Human astrovirus among hospitalized children under five years of age with acute diarrhea in Basrah, Iraq

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ABSTRACT

***Background*: Human astrovirus are considered as a common cause of infantile gastroenteritis worldwide. The importance of astrovirus in the area is not well recognized.**

***Objectives*: The study is designed to determine the frequency and importance of astrovirus in childhood gastroenteritis and to investigate the most affected age group, the predominant clinical symptoms associated with astrovirus infection.**

***Subjects and methods*: A total of 400 stool specimens (200 from symptomatic and 200 from asymptomatic healthy children) were collected during 2011-2012 and the relevant informations were obtained on special questionnaire form. Astrovirus nucleic acid was extracted from stool specimens using a spin column technique according to the instruction given by QIAamp-Mini-Elute virus spin kit for purification of virus-RNA (Qiagen-Germany). Astrovirus was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) using specific primers; Mono269-F and Mono270-R of human astrovirus.**

***Results*: Astrovirus was detected in 2.6% of acute diarrheal cases, while asymptomatic control group were all negative for astrovirus. Age group analysis revealed that astrovirus infection was more frequent among children with age of 1- < 3 years (P < 0.05). Most of astrovirus cases were observed during winter months (January and February). The dominant clinical symptoms among astrovirus infected cases were vomiting (80%), fever (60%) and dehydration and abdominal pain was found in 40% for both respectively.**

***Conclusion*: Astrovirus is frequent among children with acute diarrhea and should be taken in consideration as a causative agent in childhood gastroenteritis.**

***Keywords*: Astrovirus, acute diarrhea, viral gastroenteritis**

الحمات النجمية البشرية كمسبب للاسهال الحاد عند الاطفال في البصرة

مقدمة: الحمات النجمية البشرية تعتبر واحدة من المسببات المهمة للاسهال لدى الاطفال في العالم والتي لم تتم دراستها في المنطقة ولا توجد صورة واضحة عنها .

الاهداف: نظمت هذه الدراسة للوقوف على مديات انتشار واهمية الحمات النجمية كمسبب للاسهال في المنطقة واكتشاف اكثر الفئات العمرية تأثرا بالاصابات واي الاعراض السريرية المصاحبة للاصابات.

العينات وطرائق العمل: اجريت الدراسة على ٤٠٠ عينة براز مأخوذة من ٢٠٠ حالة اسهال حاد و ٢٠٠ عينة أخذت من الاصحاء كعينة ضابطة خلال العام ٢٠١١–٢٠١٢. استخلص الحمض النووي للحمات بواسطة تقنية سبن–كلم باستخدام عدة خاصة من شركة كيا–جين الالمانية وتم التحري عن جينات الحمات النجمية باستخدام البلمرة التسلسلية المعكوسة من خلال مبدئات نوعية متخصصة للحمات النجمية البشرية.

النتائج: رصدت الحمات النجمية عند ٢,٦% من حالات الاسهال الحاد بينما كانت جميع العينات الضابطة سالبة وكانت الحمات اكثر شيوعا عند الفئة العمرية للاطفال من ١–٣ سنوات وبوجود اعراض سريرية مصاحبة للاسهال عند الاصابة بهذه الحمات القى (٨٠%) ، ارتفاع درجة الحرارة (٦٠%)، ألام البطن والجفاف (٤٠%) على التوالي .

المحصلة: الحمات النجمية هي مسبب شائع للاسهال عند الاطفال في المنطقة ويجب الاخذ بنظر الاعتبار وجوده ضمن المسببات الاخرى المهمة.

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INTRODUCTION

Human astrovirus are recognized as a common cause of infantile acute gastroenteritis worldwide.[1] Epidemiological studies have reported the occurrence of human astrovirus infections among children hospitalized for diarrhea. The overall prevalence have ranged from 2-16% in hospital based studies and 5-17% in community-acquired gastroenteritis.[2,3] Human astrovirus have also been associated with outbreaks of diarrhea in children[4] and adults[3]; and in daycare centers.[5] Gastroenteritis associated with astrovirus like rotavirus, occurs in both developing[6] and developed countries[7], suggesting that improvement in water and sanitation are unlikely to decrease disease incidence. In developing countries, astrovirus have generally been detected in less than 10% of young children in outpatients clinics and hospitals.[8] However, in temperate climates, astrovirus diarrhea case peaks in winter[9] while the seasonality is less clear in tropical setting.[6,10] Cases of astrovirus associated gastroenteritis are most common among children less than 2 years of age and less frequent among adults.[6-8] Children may shed virus for 1-2 days prior illness and 4-5 days following illness, but shedding for 3 weeks has been reported.[4,11] Asymptomatic secretion can occur in neonates and young children and is a significant cause of infection especially in nurseries, childcare centers and hospitals.[12] Electron microscopy was the only method to detect astrovirus in fecal specimens, but was relatively insensitive and the diagnosis have been difficult since only about 10% of particles shows the typical morphological feature of astrovirus "star".[11] Enzyme immunoassays have been developed which was more sensitive, easier and less expensive to use.[12-15] Molecular diagnostic methods including reverse transcriptase-polymerase chain reaction (RT-PCR) and probes as well as virus cultivation are available in research laboratories and become routine for identification.[12-14,16] The objectives of this study are: **a)** to determine the frequency of astrovirus in acute gastroenteritis, **b)** to identify the most affected age group by astrovirus, **c)** to determine the seasonal variation and the predominant clinical symptoms associated with astrovirus gastroenteritis.

SUBJECTS AND METHODS

A total of 400 fecal specimens were collected from infants and children under five years of age in the city of Basrah during one year period (march 2011 to the end of march 2012); 200 stool specimen from symptomatic hospitalized children (including 105 males and 95 females) suffering from acute diarrhea admitted to Basrah Maternity and Children Hospital. All stool samples were collected during the first 2-3 days of disease onset. The remaining 200 stool specimens were collected from asymptomatic apparently healthy children (including 94 males and 106 females) at AlFurdos, Al-Zuhoor, Al-Amal and Al-Baraem kindergartens and nurseries as control group. Informed verbal consent was obtained from parents/caregivers for participation of children before collection of specimens. A standard structured questionnaire form was used to obtain the information regarding age, sex, season, clinical manifestation (diarrhea: within the first week of onset, fever, vomiting, abdominal pain and dehydration) and feeding practice (breast, formula or mixed) for each case. According to WHO recommendation all children were classified as specific age groups (0-2, 3-5, 6-8, 9-11, 12-17, 18-23, 24-35, and 36-48 months).[17] Spring season was from March to May, summer was from June to August, autumn from September to November and winter from December to February. Stool suspension of 20% (W/V for solid, and V/V for liquid samples) was prepared in 10% NaCl after vigorous mixing, stool suspension was clarified by centrifugation at 8000 rpm for 20 minutes at 4C.[18] Viral nucleic acid extraction (virus RNA genome)

was done from 200 ul of stool suspension using a spin-column technique according to the instructions given in the insert of the QIAamp Mini-Elute virus spin kit (Qiagene,Germany). Viral nucleic acid was recovered in 100 ul of elution buffer. Samples are lysed under highly denatured conditions at elevated temperatures. Lysis is performed in the presence of Qiagene protease and buffer AL which together ensure inactivation of RNases. Extracted viral nucleic acid examined by nanodrop instrument to determine the concentration of viral RNA (the amount of viral genome) that used in RT-PCR amplification. Specific oligonucleotide primers for astrovirus was used (Astrovirus primers sequence as: Mono269-F (4526-4545) 5-CAACTCAGGAAACAGGGTGT-3 and Mono 270-R(4974-4955) 5-TCAGATGCATTGTCATTGGT-3. After RT-PCR amplification, 2% agarose gels were prepared (w/v) and 8 ul of final PCR product were loaded on to 2% agarose gels containing 0.2 ug/ml ethidium bromide. Only those showing clear bands at electrophoresis were included in the analysis. The molecular weights were determined by comparison with DNA ladder. Samples showing specific amplicon were considered positive. Statistical package of social science (SPSS) version 15 was used to analyze data, Chi-square (X^2) test was used to assess the significance of difference between groups and variable, P-value less than 0.05 was considered to be statistically significant .

RESULTS

Astrovirus was detected in relatively small number of diarrheal cases involved 2,6% of the tested cases. None of the healthy control group were positive (**Table-1**).

Table 1. Rate of Astrovirus detection from tested stool samples

Study population	No. tested	No. positive (%)
Diarrhial cases	192/200*	5 (2.6)
Control group	122/200*	0 (0)

***192, 122 out of 200 in both test and control showed typical band at electrophoresis**

(Table-2), presents some selected demographic variables of children with acute diarrhea. As mentioned in the methodology, 200 nucleic acid were extracted but as shown in (Figure-1), 8 sampled not revealed the typical clear band and excluded from analysis. Age group analysis showed that Astrovirus infection was more frequent among children with 1-3 years of age. None of the Astrovirus cases was detected during the first 11 month of age (infancy period) and infections affecting both sexes equally.

Table 2. Selected sociodemographic variables of Astrovirus-positive children with acute diarrhea

Variables	Diarrheal group	
Age (months)	**No. positive/tested (%)**	
0 - 2	0 / 19	(0)
3 - 5	0 / 19	(0)
6 - 8	0 / 26	(0)
9 - 11	0 / 28	(0)
12 – 17	2 / 29	(7)
18 - 23	1 / 30	(3)
24 -35	2 / 22	(9)
36 – 48	0 / 19	(0)
Sex		
Male	3 / 101	(3)
Female	2 / 91	(2)
Season		
Spring	0 / 47	(0)
Summer	0 / 47	(0)
Autumn	0 / 49	(0)
Winter	5 / 49	(10)
Total	5 / 192	(2.6)

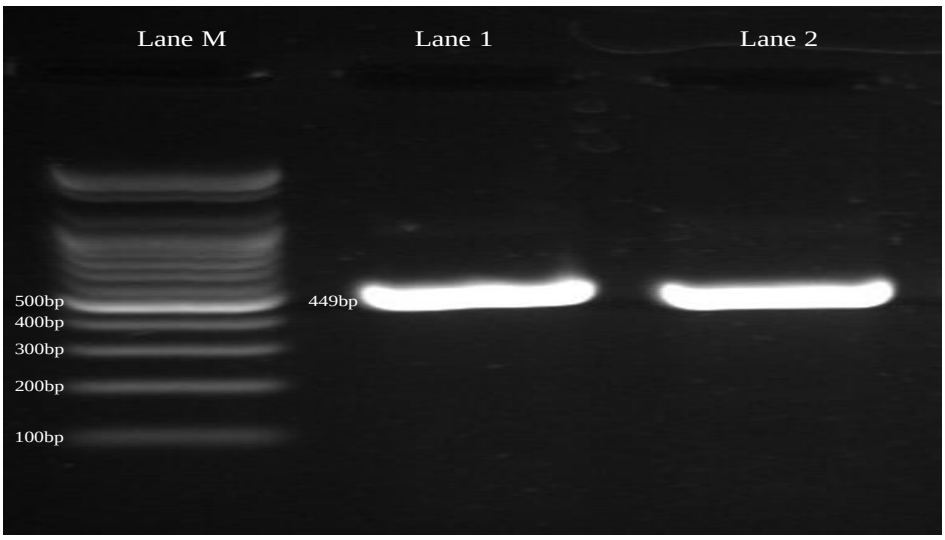

Fig 1. An agarose gel electrophoresis demonstrating specific astrovirus band at 449bp, Lanes 1 and 2 represent tested samples positive for astrovirus, Lane M 100bp DNA ladder marker (2% agarose, 100V for 30 min).

During the one year period of the study, astrovirus infections was found in winter months (January and February). February was the month with highest incidence of astrovirus infections. The rates of astrovirus infections were 10% in winter and not detected in other seasons.

Table 3. Clinical symptoms in Astrovirus infected children

Clinical symptoms	No. / total cases (%)
Vomiting	4 / 5 (80)
Fever	3 / 5 (60)
Dehydration	2 / 5 (40)
Abdominal pain	2 / 5 (40)

The clinical symptom associated with Astrovirus acute diarrhea is presented in (**Table-3)**. Vomiting (80%) was occurred at higher rate in astrovirus-positive cases, with 60% suffering from fever. Dehydration and abdominal pain was found in 40% of Astrovirus positive cases.

DISCUSSION

Epidemiological studies carried out worldwide have reported human astrovirus prevalence rates of 2-16% among hospitalized children and 5-17% among children with diarrhea in community- based studies.[2,3] The prevalence of astrovirus was 2.6% among children under five years of age who were hospitalized with acute diarrhea in Basrah, Iraq. To our knowledge there is no report about the prevalence of astrovirus diarrhea in Iraq. This is almost similar to other studies showed an incidence of astrovirus infection in children with gastroenteritis of 2% in India[19], 3% in Brazil[20] and 4.2% in Australia.[21] However, in some studies, higher rates of astrovirus infection have also been reported include 6.3% in France[7], 6.8% in USA[22], 7% in South Africa[23] and 9.2% in Saudi Arabia.[24] Our results document that the occurrence of astrovirus infection in children with acute diarrhea severe enough to require hospital admission was as low as 2.6% but astrovirus infection could be related to a mild diarrheal disease which would not require frequent hospitalization[1,25], therefore many infections due to astroviruses in this area may not detected since our study is hospital-based.

Because many diarrhea cases are treated at home and not reported to hospital; implying that the prevalence of astrovirus infection does not reflect the true picture of this infection. This highlight the need to more community-based studies in this area. Astrovirus was detected among age group of 1-3 years, while it was not detected among children less than one year and above 3 years of age. These results are similar to those observed in a 3 –year study conducted in Barcelona, Spain[26] and also in Brazil[20] where the peak incidence was found in older children aged between 2 and 4 years. However, another study showed that astrovirus infections occurred in all age groups, with a higher detection rate in children up to 24 months of age.[27] Medina, et al [25] reported that contact with astroviruses in early childhood could provide long-lasting protection against symptomatic infections. The higher median ages found in our group of patients could be due to a scarce diffusion of environmental astrovirus in our area or to the lack of protection against reinfection. Further serological studies are necessary to confirm these hypotheses. Children's gender have no effect on astrovirus rate of infection among those with acute diarrhea. A similar analysis was performed in another study conducted in Kolkata city which showed similar results.[28] Detection of astrovirus infections has been described largely in winter and rainy seasons in temperate and tropical climates respectively.[15,29] Basrah is classified as a warm and humid area. In our study in Basrah, all human astrovirus cases was detected in winter season. This seasonal pattern is consistent with other studies showed that astroviruses occur preferentially during the colder months of the year.[30] However, reports exist which describe high astrovirus incidence during spring and summer.[15] Other studies reported a year-round occurrence of astrovirus infections.[31] The reason of the different seasonal astrovirus pattern is unclear. However, because of the small number of cases detected unable to determine whether the pattern of human astrovirus infection in Basrah is seasonal. The symptoms associated with astrovirus infections was vomiting (80%), fever (60%), dehydration and abdominal pain (40%) which was almost consistent with another study[32] and higher than that reported in a study from Madrid, Spain (65%, 39% and 24% respectively) conducted at an emergency room.[33]

In conclusion, astrovirus is an important cause of diarrhea in Basrah responsible for 2.6% of hospitalized severe diarrheal cases among under 5 years children with dominance of infections in age group 1-3 years, mostly occur during winter months and the most frequent clinical symptoms associated with astrovirus infections are diarrhea, vomiting, fever and dehydration.

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