

Prevalence of Enteric Adenovirus Among Infants and Young Children Suffering From Acute Gastroenteritis In Sana'a City, Yemen

**انتشار فيروس الغداني المعوي بين الرضع والأطفال
الذين يعانون من الالتهاب المعوي الحاد
فى مدينة صنعاء-اليمن**

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Abstract :

Background: Adenoviruses, particularly enteric adenoviruses (EAd) can cause acute and severe diarrhea in young children worldwide. This study was conducted to determine the prevalence of enteric adenoviruses among infants and children suffering from acute gastroenteritis in Sana'a city and to determine the predisposing factors contributing for the virus transmission and infection.

Subjects and methods: All 326 stool specimens were randomly selected and collected from children less than 5 years of age with acute gastroenteritis attended to two public hospitals (Al-Thawrah General Hospital and El-Sabeen Maternity and Childhood Hospital) between the period from June 2008 to May 2009 in Sana'a city. The stool specimens were then tested for the enteric adenovirus antigen in stool using a commercially available enzyme-linked immunosorbent assay kit. The collected data were statistically analyzed for significance using the Epi. Info. version 6.04.

Results: Out of the 326 studied subjects, 36 (11%) were positive for EAd antigen. Males were slightly higher than females. The

highest prevalence of EAd infection occurred in the age group from 13 to 24 months which was highly statistically significant, where this prevalence decreased sharply with older age. The principal clinical symptoms among positive subjects were diarrhea (100%), abdominal pain (86.1%), vomiting (72.2%), fever (52.8%) and dehydration (41.7%). The association of positive EAd subjects with vomiting, fever and dehydration was highly statistically significant. The prevalence of EAd infection among breast-fed subjects was lower than that among mixed and bottle-fed subjects with percentages of 15.6%, 40.7% and 43.7% respectively for infants up to two years of age.

Conclusion: It can be concluded from this study, that enteric adenoviruses represented one of the most important etiological agents of acute gastroenteritis among children less than five years of age in Sana'a city, Yemen.

Key words: Enteric adenoviruses, acute gastroenteritis, children, Sana'a city, Yemen.

الملخص :

الموي حيث كانت نسبة الإصابة لدى الذكور (١٢.٧٪) مقابل (٩.٢٪) للإناث. كما بينت هذه الدراسة أن أعلى نسبة انتشار لذلك الفيروس كان في الفئة العمرية من ١٣ إلى ٢٤ شهراً بنسبة ٢٣.٩٪، وكانت هذه النتيجة ذات دلالة إحصائية مرتفعة، ثم تضاءلت تلك النسبة بشكل ملحوظ مع زيادة عمر الحالات. كما كانت الأعراض السريرية الشائعة المصاحبة للحالات المصابة بالفيروس هي إسهال وألم في البطن وتقيؤ وحمى، وأخيراً جفاف بنسب مئوية ١٠٠٪ و ٨٦.٢٪ و ٧٢.٥٪ وأخيراً ٤١.٧٪ على التوالي، وكانت هذه النتائج فيما يخص ارتباط الحالات الإيجابية بالتقيء والحمى والجفاف ذات دلالة إحصائية متقارنة. كذا كان معدل انتشار الفيروس بين الأطفال دون السنتين من العمر وفقاً لنوع الرضاعة هو بنسبة ١٥.٦٪ للرضاعة الطبيعية ونسبة ٤٣.٧٪ للرضاعة المختلطة، ونسبة ٤٠.٧٪ للرضاعة الصناعية.

الخلاصة: يستنتج من هذه الدراسة أن الفيروس الغذائي الموي هو أحد أهم المسببات لالتهابات المويحة الحادة بين الأطفال دون سن الخامسة من العمر في مدينة صنعاء - اليمن.

الكلمات المفتاحية: فيروس الغذائي الموي، التهاب موي حاد، أطفال، مدينة صنعاء، اليمن.

الهدف : الفيروسات الغذائية ومنها الفيروسات الغذائية المويحة من النوع ٤٠ والنوع ٤١ يمكن أن تسبب الإسهال الحاد والشديد عند الأطفال الصغار في جميع أنحاء العالم؛ لذلك هدفت هذه الدراسة المقطعيّة لتحديد معدل انتشار الفيروس الغذائي الموي بين الرضع والأطفال الذين يعانون من التهاب الموي الحاد في مدينة صنعاء - اليمن، وأيضاً لتحديد عوامل الأخطار المساهمة في انتشار خمج هذا الفيروس.

الطريقة: تضمنت هذه الدراسة على ٢٢٦ عينة براز إسهالي اختيارياً وجمعت في الفترة من يونيو ٢٠٠٨ م إلى مايو ٢٠٠٩ م من أطفال تقل أعمارهم عن خمس سنوات ويعانون من التهاب موي حاد، وترددوا على مستشفيين رئيسيين في مدينة صنعاء هما مستشفى الثورة العام ومستشفى السبعين للأمومة والطفولة. بعد ذلك تم اختبار هذه العينات البرازية لمستضد الفيروس الغذائي باستخدام طريقة *مُقايسة المُمثّر المناعي* المرتبط بالأنزيم المتوفرة تجارياً، أما ما يخص البيانات التي جمعت من كل حالة فقد تم تحليلها إحصائياً باستخدام برنامج حاسوبي إحصائي.

النتائج: أظهرت نتائج هذه الدراسة أن من إجمالي ٢٢٦ حالة كان ٣٦ من الحالات (١١٪) إيجابيين لمستضد الفيروس الغذائي

Introduction :

Adenoviruses are one of the most important etiological agents of serious gastroenteritis among infants and young children less than five years.^{[1],[2],[3]} Subgroup F enteric adenoviruses (EAd) apparently are second only to the rotavirus a viral cause of acute gastroenteritis in infants and young children.^{[2],[4]} According to the demographics, Three to five percent of all respiratory infections and diarrhea in the USA and a higher number in developing countries are due to Adenovirus. Antibodies to these viruses have been detected in approximately 50% of children less than five years of age in Asia, Africa, Europe, and South America with similar seropositive proportions in the different populations and are spread predominantly by the fecal-oral route.^{[3],[4]} EAd have been associated with protracted diarrhea which may contribute to infant dehydration and malnutrition in developing countries.^{[4],[5]} Usually after an incubation period of eight to ten days, periodic diarrhea occurs, with light fever, vomiting, abdominal pains and dehydration.^{[3],[6]} Adenovirus-associated diarrhea does not differ from that caused by other viruses, although the duration of symptoms may last slightly longer (3-11 days). The stools are watery, non-bloody, sometimes with bloody and with no fecal leucocytes.^{[4],[5]} 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.^{[3],[4],[5]} Moreover, it seems that infection with Adenovirus is an important hygienic issue in our country. Identifying the accurate rate of prevalence of this virus is of particular importance, as it will help in the future programming for the correct control and choosing specific treatment methods for the disease in special seasons and the time of adenovirus epidemic. Detection of Adenovirus in fecal specimens is mainly by using ELISA with species F-specific monoclonal

antibodies. The sensitivity of most Adenovirus ELISA compared to cell culture or electron microscope (EM) is >90% and specificity is usually >97%. [7]

The aims of this cross-sectional descriptive study were to determine the prevalence of EAd among infants and children suffering from acute gastroenteritis in Sana'a city and also determine the predisposing factors contributing to the virus transmission and infection.

Subjects and Methods :

This cross sectional study was carried out during a period of one year, starting in June 2008 and ending in May 2009. The study included 326 children who were less than five years of age with acute gastroenteritis attended two public hospitals (Al-Thawrah General Hospital and El-Sabeen Maternity and Childhood Hospital) in Sana'a city, Yemen. A total of 326 stool specimens were collected from children within 48 hours following their hospitalization for acute gastroenteritis in the two public hospitals. Diarrheal stool was collected in a clean - leak - proof, detergent - free and wide-necked container. All specimens were examined for routine analysis and then stored at -20°C for further analysis. All reagents and specimens were brought to room temperature (20-25 °C) before beginning the test. The specimen diluted 1:5 with the stool diluents (one gram stool to four ml of diluted wash buffer). Mixed thoroughly and left the heavy particulates to settle. The diarrheal stools diluted 1:2 with the specimen diluents, phosphate- buffered saline (PBS) (pH 7.2), in a suitable container. This fecal suspension was tested for antigens of Adenovirus using a commercial enzyme-immunoassay (ELISA-Adenovirus) (DRG international, Inc. USA) was used and absorbance of test. Then 100 µl from negative and positive controls were pipetted into different wells and diluted stool specimens were pipetted in subsequent

microwells, coated with a monoclonal antibody against the group specific antigen for all human adenoviruses and after that the wells were incubated at room temperature for 30 minutes. At the end of incubation, the microwells were well washed by the washing buffer (3X) three times. Two drops of Reagent 1, containing anti-adenovirus monoclonal antibodies with blue dye and thimerosal, were added to each well and incubated at room temperature for 5 min. The wells were washed as mentioned before. Then two drops of Reagent 2, containing anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal, were added to each well and incubated at room temperature for five min. The wells were washed. After that two drops chromogen, tetra-methylbenzidine (TMB) and peroxide, were added to each well and incubated at room temperature for five min. Finally two drops of stop solution were added into each well. The plate was mixed thoroughly by tapping strip holder and the result showed visually and the absorbance was measured at 450 nm and 620-650 nm. An absorbance equal to or greater than 0.15 unit was considered positive. The obtained results were analyzed using Epi. Info. version 6.04 statistical programme.

Results :

Adenoviruses were detected in 11 % (36/326) of the total specimens. The mean age of positive EAd subjects was 16.2 months with a SD of 8.8 months. Adenovirus infection occurred with a higher prevalence in males (12.7%: 22/173) than in females (9.2%: 14/153), these results were not statistically significant. Concerning the odd ratio, a male was nearly one and half time more at risk than a female in contracting EAd infection. The highest prevalence of EAd infection was found in the age group 13-24 months with a percentage of 23.9%, followed by the age group 7-12 months, 1-6 months, 25-36 months and 37-48 months with

percentages of 9.5%, 9.3%, 5.4% and 2.5% respectively. As regards the statistical analysis, most above mentioned results were not statistically significant, except for the age group 13-24 months with values of ($\chi^2 = 20.2$, $p = 0.000007$). Concerning the age, the age group 13-24 months was nearly 5 times more at risk than others age groups in contracting EAd infection (Table 1).

Table 1: Distribution of positive EAd subjects according to their age and gender.

Characters	Total (n = 326)		Positive EAd (n = 36)		OR	CI	χ^2	p
	No.	%	No.	%				
Gender								
Male	173	53.1	22	12.7	1.6	0.7- 3.12	1.05	0.3
Female	153	46.9	14	9.2	0.7	0.3-1.5	1.05	0.30
Age/months								
1- 6	54	16.5	5	9.3	0.8	0.3-2.3	0.21	0.064
7-12	63	19.3	16	9.5	0.8	0.3-2.2	0.18	0.66
13-24	88	27.0	12	23.9	4.7	2.2- 10.1	20.2	*0.01
25-36	56	17.2	3	5.4	0.4	0.1-1.4	2.23	0.13
37-48	40	12.3	1	2.5	0.2	0.01- 1.3	3.4	0.06
49-60	25	7.7	0	0.0	0	0-1.5	3.36	0.06
Crude Total	326	100. 0	36	11.0				

* $\chi^2 \geq 3.84$, $p < 0.05$ (Significant), OR >1 (at risk).

The principal manifestation among positive subjects for EAd infection was abdominal pain, followed by vomiting, fever and then dehydration with percentages of 86.1%, 72.2%, 52.8% and finally 41.7% respectively. There was a significant association between EAd infection and the principal manifestation (vomiting, fever and dehydration) ($\chi^2 = 37.13$, $p = 0.01$), ($\chi^2 = 4.4$, $p = 0.03$) and ($\chi^2 = 26.4$, $p = 0.01$) respectively (Table 2).

Table 2: Distribution of positive EAd subjects according to their clinical manifestations.

Clinical manifestations	Total (n = 326)		Positive EAd (n = 36)		χ^2	p
	No.	%	No.	%		
Abdominal pain	253	77.6	31	86.1	1.68	0.19
Vomiting	94	28.8	26	72.2	37.13	*0.01
Fever	120	36.8	19	52.8	4.4	*0.03
Dehydration	45	13.8	15	41.7	26.4	*0.01

* $\chi^2 \geq 3.84$, p< 0.05 (Significant).

Bottle feeding showed the highest positivity for EAd infection, while breast feeding showed the lowest positivity for infection with a percentage of 43.7% (14/32) versus 15.6% (5/32). There was a significant association between breast feeding and adenovirus infection ($\chi^2 = 6.89$, p = 0.008) (Table 3).

Table 3: Distribution of positive EAd subjects according to the type of infant's feeding.

Type of feeding	Total (n = 205)		Positive EAd (n = 32)		χ^2	p
	No.	%	No.	%		
Breast feeding	74	36.1	5	15.6	6.89	*0.01
Bottle feeding	76	37.1	14	43.7	0.72	0.39
Mixed feeding	55	26.8	13	40.7	3.7	0.05
Total	205	100.0	32	100.0		

* $\chi^2 \geq 3.84$, p< 0.05 (Significant)

Discussion :

The crude prevalence of EAd infection among the studied subjects was 11%, and this indicates that EAd infection play a role as one of the main gastroenteritis causes among infants and young children in Sana'a city, Yemen, this prevalence was nearly similar to that reported in Sweden in 1984, China in 2001 and Hungary in 2003, which were 12%, 10.8% and 11.7% respectively.^{[5],[8],[9]} In

contrast, a higher prevalence of EAd infection was reported in other studies conducted in some developing countries as Guatemala in 1990, Nigeria in 2002 and 2007 and Saudi Arabia in 2007, which were 22.2%, 17%, 22.3% and 15.5% respectively. [10],[11],[12],[13] On other hand, a lower prevalence was reported for EAd infection in some developed countries as USA in 1985, France in 1999 and Germany in 2003, which were 6.9%, 3.1% and 8.3% respectively. [14],[15],[16] The difference between these results could be attributed to the; age of subjects, the socioeconomic status, geographic location of the study area, hygiene, breast-feeding, cultural habits, climate, duration of the study and the methods used in detecting the causative agent antigen. [12],[17],[18],[19]

The prevalence of EAd infection was slightly higher among males with a percentage of 12.7% versus 9.2% for females and the odd ratio was one and half time more at risk for a male than a female. Similarly, the prevalence for EAd infection was also higher in males than females as reported in Taiwan in 2000. [18] In contrast, the prevalence of EAd in females was significantly higher than in males as stated in Iran in 2007. [20] In addition, another study stated an equal rate of infection for both sexes. [19]

In the present study, there was a significant increased prevalence for EAd infection in the age group 13-24 months which was 23.9% with an odd ratio of nearly five times more at risk than other age groups and the rate decreased sharply with older ages as seen in table 1. These results were much lower than that documented in Taiwan and Iran among infants below the age of 12 months which were 85% and 81% respectively. [18],[19] But the predominant of EAd infections among the age group 13-24 months agreed with that reported in Bangladesh, 1993. [17] In endemic developing countries in general, EAd affects predominantly infants aged 6-12 months old. [17],[19] This reason may be attributed to that most diarrheal

illness occurred in the first two years of life of infants, especially in developing countries, where malnutrition among mothers predominate the use of bottle feeding rather than breast feeding which could be a source of infection when the milk is prepared with contaminated water or under poor hygienic conditions, in addition as known, this type of feeding will not develop or improve the immune system of these infants, thus they are usually exposed to many diseases during that period of their life.

The EAd infection is a disease with specific manifestation occurring in infants and young children and affecting mostly the gastrointestinal tract. ^{[18],[19]} In this study, the principal manifestation among positive subjects for EAd infection was abdominal pain with a percentage of 86.1%, but without a statistically significant association. This result was nearly similar to that reported in Bangladesh and Iran which were 76.4% and 86.4% respectively. ^{[17],[19]} In contrast, lower results were reported in France and Saudi Arabia which were 24.2% and 20.8% respectively. ^{[13],[15]} In addition, vomiting was the second more common manifestation among positive subjects for EAd infection with a percentage of 72.2% and this result was highly statistically significant with a value of ($\chi^2 = 37.13$, $p= 0.01$). Slightly higher results were reported in Iran and Saudi Arabia which were 86.8% and 85.2% respectively. ^{[15],[19]} In contrast, lower results were documented in USA and Taiwan which were 52% and 42% respectively ^[18, 21]. Fever was accompanied with 52.8% of positive subjects for EAd infection and this result was statistically significant with a value of ($\chi^2 = 4.4$, $p= 0.03$). A nearly similar result was reported in Denmark which represented 58% of positive subjects for EAd infection. ^[22] In contrast, a higher result was reported in Iran, where fever accounted for 87.7% of positive subjects for EAd infection. ^[19] The presence of fever among

positive EAd subjects indicate the occurrence of viremia in these patients in general. [22] Finally, dehydration represented 41.7% among positive subjects for EAd infection and this result was highly statistically significant with a value of ($\chi^2 = 26.4$, $p= 0.01$) as seen in table 2. This result was nearly similar to that reported in Nigeria, where dehydration was found in 49% of positive cases for EAd infection. [12] A higher result was reported in Iran, where dehydration occurred in 88%. [19] In contrast, a lower result was reported in Saudi Arabia, where only 23% of the cases showed dehydration. [13] Thus, dehydration is considered as an important manifestation of acute diarrhea due to EAd infection.

In this study, the prevalence of the EAd among breast feeding was 15.6% for infants up to 2 years of age as seen in table 3, this result was statistically significant with value of ($\chi^2 = 6.89$, $p = 0.01$). In addition, this result was lower than the prevalence of bottle feeding and mixed feeding with percentages of 43.7% and 40.7% respectively. This finding was nearly similar to that reported in Iran in 2006, where the prevalence of breast- feeding and bottle-feeding were 19.2% and 47.8% respectively for infants up to nine months of age. [19] The low prevalent rate of EAd infection among breast-fed infants explained the acquiring of passive immunity from their mothers. [19]

Conclusion :

This study revealed that adenovirus is one of the most important etiological agents of acute gastroenteritis among infants and young children less than five years in Sana'a city, Yemen. However, the impact of these viruses in Yemen is yet to be fully studied and determined.

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