Enteric adenovirus associated with acute gastroenteritis among hospitalized and healthy children under five - years of age in Basrah, Iraq

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ABSTRACT

Background: Enteric Adenovirus are second to rotavirus as viral cause of acute gastroenteritis in infants and young children.

Aims of the study: This study aimed on determination of the incidence of enteric adenovirus-associated gastroenteritis in infants and young children below five years of age, and to detect the season and age related distribution of enteric adenovirus infections as well as the major clinical symptoms associated with these virus infections.

Objective: A total of 400 stool specimens (200 symptomatic diarrheal cases and 200 asymptomatic normal children) were collected during the period from March 2011-March 2012. All relevant informations were obtained on special questionnaire form. Viral genomic DNA was extracted from stool specimens by using a spin column technique according to the instructions given by QIAamp-MinElute virus spin kit for purification of virus genome (Qiagen, Germany).

Results: Enteric adenovirus was detected by the use of specific primers. Enteric Adenovirus was detected in 3% of diarrheal cases in hospitalized children whereas all healthy children were negative for enteric adenovirus. Age group analysis revealed that children at age groups of 9-11 and 12-17 months were more affected. The monthly distribution of enteric adenovirus cases showed to be confined to a period of 4 months (August through November). The clinical symptoms associated with adenovirus gastroenteritis was dehydration (80%), vomiting (60%) and fever (60%) while abdominal pain was not recorded.

Conclusion: Enteric adenovirus is common enteric pathogens as that for rotavirus in our community.

Key words: Enteric adenovirus, childhood gastroenteritis

الفيروس الغدي المعوي المرتبط بالتهاب المعدة والأمعاء الحاد بين الأطفال في المستشفى والأصحاء دون الخامسة من العمر في البصرة، العراق

خلفية الدراسة: الفيروس الغدي المعوي هو الثاني لفيروس الروتا كسبب فيروسي لالتهاب المعدة والأمعاء الحاد عند الرضع والأطفال الصغار.

أهداف الدراسة: هدفت هذه الدراسة إلى تحديد مدى حدوث التهاب المعدة والأمعاء المرتبط بفيروس الغدي المعوي عند الرضع والأطفال الصغار دون الخامسة من العمر، واكتشاف التوزيع المرتبط بالسن والتوزيع المرتبط بالعمر بالإضافة إلى الأعراض السريرية الرئيسية المرتبطة لهذه الالتهابات الفيروسية.

الطريقة: تم جمع ما مجموعه ٤٠٠ عينة من البراز (٢٠٠ حالة من حالات الإسهال و ٢٠٠ من الأطفال الطبيعيين بدون أعراض) خلال الفترة من مارس ٢٠١١ إلى مارس ٢٠١٢. تم الحصول على جميع المعلومات ذات الصلة في نموذج استبيان خاص. تم استخراج الحمض النووي الجينومي الفيروسي من عينات البراز باستخدام العدة الخاصة التشخيصية من QIAamp–MinElute لتنقية جينوم الفيروسات (Qiagen، ألمانيا). تم الكشف عن الفيروس الغدي المعوي عن طريق البلمرة المتسلسلة (PCR).

النتائج: تم الكشف عن الفيروس الغدي المعوي المعدي في ٣٪ من حالات الإسهال في الأطفال في المستشفى في حين أن جميع الأطفال الأصحاء كانوا سلبيين الفيروس الغدي المعوي. وكشف تحليل الفئة العمرية أن الأطفال في الفئات العمرية (٩–١١و١٢–١٧شهرا كانوا أكثر تضررا.

الاستنتاج: أظهر التوزيع الفصلي لحالات الفيروس الغدي المعوي أن يقتصر على فترة ٤ أشهر (أغسطس وحتى نوفمبر). كانت الأعراض السريرية المرتبطة بالتهاب المعدة والأمعاء في الفيروس الغدي هي الجفاف (٨٠٪) والقيء (٦٠٪) والحمى (٦٠٪) في حين لم يتم تسجيل آلام في البطن.

الكلمات المفتاحية: الفيروس الغدي المعوي، إسهال الأطفال.

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INTRODUCTION

Adenoviruses are one of the most important etiological agents of serious gastroenteritis among infants and young children less than five years.[1] They exist in all parts of the world, and are present in year-round, but are most prevalent in spring or early summer and again in midwinter in temperate climates. About 15% of all childhood diarrhea has been caused by adenoviruses.[2] By the age of 3 years most children have neutralizing anti-adenovirus 40/41, suggesting that most children have been infected by adenoviruses 40 and 41. Enteric adenoviruses generally have been detected in 1-4% of children with diarrhea in many studies.[3,4] Enteric adenoviruses were associated with 31% of hospital admissions for diarrhea[5] and have reported an incidence of 1-8% in industrialized countries, whereas 2-31% has been reported in developing countries.[6] In Iraq, enteric Adenoviruses are responsible for 20% of diarrhea that leads to hospitalization.[7] Electron microscopy (EM) was first used to detect enteric adenoviruses in fecal specimens when they are shed in large amounts (as many as $10^{[11]}$ particles/gram of feces). Since EM cannot distinguish enteric adenoviruses from no enteric serotypes, Immune electron microscopy (IEM) can enhance sensitivity and specificity of detection.[8] The introduction of molecular methods, strongly reduces the use of EM as a diagnostic tool. Application of polymerase chain reaction (PCR) assay to clinical specimens could detect all serotypes of human adenovirus with high sensitivity and allow serotype determination by sequencing of the amplicons.[9] More sensitive and faster PCR techniques are used to identify specific enteric adenovirus types 40 and 41.[10,11] The study aimed on the determination of the incidence of enteric adenovirus associated gastroenteritis in infants and young children below five years of age, and to detect the season and age related distribution of enteric adenovirus infections as well as the major clinical symptoms associated with this virus infections.

MATERIALS AND METHODS

A total of 400 fecal specimens were collected from infants and children under five years of age in the city of Basrah, south of Iraq during one year (March 2011 - February 2012): 200 stool specimens were collected from hospitalized children (including 105 male and 95 female) suffering from acute gastroenteritis admitted to Basrah Hospital for Obstetric and Child this hospital is a major referral hospital in Basrah city. The remaining 200 stool specimens were collected from nondiarrhea healthy children (including 94 male and 106 female) at Al Furdos kindergarten and nursery, Al Zohor kindergarten and nursery, and Baraem Al Amal kindergarten and nursery were studied as a control group. Informed verbal consent was obtained from parents/ caregiver of participating children before collecting the samples. A standard structured questionnaire was used to obtain the information regarding age, sex, season, clinical manifestations (diarrhea, fever, abdominal pain and dehydration) and type of feeding (breast, bottle or mixed feeding) for each case. Spring season was from March to May, summer was from June to August, autumn from September to November, and winter from December to February. According to WHO's recommendation, all children were classified in specific age groups (0–2, 3–5, 6–8, 9–11, 12–17, 18–23, 24–35, and 36–48 months).[12] Stool suspension of each specimen 20% (w/v, for the solid sample and v/v, for the liquid sample) was prepared in 10%, NaCl and after vigorously mixing, stool suspension was clarified by centrifugation at 8000 rpm for 20 min at 4C.[13] The resulting supernatants were collected and stored at -20°C until use for nucleic acid extraction. One stool specimen was collected from each child.

Viral Nucleic acid (genomic DNA) was extracted from 200µl of supernatant of the stool sample using a spin-column technique, according to the instructions given in the insert of the QIAamp MinElute virus spin kit. Viral nucleic acid was then recovered in 100µl of elution buffer. Samples are lysed under highly denaturing conditions at elevated temperatures.

Lysis is performed in the presence of QIAGEN Protease and buffer AL, which together ensure inactivation of RNases. All the extracted viral nucleic acid examined by Nano drop instrument in order to determine the concentration of viral DNA. This is important step to determine the amount of extracted viral genome will be used in the PCR and to neglect the negative sample.

Oligonucleotide Primers for PCR Amplification of Enteric adenovirus

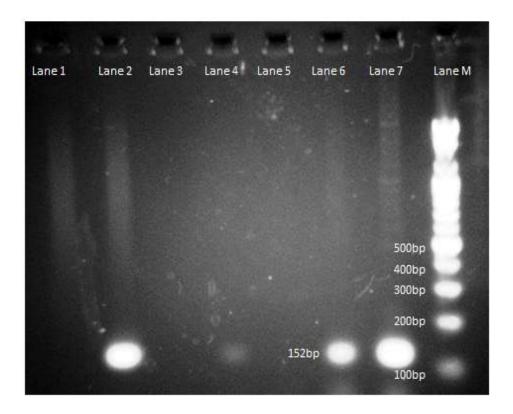| Virus	Primer a	Sequence b	Nucleotide position	Amplicon size (reference)
Adenovirus	Adeno-F	5-CACTTAATGCTGACACGGGC-3	443–462	152
	Adeno-R	5-ACTGGATAGAGCTAGCGGGC-3	597–578	Tiemessen and Nel 1996

Horizontal agarose gels were used for analysis of DNA after PCR amplification. The concentration of agarose used was 2%; gels are prepared as percentage weight/volume solutions. That is, the weight of agarose in grams per 100 ml running buffer and 8µl of the final PCR product were loaded on to 2% agarose gel containing ethidium bromide (0.2µg/ml). DNA molecular weights were determined by comparison with a 100 bp DNA ladder. Samples showing a specific amplicon were considered as positive.

Statistical Package for Social Science (SPSS) version 15 software was used to analyze the data. Chi-square (X^2) test was used to assess the significance of differences between groups and variables. P-value less than 0.05 was considered to be statistically significant and P-value less than 0.01 considered as highly significant. Enteric Adenovirus, which occurred in relatively small numbers, was detected in 3% of the stool samples studied. All the samples of healthy control group were enteric adenovirus negative (Table-1). However, the adequate amount of extracted DNA as estimated by Nanodrop were in 160 specimens out of 200 of cases and 85 of control group.

Table 1. The incidence of enteric adenovirus among diarrheal cases

Type of virus	Diarrheal group		Control group	
	No. of + ve cases / No. of tested cases	% of Positive	No. of + ve cases / No. of tested cases	% of Positive
Adenovirus	5/160	3%	0/85	0%

Agarose gel electrophoresis demonstrating Adenovirus. Lane 1, a representative of tested sample that negative for Adenovirus. Lanes 2,4.6,7 a representative of tested samples that positive for Adenovirus(152bp). Lane M, 100 bp DNA Ladder marker. (2% agarose, 100v for 30min)

Age and sex distribution of enteric adenovirus infection

(Table-2), shows distribution of adenovirus infection according to sex and age of the patients. According to table -2, of 5 positive samples, 2 (2%) specimens belonged to male and 3 (4%) to female patients, this difference was not significant (P > 0.05). Age groups analysis revealed that adenovirus infection was among children with age groups 9-11 months and 12-17 months with no significant differences between these age groups (P > 0.05).

Table 2. Age and sex distribution of adenovirus-positive in diarrheal group of children under 5 years old

Variable	Diarrheal group	
Age (months)	No. of + ve cases / No. of tested cases	% of + ve cases
0-2	0/15	0
3-5	0/20	0
6-8	0/22	0
9-11	2/21	10
12-17	3/26	12
18-23	0/24	0
24-35	0/15	0
36-48	0/17	0
Sex		
Male	2/82	2
Female	3/78	4
Total	5/160	3

Age groups X^2 = 0.18 df= 7 P>0.05 Sex X^2 = 0.67 df= 1 P>0.05

Seasonal distribution of enteric adenovirus infection

A possible relationship between the occurrence of enteric adenoviruses infection and seasons was investigated in (Table-3). The rates of enteric adenovirus infection were 5% in summer and 7% in autumn with no significant difference between these seasons (P > 0.05). The results revealed that although the fecal specimens were collected over the period of 12 months (March 2011 to 2012), Adenovirus infection was apparently confined within a period of 4 months (August through November 2011).

Table 3. Distribution of Adenovirus in diarrheal group of children under 5 years old in different season

Season	Diarrheal group	
	No. of +ve cases / No. of tested cases	% of +ve cases
Spring	0/38	0
Summer	2/41	5
Autumn	3/41	7
Winter	0/40	0
Total	5/160	3

X^2 = 0.33 df= 3 P>0.05

The study of clinical manifestations in adenovirus gastroenteritis cases showed that most children with infection had dehydration (80%), vomiting (60%) and fever (60%) while abdominal pain was not recorded (Table-4.) Dehydration (80%) was occurred at higher rate in adenovirus - positive children (P = 0.01),compared with the rate of dehydration in diarrhea cases positive with other than Adenovirus

Table 4. Distribution of clinical symptoms in diarrheal children with enteric adenovirus infections.

Type of virus	No. of cases	Symptoms							
		Vomiting		*Fever*		*Dehydration*		*Abdominal pain*	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)
Adeno	5	3	60	3	60	4	80	0	0

Vomiting: X^2 = 87.4 df = 4 P=0.01 Fever: X^2 = 69.37 df = 4 P = 0.01

Dehydration: X^2 = 72.41 df = 4 P=0.01 Abdominal pain: X^2 = 61.87 df = 4 P = 0.01

DISCUSSION

Epidemiological studies detected adenoviruses in stool samples collected from infants and young children with acute gastroenteritis in the developed and developing world.[14] Enteric adenoviruses were found in 3% of hospitalized children with acute diarrhea. Th e result of this study is consistent with the results reported from other parts of the world as the prevalence of enteric adenovirus infection has been reported to be 1.35 to 10.4% of all cases of gastroenteritis.[15-17] The prevalence rate (3%) of adenovirus infection obtained in this study is much lower than rates (20%) previously reported in other part of Iraq. [7] This difference may be due to difference in age of target population and diagnostic test; in our study the target age of population was 1-48 months and we used PCR technique while in that study done in Babylon, the age of target population was 1-12 months and they used latex agglutination technique which is less sensitive and less specific. The fact that enteric adenoviruses produced symptoms that were milder than symptoms in patients with rotavirus infection may have resulted in under-reporting, in that the number of children with adenovirus infections seeking hospital attention may have been few in number. One limitation of the present study is that true enteric adenoviruses prevalence can be higher than estimated here (3%), because in this study only hospitalized children with acute diarrhea have been evaluated and the proportion of adenoviruses infections among children with only home care or outpatient visits have not been estimated. This study provides information on the epidemiology and the prevalence of enteric adenovirus gastroenteritis in children with acute diarrhea in Basrah, Iraq. Our results highlight the importance of enteric adenovirus gastroenteritis as one of the important health problems. In this study, all infants and children with enteric adenovirus-infection were aged less than 24 months which is in agreement with previous reports from Iran [18], Turkey [19] and India. [20] This result highlights that children aged < 2 years old are at greatest risk for developing severe diarrhea from enteric adenovirus infection. The identification of adenovirus in children older than 2 years also reported, and this incompatible result may be attributed to breastfeeding, socio economic status, hygiene, culture and climate. [21] There were no significant differences between males and females of the enteric adenovirus infection ,

this result also observed in previous report in Nigeria.[22] Shimizu, et al.[14] showed a higher detection of adenovirus among male children. In our study, all enteric adenovirus detection occurred during the summer and autumn (August-November) and no cases were detected in other season, in contrast with other epidemiological studies around the world which have revealed that adenovirus diarrhea occurs throughout the year with increased incidence during the cold seasons of the year.[18] Studies conducted in other countries more cases occurring in the spring and winter with a seasonal peak observed in the months of April to May. [16,19,20] Unfortunately, the number of samples was too small to draw any significant conclusions about the seasonality of adenovirus infection. The most common sign and symptoms among infants and children infected with adenoviruses were dehydration, fever and vomiting. These findings are similar to those results reported in Nigeria[21], Iran[16,18] and Italy. [23]

In conclusion, enteric adenovirus is an important viral pathogen implicated in childhood diarrhea among hospitalized children, and children less than 2 years of age at greater risk of acquiring enteric adenovirus infections during summer and autumn. The most common clinical symptoms associated with enteric adenovirus gastroenteritis were dehydration, fever and vomiting.

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