centrifuged at 3000 rpm for 30 min, leaving it for 10 min at room temperature (RT) and finally stored at -20°C for further analysis. Stool supernatants pre-treated with chloroform before inoculation to Hep-2 cell line. In addition to removing bacteria and fungi, the chloroform pre-treatment removes potentially cytotoxic substances and dissociates virus aggregates [20].

(b) Processing of Respiratory Sample: After collection of throat swabs in 3 ml Viral Transport Media (VTM), samples were sent to the laboratory for immediate processing. Throat swabs were centrifuged at 1,500 g for 10 min at 4 °C. The supernatant was stored at -20 °C for further analysis.

(c) Elucidation of Cell Sensitivity: The sensitivity of Hep-2 cells for HAdV isolation is an important component of the laboratory's quality assurance program. It provides reassurance that a Hep-2 cell retains the ability to detect HAdVs, even if present at low titer. HAdVs titer measured on HEp-2 cells by endpoint titration as per the standard procedure. In brief, HEp-2 cells grown in 96 well ($10^{2.5}$ cells per well) tissue culture plates (Nunc, Denmark). Ten-fold serial dilutions of the virus through 10^{-1} to 10^{-9} were prepared in 2% Minimum Essential

Media (MEM; Sigma-Aldrich) and plain MEM used as a negative control.

When Cells monolayer confluence of 80-90% was reached, 100 µl of each serial dilution inoculated into four wells and plate was incubated at 36.5°C with 5% CO_2. After 5-7 days of incubation Hep-2 cell plates showed 100% Cytopathic Effect (CPE). The titer of HAdV was $10^{6.9}$ in Hep-2 cell line.

Kärber formula was used for the calculation of HAdV titer that is

$$\log CCID50 = L - d\,(S - 0.5)$$

Where: L = log of the lowest dilution used in the test, d = difference between log dilution steps, S = sum of the proportion of "positive" tests (*i.e.*, Cultures showing CPE)

$$L = -5.0;\ d = 1.0;\ S = 1 + 0.90 + 0.50 + 0 = 2.40$$

Log CCID50 = - 6.90; Virus titer = $10^{6.9}$ CCID50 / 0.1 ml in Hep-2 cell line.

(d) Cell Culture: Cell culture was performed on continuous human epithelial carcinoma cell lines (HEP-2). The monolayer of Hep-2 cells grown in cell culture flasks, using the MEM supplemented with Earle's salts, L-glutamine, Penicillin-Streptomycin, and 10% Fetal Bovine Serum. Subconfluent HEp-2 cell monolayer washed with Dulbecco's phosphate buffered saline (PBS) three times.

200 µl each of stool and throat swabs supernatant was inoculated in two separate HEp-2 cell culture tubes and incubated at 36.5 °C. The HAdV replicated and produced a cytopathic effect (CPE) in continuous human cells of epithelial origin (HEp-2) from fecal samples and throat swabs. The CPE started at 24 h after virus inoculation and reached a maximum after 24 to 48 h. The CPE consists of rounding tract formation, loss of continuity of the monolayer of HEp-2 cells.

Cytopathic effects (CPE) in the tubes were examined for 5 days from the day of incubation. If CPE was not observed on first passage, then a second blind passage was performed in the respective cell line. When 80%-100% CPE was observed, the tubes were kept at -20 °C for DNA extraction and molecular characterization.

Uninfected Hep-2 cell monolayer and cytopathic effect of HAdV showed in **Fig. 1(A)** and **1(B),** respectively. HAdV detection by PCR technique using degenerate primers for the conserved region of partial hexon gene [17].

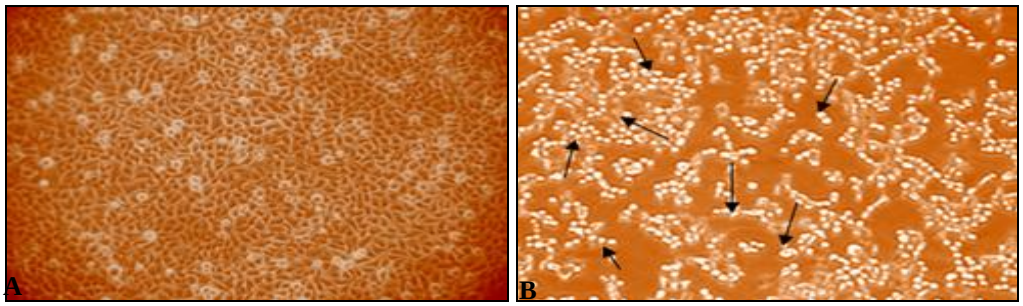

FIG. 1: (A) UNINFECTED Hep-2 CELL LINE (B) CYTOPATHIC EFFECT OF HADV IN Hep-2 CELL LINE

(e) DNA Extraction: Viral DNA extracted from 200 µl stool and throat swab supernatant separately using QIAmp DNA extraction mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. DNA extracts were stored at -80 °C for further identification.

(f) PCR Technique for the Detection of Adenovirus: For the amplification and detection of HAdV, we used 5 µl of DNA in 45 µl of the reaction mixture containing 10 mM Tris- HCl (pH 8.3), 50 mM KCL, 500 mM (each) dNTPs, 4 mM $MgCl_2$, 0.5 µl of Taq polymerase (5U/ µl) and 20 pmol of the generate forward 5'CAACACCTAYG ASTACATGAA3' and reverse primer5'KATGGG GTARAGCATGTT3' with PCR having initial denaturation step at 94 ºC for 5 min, denaturation at 94 ºC for 1 min, annealing at 50 ºC for 1 min, extension at 68 ºC for 1 min for 30 cycles and a final extension at 68 ºC for 5 min [16]. The amplified product of 475 bp was visualized on 2% agarose gel prestained with ethidium bromide along with molecular weight (MW) marker VIII (Sigma-Aldrich) under ultraviolet light, imaged with the Gel Doc XR System (Bio-Rad, Hercules, CA).

(g) Statistical Analysis: The data were evaluated for statistical significance with the Chi-square test and Fisher's exact test, where appropriate. All tests were two-tailed, and the value of $p<0.05$ was considered to represent a statistically significant difference.

(h) Ethics Statement: The study protocol was approved by the Ethics Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences Raibareli Road, Lucknow, and King George Medical University, Lucknow, U. P. Reference code: XLVIII ECM/B-P13.

RESULTS:

(a) Clinical Observations of Gastroenteritis Patients: Out of 537 fecal specimens collected between April 2013 to December 2015, 47 (8.75%) samples were HAdV positive by PCR showed in **Fig. 2**, and 32 (5.95%) samples were positive by cell culture technique.

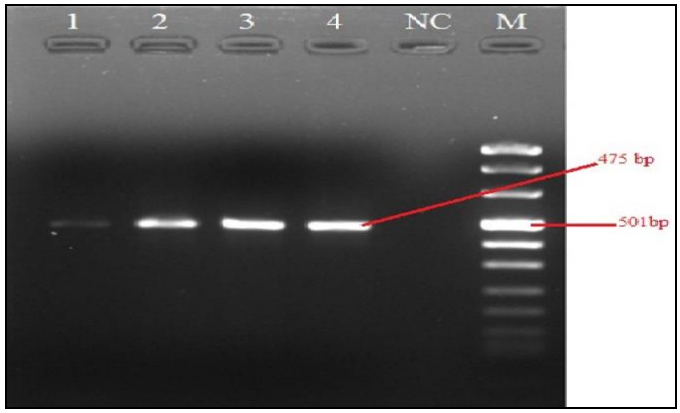

FIG. 2: GEL ELECTROPHORESIS IMAGE FOR HAdV POSITIVE ISOLATES; LANE 1 TO 4 SHOWS HAdV POSITIVE BAND; LANE 5 SHOWS NEGATIVE CONTROL; LANE 6 SHOWS DNA MOLECULAR WEIGHT MARKER VIII (SIGMA)

The total positivity for HAdV in AGE patients was 5.96% by cell culture and 8.75% by PCR technique, so increasing the rate of identification from 5.96% to 8.75% ($p>0.08$) showed in **Table 1**.

TABLE 1: TOTAL HAdV TESTING FOR GASTRO-ENTERITIS AND RESPIRATORY INFECTED CHILDREN

Gastroenteritis children	Adenovirus positive	Adenovirus negative	P value
PCR	47	490	**0.08**
Cell culture	32	505	
	Respiratory infected children		
PCR	53	680	<0.04*
Cell culture	34	699	

#Value of P≤0.05 was considered as significant and marked with an asterisk (*)

All samples (537 fecal specimens) were evaluated with two diagnostic techniques and therefore were further analyzed to determine the sensitivity, specificity, accuracy and positive and negative predictive values of the PCR. Subjects were considered to have true infection if they were culture positive. All culture-positive specimens were also PCR positive. Therefore, 32 of the 47 were considered to be true positive. Fifteen samples were PCR positive, but culture negative; hence, test characteristics were considered false positives. Given these definitions, the overall sensitivity of PCR was 100%, specificity and accuracy were 97% **Table 2**. The positive predictive value was 68%, and the negative predictive value was 100%.

TABLE 2: COMPARISON OF CELL CULTURE AND PCR RESULTS FOR HAdV INFECTION IN GASTRO-ENTERITIS AND RESPIRATORY TRACT INFECTED CHILDREN

Comparison of PCR and cell culture results for gastroenteritis children, n=47				Comparison of PCR and cell culture results for infected respiratory children, n=53			
PCR	Cell culture			PCR	Cell culture		
	Positive	Negative	Total		Positive	Negative	Total
Positive	32	15	47	Positive	34	19	53
Negative	0	490	490	Negative	0	680	680
Total	32	505	537	Total	34	699	733
Sensitivity (%)		100		Sensitivity (%)		100	
Specificity (%)		97		Specificity (%)		97	
Accuracy (%)		97		Accuracy (%)		97	

Among the positive cases, 27 (57.45%) were male, and 20 (42.55%) were female, and male/female (M/F) ratio was 1.35:1. Children between 0-2 years old represented the majority of infection 57.45%

followed by children between the age of 2-4 years at 19.15% and 23.40% infection in 4-6 years old children showed in **Table 3(A)**. The median age was 1.6 years ranging from .1 year to 5.6 years old, and mean age was 2 ± 1.6 years.

TABLE 3(A): DEMOGRAPHICAL CHARACTERISTICS AND MOST PREVALENT CLINICAL MANIFESTATIONS IN GASTROENTERITIS CHILDREN POSITIVE FOR HAdV INFECTION

Characteristics of gastroenteritis children	Gastroenteritis children with adenovirus infection N=47	Gastroenteritis children without adenovirus infection N=490	P value
Gender			
No. of male/No. of female	27/20	249/241	0.39
Age group in year			
0-2 years	27	176	
2-4 years	09	156	<0.017 *
4-6 years	11	158	
Clinical manifestations			
Watery stools	47	490	
Fever	38	499	
Abdominal cramps	27	510	
Vomiting	42	495	<0.0001*
Dehydration	25	512	
Stool with mucus and blood	0	537	

P = comparisons were made using chi-square or Fisher's exact test for categorical variables. *= shows a statically significant difference (p<0.05)

(b) Clinical Observations of Respiratory Patients: Throat swabs collected from 733 children below 6 years of age presented with symptoms of respiratory tract infection during the study period from April 2013 to December 2015. Out of 733 screened throat swabs from ARTI pediatric patients, 53(7.23%) samples were positive by PCR technique, whereas, 34(4.64%) samples were positive by cell culture technique, hence the rate of HAdV infection escalating from 4.64% to 7.23% (p<0.04*) showed in **Table 1**. All 34 culture-positive samples were also PCR positive. Therefore, 34 of the 53 were considered to be true positives. Nineteen samples were PCR positive, but culture negative; hence, test characteristics were considered false positives. Given these definitions, the overall sensitivity of PCR was 100%, specificity and accuracy were 97% **Table 2**. The positive predictive value was 64%, and the negative predictive value was 100%.

Among the positive cases, 31(58.49%) were male, and 22(41.51%) were female, and the male/female (M/F) ratio was 1.41:1. Children between 0-2 years old represented the majority of infection 56.60% followed by children between the age of 2-4 years at 22.64% and 20.75% infection in 4-6 years old children depicted in **Table 3(B)**. The median age was 1.7 years (minimum 2 years, Max 5.9 years), and mean age was 2.1 ± 1.5 years.

TABLE 3(B): DEMOGRAPHICAL CHARACTERISTICS AND MOST PREVALENT CLINICAL MANIFESTATIONS IN ACUTE RESPIRATORY TRACT INFECTED CHILDREN POSITIVE FOR HAdV INFECTION

Characteristics of ARTI children	ARTI children with adenovirus infection N=53	ARTI children without adenovirus infection N=680	P value
Gender			
No. of male / No. of female	31/22	389/291	0.85
Age group in year			
0-2 years	30	299	
2-4 years	12	186	0.20
4-6 years	11	195	
Clinical manifestations			
Fever	51	682	
Chest pain	36	697	
Cough	34	699	
Sore throat	23	710	<0.001*
Nasal discharge	21	712	
LRTI (Pneumonia and Bronchitis)	26	707	

P = comparisons were made using chi-square or Fisher's exact test for categorical variables. *= shows a statically significant difference (p<0.05)

The results of the AGE and ARTI patients analyzed, tested by PCR and cell cultures were compared considering cell culture as the gold standard method **Table 2**. The sensitivity, specificity, and accuracy parameters of PCR were obtained. Although the low sensitivity of cell culture, this test proved to be, PCR showed high accuracy and high sensitivity.

DISCUSSION: Cell culture was recognized as the "gold standard" for laboratory diagnosis of HAdV; however, HAdV replicates and produce a cytopathic effect (CPE) usually requires 2 to 14 days or sometimes more days, varying with the specimen source and with the concentration of virus in the specimen, as well as, culture requires viable virus. Sometimes HAdV can be inactivated by inadequate collection methods, inadequate transport medium, or a prolonged interval between specimen procurement and culture inoculation. Some HAdV serotypes are slow growers on cell culture, thereby requiring more passages before harvesting. So we performed 6-7 passages in Hep-2 cell lines before harvesting the virus. Although HAdV cultured in several cell lines where the enteric human adenovirus 40 and human adenovirus Ad41 are difficult to culture and do not produce clear and consistent cytopathic effects (CPE) [21]. A tentative diagnosis of HAdV infection is made based on clinical signs, symptoms, but accurate and reliable diagnostic techniques are required to confirm the HAdV infection. Young children and immunocompromised patients are especially susceptible to severe complications of HAdV infection [22-23].

Therefore, in the present study, we aimed to elucidate on comparing cell culture and PCR techniques for the detection of HAdV isolated from the children less than 6 years of age. However, we applied PCR for the detection of adenovirus, because the PCR technique is a more sensitive and reliable technique for the confirmation of HAdV. After the comparison of the positivity of HAdV by the cell culture and PCR, we found that 32 (5.96%) samples were positive by cell culture and 47 (8.75%) by PCR technique in AGE patients. In the case of ARTI patients, 34 (4.64%) were positive by cell culture, and 53 (7.23%) were positive by PCR. The present data confirm the insensitivity of viral culture for children and indicate that PCR represents a significant improvement over culture. The sensitivity and specificity of the test were excellent at 100% and 97% respectively. In AGE 15 and ARTI 19 samples considered to have false-positive PCR results may have been infected with HAdV even though negative by cell culture.

Since the PCR technique has been implemented for detecting adenoviruses, an increase in sensitivity has been confirmed, suggesting that culture as the gold standard may require modification. PCR methods of detecting HAdV in fecal specimens and conjunctival swabs have been published [24-25].

Overall, HAdV infection in AGE patients was recorded higher during the spring season peak in months from January to March and in the rainy season from May to September. General clinical features of AGE in the study participants were a watery stool, high fever, vomiting, abdominal pains, and dehydration.

The rate of HAdV infections in AGE patients was 8.75%, which is higher than previous studies compared to Tunisia 3.1% [26], 1.9% in Dhaka city, Bangladesh [27] and lower than Egypt 10.4% [28], Ghana 19.8% [29], in Northwest Nigeria 23% [30], Albania 23.2% 6, and 37.4% in Kenya [31].

HAdV infection in ARTI pediatric patients was 53(7.23%). The rate of HAdV positivity was higher than Columbia 5% [32] and Peru 6.2% [33]. Whereas, lower than Canada 7.73% [34]. HAdV infection in this study was similar to the level of infection reported from Brazil 7.1% [35], Philippines [36] and from Rajasthan, India [37]. The recorded analysis in **Table 3(A)** and **3(B)** shows the clinical and demographic profile of HAdV, infected patients in AGE and ARTI. The infection occurred mostly in the male of fewer than two years of age. Children with younger age group are likely prone to HAdV associated disease [38]. Cooper *et al*., demonstrated that pediatric patients had been infected early in life, therefore, had acquired immunity to HAdV infection [39].

The present study reported positivity of HAdV based on the signs and symptoms presented by the ARTI patients. Among the total HAdV positive samples in ARTI infected patients, fever, chest pain, cough, nasal discharge, and lower respiratory tract infection (LRTI) were the most predominant

clinical symptoms observed. HAdV associated pneumonia is a serious illness requiring hospitalization in children less than 2 years of age [38].

Our study reported that the majority of HAdV infection in ARTI infected male children as compared to female children, which is similar to a study reported from China [40]. In case of AGE male/female ratio (M/F) of HAdV infected pediatric patients were 1.35:1, similar to Taiwan 1.30:1 [41], whereas lower than Israel 1.48:1 [42].

To the best of our knowledge, this is the first study from India on North Indian pediatric patients about the detection of HAdV by cell culture and PCR techniques. Studies that compared the PCR and cell culture techniques by using clinical specimens have shown that, despite the advantages of detecting viral DNA by PCR, such as speed, sensitivity, ability to detect nonviable viruses and potential elimination of the toxic effects of the specimen, there were a large number of positive samples through the PCR method [43]. We found that, on comparing cell culture with PCR, the detection rates for HAdV in throat samples obtained from young children with ARTI was 4.64% and 7.23% respectively, which was significantly greater ($p<0.04$*) by PCR technique. PCR technique was found to be more reliable and useful for rapid diagnosis and is a highly sensitive and specific tool to detect HAdV in the AGE and ARTI patients as compared to cell culture. It can be used to determine whether the virus is reactivated and shed without causing pathogenic effects.

CONCLUSION: In conclusion, PCR is a reasonably sensitive and highly specific method for the diagnosis of HAdV infection in children. The use of PCR should provide the resources to perform accurate epidemiological studies as well as to investigate the immune response to infection and to study potential antiviral therapeutics for children. Molecular diagnostic techniques significantly increase the detection rate of respiratory viruses in patients with pneumonia and bronchitis as compared with traditional cell culture methods. However, prospective surveillance studies are still required to further establish the clinical value of these techniques. Rapid and highly sensitive molecular methods used for the detection of HAdV infectivity are needed because of their rapid spread and high hospital mortality rate, so the molecular method will be important for the control and treatment of infection. This technique very useful for the routine laboratory investigations in hospitalized patients will assist in the introduction of preventive measures to avoid viruses' transmission and to diminish the misuse of broad-spectrum antibiotics in this environment.

ACKNOWLEDGEMENT: This study supported by an ICMR-SRF fellowship awarded to Mrs. Rambha Tripathi from Indian Council of Medical Research, Government of India, New Delhi (Ref. No. 80/840/2013ECD-I). I acknowledge Richa Kapoor for technical assistance.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES:

1. Rowe WP, Huebner RJ and Gilmore LK: Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proceedings of the Society for Exp Biol and Med 1953; 84: 570-573.
2. Echavarría M: Adenoviruses. eLS 2009.
3. Lu L, Zhong H and Su L: Detection and Molecular Characterization of Human Adenovirus Infections among Hospitalized Children with Acute Diarrhea in Shanghai, China, 2006–2011. Canadian Journal of Infectious Diseases and Medical Microbiology 2017; Article ID 9304830: 6.
4. Moyo SJ, Hanevik K and Blomberg B: Prevalence and molecular characterisation of human adenovirus in diarrhoeic children in Tanzania; a case control study. BMC Infectious Diseases 2014; 14: 666.
5. Walker CLF, Rudan I and Liu L: Global burden of childhood pneumonia and diarrhoea. The Lancet 2013; 381: 1405-1416.
6. La Rosa G, Della Libera S and Petricca S: Genetic diversity of human adenovirus in children with acute gastroenteritis, Albania, 2013–2015. BioMed Research International 2015; 2015: 142912.
7. Chen RF and Lee CY: Adenoviruses types, cell receptors and local innate cytokines in adenovirus infection. International Reviews of Immunology 2014; 33: 45-53.
8. Ruuskanen O, Metcalf JP and Meurman O: Adenoviruses. Clinical Virology, Third Edition. American Society of Microbiology 2009: 559-79.
9. Sriwanna P, Chieochansin T and Vuthitanachot C: Molecular characterization of human adenovirus infection in Thailand, 2009-2012. Virology Journal 2013; 10: 1.
10. Williams BG, Gouws E and Boschi-Pinto C: Estimates of world-wide distribution of child deaths from acute respiratory infections. The Lancet infectious diseases 2002; 2: 25-32.
11. Murtagh P, Giubergia V and Viale D: Lower respiratory infections by adenovirus in children. Clinical features and risk factors for bronchiolitis obliterans and mortality. Pediatric Pulmonology 2009; 44: 450-56.

12. Scott MK, Chommanard C and Lu X: Human Adenovirus Associated with Severe Respiratory Infection, Oregon, USA, 2013–2014. Emerging Infec Dis 2016; 22: 1044.
13. Chen Y, Liu F and Wang C: Molecular identification and epidemiological features of human adenoviruses associated with acute respiratory infections in hospitalized children in Southern China, 2012-2013. PloS one 2016; 11: e0155412.
14. Hage E, Liebert UG and Bergs S: Human mastadenovirus type 70: a novel, multiple recombinant species D mastadenovirus isolated from diarrhoeal faeces of a hematopoietic stem cell transplantation recipient. Journal of General Virology 2015; 96: 2734-42.
15. Hurst JC, Benton WH and Stetler RE: Detecting viruses in water. Jou of the Ame Water Works Asso 1989; 81: 71-80.
16. Ferone EA, Berezin EN and Durigon GS: Clinical and epidemiological aspects related to the detection of adenovirus or respiratory syncytial virus in infants hospitalized for acute lower respiratory tract infection. Jornal de Pediatria 2014; 90: 42-49.
17. Casas I, Avellon A and Mosquera M: Molecular identification of adenoviruses in clinical samples by analyzing a partial hexon genomic region. Journal of Clinical Microbiology 2005; 43: 6176-82.
18. Heim A, Ebnet C and Harste G: Rapid and quantitative detection of human adenovirus DNA by real-time PCR. Journal of Medical Virology 2003; 70: 228-39.
19. Ebner K, Suda M and Watzinger F: Molecular detection and quantitative analysis of the entire spectrum of human adenoviruses by a two-reaction real-time PCR assay. Journal of Clinical Microbiology 2005; 43: 3049-53.
20. Organization WH. Polio Laboratory Manual. 2004. Geneva, Switzerland 2004.
21. Tiemessen CT and Kidd AH: The subgroup F adeno-viruses. Journal of General Virology 1995; 76: 481-97.
22. Kojaoghlanian T, Flomenberg P and Horwitz MS: The impact of adenovirus infection on the immuno-compromised host. Reviews in Medical Virology 2003; 13: 155-171.
23. Walls T, Shankar A and Shingadia D: Adenovirus an increasingly important pathogen in paediatric bone marrow transplant patients. The Lancet Infectious Diseases 2003; 3: 79-86.
24. Allard A, Girones R and Juto P: Polymerase chain reaction for detection of adenoviruses in stool samples. Journal of Clinical Microbiology 1990; 28: 2659-67.
25. Jackson R, Morris D and Cooper R: Multiplex polymerase chain reaction for adenovirus and herpes simplex virus in eye swabs. Jou of Virological Methods 1996; 56: 41-48.
26. Fodha I, Chouikha A and Peenze I: Identification of viral agents causing diarrhea among children in the Eastern Center of Tunisia. Jou of Med Virol 2006; 78: 1198-03.
27. Dey SK, Shimizu H and Phan TG: Molecular epidemiology of adenovirus infection among infants and children with acute gastroenteritis in Dhaka City, Bangladesh. Infection, Genetics and Evol 2009; 9: 518-22.
28. Kamel AH, Ali MA and El-Nady HG: Predominance and circulation of enteric viruses in the region of Greater Cairo, Egypt. Jou of Clin Microbiology 2009; 47: 1037-45.
29. Silva PA, Stark K and Mockenhaupt FP: Molecular characterization of enteric viral agents from children in northern region of Ghana. Journal of Medical Virology 2008; 80: 1790-98.
30. Aminu M, Ahmad A and Umoh J: Adenovirus infection in children with diarrhea disease in Northwestern Nigeria. Annals of African Medicine 2007; 6.
31. Magwalivha M, Wolfaardt M and Kiulia NM: High prevalence of species D human adenoviruses in fecal specimens from Urban Kenyan children with diarrhea. Journal of Medical Virology 2010; 82: 77-84.
32. Rojas L, Jaramillo C and Mojica M: Molecular typing of adenovirus circulating in a Colombian pediatric population with an acute respiratory infection. Epidemiology and Infection 2012; 140: 818-22.
33. Ampuero JS, Ocaña V and Gómez J: Adenovirus respiratory tract infections in Peru. PLoS One 2012; 7: e46898.
34. Yeung R, Eshaghi A and Lombos E: Characterization of culture-positive adenovirus serotypes from respiratory specimens in Toronto, Ontario, Canada: September 2007–June 2008. Virology Journal 2009; 6: 1.
35. Luiz LN, Leite JPG and Yokosawa J: Molecular characterization of adenoviruses from children presenting with acute respiratory disease in Uberlândia, Minas Gerais, Brazil, and detection of an isolate genetically related to feline adenovirus. Memórias do Instituto Oswaldo Cruz 2010; 105: 712-16.
36. Yamamoto D, Okamoto M and Lupisan S: Impact of human adenovirus serotype 7 in hospitalized children with severe fatal pneumonia in the Philippines. Japanese Journal of Infectious Diseases 2014; 67: 105-10.
37. Swamy MMA, Malhotra B and Reddy PJ: Human Adenovirus in Hospitalised Children at a Tertiary Care Center in Jaipur. Indian Jou of Applied Research 2016; 5.
38. Ferone EA, Berezin EN and Durigon GS: Clinical and epidemiological aspects related to the detection of adenovirus or respiratory syncytial virus in infants hospitalized for acute lower respiratory tract infection. Jorn de Pediatria (Vers ٱ o em Portugu˜ s) 2014; 90: 42-49.
39. Cooper R, Hallett R and Tullo A: The epidemiology of adenovirus infections in Greater Manchester, UK 1982-96. Epidemiology and Infection 2000; 125: 333-45.
40. Lu MP, Ma LY and Zheng Q: Clinical characteristics of adenovirus-associated lower respiratory tract infection in children. World Journal of Pediatrics 2013; 9: 346-49.
41. Tsou T: Community Outbreak of Adenovirus, Taiwan, 2011-Volume 18, Number 11-November 2012-Emerging Infectious Disease Journal-CDC 2012.
42. Mandelboim M, Dror P and Azar R: Adenovirus infections in hospitalized patients in Israel: epidemiology and molecular characterization. Journal of Clinical Microbiology 2011; 49: 597-01.
43. Echavarria M, Forman M and Ticehurst J: PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. Journal of clinical Microbiology 1998; 36: 3323-26.

How to cite this article:
Tripathi R, Awasthi S, Zia A, Saxena S, Singh D, Pandey A, Bhattacharya P and Dhole TN: Comparison of PCR and cell culture for human adenovirus detection in gastroenteritis and respiratory tract infected North Indian children. Int J Pharm Sci & Res 2019; 10(6): 2884-91. doi: 10.13040/IJPSR.0975-8232.10(6).2884-91.