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A Trend of Seasonality of Enteric Adenoviral Gastroenteritis in Pediatric Patients Less than Five Years from Baghdad

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ABSTRACT

Background: In developing countries, viral agents are the most commonly identifiable causes in children with gastroenteritis. Some of the viral infections have showed seasonal tendencies in relation to geographical regions. Enteric types of adenoviruses (Ad 40, Ad41) are among the most common and important etiological agents associated with sporadic cases and outbreaks of infantile acute gastroenteritis.
Objectives: The purpose of this study was to determine the prevalence and seasonal tendency as well as the diagnostic value of the related clinical and laboratory findings of Adenovirus- associated diarrhea in children up to five years of age.
Study Design: A prospective study along one–year period (February, 2013 till January, 2014) was carried out in the outpatient clinics of AL-Elweya Pediatric Teaching Hospital and Welfare Children Teaching Hospital at Baghdad. Stool samples from a total of 807 children (aged between 5-60 months) with acute gastroenteritis, were enrolled in this study. Stool samples were tested by immunochromatographic assay for enteric adenoviruses. This sample of children, and according to their age, was categorized into four groups: >12 months, 12->24 months, 24->36months and <36 months. Total monthly numbers among these age groups formed the basis for assessing seasonal tendency of adenovirus infections.
Results: Less than 5 years children with acute gastroenteritis tested for Adenovirus infection have a mean of 11.1±10.1 months and the males comprised 57.9% (467 out of 807) while females 42.1% (340 out of 807). Shedding of Adenovirus was detected in 14.6% of acute diarrheal stool samples, among them 58.5% were males with a male to female ratio of 1.4: 1. Children with acute Adenoviral diarrhea were younger than those without viral infection, yet the association was statistically not significant. Adenovirus-positive cases showed two ratal peaks; the highest one was during January (25 cases) and the second was during September (22 cases). During the hottest months in Iraq, July and August, the number of cases in each was dropped to three cases while two cases were reported during each of November and December.
Conclusions: The present data indicate that the infection rate was low in November and December (had peaked equally in Autumn and early Winter), while high in January.

Key words: Children gastroenteritis, Enteric adenoviruses, Seasonality, immune-chromatographic assay (ICA)

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INTRODUCTION

Acute gastroenteritis is one of the most globally common diseases [1]. The greatest morbidity and mortality of acute gastroenteritis are targeting the extremes of age: children and the elderly [2]. More than 700 million/Year acute diarrheas with 3-5 million/Year mortalities had been reported in children less than five years of age, the majority in developing countries [3]. However, deaths from diarrhea are less common in developed countries, although acute gastroenteritis has led to medical bills due to many hospitalizations or doctor visits [2].

Worldwide, infectious diarrheal diseases are the third leading cause of death and viral gastroenteritis cases have either gradually increased in rates among young children in developed countries or are among the

leading causes of illness in young children in developing countries [4-6].

Sanitary improvements in the developed countries significantly decreased bacterial and parasitic gastroenteritis cases but have little effect on those due to viral types and as such, viral gastroenteritis has gradually increased in these regions [7]. However, viral gastroenteritis is affecting all children in their first few years of life regardless their level of hygiene, quality of water, food or sanitation, or type of behavior [2].

The majority of pediatric gastroenteritis was related to Rotaviruses, Caliciviruses, Astroviruses and Adenoviruses, yet globally, Rotaviruses and enteric adenovirus types 40 and 41 were found to be the most prevalent etiological agents of acute severe viral gastroenteritis in pediatric patients associated with high mortality rates in the developing countries [2,8-10]. However, highly effective Rotavirus vaccine has prevented severe diarrheal cases as well as reduced the attributable mortalities [1]. A three-year passive surveillance of gastroenteritis found that infections with either Astroviruses or Rotaviruses has peaked simultaneously in winter months [11]. However, another study noted an increase in Rotaviruses infections in autumn-winter season but a trend of either seasonal pattern or prevalence was neither observed in Adenoviruses nor Astroviruses infections [4].

Viral gastroenteritis are among the leading causes of diseases in young children in developing countries [5] and for this respect, the current study was designed for the purpose of enrichment the present data dealing with Adenovirus infections to determine the prevalence of acute gastroenteritis associated with such viral infections in Baghdad and we think that it is reasonable to reveal the possibility of a seasonality for Adenovirus infections in children under two- years in our country.

MATERIALS AND METHODS

Study population

This prospective study was conducted during the period between February 2013 to January 2014, on a total number of 807 infants and young children less than 5 years old with acute diarrhea presented to the outpatient clinics of 2 pediatric hospitals in Baghdad (AL-Elweya Pediatric Hospital and Children Welfare Teaching Hospital, CWTH).

Macroscopic and microscopic stool laboratory examinations were done. As an inclusion criteria, this study included those children with watery diarrhea that were not lasting more than seven days after the onset of illness, and as such, the exclusion criteria of this research was neither to include children hemorrhagic diarrhea nor those stool samples containing parasitic agents.

An immunochromatographic assay used in this study was purchased from (CerTest, Spain) and stool samples were collected in a labeled screw- cap clean container and were tested for antigenic detection of Adenovirus and according to instructions of the manufacturers.

The Adenovirus is a qualitative immuochromatographic assay for determination adenovirus in fecal samples, where mouse monoclonal antibodies against the viral antigens on the test band region are pre-coated. During testing, the samples are allowed to react with the colored conjugate (anti-adenovirus mouse monoclonal antibodies-blue microspheres) which was pre-dried on the test region. This mixture moves upward across the membrane according to the capillary action. The specific antibodies present on the membrane will capture these colored particles where blue colored lines will be visible. Again, when this mixture moves upward to the immobilized antibody placed in the control band region, a green-colored band appears.

Regarding the Adenovirus, approximately 100 mg or 100 µl of stool sample was put and shaken in collection tube containing the diluents. Four drops or 100 µl was dispensed in S circular window in the card and appearance of blue and green-colored bands were noticed after 10 minutes.

Negative results indicated by the appearance of only one green band (control line). A total absence of control band on the card, regardless the resulted test lines, was regarded as an invalid result.

The T, ANOVA, and Chi square statistical tests were applied for analysis of the obtained results by using the SPSS program (version-10) and Excel application.

RESULTS

During a period of one year, 807 cases of acute diarrhea among children aged ≤ 5 years were studied. Their age ranged from 0.5-60 months with a mean ± SD of 11.1 ± 10.1 months, among these children. 467 (57.9%) were males with a male: female ratio of 1.4: 1 (Figure 1).

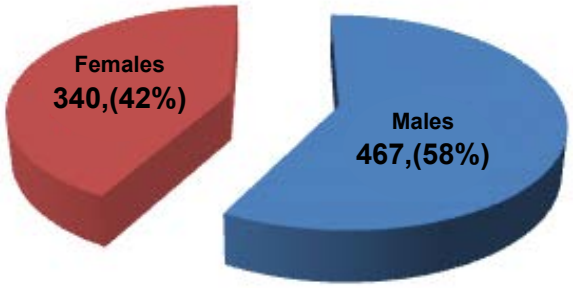

Figure 1: Distribution of the studied children by gender

Immuno-chromatographic assay for virology examination of Adenoviral antigen in the studied fecal samples has revealed that Adenovirus was responsible for their diarrhea in 14.6% of the children with acute gastroenteritis (Figure 2), and among them 58.5% were males with a male to female ratio of 1.4: 1 (Table 1).

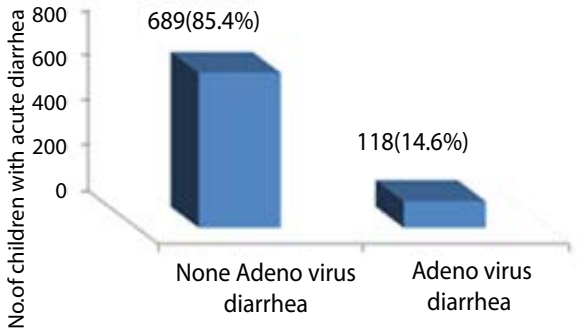

Figure 2: The studied by cause of diarrhea

Table 1: Children with diarrhea by gender and presence of Adenovirus

Viruses	Males		Females		Total	
	No.	%	No.	%	No.	%
Adenovirus Diarrhea	69	58.5	49	41.5	118	14.6
None Adenovirus Diarrhea	398	57.8	291	42.2	689	85.4
Total	467	57.9	340	42.1	807	100

Although children with acute diarrhea due to Adenovirus were younger (mean ± SD of 10.7 ± 10.3 months) than those without Adenovirus infection (mean ± SD of 11.1 ± 10.1 months) yet the difference in mean age in months was statistically not significant (Students' T Test, df=805, P=0.44) (Tables 2 and 3), even when the children were classified into age groups (in months) the association was found to be statistically not significant (χ^2=2.76, df=3, P=0.43) (Table 3).

Table 2: Children with diarrhea by age (in months) and cause of diarrhea

Age (in months)	Children with Adenovirus Diarrhea (No=118)	Children with none Adenovirus Diarrhea (No=689)	P Value
Range	0.5-108	0.5-102	0.44*
Mean ± SD	10.7 ± 10.3	11.1 ± 10.1	

*Difference in mean age is statistically not significant (Students' T Test, df=805, P=0.69)

Table 3: Children with diarrhea by age groups (in months) and cause of diarrhea

Viruses	Adenovirus Diarrhea (No=118) No.	%	None Adenovirus Diarrhea (No= 689) No.	%	P value
<12	86	15.9	454	84.1	0.43*
≥ 12-<24	18	10.8	149	89.2	
≥ 24-<36	9	13.6	57	86.4	
≥ 36	5	14.7	29	85.3	

*The association is statistically not significant (χ^2=2.76, df=3, P=0.43)

Most of the children with acute diarrhea developed fever, yet those positive to Adenovirus developed fever more significantly (90.7%) than those without Adenovirus (74.6%) (χ^2=14.7, df=1, P=0.0001) (Figure 3). On the other hand, nearly most of the children with Adenoviral diarrhea developed vomiting (96.6%) compared to only 85.4% of those without Adenoviral diarrhea and the association between cause of diarrhea and developing vomiting was statistically significant (χ^2=11.1, df=1, P=0.0009) (Figure 3).

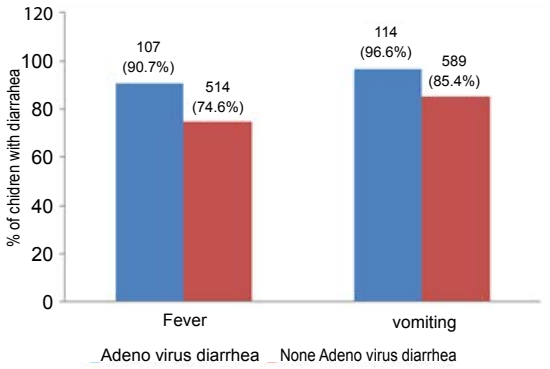

Figure 3: Percentage distribution of children who suffered from fever* and vomiting by type of infection**
The association was statistically significant (74.6%) (χ^2=14.7, df=1, P=0.0001)
****The association was statistically significant (χ^2=11.1, df=1, P=0.0009)**

Examination of stool specimens has revealed that in 88.2% of children with Adenovirus diarrhea, ≥ 3+fatty drops was found compared to only 52.9% of children with non-Adenovirus diarrhea, whereas 27.7% of those with non-Adenovirus diarrhea had no fatty drops in their stool specimens compared to only 4.2% of children with Adenovirus diarrhea and the association between the amount of fatty drops in stool specimen and cause of diarrhea was statistically significant (χ^2=52.1, df=2, P=0.0000) (Table 4).

Table 4: Children with diarrhea by finding fatty drops in stool specimens and presence of Adenovirus

Amount of fatty drops found in stool specimens	Children with Adenovirus Diarrhea		Children with non-Adenovirus Diarrhea		Total		P Value
	No.	%	No.	%	No.	%	
None	5	4.2	191	27.7	196	24.3	0.0000*
+/++	9	7.6	133	19.4	142	17.6	
≥ +++	104	88.2	365	52.9	469	58.1	
Total	118	14.6	689	85.4	807	100	

*Statistically significant association (χ^2=52.1, df=2, P=0.0000)

Figure 4 shows the distribution of cases with acute diarrhea due to Adenovirus by month of occurrence. In this Figure, two peaks were revealed where the highest one was during January (25 cases) and the second was during September (22 cases). It also showed that the lowest number of cases (two cases) was recorded during November and December.

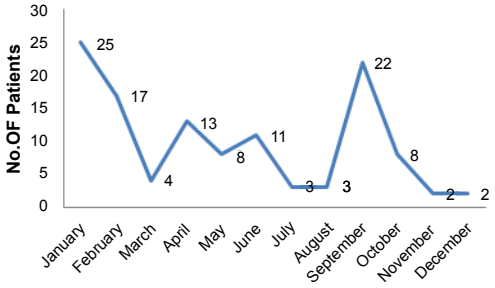

Figure 4: Distribution of cases with acute diarrhea due to Adenovirus by month of occurrence

In the present research the pattern of annual variation for Adenovirus was studied by applying the Edwards' test to detect the seasonal variation in twelve months' periods. The total number of Adenovirus cases was 118, the monthly distributions of these cases is illustrated in Figure 4 while the relationship between months of occurrence (k) and frequency of cases (fi) for the twelve months' periods are shown in Figure 5.

The curve in Figure 5, showing the relationship between (k) and (fi) has one peak during the year, indicating poor seasonal variation.

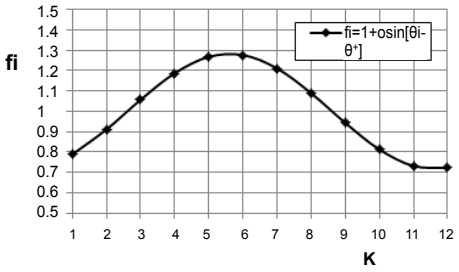

Figure 5: Edwards test; yearly distribution

DISCUSSION

Infectious-related diarrhea is still one of the leading causes of young children illnesses in developing countries and is gradually increasing in developed nations [4-6].

Five types of viruses have been usually attributed to cause childhood gastroenteritis over the world; Rotaviruses, Adenoviruses, Astroviruses, Noroviruses and Caliciviruses [9,12].

Among the most recognizable etiologies in acute gastroenteritis affecting Infants and young children less than two years of age in both developed and developing are Rotaviruses followed by Adenoviruses types (Ad 40, Ad 41) [13,14] .

This study aimed to evaluate the frequencies of adenovirus infections in children with acute gastroenteritis in the central regions of Baghdad according to their age, gender and seasonal features so as this could help, in part, for future development of a vaccine for such agents. A total number of 807 children, their age between 0.5-60 months with diarrhea, were enrolled in this study during the period from February, 2013 till January, 2014.

Stool samples from children with diarrheal diseases are preferable for the detection of viral agents since they are shed in high concentration [15]. Accordingly, this research work has used stool as a sample for the antigen-based Immuno-chromatographic assay (ICA) to diagnose Adenovirus [16] while the clinical data of these children were abstracted from children families to be recorded (and then analyzed) in a special questionnaire formats prepared for this purpose.

Shedding of Adenovirus in this study was detected in 14.6% of acute diarrheal stool samples whereas studies in many other countries, as Italy, China, KSA, Singapore, Indonesia, Botswana, Brazil, Iran, Turkey have detected enteric adenovirus in stools of 2.6%, 2.5%, 5.3%, 3%, 2.3%, 2%, 3.6%, 5%-15%, 23.6% pediatric patients with gastroenteritis, respectively [4,9,12,14,17-19].

Adenoviruses were detected predominantly in those 2-5 years children with a prevalence of 3.5% [12]. Adenoviral gastroenteritis in infants aged less than 6 months were relatively more important causes than in toddlers aged 12 months or more [20].

However, neither mean (10.7 ± 10.3 months) nor age stratifications of children (in months) with acute diarrhea due to Adenovirus showed statistically significant (Students' T Test, df=805, P=0.44), (χ^2=2.76, df=3, P=0.43) (Tables 2 and 3 respectively).

Adenovirus was detected in 58.5% of acute diarrheal stool samples of males, constituting a male to female ratio of 1.4: 1. This result is consistent with Motamedifar et al. results who found Adenovirus gastroenteritis among children in Shiraz, Iran more in males than females [21].

It was stated previously that, in comparison to rotavirus infections, high fever was less frequently observed in adenovirus type 40 and 41 gastroenteritis [22].

In the present study, most of the children with acute diarrhea developed fever, yet those positive to Adenovirus developed fever more significantly (90.7%) than those without Adenovirus (74.6%) (χ^2=14.7, df=1, P=0.0001) (Figure 3). The current results are consistent with the results of 7. Akan H et al. and Modarres S et al. [7,14] who found that fever has a significant relationship to adenovirus (in contrast to rotavirus) infection so as to be a beneficial symptom to distinguish between these two infections.

However, Hamkar et al. in Iran as well as Coffin et al. in the United States had found no fever in patients with enteric adenovirus infections [12,23].

On the other hand, nearly most of the studied children with Adenoviral diarrhea in this study developed vomiting (96.6%) compared to 85.4% of those without Adenoviral diarrhea and the association between cause of diarrhea and developing vomiting was statistically significant (χ^2=11.1, df=1, P=0.0009) (Figure 3).

It appeared that the detected prevalence in our Iraqi pediatric patients with gastroenteritis could referred to that families were seeking hospital care for their children with adenoviral infections since their symptoms were more severe than those without adenovirus infection, and these finding are clearly in contrast to those reported by Hamkar et al. [12] for under-reporting as well as lower numbers of parents seeking hospital care for their children with adenoviral infections. By applying the Edwards' test in the present research, the pattern of annual variation for Adenovirus was studied to detect any seasonal variation in twelve months' periods. The total number of Adenovirus cases was 118, the monthly distributions of these cases is illustrated in Figure 4 while the relationship between months of occurrence

and frequency of cases for the twelve months' periods are shown in Figure 5.

In previous report in China had revealed that adenovirus was detected throughout the year and there was no seasonal pattern or any peak in frequency of adenovirus through the year [24].

In addition, Enteric Adenoviral gastroenteritis in most parts of the world was documented throughout the entire year and does not display seasonal distribution [14,25].

Adenoviruses prevalence showed also no seasonal trend in Bates et al. study [20] as well as in Gaborone by an African study done by Basu et al. [4].

Also during this study, and similarly in an Iranian study by Modarres et al. Adenovirus (Ad 40, Ad 41) infections had occurred throughout the relevant years of both studies, however data of our study indicated an outbreaks of adenovirus infections in winter (January) and Autumn (September) whereas in that Iranian study more than 81.5% of these infections occurred in winter and spring seasons (December to June) [14]. Walter et al. noted a peak in frequency of adenovirus in July and October [26]. In Turkey, Ozdemir and co-workers [9] have reported acute diarrhea due to adenovirus infection during fall and winter months, while no cases were detected between May to August. In Northern Iran, adenoviral gastroenteritis has peaked in frequency in July and October, yet 77.8% was reported in winter season [12].

In view of these findings, it was noticed that Iran and Turkey have close geographical conditions which might be a factor for this similarity in Adenoviral trend timing, yet these findings are, in turn, closely quite different from the hot climates dominated in our country nearly during the majority of year-round months.

In Iraq, Ali et al. [27] found significant prevalence of gastroenteritis due to Rotavirus, Adenovirus, Astrovirus and Enterovirus and mixed viral infections was observed to constitute a respective bulk among Iraqi pediatric patients with gastroenteritis less than five years in Baghdad. In Iraq also Ali et al. [28] found that Rotavirus infections have also showed two peaks in similar trending to what we observed in the present study on the possibility of seasonal trending of Adeno infections; one was during September (61 cases) and a second during January (52 cases). Also and similar to Rotavirus infections studied in [28], the association between the amount of fatty drops in stool specimen and cause of diarrhea due to Adeno infections was statistically significant (χ^2=52.1, df=2, P=0.0000) (Table 4). This finding has complicated the subject and did fail to reach the level of using fatty drops amount in stool specimens as a biomarker for differentiation between these two viruses.

CONCLUSION

This study explored the medical significance of testing Adenoviral antigens as a clinical-guided approach in relation to age-related prevalence and the seasonality of adenovirus infection in pediatric patients seek medical care for diarrhea. Such knowledge could have a relevancy to the control planning strategies of enteric adenoviral diarrhea in our country as well as future vaccine development for adenoviral diarrheal diseases.

CONFLICT OF INTEREST

The authors' declares that they have no conflict of interest.

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