

Detection of Adenovirus Type 40/41 Among Children With Diarrhea, In Al-Najaf province, Iraq

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Abstract

Adenoviruses type 40/41 are from the most important etiologic agents in acute gastroenteritis of children. The aim of this study was to detect of Adenovirus type 40/41 among children with diarrhea admitted to Al-Najaf province, in AL- Zahra teaching hospital for maternity and children and Al-Hakeem hospital, during the period extended from November, 2016 to February, 2017 including 90 stool specimen from children with diarrheal, their age range (from 6 to 48 months). All stool specimen were subjected to rapid test specific of Adenovirus antigens using immunochromatographic assay, which showed prevalence of (21 cases 23.33 %). The results showed the majority of infections with adenovirus were recorded among children age (6-30) months. Five specimen (23.8 %) of 21 stool samples were detected by Real-time PCR technique . The positive cases were higher among infants fed with artificial dry milk 18 cases (85.71 %) compared with infants fed with mother's milk 3 cases (14.28%). All of the cases complained of watery or mucosal consistence stool , abdominal pain vomiting, and fever. The results of this study showed that adenovirus is one of the main factors causing acute gastroenteritis in infants and children in developing countries.

Keywords: Adenovirus, Gastroenteritis, Immunochromatography

الخلاصة

الفيروس الغدي نوع 41/40 من العوامل المسببة في التهاب المعدة والأمعاء الحاد للأطفال. وكان الهدف من هذه الدراسة الكشف عن نوع فيروس العدوى 41/40 بين الأطفال المصابين بالإسهال الراقدين في مستشفى الزهراء التعليمي للأمومة والأطفال ومستشفى الحكيم في محافظة النجف خلال الفترة الممتدة من تشرين الثاني 2016 إلى شباط 2017 تتضمن 90 عينة من براز الأطفال الذين يعانون من الإسهال، معدل العمر (من 6 إلى 48 شهرا). كل عينات البراز خضعت لاختبار سريع محدد لمضادات الفيروسات باستخدام الاختبار المناعي اللوني، والتي أظهرت انتشار (21 حالة 23.33%). وأظهرت النتائج أن معظم الإصابات مع اتش اتش بين الأطفال في سن (6-30) أشهر. تم الكشف عن خمسة عينات (23.8%) من 21 عينة براز بواسطة تقنية الوقت الحقيقي-Real-time PCR. وكانت الحالات الإيجابية عالية بين الرضع الذين يتغذون على الحليب الجاف الصناعي 18 حالة (85.71%) مقارنة بالرضع الذين يتغذون على حليب الأم (3 حالات 14.28%). ويشكو جميع الحالات من البراز المائي أو المخاطية، والتقيؤ ألم في البطن، والحمى. وأظهرت نتائج هذه الدراسة أن الفيروس الغدي هو واحد من العوامل الرئيسية المسببة لالتهاب المعدة والأمعاء الحاد عند الرضع والأطفال في البلدان النامية.

الكلمات المفتاحية : الفيروس الغدي، التهاب المعاء الحاد، المناعي اللوني

Introduction

Many different agents including viruses, bacteria, and parasites can cause acute gastroenteritis associated with acute diarrhea. Acute gastroenteritis remains a major public health problem. According to the World Health Organization (WHO), 2 million cases of diarrhea were seen in children below the age of five years were died annually because of acute gastroenteritis Celik, *et al.* (2015). Viral gastroenteritis is a common cause of morbidity and mortality worldwide. More than 20 different types of viruses have been identified as etiological agents for gastroenteritis Al-Marzoqi, *et al.* (2010) Adenoviruses (members of the family Adenoviridae) are

medium-sized (90–100 nm), nonenveloped (without an outer lipid bilayer) viruses with an icosahedral nucleocapsid containing a double stranded linear, non-segmented DNA genome that is between 26 and 48 Kbp. Their name derives from their initial isolated of human adenoids in 1953 Rowe,*et al.* (1953), Francesca,*et al.* (2015).. Human adenoviruses (HAdVs) were major causes of a number of different clinical syndromes including gastroenteritis, respiratory disease, conjunctivitis, hemorrhagic cystitis, and exanthema. They comprise 51 different serotypes grouped into 6 species, A to F. The enteric serotypes were mostly associated with gastroenteritis were Ad-40 and 41 which belong to species (F).Enteric adenoviruses associated with protracted diarrhea which may contribute to infant dehydration and malnutrition in developing countries, Al-Sayidi,*et al.* (2014) spread predominantly by the fecal-oral route M. Asilova, (2012). Usually, after an incubation period of 8 up to 10 days, periodic diarrhea occur, with low grade fever, vomiting, abdominal pains, and dehydration,González *et al.*(2011).The aim of this study was to detect human adenovirus (HAdV- 40 and 41)the major enteric viral pathogens in children hospitalized in Al-Najaf Governorate, Iraq.

Methods

1-Specimen collection.

A total of 90 stool specimen were collected from patients exhibiting symptoms of diarrhea and/or vomiting attending to AL- Zahra teaching hospital for maternity and children and Al-Hakeem hospital in Al-Najaf governorate over period between November, 2016 to February, 2017, each patient were collected stool amount from"3gm " in disposable containers. In stool specimen adenovirus type 40 and 41 were done by direct chromatography technique by using Coris BioConcept company, Zlateva, *et al.*(2005). Ten stool specimen received at the laboratory from pediatric patients not exhibiting symptoms of gastroenteritis were similarly divided into aliquots and stored and used as controls for the purpose of this study.

2- Extraction of Viral DNA

Viral DNA was extracted by using extract QIAamp DNA stool Mini Kit (QIAGEN, Hilden, Germany) according to the kit protocol.

Results

A viral detection in stool by :-

1-Immunochromatographic test .

Identification of virus by method according to the presence of red colored line in the test reaction zone(T1 and T2) and blue control reaction zone indicating to positive result as shows in figure 1-B . While it is absent according to the red color in the test reaction zone for other specimen of but a blue colored line was visible in the control reaction zone indicating to control only or negative result figure 1-A.



Figure (1): Adenovirus rapid test. A- the only one red colored indicate negative sample. B-the two red colored indicate positive sample.

Incidence of Adenovirus:

Out of 90 patients of the specimen were collected of children under five years of age with gastroenteritis symptoms, were screened based on Immunochromatographic test, among them 21 children (23.33 %) were positive for adenovirus while 69(76.66%) patients gave negative result as shown in figure 2.

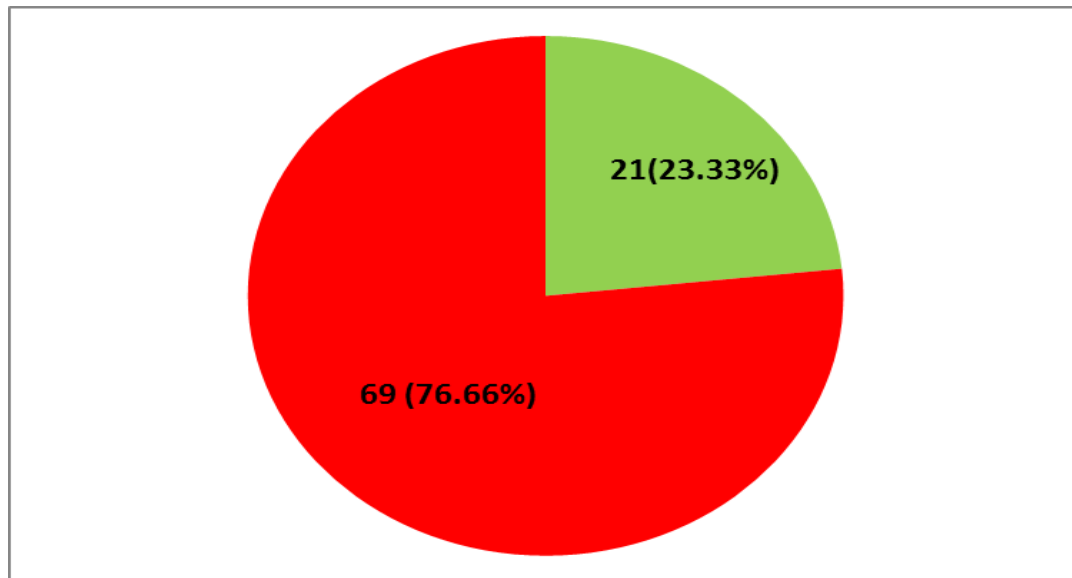


Figure (2): Incidence of adenoviruses among diarrheic children .

The highest incidence of diarrhea caused by Ad-40 and Ad-41 was in children between 6 and 12 months of age 7 cases (33.33%) and age group 18- 24 months of age 4 cases (19.04%) and the lowest infection was in children between 42-48 months of age 1 cases (4.76%) (figure 3), male children more exposure to viral infection than female about 11 (52.38%) of positive cases for Adenovirus infection in boys and 10 cases (47.61%) in girls, table(1) .

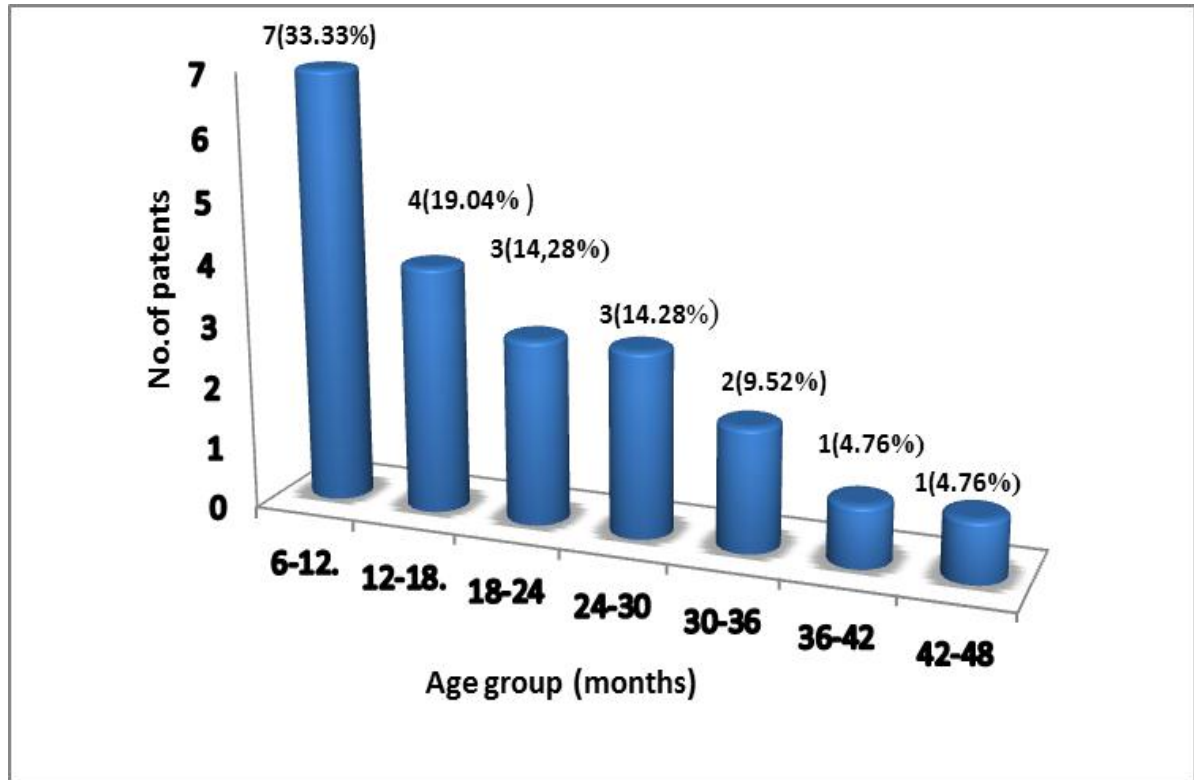


Figure (3). Distribution the positive cases of adenovirus according to age groups of children with diarrheal infections.

2- Molecular detection (RT- PCR technique).

Positive specimen were diagnosed for HAdV-40 and HAdV-41, using a specific Real-time polymerase chain reaction (RT-PCR) method, among 21 children with nonbacterial acute gastroenteritis ,enteric adenovirus were identified in 5 cases out of 21 infected children (23.8%), figure (4) ,table(1).

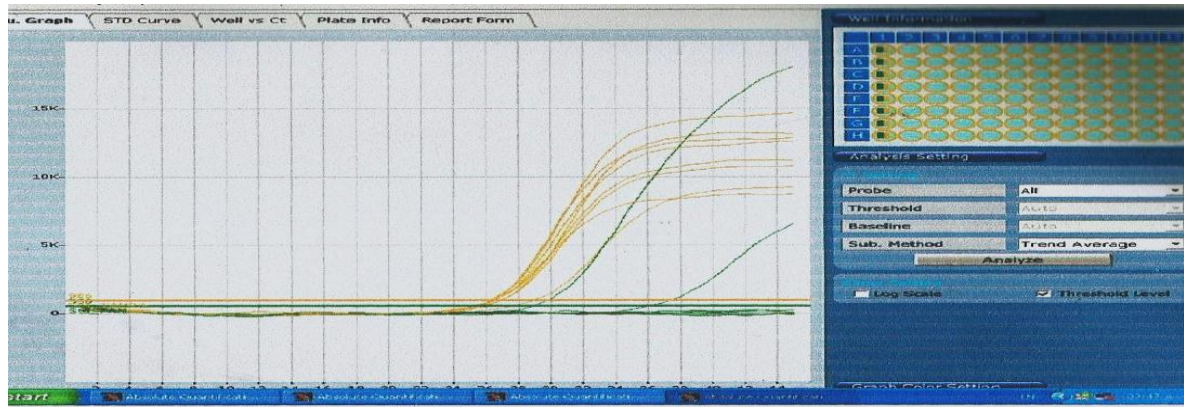


Figure (4) Amplification plot of Real-time PCR of commercial standards and specimens ,internal control positive for the quantitative detection of HAdV-40 and HAdV-41.

Table(1): Distribution the positive cases of adenovirus with age of children with diarrheal infections.

Age group (Months)	Male	Female	No. of Patients	Type of feeding		RT-PCR
				mother's milk	dried milk	
6-12	4	3	7 (33.33%)	1	6	3 (14%)
12-18	2	2	4 (19.04%)	1	3	1(4.9%)
18-24	1	2	3 (14.28%)	1	2	1(4.9%)
24-30	1	2	3 (14.28%)	0	3	0
30-36	2	0	2 (9.52%)	0	2	0
36-42	1	0	1 (4.76%)	0	1	0
42-48	0	1	1 (4.76%)	0	1	0
total	11	10	21	3	18	5

Discussion

Adenovirus type 40/41 has been found to be associated with acute gastroenteritis and is responsible for 1 to 20% of the cases of diarrheal disease in children, also adenovirus has detected in gastroenteritis studies in developing and developed countries with a incidence ranging from "2-35%" Muller,*et al.* (2010). .In this study, only 21cases (23.33%) of acute gastroenteritis in children was due to the enteric adenoviruses, most positive cases of Adenovirus infection in children less than 24 months old were determined with 14 cases(66.66 %),studies performed in developing countries have almost reported the same age distribution regarding the Adenovirus infection , because of decreased protection from maternal antibodies which starts to decline at this age as well as using solid food which may be contaminated from environment , were in accordance with Samarbaf-Zadeh *et al.*(2010) & Liu *et al.* (2014) , who found that the positive rate lower in infants under 6 months of age compared to the positive rates of the other age groups. In the current study, I reported that male children more exposure to viral infection than female table (1), simulates to

Anahita,*etal* (2016),study that record males and females constituted 62.87% and 37.1% of the patients with positive result, respectively. The research show the viral DNA was detected in only 5 cases (23.8%) have acute gastroenteritis in children by using Real-time PCR technique table(1), RT-PCR is important to diagnose viral DNA for high speed, more sensitive than the commercial kit accessible for clinical diagnosis, accessible commercial kits such as ELISA and Immune Chromatography Anahita,*etal* (2016). In addition, agreed with Alameedi study (2015), most positive cases of gastroenteritis in children are recognized in the cold and middle months of years. Findings showed that most gastroenteritis cases were reported in children fed with dried milk 18 cases (85.71 %) and cases occurred in children fed with mother's milk 3 cases (14.28%) , because transmission this agents by direct person to person contact , aerosolization and contaminated water or food in the bottle used to feeding ,also these viruses can be transmitted by droplet through vomitus infected person in rooms, and the lowest infection with breast-fed children indicated that antibodies produced by mothers play a security role against adenovirus, also the data of this study were in accordance with Al-Dahmoshi *et al.* (2013),who found that majority of infections with norovirus and adenovirus were recorded in children feeding on dried milk alone or plus mother's milk.According to the results, diarrhea with three parameters of abdominal pain, vomiting and fever were the main clinical feature of viral gastroenteritis among adenovirus positive patients in the present study, the clinical features of these patients are similar to other studies conducted in different countries, including Iraq Al-Khafaji, (2015). and Brazil Maria *et al.* (2015). .

Conclusions

Human adenovirus type 40/41 is one of most viral gastroenteritis in developing countries and case acute diarrhea occurring annually in children aged less than five years throughout the world.

Recommendations

Further research is needed to understand the correlation between prolonged shedding of Human adenovirus type 40/41 and the risk of infection to susceptible patients

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